Graduate thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences

Karen Haugvik Francke

Organ dysfunction and biochemical profiles in infants with hypoxic-ischemic encephalopathy

Secondary analyses of a randomized controlled trial on therapeutic hypothermia

Graduate thesis in Medisin
Supervisor: Karoline Aker
Co-supervisor: Ragnhild Støen
January 2023

NTNU
Norwegian University of
Science and Technology

Karen Haugvik Francke

Organ dysfunction and biochemical profiles in infants with hypoxic-ischemic encephalopathy

Secondary analyses of a randomized controlled trial on therapeutic hypothermia

Graduate thesis in Medisin
Supervisor: Karoline Aker
Co-supervisor: Ragnhild Støen
January 2023

Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences

NTNU
Norwegian University of
Science and Technology

ORGAN DYSFUNCTION AND BIOCHEMICAL PROFILES IN INFANTS WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY: SECONDARY ANALYSES OF A RANDOMIZED CONTROLLED TRIAL ON THERAPEUTIC HYPOTHERMIA

Authors:

Karen Haugvik Francke, medical student
Faculty of Medicine and Health Science, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
karenhf@ntnu.no

Prof. Ragnhild Støen. MD, PhD
Department of Paediatrics, St. Olavs Hospital, Trondheim University Hospital
Department of Clinical and Molecular Medicine, NTNU, Trondheim, Norway
ragnhild.stoen@ntnu.no

Prof. Niranjan Thomas
Department of Neonatology, Christian Medical College, Vellore, India
Department of Neonatology, Joan Kirner Women's and Children's at Sunshine Hospital, Melbourne, 3021, Australia
niranjanawt@gmail.com

Dr. Karoline Aker, MD, PhD
Department of Clinical and Molecular Medicine, NTNU, Trondheim, Norway
karoline.aker@ntnu.no

Research group:

NTNU Neo

Abstract

Background

Results from trials on therapeutic hypothermia (TH) for infants with moderate to severe hypoxic-ischemic encephalopathy (HIE) after perinatal asphyxia in low- and middle-income countries (LMICs) are contradictory, and lack of proper intensive care may, at least partly, explain these differences. We investigated biochemical profiles, clinical markers of organ dysfunction and supportive treatments in infants randomized to TH or standard care with normothermia (SC) in an RCT in a level III neonatal intensive care unit (NICU) in south India.

Methods

This is a substudy of the Therapeutic Hypothermia in India (THIN)-study. Fifty infants with moderate to severe HIE were recruited between 2013 and 2015 and randomized to TH (n=25) or SC (n=25). Data from the first admission as well as outcome at 18 months were collected prospectively.

Results

Infants in the TH-group had lower pH than the SC-group at 6-12 hours (median (IQR) 7.28 (7.20-7.32) vs 7.36 (7.31-7.40), respectively, p=0.003) and 12-24 hours (median (IQR) 7.30 (7.24-7.35) vs 7.41 (7.37-7.43), respectively, p<0.001). There was no significant difference in the use of vasopressors (56% and 68% of infants in TH- and SC-group, respectively, p=0.38) or bicarbonate (20% and 32% in TH- and SC-group, respectively, p=0.70). There was also no significant difference in occurrence of thrombocytopenia (16% and 8% in TH- and SC-group, respectively, p=0.67). Fresh frozen plasma was given to 32% and 40% of infants in TH- and SC-group, respectively (p=0.56). Urine output <1ml/kg/h was more common in SC- compared to TH-group during the first 24 hours of life (16/23 (70%) vs. 7/25 (28%), respectively, p=0.004) with no difference in maximum creatinine. Anuria/oliguria beyond 24 hours of life, need for mechanical ventilation, vasopressor and ≥2 anticonvulsants were all associated with an increased risk of adverse outcome at 18 months of age.

Conclusion

In this study from a level III NICU in south-India, biochemical and clinical markers of organ dysfunction were similar in infants randomized to TH compared to standard care.

Keywords

Therapeutic hypothermia, hypoxic-ischaemic encephalopathy, organ dysfunction, biochemical profile

Sammendrag

Bakgrunn

Det er funnet motstridende resultater i studier som har undersøkt behandling med terapeutisk hypotermi (TH) hos nyfødte med moderat til alvorlig hypoksisk-iskemisk encefalopati (HIE) etter perinatal asfyksi i lav- og mellominntektsland, og mangel på tilstrekkelig intensivpleie kan, i alle fall delvis, forklare disse forskjellene. Vi undersøkte biokjemisk profil, kliniske markører for organ-dysfunksjon og støttebehandling hos nyfødte som ble randomisert til enten TH eller standard behandling for normotermi (SB) i en randomisert kontrollert studie fra en nyfødtintensivavdeling (level III) i sør-India.

Metoder

Dette er en substudie av THIN (Therapeutic Hypothermia in India)-studien. Femti nyfødte med moderat til alvorlig HIE ble rekruttert mellom 2013 og 2015, og randomisert til TH (n=25) eller SB (n=25). Det ble samlet data fra den første innleggelsen, samt utfall ved 18 måneder.

Resultater

Nyfødte i TH-gruppa hadde lavere pH enn SB-gruppa ved 6-12 timer (median (IQR) 7.28 (7.20-7.32) vs. 7.36 (7.31-7.40), henholdsvis, p=0.003) og 12-24 timer (median (IQR) 7.30 (7.24-7.35) vs. 7.41 (7.37-7.43), henholdsvis, p<0.001). Det var ingen forskjell i bruk av vasopressorer (henholdsvis 56% og 68% av nyfødte i TH- og SB-gruppa, p=0.38) eller bikarbonat (henholdsvis 20% and 32% in TH- and SB-gruppa, p=0.70). Seks (12%) nyfødte hadde et trombocytt-tall <100 000 per µl, med ingen forskjell mellom gruppene. Det var ingen forskjell i bruken av ferskt frosset plasma (32% og 40% av nyfødte i TH- og SB-gruppa, henholdsvis, p=0.56). Diurese <1ml/kg/t var mer vanlig i SB-sammenlignet med TH-gruppa i løpet av det første levedøgnet (henholdsvis 16/23 (70%) vs. 7/25 (28%), p=0.004), med ingen forskjell i høyeste nivå av kreatinin. Anuri/oliguri utover det første levedøgnet, samt behov for mekanisk ventilasjon, behandling med vasopressorer og ≥2 antikonvulsanter, var assosiert med en økt risiko for alvorlig utfall ved 18 måneders alder.

Konklusjon

I denne studien fra intensivavdeling for nyfødte i sør-India, var biokjemiske og kliniske markører for organ-dysfunksjon like hos nyfødte randomisert til TH sammenlignet med de som fikk standard behandling.

Introduction

Therapeutic hypothermia (TH) reduces mortality and long-term disability (RR (95% CI) = 0.75 (0.68, 0.83)), and has been standard treatment for infants with moderate to severe hypoxic ischaemic encephalopathy (HIE) since 2010 [1, 2]. Although controversy remains if TH should be provided in resource-limited settings [3-6], the latest international guidelines on Neonatal Life Support recommends TH to be implemented in settings with limited resources if certain intensive care facilities like intravenous therapy, respiratory support, pulse oximetry, antibiotics and anticonvulsants are available [7]. A recent systematic review and meta-analysis found disability and cerebral palsy in infancy to be reduced by TH independent of setting, but mortality at 18-24 months was only reduced in high-income countries (HICs) [8].

Research on accidental hypothermia has shown that metabolic acidosis and coagulopathy may result from moderate hypothermia, which is similar to what is achieved during TH [9-11]. In the hypothermia for moderate or severe neonatal encephalopathy in low-income and middle-income countries (HELIX)-study [12] , which is the largest randomized controlled trial (RCT) on TH yet to be performed, results showed no beneficial effect on the combined outcome of death or disability, and TH increased the risk of death. Infants in the TH-group had more complications with persistent metabolic acidosis, persistent hypotension, prolonged blood coagulation, gastric bleeding and severe thrombocytopenia compared to the standard care group, suggesting that cooling had a negative impact on multiple organ systems. Based on their results, the HELIX trial group have recommended that TH should not be provided in LMICs [12]. Though several of the large RCTs from HIC have also reported a higher incidence of thrombocytopenia in the TH-group [13-15], no difference is seen in the occurrence of hypotension, haemorrhage or coagulopathies [16-20]. In one single centre study, TH more than doubled the risk of persistent pulmonary hypertension of the newborn (PPHN) [21], and in the TH-group, infants with PPHN required more supportive treatment and had a higher early mortality-rate even though they did not have more brain injury on MRI. Hypothermia may also affect cardiac, liver and renal function, but such organ dysfunction may, however, be indistinguishable from the effect of the hypoxic-ischemic insult in itself [14, 22]. Although most guidelines recommend that TH is provided in facilities with a certain level of intensive care [7, 23, 24], it is still unclear to what extent supportive treatments for respiratory, hemodynamic and metabolic control are needed for TH to be both effective and safe.

There are few recent studies with a normothermic control group after the implementation of TH as standard care to infants with moderate to severe HIE. Detailed biochemical and clinical markers of organ dysfunction during TH is important to understand conflicting results from different settings and to optimize treatment across settings. The main purpose of this study is to describe organ dysfunction and biochemical profile in cooled and non-cooled infants with moderate to severe HIE in an LMIC

setting as a substudy of a small single-centre RCT from India. We also explore associations between organ dysfunction in the neonatal period and outcomes at 18 months.

Methods

This is a substudy of the Therapeutic Hypothermia in India (THIN)-study, a single-centre RCT of infants admitted with moderate to severe HIE to the neonatal intensive care unit (NICU) at the Christian Medical College Vellore, a tertiary care teaching hospital in rural south India. Fifty infants at or near term admitted before 5 hours after birth with signs of moderate to severe HIE were recruited between September 2013 and October 2015. Included infants were randomly assigned to hypothermia with target core temperature 33.5°C±0.5°C for 72 hours induced by a phase changing material-based cooling device (MiraCradle Neonate Cooler, Pluss Advanced Technologies, India) or standard care with normothermia. Full description of the trial is published elsewhere [25].

This substudy include demographics, biochemical data on kidney function, infection parameters and coagulation, urine output, seizures, adverse events, treatment data and adverse outcomes at 18 months. Cord blood results were only available in inborn infants. Blood gas, kidney function, liver enzymes, coagulation parameters and full blood counts were monitored per study protocol, and investigation for infection was done as clinically indicated. Data on liver enzymes was only registered directly after birth and were thus not included in the analyses. All treatments including medications were as per existing treatment protocols. Blood samples measuring coagulation parameters were most often taken from arterial lines, which would run heparin and therefore could interfere with the actual values of activated partial thromboplastin time (APTT), even if protocol was to withdraw at least 5 mL of blood before taking samples. We defined elevated levels of international normalized ratio (INR) as >1.8 and APTT as >43 seconds [26]**.** Anuria was defined as urine output <0.5 ml/kg/h, and oliguria as urine output <1 ml/kg/h. Mechanical ventilation was provided for infants with respiratory failure. Seizures were managed with a loading dose of phenobarbitone. Second-line anticonvulsant was phenytoin and third was levetiracetam. Adverse outcome was defined as death, cerebral palsy with GMFCS (Gross Motor Function Classification System) level 3-5 or Bayley-III cognitive and/or motor composite score <85 at 18 months age [27]. One infant had outcome evaluated by Griffith Mental Developmental Scales (GMDS) at 48 months, and one based on phone assessment, and both were classified as having good outcome.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 27 and 29. The data is presented in tables, and data showing development over time are presented as line plots. Counts with proportions were used for dichotomous variables and median with interquartile range (IQR) was used for continuous variables, as we had a small sample size, and did not expect the variables to be normally

distributed. When comparing the groups by randomization (TH vs. standard care with normothermia (SC)) or outcome (adverse vs. good), Chi^2-test, Fisher's exact test and linear-by-linear association were used as appropriate to find p-values for dichotomous variables. Mann Whitney U-test was performed to find the p-values for continuous variables that were not normally distributed. A p-value <0.05 was considered statistically significant for all analyses. Unadjusted odds ratios for an adverse outcome were calculated for significant exposures.

Results

Fifty infants were included, 25 receiving TH and 25 receiving standard care. Demographics, neonatal characteristics and outcome are shown in table 1. Data on biochemical profile, organ dysfunction and treatment according to randomization group are presented in table 2. Longitudinal data on pH, platelet count, INR, APTT, creatinine, urea, troponin T and interleukin (IL)-6 are shown in figure 1. Infants in the TH-group had significantly lower pH at 6-12 hours (median (IQR) in TH- and SC-group 7.28 (7.20-7.32) vs 7.36 (7.31-7.40), respectively, p=0.003) and 12-24 hours after birth (median (IQR) in TH- and SC-group 7.30 (7.24-7.35) vs 7.41 (7.37-7.43), respectively, p<0.001). No infant had a persisting pH-value <7.2 for more than 12 hours.

During the first 24 hours of life, significantly more infants in the SC-group had anuria/oliguria, compared to the TH-group (16/23 (70%) vs 7/25 (28%), respectively; p=0.004, table 2). When excluding three infants with global brain injury on MRI (there was four in total, but one had missing data),all in SC-group, there was still a significantly higher occurrence of anuria/oliguria the first 24 hours of life in the SC-group compared to the TH-group (13/20 (65%) vs 7/25 (28%), p=0.013). Fourteen (28%) infants received mechanical ventilation, and two of these (one in each group) were on high frequency oscillation. No infant was on mechanical ventilation longer than three days. All, except one infant in SC-group, were treated with antibiotics, and twenty-one (11 in TH- and 10 in SC-group) were treated with antibiotics for more than 72 hours. No infant had culture-positive sepsis.

Twice as many infants in the cooled group had thrombocytopenia compared to the non-cooled group (16% vs. 8%, respectively, table 2), though this difference was not significant. No infant had severe thrombocytopenia defined as a platelet count <25 000 per μl or <50 000 per μl with active bleeding. 60% of cooled infants and 40% of non-cooled infants had elevated INR (p=0.16), and all infants except 2 (SC-group) had elevated APTT. Fresh frozen plasma was the most frequently used blood product during the intervention (8/25 (32%) in the SC-group and 10/25 (40%) in the TH-group). Only 2 infants in the TH-group and one in the SC-group received packed red blood cells (8% vs 4%, respectively, p=1.0), and none were given a platelet transfusion. Two infants (SC-group) had significant haemorrhage (both had subgaleal bleeds). No infants in the study suffered subcutaneous fat necrosis.

Outcome at 18 months were registered for 47 infants (1 infant in the TH-group and two in the SC-group were lost to follow up), of which 17 (36%) had an adverse outcome. Of these, eight died (two in TH- and six in the SC-group), and nine had a Bayley-III cognitive and/or motor CS <85 (three in TH- and six in SC-group). Fewer infants in the TH-group had an adverse outcome at 18 months than in the SC-group (5/24 (21%) vs 12/23 (52%), respectively, p=0.025, table 1). Anuria/oliguria at 24-48 hours, as well as treatment with vasopressors, mechanical ventilation and ≥2 anticonvulsants were all statistically significant associated with an adverse outcome, as presented in table 3.

Discussion

In this secondary analysis of the THIN-study, there were no differences in respiratory and haemodynamic support or adverse events between cooled and non-cooled infants. No infants had persistent metabolic acidosis or culture proven sepsis, but cooled infants had a lower occurrence of anuria/oliguria compared to non-cooled infants. Renal dysfunction and treatment with vasopressors, mechanical ventilation and ≥ 2 anticonvulsants were all associated with an adverse outcome at 18 months.

Cooled infants had a statistically significant lower pH than non-cooled infants at some time points during the intervention, but still within acceptable range. This differs from the HELIX-study, where almost a quarter of infants in the hypothermia-group had persistent metabolic acidosis, (23% among cooled vs 12% among non-cooled infants, p=0.0029) [12]. Persistent hypotension despite maximum inotropic support was also more common in cooled infants in the HELIX-trial, however, blood pressure was only measured non-invasively according to the study protocol [12, 28]. All infants in the THIN-study had arterial line for continuous monitoring, but BP-measures were, unfortunately, not registered. However, there was no difference in the use of vasopressors between the two groups. Hypothermia may result in metabolic acidosis via different pathways [9], and hemodynamic control with close monitoring of blood pressure is key to avoid persistent hypotension and metabolic acidosis due to poor perfusion during TH [29]. Similar to our findings, RCTs from HICs have not found increased risk of hypotension or persistent metabolic acidosis with TH [16-20], probably reflecting the level of intensive care and monitoring provided [29].

A higher incidence, although not statistically significant, of thrombocytopenia among cooled compared to non-cooled infants in the present study, is in line with what has been reported by others [5, 14, 15]. Similarly, more cooled infants had elevated INR, but this difference was also not significant. The very high proportion of infants with elevated APTT in both groups is most likely due to samples taken from arterial lines with heparin. More importantly, there were no indications of more severe coagulopathies among cooled infants, which is in line with studies from HICs [16-20]. In contrast, the HELIX-study found significantly more severe thrombocytopenia, prolonged blood coagulation and gastric bleeding in the hypothermia-group [12].

In the precooling era, renal dysfunction is described as the most common organ system affected after perinatal asphyxia [30]. We found less anuria/oliguria in cooled than non-cooled infants, also when excluding 3 infants in the SC-group with global brain injury on MRI. Both serum-creatinine and -urea were also lower in the TH-group, although not statistically significant. As a transient increase in creatinine can also be seen in healthy new-borns [31], one should be careful using this parameter alone to evaluate renal function. Even though four large RCTs on cooling in HICs did not report any significant differences in renal dysfunction [16-19], the possible reno-protective effect of TH in our study is supported by a meta-analysis reporting significantly less acute kidney injury in cooled compared to non-cooled infants [32].

No infant in our study had culture-proven sepsis, and there was no significant difference between the groups in infection parameters. This finding does not support a higher prevalence of sepsis, which has been used as an argument against TH in LMICs [33, 34]. There has been a concern that early-onset sepsis may alter the effect of TH [35], but recent studies have reported similar mortality and a beneficial effect on neurodevelopment with TH, even in the presence of infection [36].

We report that infants treated with TH had a lower occurrence of adverse outcome, which is in line with studies from both high- and low-income countries [1, 6]. Even though LMICs carry the greatest burden of neonatal deaths due to perinatal asphyxia and HIE, controversy still remains regarding the safety and efficacy of TH in these settings [3-5, 37-39]. Our findings on similar biochemical profiles and level of supportive treatments supports that TH was safe in the context of this single-centre study in India. Although there is no consensus on the optimal supportive care to infants receiving TH, respiratory support, continuous monitoring and preservation of hemodynamic stability, 24/7 imaging services etc. are standard in centers offering cooling in high-income countries [7, 24]. These requirements pose a challenge in many low-resource settings, where the access to neonatal intensive care and transport services vary greatly. Most guidelines on TH recommend centralization to so-called “cooling centres” where staff has received special training and equipment to provide advanced intensive care is available [23, 24, 40]. Clinical guidelines and recommendations for supportive treatments, as well as organization of services including transport, is needed and should be the focus of future research.

Limitations of the present study are mainly the small cohort and missing data on blood pressure and some of the biochemical measurements. The small sample size increases the chance of selection bias despite a proper randomization procedure. The primary outcome of this RCT was a sensitive MRI biomarker for developmental outcome [25], which was chosen to reduce sample size. This limits the possibility to make prediction models for outcome based on organ dysfunctions and/or biochemical profiles during cooling [41]. Despite these limitations, we believe our study supports that the safety

and efficacy of TH depends on the level of intensive care available and not population differences as previously suggested [12, 42, 43].

Conclusion

In this single-centre RCT from India, cooled and non-cooled infants with moderate to severe HIE had similar occurrence of organ complications and received the same level of intensive care. This suggests that the level of intensive care provided during TH may explain the lack of safety and efficacy which have been reported in some studies from low-resource settings.

List of abbreviations:

- APTT – activated partial thromboplastin time
- HELIX – hypothermia for moderate or severe neonatal encephalopathy in low-income and middle-income countries
- HICs – high-income countries
- HIE – hypoxic-ischaemic encephalopathy
- INR – international normalized ratio
- LMICs – low- and middle-income countries
- NICU – neonatal intensive care unit
- PPHN – persistent pulmonary hypotension of the new-born
- RCT – randomized controlled trial
- SC – standard care with normothermia
- TH – therapeutic hypothermia
- THIN – therapeutic hypothermia in India

Declarations

Ethics approval and consent to participate

All parents of included infants gave written consent after being informed about the trial with both an information leaflet and oral explanation. The study was approved by the Institutional Review Board at the Christian Medical College (number 2013/8223) and the Regional Committee for Medical and Health Research Ethics in central Norway (number 2013/2167, reference: 18557).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Anonymised data (including data dictionaries) will be made available on

request to researchers who provide a methodologically sound proposal for use in achieving the goals of the approved proposal.

Competing interests

NT has a patent 1796/DEL/2013 Life cradle device for inducing neonatal hypothermia issued.

Funding

There was no funding for this substudy. Funding for the THIN-study was provided by Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU).

Authors' contributions

NT was the PI of the THIN-study, participated in the conceptualization and design of the study and data collection and revised and reviewed the manuscript. KHF analysed the data and drafted the initial manuscript. KA, RS and KHF reviewed and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors thank all the staff of the Department of Neonatology, CMC, Vellore, who took care of the study babies and the research officers for adequate recruitment and collection of clinical data. We thank members of the Data Safety Monitoring Board at the CMC for monitoring the study.

Tables and figures

Table 1: Demographics, neonatal characteristics and outcome

	Therapeutic hypothermia (n=25)	Standard care (n=25)	Total (n=50)
Inborn, n/N (%)	13/25(52)	17/25(68)	30/50 (60)
Mode of delivery, n/N (%)			
Normal vaginal	9/25(36)	10/25(40)[1]	19/50 (38)
Operative vaginal	5/25 (20)	5/25 (20)	10/50 (20)
CS	11/25 (44)	10/25 (40)	21/50 (42)
Male gender, n/N (%)	17/25 (68)	16/25 (64)	33/50 (66)
GA (weeks), mean (SD)	39.1(1.3)	39.2 (1.4)[2]	39.1(1.3)
Birth weight (g), mean (SD)	2911(483)	2960(553)	2935 (515)
SGA[3], n/N(%)	7/25 (28)	7/24 (29)	14/49 (29)
Apgar-score, median (IQR)[4]			
1-minute	3(1-3)	3(2-4)	3(1-4)
5-minute	4(4-5)	6(5-7)	5(4-7)
10-minute	7(5-7)	7(6-8)	7(6-7,75)
Cord/blood<60 min pH, median (IQR) [5], [range]	6.81(6.69-6.93) [0.32]	6.95(6.79-7.08) [0.59]	6.88 (6.73-7.02) [0.59]
Cord/blood<60 min BD, median (IQR) [6], [range]	20.0 (22.1-18.7) [10.7]	16.4 (20.0-12.4) [16.6]	19.2 (20.9-14.0) [16.6]
Blood 1-6h pH, median (IQR)[7], [range]	7.25 (7.13-7.36) [0.41]	7.24 (7.17-7.32) [0.36]	7.25 (7.15-7.34) [0.41]
Blood1-6h BD, median (IQR)[8], [range]	9.3 (16.5-6.8) [18.4]	12.7 (15.9-6.7) [23.6]	10.8 (15.9-6.8) [23.6]
Clinical seizures before randomization, n/N (%)	13/25 (52)	13/25 (52)	26/50 (52)
HIE-stage, n/N(%)			
HIE grade 2	24/25(96)	24/25(96)	48/50 (96)
HIE grade 3	1/25(4)	1/25(4)	2/50 (4)
Moderate/severe injury on MRI[9], n/N (%)	2/23 (9)	10/23 (44)	12/46 (26)
Adverse outcome[10], n/N (%)	5/24 (21)	12/23 (52)	17/47 (36)

[1] 1 infant was breech delivery
[2] 2 missing (SC)
[3] Small for gestational age, defined as birth weight less than the 10th percentile according to the Intergrowth 21st chart
[4] 1-minute: 8 missing, 5-minute: 7 missing, 10-minute: 22 missing
[5] Data was unavailable for 13 infants in the TH-group and 7 in the SC-group
[6] Data was unavailable for 14 infants in the TH-group and 8 in the SC-group
[7] Data was unavailable for 8 infants in the TH-group and 2 in the SC-group
[8] Data was unavailable for 8 infants in the TH-group and 2 in the SC-group
[9] Normal/Mildly abnormal MRI was defined as normal/mild basal ganglia and thalami score and/or normal/mild/moderate white matter score. Moderately/Severely abnormal MRI was defined as moderate/severe basal ganglia and thalami score and/or absent posterior limb of the internal capsule and/or severe white matter score. P-value of 0,007 (Chi^2-test)
[10] Adverse outcome was defined as BSID (Bayley Scales of Infant and Toddler Development) < 85 (motor/cogn comp score), GMFCS (Gross Motor Function Classification System) 3-5, or death. P-value of 0.025 (Chi^2-test)

Table 2: Biochemical data, organ dysfunction and treatment during admission

Randomization	Therapeutic hypothermia (n=25)	Standard care (n=25)	Total (n=50)	p-value
Biochemical data				
Creatinine max (umol/L), median (IQR), [range]	65 (54-82) [39-194]	73 (55-111) [31-314]	68 (55-87) [31-314]	0.35
Urea max (mmol/L), median (IQR), [range]	3.0 (2.5-4.3) [1.8-7.3]	3.8 (2.5-6.5) [1.5-11.3]	3.2 (2.5-5.0) [1.5-11.3]	0.17
CRP max (mg/dL), median (IQR)[11] [range]	8.6(4.2-26.1) [0.0-80.1]	10.0 (5.3-17.9) [0.0-66.9]	8.6 (4.3-21.3) [0.0-80.1]	0.92
IL-6[12] max (pg/mL), median (IQR), [range]	13 (12-15) [10-17]	13 (12-15) [7-19]	13 (12-15) [7-19]	0.85
Troponin T max (pg/mL), median (IQR), [range]	170 (109-257) [59-841]	256 (126-345) [75-1180]	193 (126-310) [59-1180]	0.24
Platelet count				
Minimum ($x10^9$/L), median (IQR), [range]	153 (127-177) [54-252]	169 (110-219) [39-325]	160.5 (121-189) [39-325]	0.19
<100 000 per µl, n/N(%)[13]	4/25(16)	2/25(8)	6/50(12)	0.67
INR[14]				
Maximum, median (IQR), [range]	1.9 (1.6-2.4) [1.4-4.9]	1.7 (1.5-2.5) [1.1-5.2]	1.8 (1.5-2.4) [1.1-5.2]	0.28
>1.8, n/N (%)	15/25 (60)	10/25 (40)	25/50 (50)	0.16
APTT[15]				
Maximum (sec), median (IQR), [range]	74 (63-85) [44-180]	61 (49-84) [34-179]	68 (58-85) [34-180]	0,097
>43 sec, n/N (%)	25/25 (100)	23/25 (92)	48/50 (96)	0.49
Organ dysfunction and treatment				
Anuria/oliguria, n/N (%)[16]				
0-24 hours[17]	7/25 (28)	16/23 (70)	23/48 (52)	0.004
24-48 hours	1/23 (4)	5/23 (22)	6/46 (13)	0.19
48-72 hours	0/22 (0)	1/19 (5)	1/41 (2)	0.46
>72 hours	0/21 (0)	1/20 (5)	1/41 (2)	0.49
Number of vasopressors				0.38[18]
0	11/25 (44)	8/25 (32)	19/50 (38)	
1	10/25 (40)	12/25 (48)	22/50 (44)	
2 or more	4/25 (16)	5/25 (20)	9/50 (18)	
Intravenous bicarbonate, n/N(%)	5/25(20)	3/25 (12)	8/50(16)	0.70
Highest level of respiratory support, n/N (%)				0.53[19]
Intermittent mandatory ventilation	5/25 (20)	7/25 (28)	12/50 (24)	
High frequency oscillation	1/25 (4)	1/25 (4)	2/50 (4)	
Duration of antibiotics (days), median (IQR) [range][20]	3 (3-5.75) [2-15]	3 (3-5) [2-7]	3 (3-5) [2-15]	0.501
Clinical seizures, n/N (%)	20/25 (80)	22/25 (88)	42/50 (84)	0.70
Number of anticonvulsants, n/N (%)				0.17
0	6/25 (24)	3/25 (12)	9/50 (18)	
1	10/25 (40)	10/25 (40)	20/50 (40)	
2	6/25 (24)	6/25 (24)	12/50 (24)	
3	3/25 (12)	5/25 (20)	8/50 (16)	
4	0/25 (0)	1/25 (4)	1/50 (2)	
Any blood products, n/N(%)	10/25 (40)	9/25(36)	19/50 (38)	0.77

[11] Data was unavailable for 1 infant in the TH-group and 1 in the SC-group
[12] Interleukin-6
[13] Only one infant had a platelet count <50 000 per µl; this infant had a platelet count of 39 000 and platelet clumps seen in smear, but no active bleeding.
[14] International Normalized Ratio
[15] Activated Partial Thromboplastin Time
[16] Anuria was defined as urine output <0,5 ml/kg/h. Oliguria was defined as urine output <1 ml/kg/h.
[17] A minimum of 12 hours within the first 24 hours of life.
[18] P-value based on Chi2-test on infants who received treatment with vasopressors vs. infants who did not.
[19] P-value based on Chi^2-test on infants who were treated with mechanical ventilation vs. infants not treated with mechanical ventilation.
[20] Data was unavailable for 1 infant in the TH-group and 1 in the SC-group. 1 infant in the SC-group was not treated with antibiotics and is not included in estimation of median and IQR.

Table 3: Organ dysfunction and treatments by outcome at 18 months[21]

Outcome	Good (n=30)	Adverse (n=17)	p-value	OR	CI (95%)
Anuria/oliguria[22] 24-48 hours, n/N (%)	1/27 (4)	5/16 (31)	0.02	11.82	1.23, 113.23
Vasopressors, n/N (%)	14/30 (46)	14/17 (82)	0.017	5.33	1.27, 22.48
Mechanical ventilation, n/N (%)	3/30 (10)	8/17 (47)	0.009	8.00	1.74, 36.81
≥2 anticonvulsants, n/N (%)	8/30 (27)	13/17 (77)	<0.001	8.94	2.24, 35.61

[21] Adverse outcome was defined as BSID (Bayley Scales of Infant and Toddler Development) < 85 (motor/cogn comp score), GMFCS (Gross Motor Function Classification System) 3-5, or death. P-value of 0.025 (Chi^2-test)

[22] Anuria was defined as urine output <0,5 ml/kg/h. Oliguria was defined as urine output <1 ml/kg/h.

Figure 1: Trendlines for biochemical data in cooled and non-cooled infants[23]

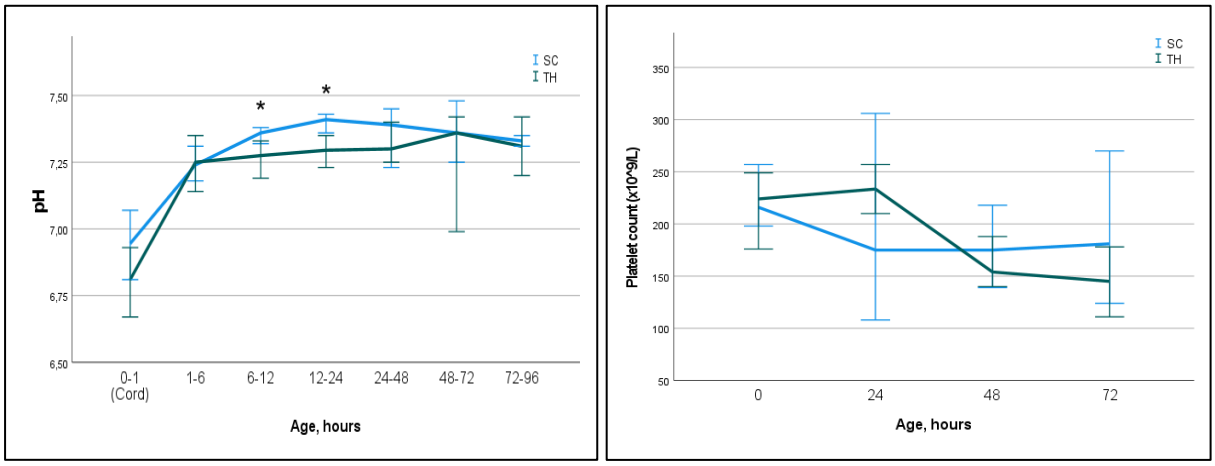


*Figure 1a: median pH[24] (lowest measured) during the first 96 hours of life and 1b: median platelet count[25] during the first 72 hours of life (Error Bars: 95% CI). *There is a statistically significant difference between the groups from 6-12 hours (p=0.003) and from 12-24 hours (p<0.001).*

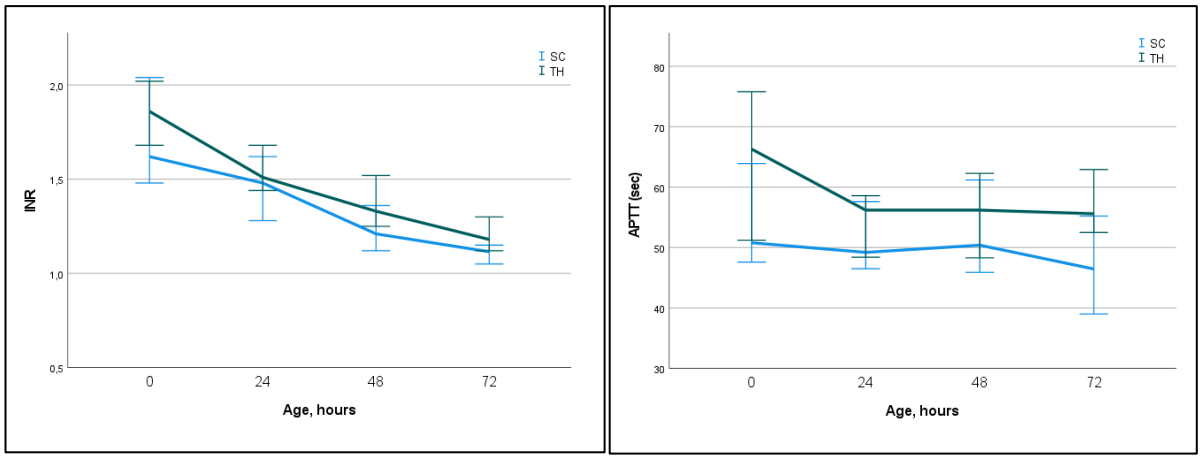


Figure 1c: median INR[26] and 1d: median APTT[27] during the first 72 hours of life (Error Bars: 95% CI)

[23] TH=therapeutic hypothermia, SC=standard care with normothermia
[24] Cord: 20 missing, 1-6 hours: 10 missing, 6-12 hours: 17 missing, 12-24 hours: 26 missing, 24-48 hours: 33 missing, 48-72 hours: 39 missing, 72-96 hours: 45 missing
[25] 0 hours: 2 missing, 24 hours: 42 missing, 48 hours: 4 missing, 72 hours: 21 missing
[26] 0 hours: 2 missing, 24 hours: 1 missing, 48 hours: 5 missing, 72 hours: 7 missing
[27] 0 hours: 2 missing, 24 hours: 2 missing, 48 hours: 5 missing,72 hours: 7 missing

Figure 1 (continuation)

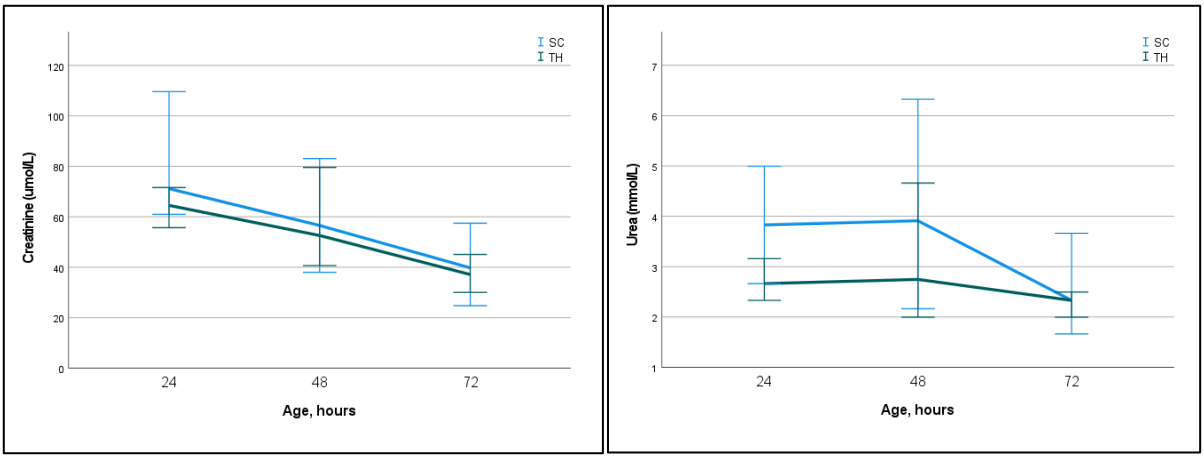


Figure 1e: median creatinine[28] and 1f: median urea[29] during the first 72 hours of life (Error Bars: 95% CI).

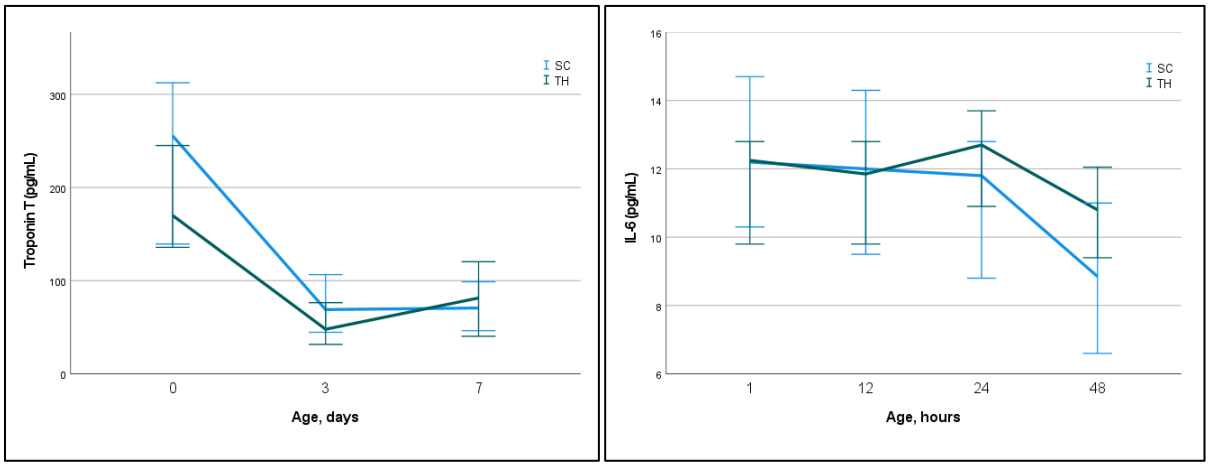


Figure 1g: median troponin T[30] during the first 7 days of life and 1h: median IL-6[31] during the first 48 hours of life (Error Bars: 95% CI)

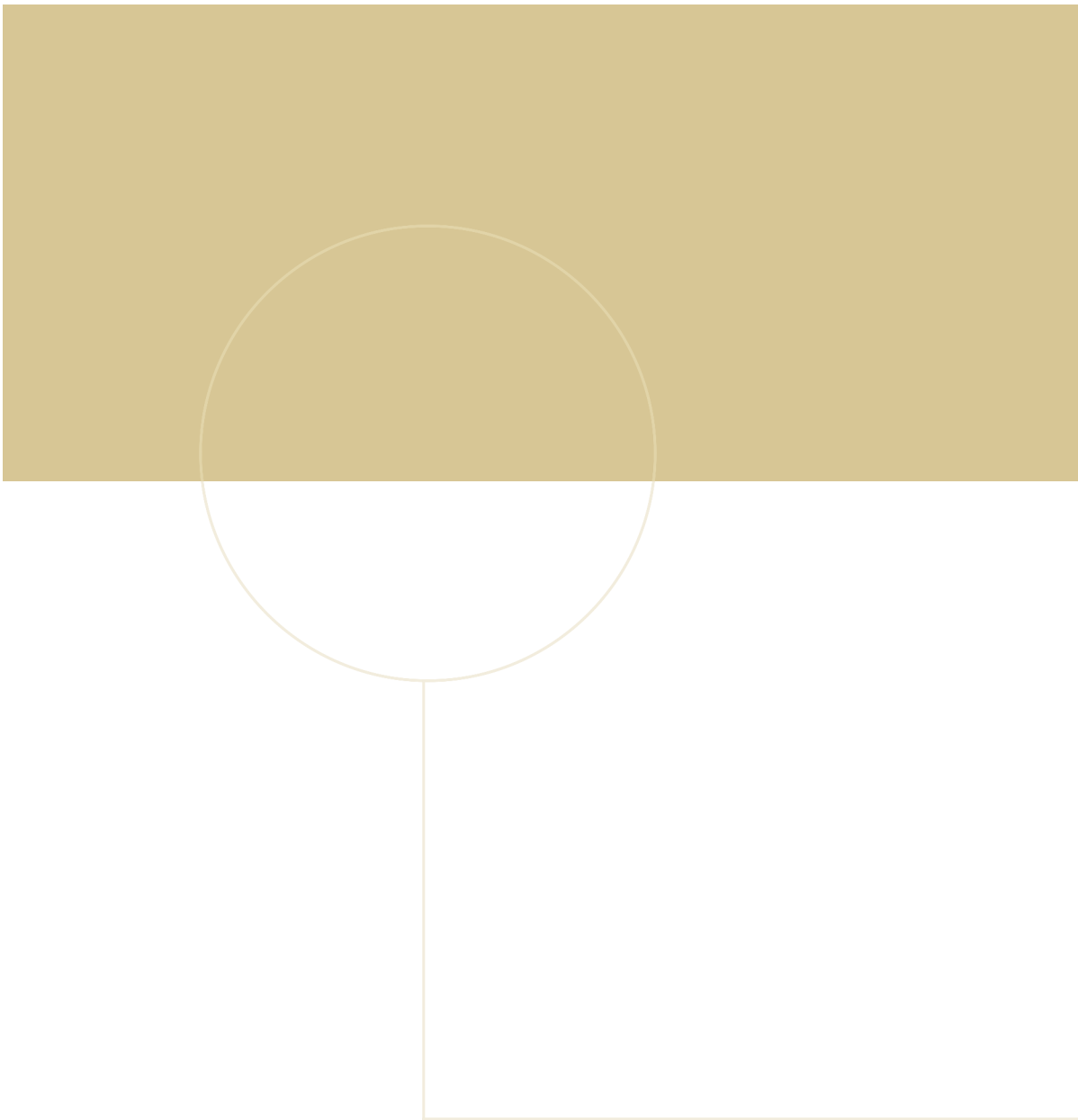
[28] 24 hours: 1 missing, 48 hours: 22 missing, 72 hours: 5 missing
[29] 24 hours: 2 missing, 48 hours: 24 missing, 72 hours: 8 missing
[30] 0 days: 1 missing, 3 days: 5 missing, 7 days: 14 missing
[31] 1 hour: 1 missing, 12 hours: 1 missing, 24 hours: 2 missing, 48 hours: 3 missing

References:

1. Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG: **Cooling for newborns with hypoxic ischaemic encephalopathy**. *Cochrane Database Syst Rev* 2013, **2013**(1):Cd003311.
2. Perlman JM, Wyllie J, Kattwinkel J, Atkins DL, Chameides L, Goldsmith JP, Guinsburg R, Hazinski MF, Morley C, Richmond S *et al*: **Part 11: Neonatal resuscitation: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations**. *Circulation* 2010, **122**(16 Suppl 2):S516-538.
3. Thayyil S: **Cooling therapy for the management of hypoxic-ischaemic encephalopathy in middle-income countries: we can, but should we?** *Paediatr Int Child Health* 2019, **39**(4):231-233.
4. Montaldo P, Pauliah SS, Lally PJ, Olson L, Thayyil S: **Cooling in a low-resource environment: lost in translation**. *Semin Fetal Neonatal Med* 2015, **20**(2):72-79.
5. Bellos I, Devi U, Pandita A: **Therapeutic Hypothermia for Neonatal Encephalopathy in Low- and Middle-Income Countries: A Meta-Analysis**. *Neonatology* 2022, **119**(3):300-310.
6. Diggikar S, Krishnegowda R: **Therapeutic Hypothermia for Neonatal Encephalopathy in Low- and Middle-Income Countries: A Literature Review**. *J Trop Pediatr* 2022, **68**(2).
7. Wyckoff MH, Wyllie J, Aziz K, de Almeida MF, Fabres J, Fawke J, Guinsburg R, Hosono S, Isayama T, Kapadia VS *et al*: **Neonatal Life Support: 2020 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations**. *Circulation* 2020, **142**(16_suppl_1):S185-s221.
8. Mathew JL, Kaur N, Dsouza JM: **Therapeutic hypothermia in neonatal hypoxic encephalopathy: A systematic review and meta-analysis**. *J Glob Health* 2022, **12**:04030.
9. Haverkamp FJC, Giesbrecht GG, Tan E: **The prehospital management of hypothermia - An up-to-date overview**. *Injury* 2018, **49**(2):149-164.
10. Paal P, Brugger H, Strapazzon G: **Accidental hypothermia**. *Handb Clin Neurol* 2018, **157**:547-563.
11. Zafren K, Mechem CC: **Accidental hypothermia in adults**. In. UpToDate; 2022.
12. Thayyil S, Pant S, Montaldo P, Shukla D, Oliveira V, Ivain P, Bassett P, Swamy R, Mendoza J, Moreno-Morales M *et al*: **Hypothermia for moderate or severe neonatal encephalopathy in low-income and middle-income countries (HELIX): a randomised controlled trial in India, Sri Lanka, and Bangladesh**. *Lancet Glob Health* 2021, **9**(9):e1273-e1285.
13. Yan ES, Chock VY, Bonifacio SL, Dahlen A, Guimaraes CV, Altit G, Bhombal S, Van Meurs K: **Association between multi-organ dysfunction and adverse outcome in infants with hypoxic ischemic encephalopathy**. *J Perinatol* 2022, **42**(7):907-913.
14. Di Salvo ML, Hargett SL, Jnah A, Newberry D: **System Specific Effects of Therapeutic Hypothermia with Neonatal Encephalopathy: Pearls for Clinicians**. *Neonatal Netw* 2020, **39**(4):205-214.
15. Boutaybi N, Razenberg F, Smits-Wintjens VE, van Zwet EW, Rijken M, Steggerda SJ, Lopriore E: **Neonatal thrombocytopenia after perinatal asphyxia treated with hypothermia: a retrospective case control study**. *Int J Pediatr* 2014, **2014**:760654.
16. Jacobs SE, Morley CJ, Inder TE, Stewart MJ, Smith KR, McNamara PJ, Wright IM, Kirpalani HM, Darlow BA, Doyle LW: **Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial**. *Arch Pediatr Adolesc Med* 2011, **165**(8):692-700.
17. Simbruner G, Mittal RA, Rohlmann F, Muche R: **Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT**. *Pediatrics* 2010, **126**(4):e771-778.
18. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, Fanaroff AA, Poole WK, Wright LL, Higgins RD *et al*: **Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy**. *N Engl J Med* 2005, **353**(15):1574-1584.

19. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, Polin RA, Robertson CM, Thoresen M, Whitelaw A *et al*: **Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial**. *Lancet* 2005, **365**(9460):663-670.
20. Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, Kapellou O, Levene M, Marlow N, Porter E *et al*: **Moderate hypothermia to treat perinatal asphyxial encephalopathy**. *N Engl J Med* 2009, **361**(14):1349-1358.
21. Joanna RGV, Lopriore E, Te Pas AB, Rijken M, van Zwet EW, de Bruine FT, Steggerda SJ: **Persistent pulmonary hypertension in neonates with perinatal asphyxia and therapeutic hypothermia: a frequent and perilous combination**. *J Matern Fetal Neonatal Med* 2022, **35**(25):4969-4975.
22. Groenendaal F, van Bel F: **Perinatal asphyxia in term and late preterm infants**. In. UpToDate; 2021.
23. Lemyre B, Chau V: **Hypothermia for newborns with hypoxic-ischemic encephalopathy**. *Paediatr Child Health* 2018, **23**(4):285-291.
24. Deierl A, Tusor N, Ben-Sasi K, Bate T: **Hypoxic Ischaemic Encephalopathy Management and Therapeutic Hypothermia pathway in North West London Perinatal Network neonatal units**. In. North West London Perinatal Operational Delivery Network; 2021.
25. Aker K, Støen R, Eikenes L, Martinez-Biarge M, Nakken I, Håberg AK, Gibikote S, Thomas N: **Therapeutic hypothermia for neonatal hypoxic-ischaemic encephalopathy in India (THIN study): a randomised controlled trial**. *Arch Dis Child Fetal Neonatal Ed* 2020, **105**(4):405-411.
26. Nielsen ST, Strandkjær N, Juul Rasmussen I, Hansen MK, Lytsen RM, Kamstrup PR, Rode L, Goetze JP, Iversen K, Bundgaard H *et al*: **Coagulation parameters in the newborn and infant - the Copenhagen Baby Heart and COMPARE studies**. *Clin Chem Lab Med* 2022, **60**(2):261-270.
27. Aker K, Thomas N, Adde L, Koshy B, Martinez-Biarge M, Nakken I, Padankatti CS, Støen R: **Prediction of outcome from MRI and general movements assessment after hypoxic-ischaemic encephalopathy in low-income and middle-income countries: data from a randomised controlled trial**. *Arch Dis Child Fetal Neonatal Ed* 2022, **107**(1):32-38.
28. Thayyil S, Oliveira V, Lally PJ, Swamy R, Bassett P, Chandrasekaran M, Mondkar J, Mangalabharathi S, Benkappa N, Seeralar A *et al*: **Hypothermia for encephalopathy in low and middle-income countries (HELIX): study protocol for a randomised controlled trial**. *Trials* 2017, **18**(1):432.
29. Thoresen M, Whitelaw A: **Cardiovascular changes during mild therapeutic hypothermia and rewarming in infants with hypoxic-ischemic encephalopathy**. *Pediatrics* 2000, **106**(1 Pt 1):92-99.
30. Carter BS, McNabb F, Merenstein GB: **Prospective validation of a scoring system for predicting neonatal morbidity after acute perinatal asphyxia**. *J Pediatr* 1998, **132**(4):619-623.
31. Manzar S, Al-Umran K, Al-Awary BH, Al-Faraidy A: **Changes in plasma creatinine in first 72 hours of life**. *Arch Dis Child Fetal Neonatal Ed* 2001, **85**(2):F146-147.
32. van Wincoop M, de Bijl-Marcus K, Lilien M, van den Hoogen A, Groenendaal F: **Effect of therapeutic hypothermia on renal and myocardial function in asphyxiated (near) term neonates: A systematic review and meta-analysis**. *PLoS One* 2021, **16**(2):e0247403.
33. Pauliah SS, Shankaran S, Wade A, Cady EB, Thayyil S: **Therapeutic hypothermia for neonatal encephalopathy in low- and middle-income countries: a systematic review and meta-analysis**. *PLoS One* 2013, **8**(3):e58834.
34. Thayyil S, Shankaran S, Cowan F, Ayer M, Price D, Bainbridge A, Cady E, Marlow N, Costello A, Robertson N *et al*: **Brain Injury Following Whole Body Cooling after Neonatal Encephalopathy in a South Indian Neonatal Unit**. *Pediatric Research* 2011, **70**(5):33-33.
35. Wintermark P, Boyd T, Gregas MC, Labrecque M, Hansen A: **Placental pathology in asphyxiated newborns meeting the criteria for therapeutic hypothermia**. *Am J Obstet Gynecol* 2010, **203**(6):579.e571-579.

36. Hakobyan M, Dijkman KP, Laroche S, Naulaers G, Rijken M, Steiner K, van Straaten HLM, Swarte RMC, Ter Horst HJ, Zecic A *et al*: **Outcome of Infants with Therapeutic Hypothermia after Perinatal Asphyxia and Early-Onset Sepsis**. *Neonatology* 2019, **115**(2):127-133.
37. Prashantha YN, Suman Rao PN, Nesargi S, Chandrakala BS, Balla KC, Shashidhar A: **Therapeutic hypothermia for moderate and severe hypoxic ischaemic encephalopathy in newborns using low-cost devices - ice packs and phase changing material**. *Paediatr Int Child Health* 2019, **39**(4):234-239.
38. Lehtonen L, Gimeno A, Parra-Llorca A, Vento M: **Early neonatal death: A challenge worldwide**. *Semin Fetal Neonatal Med* 2017, **22**(3):153-160.
39. Shankaran S: **Outcomes of hypoxic-ischemic encephalopathy in neonates treated with hypothermia**. *Clin Perinatol* 2014, **41**(1):149-159.
40. Azzopardi D, Brocklehurst P, Edwards D, Halliday H, Levene M, Thoresen M, Whitelaw A: **The TOBY Study. Whole body hypothermia for the treatment of perinatal asphyxial encephalopathy: a randomised controlled trial**. *BMC Pediatr* 2008, **8**:17.
41. Riley RD, Ensor J, Snell KIE, Harrell FE, Jr., Martin GP, Reitsma JB, Moons KGM, Collins G, van Smeden M: **Calculating the sample size required for developing a clinical prediction model**. *Bmj* 2020, **368**:m441.
42. Krishnan V, Kumar V, Shankaran S, Thayyil S: **Rise and Fall of Therapeutic Hypothermia in Low-Resource Settings: Lessons from the HELIX Trial**. *Indian J Pediatr* 2021.
43. Krishnan V, Kumar V, Variane GFT, Carlo WA, Bhutta ZA, Sizonenko S, Hansen A, Shankaran S, Thayyil S: **Need for more evidence in the prevention and management of perinatal asphyxia and neonatal encephalopathy in low and middle-income countries: A call for action**. *Semin Fetal Neonatal Med* 2021, **26**(5):101271.



NTNU

Norwegian University of
Science and Technology