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Temperature dysregulation during therapeutic hypothermia predicts long-term outcome in neonates with HIE

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Abstract

Few reliable or easily obtainable biomarkers to predict long-term outcome in infants with hypoxic-ischemic encephalopathy (HIE) have been identified. We previously showed that mattress temperature (MT), as proxy for disturbed temperature regulation during therapeutic hypothermia (TH), predicts injury on early MRI and holds promise as physiologic biomarker. To determine whether MT in neonates treated with TH for moderate-severe HIE is associated with long-term outcome at 18–22 months, we performed a secondary analysis of the Optimizing Cooling trial using MT data from 167 infants treated at a core temperature of 33.5°C. Median MTs from four time-epochs (0–6 h, 6–24 h, 24–48 h, and 48–72 h of TH) were used to predict death or moderate-severe neurodevelopmental impairment (NDI), using epoch-specific derived and validated MT cutoffs. Median MT of infants who died or survived with NDI was consistently 1.5–3.0°C higher throughout TH. Infants requiring a median MT above the derived cut-offs had a significantly increased odds of death or NDI, most notably at 0–6 h (aOR 17.0, 95%CI 4.3–67.4). By contrast, infants who remained below cut-offs across all epochs had 100% NDI-free survival. MTs in neonates with moderate-severe HIE during TH are highly predictive of long-term outcome and can be used as physiologic biomarker.

Keywords

Hypoxic-ischemic encephalopathy, therapeutic hypothermia, thermoregulation, biomarker, neurodevelopmental outcome

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Introduction

Hypoxic-ischemic encephalopathy (HIE) is the most common cause of acute neonatal encephalopathy, affecting 1–4 per 1,000 live births in high resource countries.[1,2] Therapeutic hypothermia (TH, 72 h at 33.5°C), initiated within 6 hours of birth, remains the only therapy that improves outcomes in infants with moderate-severe HIE.[3–8] Predicting the severity of HIE injury and its impact on outcomes is difficult, particularly prior to completion of TH treatment. Due to the poor prognostic performance of traditional clinical markers such as Apgar score and day of life 1 Sarnat exam or Thompson score, significant research has been conducted to identify markers of injury with predictive value on outcome.[9–11] A multitude of candidates have been identified, but so far no single easily obtainable and interpretable marker has been consistently reliable.[12–16] To date, electroencephalography (EEG) background pattern has been the most reliable marker for long-term outcome. In particular,

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correlation with a normal outcome is high when the EEG background is normal within the first six hours and stays normal.[17] Similarly, highly abnormal resistive indices or cerebral blood flow measured on transcranial doppler prior to initiation of hypothermia may be able to predict death or severe disability.[18] However, both EEG and transcranial doppler technology still require expensive additional equipment and trained staff to read and accurately interpret the recordings.

Asphyxiated newborns and uncooled infants with moderate-severe HIE demonstrate disturbed temperature regulation.[19,20] Previously, our group used the cooling device water output temperature, from now on referred to as mattress temperature (MT), during servo-controlled cooling as a proxy for impaired thermoregulation and found an association with increased MRI injury and unfavorable short-term outcome, concluding that thermoregulatory assessment offers promise as a physiologic biomarker for predicting outcome by reflecting severity of injury.[21] Based on our prior work, we hypothesized that infants with a worse long-term outcome would show a greater degree of temperature dysregulation and therefore require a higher MT (less active cooling) during TH to maintain a core temperature around 33.5°C. We also postulated that MT cut-offs could be derived to predict long-term outcome as early as 6 hours into the treatment course.

Methods

We performed a secondary analysis of data from infants enrolled in the Optimizing Cooling (OC) trial, which randomized 364 infants with moderate-severe HIE to variations in length (72 vs 120 hours) and depth (33.5°C vs 32.0°C) of TH. We obtained written permission from the Data and Specimen Hub (DASH) of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) to obtain and use the publicly available data from the OC trial for re-analysis and publication. The institutional review boards at the University of Washington (UW) and Children's Hospital of Philadelphia (CHOP) determined that the study protocol (UW IRB ID: STUDY00011104; CHOP IRB 21-018646) was exempt from review.

We analyzed the first 72 hours of temperature data from infants randomized to a target esophageal core temperature of 33.5°C for either 72 hours (n = 95) or 120 hours (n = 96). Infants treated at 32°C were excluded. Infants were cooled using either the Blanketrol II or the Blanketrol III device (Cincinnati Sub-Zero Product LLC, Gentherm, Cincinnati, OH). Mattress and esophageal core temperatures were recorded every 15 minutes for the first 4 hours of cooling, hourly for the next 8 hours, and every 4 hours for the remaining duration of TH.

To understand the relationship between MT and injury most accurately, outcomes were analyzed by the intervention provided rather than by intention-to-treat. For instance, if a subject was randomized to 32.0°C but treatment occurred at 33.5°C, the subject was included in this study and vice versa. Subjects were excluded if they were treated with extracorporeal membrane oxygenation (ECMO), if less than 50% of the expected temperature data points were available, if they were re-warmed prior to 72 hours of TH, or if no outcome data were available.

HIE severity based on the Sarnat examination at randomization was re-scored according to contemporary criteria. The Sarnat exam used at the time this trial was conducted considered hypotonia and hypertonia equally as signs of moderate encephalopathy; however, further evolution of the Sarnat Exam now classifies hypertonia as a feature of mild encephalopathy.[22] The dataset was therefore adjusted accordingly to reflect the more contemporary descriptions of the Sarnat stages for mild, moderate, and severe encephalopathy. Two subjects were adjusted from severe to moderate, 6 from moderate to severe, and 6 from moderate to mild based on the data provided.

An organ dysfunction score was also derived to examine evidence of peripheral injury that might confound thermoregulation and outcome. End-organ involvement as well as documented clinical and/or EEG seizures were abstracted from the original dataset. The following parameters were included: cardiac dysfunction, defined as diagnosis by echocardiography and/or hypotension, respiratory failure requiring mechanical ventilation or continuous positive pressure ventilation, disseminated intravascular coagulopathy and/or thrombocytopenia, renal dysfunction defined as oliguria and/or anuria, hypoglycemia defined as blood glucose <50 mg/dL, and hepatic dysfunction defined as alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) >100 IU/L; end-organ involvement was categorized from 0–6 based on the number of systems involved.

Our primary outcome was the composite of death or moderate-severe neurodevelopmental impairment (NDI) at 18–22 months, as defined in the original trial (supplemental Table s1). Detailed study descriptions of the OC trial have been published previously.[23,24]

Statistical analysis

Descriptive statistics were used to characterize the selected cohort compared to the original trial cohort, including maternal and infant factors as well as

incidence of the primary outcome. For ultimate statistical inference, generalized estimating equations (GEE) with robust standard errors were used to appropriately account for potential clustering of outcomes by study site, as performed in the original trial outcome analyses.[24]

Graphical illustrations were used to display MTs over time for the included cohort, stratified by primary and secondary outcomes (death and NDI separately). Average MT over time by outcome group was depicted graphically using the LOESS (locally estimated scatterplot smoothing) method. Epochs of TH were determined based on visual examination of MT trajectories, and median MT was derived for each infant within each epoch. Infants who had <50% of the expected temperature measurements within an epoch recorded were not included in the analyses for that epoch (n = 4–6 infants excluded per epoch).

As multiple clinical variables could potentially confound the relationship between MT and outcome, medication exposure and severity of illness parameters were compared by outcome group (NDI-free survival vs moderate or severe NDI vs death; Table 2). Medication exposure and severity of illness parameters that were significantly different across groups were included as covariates in later models examining MT by outcome as well as to predict outcome using MT. As a result, all GEE models examining MT and/or outcome groups were adjusted for TH treatment duration (72 vs 120 hours), severity of encephalopathy at enrollment (severe vs moderate), documented seizures, exposure to phenobarbital, morphine, or inotropes (yes vs no), and degree of end-organ dysfunction on a 0–6 continuous scale.

Within each epoch, differences in mean adjusted MT (with 95% confidence interval, CI) by outcome were determined using GEE linear regression models. To determine predictive MT cut-offs within each epoch, a modified 5-fold cross-validation approach was used. The data were randomly split into five folds such that each infant appeared in four training folds and one validation fold. Within each epoch, MT cut-offs were determined using the cutpointr library in R, optimizing for the sum of sensitivity and specificity to predict primary outcome (death or NDI) in unadjusted models. The sensitivity and specificity of the cut-off in the validation fold was then examined. To select a final cut-off for each epoch for outcome prediction, the cut-off identified in the most folds was selected. In the 24–48 h epoch, two different MT cut-offs were selected in the same number of training folds (n = 2 each), so the cut-off that appeared to have higher sensitivity for predicting the primary outcome in the validation folds was selected. Using GEE logistic regression models, adjusted odds ratios (aOR) with 95% confidence intervals (CI) for primary outcome based on being above the derived cut-off within an epoch were derived in the validation datasets.

Finally, exploratory analyses were then performed using a combination of the derived cut-offs across the entire dataset. Infants were combined into trajectory groups based on whether they were above or below cut-offs within multiple epochs, and aORs for the primary outcome were determined within those trajectory groups for both the cohort overall and those surviving to discharge, as well as when split by severity of encephalopathy. A sensitivity analysis was performed by applying the same trajectory analyses to the entire cohort of 191 infants cooled to 33.5°C as presented in the OC dataset in an intention-to-treat manner without further selection or adjustment of participants based on reassessment of severity of encephalopathy or availability of MT data.

Analyses were conducted in RStudio using the R statistical package (Version 4.1.2, Foundation for Statistical Computing, Vienna, Austria).[25] P-values <0.05 were considered significant.

Results

Patient characteristics

Characteristics of the 167 included infants are displayed in Table 1, including comparison to the overall cohort of the original trial. Final cohort selection information and CONSORT diagram are shown in Figure 1. Of the 364 patients enrolled in the OC trial, 191 were randomized to receive TH to a target core temperature of 33.5°C. After applying our inclusion and exclusion criteria and excluding a further 6 subjects with mild encephalopathy according to contemporary Sarnat staging, 167 infants remained for analysis (Figure 1). The infants included in our analysis appeared to closely reflect the overall OC cohort; the only detectable difference between the final sub-cohort and the original OC cohort was a more balanced distribution of infant sex (51.5% vs 58.2% males, p = 0.001) and a slightly lower incidence of histologic chorioamnionitis (12.6% vs 17.6%, p = 0.02).

Primary analyses by dichotomous primary outcome

Mattress temperature over time by primary outcome

Of the included infants, n = 55 (33%) either died (n = 23, 14%) or survived with NDI (n = 32, 19%). Of the 23 deaths, 20 (87%) occurred during the initial hospitalization at a median age of 134 hours (IQR 89, 208), and 3 (13%) died after discharge. Infants with the

Table 1. Demographics of included infants compared to all infants in the original trial cohort.

	n (%) / median (IQR)		
	Current cohort	Original trial cohort	p-Value
Total number of included infants	**167**	**364**	
Maternal characteristics			
Age	29.0 (23.0–33.5)	28.0 (22.0–33.0)	0.15
Pregnancy complications			
Diabetes	20 (12.0)	46 (12.6)	0.54
Hypertension/preeclampsia	31 (18.6)	73 (20.1)	0.55
Mode of delivery			
Vaginal delivery	54 (32.3)	117 (32.1)	0.93
Cesarean section	113 (67.7)	247 (67.9)	
Labor complications			
Decelerations/loss of fetal heart tones	135 (80.8)	282 (77.5)	0.07
Cord accident	26 (15.6)	49 (13.5)	0.24
Antepartum hemorrhage	20 (12.0)	40 (11.0)	0.45
Maternal hemorrhage	29 (17.4)	55 (15.1)	0.16
Shoulder dystocia	11 (6.6)	29 (8.0)	0.18
Clinical chorioamnionitis	11 (6.6)	28 (7.7)	0.40
Placental problems	38 (22.8)	80 (22.0)	0.67
Uterine rupture	8 (4.8)	22 (6.0)	0.23
Placenta			
Placenta pathology performed	80 (47.9)	188 (51.6)	0.07
Histologic chorioamnionitis	21 (12.6)	64 (17.6)	0.02*
Infant characteristics			
Inborn	56 (33.5)	130 (35.7)	0.18
Gestational age (weeks)	39 (37–40)	39 (38–40)	0.84
Male sex	86 (51.5)	212 (58.2)	0.001*
Birth weight (grams)	3,270 (2,891–3,788)	3,310 (2,949–3,784)	0.33
Apgar			
1 minutes	1 (0–2)	1 (0–2)	0.47
5 minutes	2 (1–3)	2 (1–3)	0.33
10 minutes	4 (2–6)	4 (3–6)	0.46
Resuscitation			
Positive pressure ventilation	161 (96.4)	343 (94.2)	0.15
Intubation	132 (79.0)	286 (78.6)	0.68
Chest compressions	75 (44.9)	160 (44.0)	0.71
Continued resuscitation at 10 min	146 (87.4)	315 (86.5)	0.71
Cord blood			
pH	6.9 (6.8–7.0)	6.9 (6.8–7.1)	0.24
Base deficit	16 (11–20)	16 (11–20)	0.83
Cord or first blood gas within 1 hour of life			
pH $\leq$7.0	118 (70.7)	264 (72.5)	0.23
Base deficit $\geq$16	113 (67.7)	255 (70.1)	0.28
Sarnat stage at enrollment			
Moderate	133 (79.6)	280 (76.9)	0.15
Severe	34 (20.4)	84 (23.1)	
Clinical seizures present at randomization	53 (31.7)	105 (28.8)	0.11
Infant sedated during qualifying exam	49 (29.3)	98 (26.9)	0.48
Age cooling started (hours)	4.1 (3.1–5.0)	4.1 (3.1–5.0)	0.71
Outcomes			
Survival to discharge	147 (88.0)	315 (86.5)	0.40
Outcomes at 18–22 months			
Death	23 (13.8)	56 (15.4)	0.33
Moderate-severe disability	32 (19.2)	54 (14.8)	0.20
Disability-free survival	112 (67.1)	237 (65.1)	0.72

*Indicates a significant difference between cohorts. IQR: interquartile range.

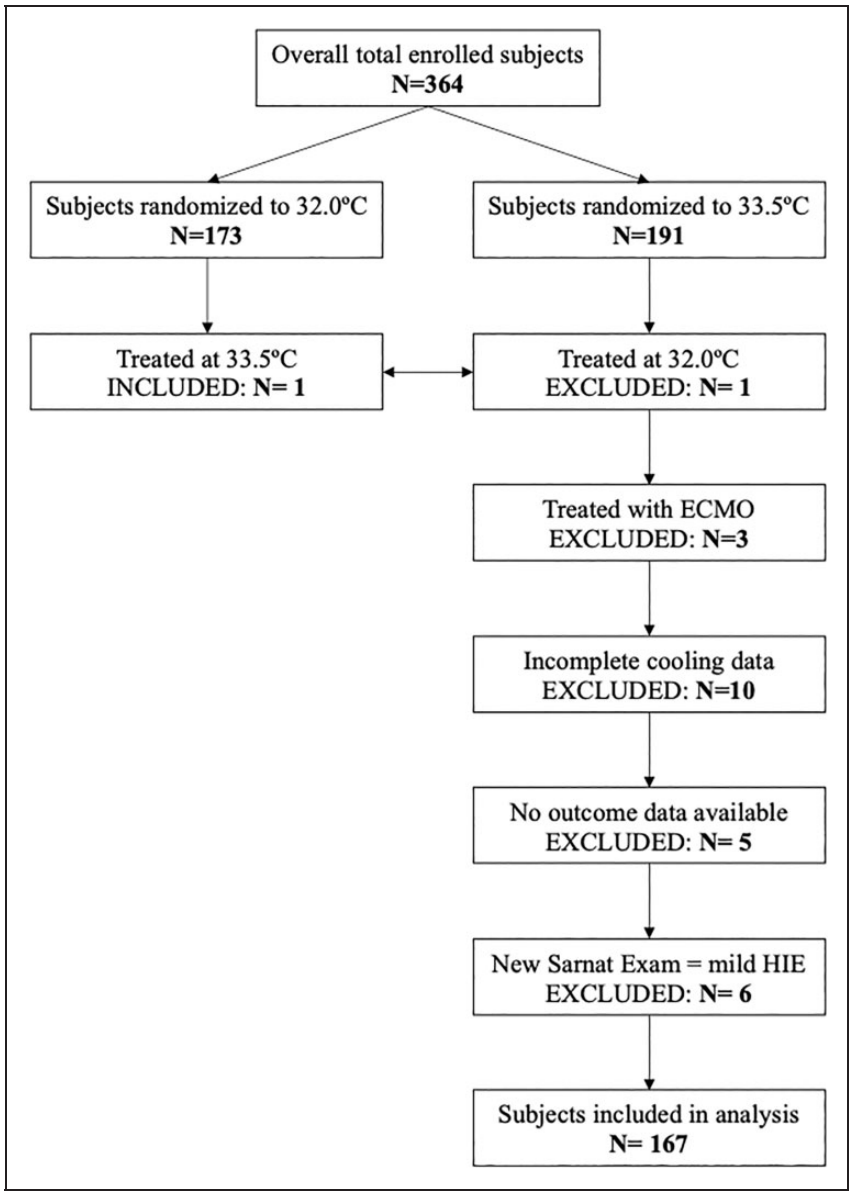

Figure 1. Patient inclusion and exclusion criteria. One subject was not treated as intended (therapeutic hypothermia at 32.0°C instead of 33.5°C) and excluded from this analysis, and one subject from the 32.0°C group was treated at 33.5°C and was included. ECMO = extracorporeal membrane oxygenation, HIE: hypoxic-ischemic encephalopathy.

composite primary outcome of death or NDI required a significantly higher MT to maintain a core body temperature of 33.5°C compared to infants who survived without NDI. Visual examination of MTs over time showed that average MT trajectories during TH followed a similar pattern with three distinct phases: 1) an increase over the first 6–12 h, 2) a decrease in the period around 12–30 h, and 3) a slow increase over time until 72 h (Figure 2). The average MT of infants who died or survived with NDI was consistently 2–3°C higher throughout the entire 72 h of TH (Figure 2(a)). While infants who died followed a similar trajectory as those who survived with NDI for the first 6 h of cooling, after 24 h the MT of those who died remained approximately 1°C higher compared to neonates who survived with NDI (Figure 2(b)).

Selection of independent confounding outcome predictors

Based on the temporal changes in average MT that were seen and the expected physiologic changes occurring in HIE, the 72 h of TH were divided into four distinct epochs. A median MT was determined for each infant within each TH epoch: 0–6 h, 6–24 h, 24–48 h, and 48–72 h. Infants who died or survived with NDI had

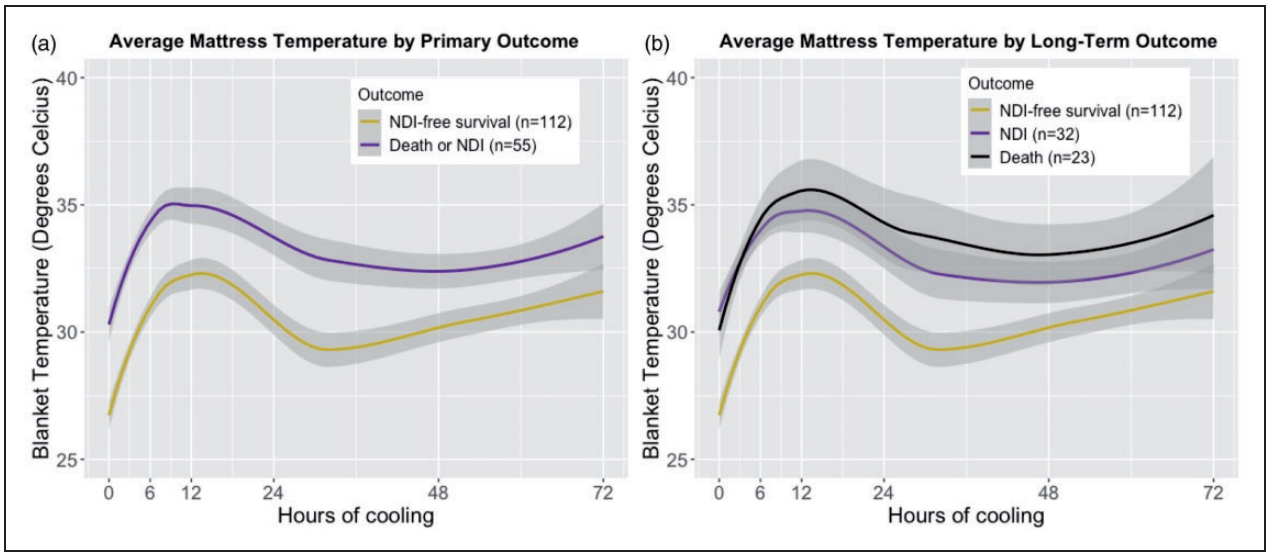

Figure 2. Average mattress temperature with 95% CI by combined primary outcome death or neurodevelopmental impairment (a) and split primary outcome (b) over the first 72 h of cooling, determined using the LOESS (locally estimated scatterplot smoothing) method.

significantly lower esophageal temperatures at initiation of TH as well as higher average MTs across all epochs. They were also more likely to have severe encephalopathy at randomization, documented seizures, evidence of end-organ dysfunction, and a higher likelihood of being exposed to anticonvulsant medications, particularly phenobarbital, inotropes, and morphine (Table 2). As many of these clinical factors may confound the association between MT and outcome, later analyses were adjusted for severity of encephalopathy, organ dysfunction score, documented seizures, exposure to phenobarbital, morphine, and inotropes, and treatment duration of TH.

Mattress temperature by outcome across cooling time epochs

Among infants who died or survived with NDI, adjusted average MTs were significantly higher across all epochs compared to the NDI-free survival group (supplemental Table s2). In the 0–6 h period, average adjusted MT was 3.4°C higher in the death/NDI group (95% CI 1.5–5.3°C, $p < 0.001$), and remained 1.5–2.1°C higher in the later epochs. Full model outputs for adjusted average MT within each epoch including the coefficients for all covariates are shown in supplemental Table s2.

Secondary analyses by temperature trajectories and outcomes

Epoch-specific cut-offs for outcome prediction

Epoch-specific final optimal MT cut-offs including the sensitivity and specificity for predicting outcome in the validation fold is shown in Table 3. In the 24–48 h epoch, two different MT cut-offs were selected in the same number of training folds, so the cut-off of ≥31.0°C, which had the higher sensitivity for predicting outcome in the associated validation fold (80% and 85% vs 29% and 45%), was selected. The final cut-offs for each epoch were ≥33.0°C (0–6 h), >31.0°C (6–24 h), >31.0°C (24–48 h), and ≥32.0°C (48–72 h). In fully adjusted models using the entire cohort, having a median MT above the selected cut-off in the 0–6 h, 24–48 h, and 48–72 h epochs was associated with a significantly increased aOR (95% CI) for death or NDI; 0–6 h: 17.0 (4.3–67.4); 24–48 h: 3.1 (1.3–7.5), and 48–72 h: 3.3 (3.3–8.4) (Table 4). Full model outputs including aORs for all variables in each model are displayed in supplemental Table s3.

Trajectories of mattress temperature over time and long-term outcome

In an exploratory attempt to predict outcome based on MT trajectories, we considered whether being above or below target cut-offs at multiple time points might provide greater granularity in outcome prediction. Infants were thus categorized based on being above or below the cut-offs within the 0–6 h, 24–48 h, and 48–72 h epochs. Infants were classified into four groups based on whether they were: 1) below the cut-off at all three time points (n = 28), 2) below the cut-off at 0–6 h but above one or both of the 24–48 h and 48–72 h cut-offs (n = 29), 3) above the 0–6 h cut-off but below at least one of the 24–48 h or 48–72 h cut-offs (n = 48), and 4) above all three cut-offs (n = 58) (Figure 3(a)). Those in Group 1 had 100% NDI-free survival, with increasing

Table 2. Temperature, encephalopathy, and organ dysfunction by outcome.

Outcome group (n=)	n (% of available data)/Median (IQR)		p-value vs no NDI
	NDI-free survival (n = 112)	Death/NDI (n = 55)	
Encephalopathy parameters			
Severe Sarnat at randomization	16 (14.3)	**23 (41.8)**	**0.0001**
Documented seizures	46 (41.4)	**40 (72.7)**	**0.0001**
Esophageal temperature at TH initiation (°C)	34.5 (33.5–36.1)	**33.5 (32.7–36.0)**	**0.007**
Anticonvulsant medications			
Phenobarbital	39 (34.8)	**37 (67.3)**	**0.0002**
Lorazepam (as anticonvulsant)	14 (12.5)	8 (14.5)	0.70
Levetiracetam	6 (5.4)	7 (12.7)	0.11
Other anticonvulsant	8 (7.1)	6 (10.9)	0.39
Analgesic medications			
Morphine	39 (34.8)	**33 (60.0)**	**0.002**
Fentanyl	21 (18.8)	10 (18.2)	0.90
Midazolam	33 (29.5)	15 (27.3)	0.77
Other analgesic	15 (13.4)	9 (16.4)	0.62
Inotrope exposure	45 (40.2)	**33 (60.0)**	**0.016**
Median mattress temperature by epoch			
0–6 h	33.0 (29.0–35.0)	**34.5 (33.8–36.0)**	**<0.0001**
6–24 h	32.0 (30.0–34.5)	**33.5 (32.0–36.2)**	**<0.0001**
24–48 h	30.5 (28.0–32.5)	**33.0 (31.0–34.5)**	**<0.0001**
48–72 h	32.0 (29.0–34.0)	**33.0 (32.0–34.5)**	**0.002**
Organ involvement			
Oliguria/anuria	47 (42.0)	**44 (80.0)**	**<0.0001**
Liver dysfunction	69 (61.6)	**43 (78.2)**	**0.038**
DIC	38 (33.9)	**31 (56.4)**	**0.005**
Hypoglycemia	30 (26.8)	20 (36.4)	0.17
Hypotension	36 (32.1)	26 (47.3)	0.056
Respiratory support	96 (85.7)	**54 (98.2)**	**0.025**
Total organ involvement score	3 (2–4)	**4 (3–5)**	**<0.0001**
Sarnat after rewarming			
Mild	72 (66.1)	**6 (10.9)**	**<0.0001**
Moderate	34 (31.2)	**26 (47.3)**	
Severe	3 (2.8)	**15 (27.3)**	
Missing/Died[a]	3 (2.8)	8 (14.5)	–

Comparison of average mattress temperatures (MT) by epoch of therapeutic hypothermia (TH) as well as evidence of encephalopathy and organ dysfunction by long-term outcome. Infants who died or survived with moderate-severe neurodevelopmental impairment (NDI) were more likely to have severe encephalopathy at randomization, documented seizures, evidence of end-organ dysfunction, a higher likelihood of being exposed to anticonvulsant medications, particularly phenobarbital, as well as morphine and inotropes. Infants who died or survived with NDI also had significantly higher average MTs across all time epochs, though only a trend was seen between survivors at 48–72 h. Bolded values indicate p-value <0.05 compared to the NDI-free survival group after adjustment for duration of cooling, where those that survived with NDI or died were considered as separate factors within the same regression models. Binary outcomes were compared with generalized estimated equations (GEE) logistic regression, and continuous outcomes by GEE linear regression. All comparisons are unadjusted except for TH treatment duration.

[a]Sarnat exam was not documented after rewarming in 11 infants: 3 without NDI, 2 with NDI, and 5 who died. MT: mattress temperature; IQR: interquartile range; NDI: neurodevelopmental impairment; ALT: alanine aminotransferase; AST: aspartate aminotransferase; DIC: disseminated intravascular coagulopathy.

risk of death or NDI across each subsequent group (Figure 3(b)). Using Group 4 as the reference, Group 1 had a significantly lower aOR for death or NDI (0.0, 95% CI 0.0–0.0) as did Group 2 (0.06; 0.01–0.38), with a non-significantly decreased aOR in Group 3 (0.45; 0.18–1.15).

Similar results were seen in the 146 infants surviving to discharge (Figure 3(c)). Three infants died after discharge, all of whom were in Group 4, meaning that they were all above all three MT cut-offs. When examining trajectory group and outcome by level of encephalopathy (Figure 3(d) to (e)), infants who were below all three cut-points had 100% NDI-free survival regardless of level of encephalopathy. The ability of trajectory group to separately predict outcome in infants with moderate encephalopathy (n = 129, Figure 3(d)) and severe encephalopathy (n = 37, Figure 3(e)) mirrored that of the cohort as a whole.

Table 3. Epoch-specific cut-ff cross-validation.

	0–6 h			6–24 h			24–48 h			48–72 h		
Fold	Cut-off (80% Training)	Sens (20% Validation)	Spec	Cut-off (80% Training)	Sens (20% Validation)	Spec	Cut-off (80% Training)	Sens (20% Validation)	Spec	Cut-off (80% Training)	Sens (20% Validation)	Spec
1	33.0°C	92%	50%	31.0°C	75%	27%	32.5°C	45%	59%	32.0°C	64%	50%
2	33.0°C	83%	30%	35.0°C	25%	76%	29.0°C	83%	24%	32.0°C	64%	36%
3	33.0°C	88%	45%	31.0°C	100%	29%	32.5°C	29%	75%	31.5°C	100%	45%
4	33.0°C	100%	57%	31.0°C	100%	38%	31.0°C	80%	57%	32.0°C	90%	57%
5	33.0°C	100%	58%	31.0°C	100%	0%	31.0°C	85%	50%	31.5°C	85%	38%
	Cut-off	**Sens**	**Spec**	**Cut-off**	**Sens**	**Spec**	**Cut-off**	**Sens**	**Spec**	**Cut-off**	**Sens**	**Spec**
Overall	33.0°C	93%	49%	31.0°C	91%	30%	31.0°C	77%	54%	32.0°C	79%	48%

Epoch-specific cut-offs to predict the primary outcome were developed using 5-fold cross-validation. The data were randomly split into five folds such that each infant for whom at least 50% of expected MTs were available in that epoch appeared in four training folds and one validation fold. Within each fold in each epoch, average MT cut-offs were selected optimizing for the sum of sensitivity and specificity to predict outcome in unadjusted models. For each of the five training folds, the optimal MT cut-off and the sensitivity and specificity for predicting outcome in the validation fold is shown. To select a final cut-off for each epoch for outcome prediction, the cut-off identified in the most folds was selected. In the 24–48 h epoch two different MT cut-offs were selected in the same number of training folds ($\geq$32.5°C and $\geq$31°C, n = 2 each), so the cut-off of $\geq$31°C was selected as it had a higher sensitivity for predicting the primary outcome in the associated validation folds. Overall sensitivity and specificity for the cut-offs across the entire cohort are also shown.

Table 4. Epoch-specific mattress temperature (MT) cut-offs to predict primary outcome.

	Optimal cut-off	Infants above cut-point (all data)	
Time epoch	Temperature (°C)	n = (%)	OR for death or NDI (95% CI)
0–6 h	$\geq$33.0	107 (65.2)	**17.0 (4.3–67.4)**
6–24 h	$\geq$31.0	104 (77.0)	3.3 (0.9–12.3)
24–48 h	$\geq$31.0	92 (56.4)	**3.1 (1.3–7.5)**
48–72 h	$\geq$32.0	99 (60.7)	**3.3 (1.3–8.4)**

Epoch-specific cut-offs of average MT to predict primary outcome were determined within each epoch separately due to the fluctuating nature of average MT over time (Figure 2). Cut-offs were determined using the cutpointr library in R with five-fold cross validation. Adjusted odds ratios (aOR) with 95% CI are provided for the whole dataset after adjusting for severity of encephalopathy at enrollment, length of TH treatment, exposure to phenobarbital, morphine, or inotropes, and degree of organ dysfunction. aORs with the confidence intervals that does not cross 1 are highlighted in bold. NDI: neurodevelopmental impairment.

A sensitivity analysis including all 191 infants treated at 33.5°C, adjusting for level of encephalopathy in the original trial documentation, did not alter the results (supplemental Figure s1): using Group 4 as the reference, 100% NDI-free survival was seen in Group 1, a significant 92% reduction in odds of death/NDI was seen in group 2 (aOR 0.08; 95% CI 0.01–0.44), and a non-significant 56% reduction in odds of death/NDI was seen in Group 3 (aOR 0.44; 95% CI 0.17–1.13).

Full model outputs including coefficients for all covariates from the results presented in Figure 3 and supplemental Figure s1 are presented in supplemental Table s4.

Discussion

This secondary analysis of the OC trial adds further evidence to the emerging literature supporting temperature dysregulation as a strong biomarker of outcomes for newborns with HIE. Infants with a combined outcome of death or moderate-severe NDI required less active cooling and therefore a significantly higher MT during TH compared to those who survived without NDI. This association was detectable and strongly predictive of outcome in fully adjusted multivariable models within the first 6 h of cooling. Infants who required a MT of 33.0°C or higher during the first 6 h of TH had an aOR of 17.0 for death or moderate-severe NDI even after adjusting for severity of overall illness including additional end-organ involvement, highlighting that MT may provide a novel biomarker that is strongly predictive of outcome without requiring any additional measurements or sample collection. Importantly, however, it must be acknowledged that this secondary analysis of a convenience dataset using older cooling technologies is not intended to provide a

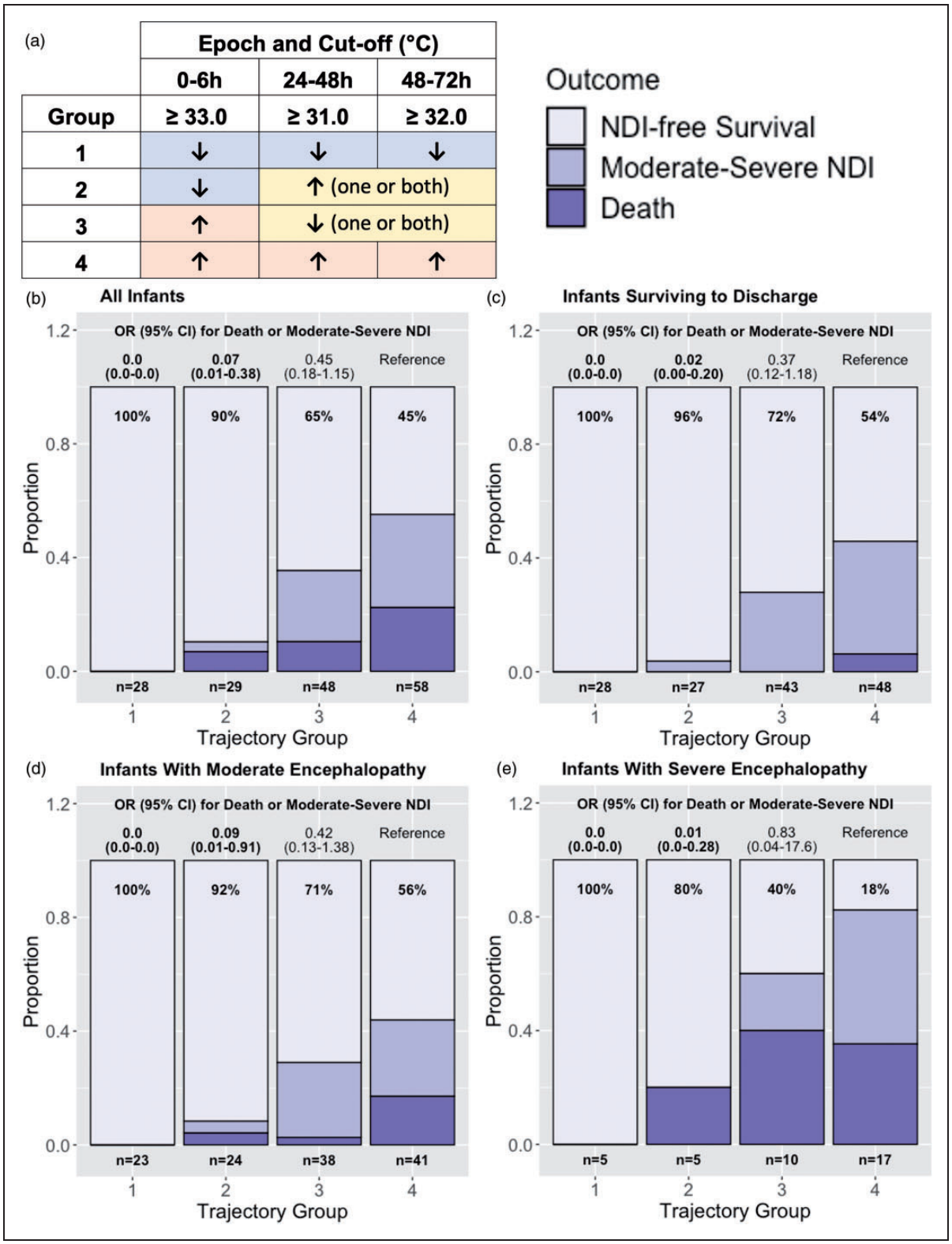

Figure 3. (a) Infants were classified into four groups: 1) those that were below the cut-off at all three time points, 2) below the cut-off at 0–6 h but above one or both the 24–48 h and 48–72 h epochs, 3) above the 0–6 h cut-off but below at least one of the 24–48 h or 48–72 h cut-offs, and 4) above all three cut-offs. Adjusted odds ratio (aOR) for death or moderate-severe neurodevelopmental impairment (NDI) by 0–6 h, 24–48 h, and 48–72 h temperature cut-offs in all infants (b), in those surviving to discharge (c), and separately in those with moderate (d) and severe (e) encephalopathy. Percentages indicate those with NDI-free survival within each

Continued.

comment on the exact MT cut-offs that may predict outcome in all infants undergoing TH in modern NICUs. Instead, it provides greater support for the development of thermoregulatory biomarkers using modern equipment in future appropriately designed clinical trials.

We have previously shown in a smaller cohort that MT during TH correlates with MRI injury and short-term outcome; however, correlation with standardized long-term outcome was not available.[21] Instead, the Weeke MRI injury score, which correlates with neurodevelopmental outcome at 2 years of age, was used as a surrogate.[21,26,27] To validate our findings, we applied the same hypothesis to a larger dataset with standardized long-term outcome data and were able to confirm our initial findings. We then examined the cohort with an unfavorable outcome in more detail to better understand whether the higher MT trajectory was mainly driven by infants who died. Our analysis showed that while infants who died required the highest MTs, newborns who survived with moderate-severe NDI also required significantly higher MTs compared to those who survived NDI-free. Similarly, when predicting death or NDI in infants with moderate or severe encephalopathy separately, MT temperature trajectory remained predictive of outcome regardless of grade of encephalopathy, particularly in those whose MT remained below the cut-offs throughout the entire 72 h period who had 100% NDI-free survival.

There is strong biologic plausibility that temperature dysregulation reflects the degree of brain injury and thus by proxy could predict later outcome, as thermoregulatory circuits are located adjacent to the central deep grey matter, an area that is commonly affected in severe HIE.[28–30] In human infants with perinatal asphyxia, a spontaneous decrease in core temperature within a few hours after birth followed by a slow recovery period was first described by Burnard and Cross in 1958.[19] This observation was confirmed in a more recent HIE cohort by Enweronu-Laryea *et al*., with a greater degree of spontaneous hypothermia in neonates with an abnormal outcome, and even greater disturbances in infants who died.[20] Similar relationships between spontaneous hypothermia and degree of brain injury had been shown in animal models. For instance, rat pups exposed to hypoxic-ischemic brain injury had a spontaneous decrease of their core body temperature 1 h after the insult that slowly recovered within 24 h, and their degree of hypothermia was associated with neuropathological injury on day 7.[31]

Despite the supporting evidence, the exact physiologic mechanisms that drive the differences in MT by outcome group are not entirely clear. Healthy infants underdoing the fetal-neonatal transition maintain body temperature after exposure to the cold outside environment in large part by activating non-shivering thermogenesis (NST) via the activation of brown adipose tissue (BAT). [32] Heat production by BAT requires oxygen in order for the electron transport chain to pump protons into the intermembrane space before they can be dissipated by uncoupling proteins, which generates heat.[33] A loss of core body temperature early after asphyxia may therefore be explained by initial hypoxia. However, later disruptions in thermoregulation in infants with HIE are likely due to some combination of direct injury to the thermoregulatory centers integral to the hypothalamic-pituitary-adrenal axis, loss of sympathetic tone to drive NST (through noradrenaline), and loss of muscle tone and capacity for shivering.[34] Others have also shown that more severe encephalopathy is associated with higher central brain temperatures, which may result in a reduction in the temperature set-point in the hypothalamus.[35] However, MTs were not provided in the brain temperature studies, therefore it remains unclear if the higher brain temperatures in infants with more severe encephalopathy is due to endogenous (patho)physiology or because the cooling mattress the infants were lying on had a higher temperature, as would be expected based on our data.

Another important area to consider when examining the relationship between thermoregulatory parameters (or their proxies, such as MT) and outcome in infants with HIE are medications administered to infants with encephalopathy, particularly anticonvulsants. Perhaps the best example of this is phenobarbital, which was by far the most common anticonvulsant used in the dataset. In animal models, low doses of GABA agonists such as barbiturates (e.g. phenobarbital) and benzodiazepines (e.g. diazepam) produce increases in body temperature, with decreases seen at higher doses.[36] As first-line anticonvulsant drugs are often GABA-agonists, this may be an ongoing confounder with respect to the relationship between MT and outcome unless it is appropriately accounted for. Treatment with phenobarbital has also been associated with more rapid

Figure 3. Continued.
trajectory group. Using Group 4 as the reference group, Groups 1 and 2 both had significantly reduced odds for death or NDI in all infants as well as those surviving to discharge, with a nonsignificant decrease in odds in Group 3. Regardless of severity of encephalopathy, being below all three cut-offs (Group 1) was associated with 100% NDI-free survival. All models were adjusted for exposure to phenobarbital, inotropes, and morphine, documented seizures, severity of encephalopathy (except D and E), duration of therapeutic hypothermia treatment, and degree of organ dysfunction.

cooling during the induction phase, but even after adjusting for phenobarbital exposure in all final MT models, MT was still strongly predictive of outcome.[37]

Though spontaneous hypothermia appears to correlate with degree of injury in infants with HIE, it is difficult to routinely measure this in the modern era of TH in which core temperature is actively controlled as soon as possible after birth. Instead, the MT required to achieve a standardized TH temperature can be used as an indirect measurement of these physiologic disturbances. Due to the evolving nature of the injury, the MT required to achieve a core temperature of 33.5°C also evolves over the TH period, and the cut-offs derived to predict abnormal outcome changed as well. In our work, the time epoch between 6–24 h appeared to be the least clear with respect to outcome prediction, which may be due to inter-individual differences in timing of onset of secondary injury phases.[21,38–40] The distinction became more robust with a stronger predictive signal during the 24–48 h and 48–72 h epochs, when infants with a MT above the cut-offs had a significantly higher aOR of 3.1 and 3.3, respectively, for an unfavorable outcome. While both the MT difference by outcome and aOR for death or NDI were most notable in the 0–6 h epoch when applied to the entire cohort, the predictive signal in the later time epochs was still remarkably strong relative to other studies of biomarkers in neonates with HIE.[41]

In addition to discrete cut-offs within epochs, we observed that MT trajectory over time was strongly predictive of outcome. Infants who maintained MTs below the cut-offs throughout all time epochs had a universally favorable outcome, which is a rarity in studies of HIE biomarkers. In fact, this meant that we were unable to use this group as the reference group in our trajectory analyses, as it is impossible to calculate an aOR with a denominator of zero. By comparison, infants who maintained their MTs above all cut-offs had a more than 50% chance of adverse outcome. Infants who had MTs both above and below various cut-offs had intermediate risk, though the 0–6 h cut-off appeared to have the greatest predictive ability with those above that cut-off having a worse outcome than those below it regardless of later temperatures. This observation highlights the weight of critical time periods following an acute perinatal event, emphasizes the significant role the first hours of TH play, and potentially directs future investigations toward this time-period for additional adjunct neuroprotective interventions.

Our results are in line with a recent study that posed a similar question, utilizing data from the original NICHD Hypothermia trial combined with a smaller subset of OC trial participants to demonstrate an association between blanket temperatures during TH and outcome at 18–22 months by analyzing outcomes based on number of MT readings above 33.5°C during the maintenance phase of TH only.[42] Critically, this replication of findings is important if MT is to be developed as a prognostic marker in clinical practice. Though there is some overlap in the data used, our approach was much more stringent as we opted to only include infants from one trial to minimize the effect of changing care practice over time, and we chose a different analytic approach.[43] This included: 1) taking into account the entire duration of TH, 2) including all MT values from all infants, 3) developing and validating epoch specific cut-offs based on the natural evolution of MTs and injury over time, and 4) evaluating the prognostic value of simple combinatorial trajectories. We also found our most notable predictive signal during the 0–6 h cooling period that was excluded in the other analysis. We therefore believe that this is the first analysis demonstrating the strong ability of MTs to predict long-term outcome as early as 6 hours into the treatment course, highlighting its potential as a biomarker of injury.

The ideal biomarker should be quantifiable, reliable, reproducible, easy to obtain and interpret with minimal training, and in the case of neonates with HIE provide an early window into severity of injury and long-term outcome. The search for early biomarkers in neonates with HIE has been the subject of numerous studies, but accurate biomarkers have been notoriously difficult to obtain, are too non-specific, or have poor reproducibility across different studies.[10,44] EEG characteristics have emerged as promising physiologic biomarkers, particularly EEG background pattern during TH which when normal at 6 h and throughout the first 24 h of life is universally associated with a good outcome, whereas a lack of normal background by 72 h is highly associated with an unfavorable outcome.[17,45,46] Chalak *et al.* studied the impact of seizures during rewarming on long-term outcome in a sub-cohort of the OC trial and found that infants with seizures during rewarming were more likely to have an abnormal outcome at 18–22 months compared to those who did not (46% vs 25%) with a risk ratio of 1.72.[47] Similarly, the Lactate to N-Acetyl Aspartate ratio measured by magnetic resonance spectroscopy has shown promising results as a predictor of outcome.[48] However, both, EEG monitoring and MRS require additional equipment and expert interpretation, which limits their use as biomarkers.

Our work shows strong and early predictive ability using MT as a physiologic biomarker. It is also inexpensive and easily interpretable by non-experts, and therefore applicable in lower-resource settings. Using MT alone at single time points is clearly not going to

be adequate for predicting long-term outcome; however, when coupled with simple clinical variables and modeled as temperature trajectories over time, predictive accuracy improved. For example, a recent study showed that adding measures of lactate dehydrogenase (LDH) provided additional prognostic ability when combined with MRI data.[49] While not all necessary information may be available in the first 0–6 h of cooling, a risk calculator including other clinical variables could be updated with MTs in real time, providing useful prognostic information within the standard TH period.

This secondary analysis has several limitations. This dataset is from a trial in a different era of TH during which MRI was not performed in all patients, EEG recording throughout cooling was not standard of care, and outcome was only available at 18–22 months of age. Furthermore, while a large number of confounding factors could be taken into account as having an effect on temperature regulation and perfusion, the dataset did not provide hemodynamic information that is now more accessible, such as serial assessments of cardiac function by bedside echocardiography. In addition, MT recording intervals in this dataset were protocolized to capture overcooling within the first few hours of TH rather than to track physiologic changes in the neonate. This resulted in more sampling during the first 24 hours, particularly the first 6 hours, which could have potentially favored predictive ability in the earlier time window due to a greater density of available data. Lastly, the devices used during the OC trial have been largely replaced by more contemporary technology which now allows for shorter feedback loops and tighter temperature control. The longer feedback loops and larger temperature swings observed historically could have confounded the derived cut-points to a certain degree, especially during the later stages of TH when temperatures where only recorded every 4 hours. However, given that we were able to replicate findings obtained from a smaller cohort using continuous patient and MT recordings, the findings shown in this study provide robust evidence for use of MT measurement as an indirect physiologic biomarker and strong predictor of long-term outcomes.

Conclusion

This study confirms the value and utility of cooling MT as an early prognostic physiologic biomarker in neonates undergoing TH with moderate-severe HIE, providing a free and easily accessible metric to assist clinical decision making and longer-term prognostication for clinicians and families.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

Data from the Optimizing Cooling Trial are available through the Eunice Kennedy Shriver National Institute of Child Health and Human Development Data and Specimen Hub (https://dash.nichd.nih.gov/study/228264).

Authors' contributions

UM and TW developed the concept and designed of the project, acquired, analyzed, and interpreted the data, drafted and critically revised the article for important intellectual content, and approved the final.
JF contributed to the concept of the project, data acquisition and interpretation, critically revised the article for important intellectual content, and approved the final version.
JL and MPD contributed interpreting the data and critically revised the article for important intellectual content and approved the final version.
SJ critically revised the article for important intellectual content and approved the final version.

Supplementary material

Supplemental material for this article is available online.

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