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Outcome of Infants with Therapeutic Hypothermia after Perinatal Asphyxia and Early-Onset Sepsis

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Keywords
Early-onset sepsis · Perinatal asphyxia · Therapeutic hypothermia · Cerebral palsy · Neurodevelopmental impairment · Group B streptococcus

Abstract
Background: Animal models suggest that neuroprotective effects of therapeutic hypothermia (TH) after perinatal asphyxia are reduced in infants with early-onset sepsis. ***Objectives:*** To assess the outcome of infants with perinatal asphyxia, neonatal encephalopathy, and TH in the presence of early-onset sepsis. ***Methods:*** In a retrospective cohort of 1,084 infants with perinatal asphyxia and TH, the outcome of 42 infants (gestational age 36.1–42.6 weeks and birth weight 2,280–5,240 g) with proven sepsis ($n = 14$) and probable sepsis ($n = 28$) was analyzed. Death, cerebral palsy, or a delayed development at 2 years was considered an adverse outcome. ***Results:*** Sepsis was caused mostly by group B streptococci ($n = 17$), other Gram-positive bacteria ($n = 5$), and *Candida albicans* ($n = 1$). Of the 42 infants, 9 (21.4%) died, and 5 (11.9%) showed impairments on follow-up. The outcome is comparable to the previously reported outcome of infants with TH without early-onset sepsis. ***Conclusion:*** A good outcome was reported in the majority of infants with perinatal asphyxia, TH, and early-onset sepsis. Cooling should not be withheld from these infants.

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Introduction

Neonatal encephalopathy (NE) following perinatal asphyxia in term neonates is still a common and serious condition. The prevalence of NE after perinatal asphyxia is approximately 1–6 per 1,000 full-term live births [1, 2]. It is well known that infants with moderate-to-severe NE carry a high risk of adverse outcome, such as cerebral palsy (CP), neurodevelopmental impairment, or mortality, even after therapeutic hypothermia (TH) [1, 3, 4]. In addition, early-onset sepsis which is mostly caused by group B streptococcus (GBS) or Gram-negative organisms, such as *Escherichia coli*, carries a high risk of an adverse outcome [5]. The outcome of infants with perinatal asphyxia, early-onset sepsis, and TH has not been reported in much detail. It has been suggested that encephalopathic newborns with early-onset sepsis may have a worse outcome compared to nonseptic neonates [6]. Studies in adults with sepsis did not show benefits of hypothermia [7]. In addition, in the study by Geurts et al. [8] an increased risk for pneumonia and sepsis was observed, although the overall infection risk was not significantly higher. At present, little is known about the interplay of hypothermia and sepsis. Several animal models have examined the neuroprotective effect of TH in the presence of bacterial infections and results are inconclusive [9–12].

In the present study, the outcome of infants with perinatal asphyxia, NE treated with hypothermia, and early-onset sepsis was assessed.

Subjects and Methods

Infants with a gestational age between 36 + 0 and 42 + 0 weeks with perinatal asphyxia, NE, and TH admitted to one of the level III participating Neonatal Intensive Care Units (NICU) in the Netherlands or Flanders, Belgium, between January 2008 and December 2016 were included. During this period, 1,084 infants were treated with TH in the participating hospitals. Retrospectively, data were collected from the medical files. Growth percentiles were calculated according to the Netherlands Perinatal Registry Birth Weight centiles (www.perined.nl) [13].

Infants with positive blood cultures within 48 h after birth and clinical signs of sepsis were considered to have a proven sepsis. Infants with clinical signs of early-onset sepsis and an elevated CRP (≥50 mg/L) or positive surface cultures, but no positive blood culture, were considered to have a probable sepsis. All infants were treated with antibiotics for at least 7 days, and all had signs of multi-organ failure. Most infants were too ill to undergo lumbar punctures.

The severity of encephalopathy was graded according to Sarnat. TH was used as described previously [3]. Although 3 infants appeared to have a mild encephalopathy, aEEG showed a suppressed background pattern and TH was applied. In 3 infants with a good aEEG background pattern on admission, TH was started because of a high Thompson score.

In all infants, aEEG was used routinely, and patterns were analyzed as described previously [14]. Clinical and/or aEEG-detected seizures were treated according to the Dutch/Flemish neonatal seizure protocol which includes phenobarbital with midazolam and/or lidocaine as add-on therapy [15]. Brain imaging (cranial ultrasound and MRI) was collected from the files. MRI abnormalities were reported as watershed lesions, lesions in the basal ganglia and thalamus (BGT), or near total injury [16].

Outcome

After discharge, follow-up assessments were performed in the participating hospitals at regular intervals up to at least 18 months in the routine follow-up program. Death, CP, neurodevelopmental impairment of >3 months, a Griffiths' developmental quotient <88 (–1 standard deviation, SD), or a score on the Bayley Scales of Infant and Toddler Development-III <85 (–1 SD) were all considered an adverse outcome. In addition, infants ($n = 4$) with a normal MRI at birth and having no neurological abnormalities at the age of 6 months, and 2 additional infants with a normal MRI and no follow-up data were categorized in the group with no adverse outcome.

Statistical Analysis

Mortality and adverse neurodevelopmental outcome data were compared to the data reported previously in our units [3] and a Cochrane review [4], using χ^2 tests, Fisher tests, or analysis of variance (ANOVA) where appropriate. Data were expressed as mean ± SD, median with interquartile range (IQR), or in percentages. With the number of 42 patients, it would be possible to compare neuroprotective effects of hypothermia in septic patients (both proven and probable sepsis combined) with all hypothermia patients presented in the studies mentioned above [3, 4] with an alpha of 0.05 and a power of 0.80. This retrospective study was approved by the local ethics committee, and the requirement to obtain informed consent for this study with anonymous data analysis was waived according to national regulations.

Results

Between January 2008 and December 2017, 42 infants with perinatal asphyxia and TH showed early-onset sepsis. Of these 42 infants, 14 infants had proven sepsis and 28 probable sepsis. Clinical data of our patients are presented in Table 1. Clinical data were not significantly different between the proven and probable sepsis groups. Gestational age and birth weight were lower in the neonates who died ($n = 9$) compared to the ones who survived ($n = 33$); however, the 5th and the 10th percentile birth weights were similar. Infants who died had a higher Thompson score and a more severe encephalopathy. Clinical data of the patients with sepsis, such as gestational age, birth weight, and severity of encephalopathy were comparable to those reported in other studies of

Table 1. Clinical data

Characteristics	All early-onset septic infants (n = 42)	Probable sepsis (n = 28)	Proven sepsis (n = 14)	p value*	Survived (n = 33)	Died (n = 9)	p value+
Gestational age, weeks	40.1±1.55	40.0±1.49	40.2±1.72	0.75	40.3±1.45	39.1±1.65	**0.04**
Female	21 (50.0)	14 (50.0)	7 (50.0)	1.00	16 (48.5)	5 (55.6)	0.71
Birthweight	3,659±662	3,660±726	3,658±534	0.99	3,814±619	3,094±502	**0.003**
P5 for SGA	4 (9.5)	3 (10.7)	1 (7.1)	1.00	2 (6.1)	2 (22.2)	0.20
P10 for SGA	6 (14.3)	4 (14.3)	2 (14.3)	1.00	3 (9.1)	3 (33.3)	0.10
Mode of birth (n = 39[a])				0.56			0.42
Section	16 (41.0)	10 (37.0)	6 (50.0)		12 (40.0)	4 (44.4)	
SVD	18 (46.2)	14 (51.9)	4 (33.3)		13 (43.3)	5 (55.6)	
Vacuum extraction	5 (12.8)	3 (11.1)	2 (16.7)		5 (16.7)	0 (0.0)	
Meconium (n = 34[a])	19 (55.9)	11 (50.0)	8 (66.7)	0.35	14 (51.9)	5 (71.4)	0.35
Grade of encephalopathy on admission (n = 21[b])				0.75			**<0.0001**
Mild	3 (14.3)	2 (16.7)	1 (11.1)		3 (18.8)	0 (0.0)	
Moderate	12 (57.1)	6 (50.0)	6 (66.7)		12 (75.0)	0 (0.0)	
Severe	6 (28.6)	4 (33.3)	2 (22.2)		1 (6.3)	5 (100)	
Apgar score (n = 41[a])							
1 min	1 [2]	1 [2]	1.5 [2]	0.57	1 [2]	0 [2]	0.14
5 min	3 [3]	3 [3]	3.5 [3]	0.39	3.5 [2]	2 [5]	0.4
pH[c] (n = 34[a])	6.98±0.20	7.03±0.20	6.91±0.17	0.07	6.98±0.21	7.0±0.13	0.93
Thompson score (n = 35[a])	10 [3]	10 [3]	10 [2]	0.89	10 [3]	13 [3]	**0.02**
Antiepileptic drugs ≥1 (n = 37[a])	26 (70.3)	15 (60.0)	11 (91.7)	0.06	20 (71.4)	6 (66.7)	0.79
Highest CRP during hypothermia (n = 39[a])	106±72.5	94±54.5	135±103	0.11	103±69.0	116±89.5	0.65
aEEG on admission (n = 33[a])				0.58			0.06
CNV	3 (9.1)	3 (13.6)	0 (0.0)		3 (12.0)	0 (0.0)	
DNV	6 (18.2)	4 (18.2)	2 (18.2)		6 (24.0)	0 (0.0)	
BS	13 (39.4)	7 (31.8)	6 (54.5)		11 (44.0)	2 (25.0)	
LV	5 (15.2)	4 (18.2)	1 (9.1)		2 (8.0)	3 (37.5)	
FT	6 (18.2)	4 (18.2)	2 (18.2)		3 (12.0)	3 (37.5)	
Mortality	9 (21.4)	6 (21.4)	3 (21.4)	1.00	–	–	–

Values are expressed as means ± SD, numbers with percentage in parentheses, or medians with IQR in square brackets. Bold values are $p < 0.05$. SGA, small for gestational age; P5, 5th percentile; P10, 10th percentile; SVD, spontaneous vaginal delivery; aEEG, amplitude-integrated electroencephalography; CNV, continuous normal voltage; DNV, discontinuous normal voltage; BS, burst suppression; LV, low voltage; FT, flat trace.

[a] Percentages are based on data that were available from the number of infants in the institutions. It means that some data were missing. [b] Data were missing for 21 infants. [c] The umbilical arterial pH was not reported in 8 infants. * p values were calculated between probable and proven sepsis. + p values were calculated between infants who survived and those who died.

TH in the Cochrane review by Jacobs et al. [4] and in Groenendaal et al. [3].

Microbiology

The children with proven sepsis showed a positive blood culture with Gram-positive bacteria, which included GBS (n = 10), *Actinomyces* (n = 1), coagulase-negative staphylococci (n = 1), *Streptococcus viridans* (n = 1), and *Streptococcus milleri* (n = 1, Table 2). The infant with *S. viridans*-proven sepsis died. The infants with proven sepsis who survived developed no neurological impairments. Some neonates in the probable sepsis group had no surface cultures taken (n = 19). These infants were diagnosed with probable sepsis based on their high CRP values and clinical symptoms, leaving 9 neonates with a positive surface with Gram-positive bacteria (GBS, n = 7, and *Enterococcus hirae*, n = 1) or fungus (*Candida*, n = 1). There was no significant difference in adverse outcome, considering the type of organism found in blood or surface culture (Table 2).

Table 2. Bacteria cultured in infants with a proven or probable sepsis

	Proven sepsis (n = 14)	Probable sepsis (n = 28)	Adverse outcome
Surface or blood culture[a]			
GBS	10 (71.4)	7 (77.8)	6
CNS	1 (7.1)	–	0
Actinomyces oris	1 (7.1)	–	0
Streptococcus milleri	1 (7.1)	–	0
Streptococcus viridans[b]	1 (7.1)	–	1
Enterococcus hirae	–	1 (11.1)	0
Candida	–	1 (11.1)	0
Unknown[c]	–	19 (45.2)	7

Values are expressed as numbers with percentages in parentheses. GBS, group B streptococcus; CNS, coagulase-negative staphylococcus.

[a] Surface culture was taken from the ear and/or umbilicus, with missing data for 19 infants. [b] This infant with *S. viridans* sepsis developed a coinfection with CNS at birth. [c] These infants were included in the probable sepsis group due to clinical signs of sepsis and a CRP >50 mg/L.

Table 3. MRI data and survival

Characteristics	Probable sepsis (n = 28)	Proven sepsis (n = 14)	Survived (n = 33)	Died (n = 9)
MRI (n = 35)[a]				
Normal	13 (46.4)	5 (35.7)	18 (54.5)	0 (0.0)
WS	5 (17.8)	2 (14.3)	7 (21.2)	0 (0.0)
BGT	4 (14.3)	2 (14.3)	5 (15.2)	1 (11.1)
NT	3 (10.7)	1 (7.1)	1 (3.0)	3 (33.3)
Not performed	3 (10.7)	4 (28.6)	2 (6.1)	5 (55.6)

Values are expressed as numbers with percentages in parentheses. MRI, magnetic resonance imaging; WS, watershed pattern of injury; BGT, basal ganglia and thalamus pattern of injury; NT, "near total" pattern of injury.

[a] MRI was not performed in 7 infants, of whom 5 died.

Outcome

Imaging

Findings of cranial MRI examinations at follow-up are presented in Table 3. The MRI showed no abnormalities in 51.4% of the infants with sepsis. Infants who died had more severe MRI abnormalities ($p < 0.0001$). Four infants (11.4%) had a near total pattern on the MRI. Of the 4 infants with a near total pattern, 3 died and 1 survived but developed neurological disabilities. The aEEG of these 4 neonates showed a flat trace or continuous low voltage and 2 had a Thompson score of >11. Furthermore, 6 neonates (17.1%) had BGT involvement on the MRI, and 7 neonates (20%) had a watershed-type injury. One infant with a BGT pattern died and 2 developed neurological disabilities. Finally, there was no difference in MRI results between proven and probable sepsis ($p = 0.992$).

Mortality

The overall mortality among septic infants with TH after asphyxia was 21.4%. Two infants died shortly after admission due to severe sepsis, 7 others died after redirection of care following severe brain injury which was demonstrated using MRI. Postmortem examination was performed in 2 infants, confirming the multi-organ involvement and MRI findings. No significant difference was found in mortality between the proven and probable sepsis groups. The mortality in the present study (21.4%) was comparable to the previous study (31.8%) and the Cochrane review (26.8%; Table 4).

Follow-Up

Outcome data on Neurodevelopmental disabilities or CP are presented in Table 4. Of the 42 neonates, 33 (78.6%) infants survived. Among the survivors, 5 (15.1%) had neurodevelopmental impairment including CP. Three infants were too young to be formally tested or had no follow-up. The remaining 25 infants with perinatal asphyxia and early-onset sepsis were normal (59.5%). Hypothermia-treated survivors with sepsis had no difference in the incidence of adverse outcome compared to the previous TH studies.

Discussion

In the present study, the outcome of septic neonates who underwent TH was reported. During the study period, 42 of the 1,084 infants (3.9%) had proven or probable early-onset sepsis. Whereas one-third had an adverse outcome, more than 60% was normal at 18 months or later. An additional 2 younger infants were too young to be formally tested but were normal at this younger age. These outcomes are comparable to the data reported in large RCTs and the results of previously reported patients in the Netherlands and Flanders, Belgium, without sepsis [4, 17–20].

Table 4. Outcome of septic neonates with TH after asphyxia compared to previous studies [3, 4]

Outcome	Early-onset sepsis (n = 42)	Groenendaal et al. [3] (n = 308)	p value*	Cochrane review [4] (n = 678)	p value^{+}
Normal outcome	26 (61.9)	168 (54.5)	0.37	366 (54.0)	0.32
Neurodevelopmental impairment or CP	5 (11.9)	42 (13.6)	0.76	130 (19.2)	0.24
Mortality	9 (21.4)	98 (31.8)	0.17	182 (26.8)	0.44
Adverse outcome	14 (33.3)	140 (45.5)	0.14	312 (46.0)	0.11
Too young to be tested	2 (4.8)	–	–	–	–

Values are expressed as numbers with percentages in parentheses. * Significant difference was calculated between the early-onset sepsis group and Groenendaal et al. [3]. $^{+}$ Significant difference was calculated between the early-onset sepsis group and the Cochrane review [4].

Infections with GBS are still an important cause of serious morbidity in neonates [21]. In the present study, the outcome of infants with infections caused by GBS was not different from infections caused by other organisms. Infections with Gram-negative organisms were not seen in the present study. In the Netherlands, early-onset sepsis with Gram-negative organisms in full-term infants is very rare (data from the Netherlands Perinatal Registry, www.perined.nl).

TH has a neuroprotective effect by influencing different pathways including metabolism, cerebral blood flow, the release of excitatory amino acids, and apoptosis. Furthermore, TH has an antioxidant effect, the ability to block the proinflammatory cascade and reduce ATP loss [6, 22]. During sepsis, metabolic demands in different organs are high due to the inflammation response, which may increase neuronal apoptosis and subsequent neurological damage. Based on this theory, TH could also be effective in infants with early-onset sepsis. In contrast, hypothermia may suppress the potentially protective inflammatory cascade and may result in functional immune compromise, leading to an adverse outcome in infants with sepsis [23]. Animal experiments have described conflicting results in models of perinatal asphyxia and infections. Neuroprotective effects have been described in neonatal models of Gram-positive sepsis and TH [11], whereas a lack of effects has been detected in neonatal models of Gram-negative sepsis and TH [9, 10]. In contrast, prolonged survival in Gram-negative sepsis was documented in adult models of Gram-negative and Gram-positive sepsis and TH [12, 24].

The large trials of TH in perinatal asphyxia and NE have not described the effects of TH in infants with early-onset sepsis in much detail. TH may increase the risk of infections [23]. A meta-analysis in adults strongly suggested an association between TH and the risk of pneumonia and sepsis [8]. In main randomized trails, solely 5–11.3% of infants developed sepsis [17, 18, 20, 25]. However, early-onset sepsis has not been defined in much detail in most trials, and in many studies no difference was reported between early and late-onset sepsis. In the present study, 14 of the infants developed late-onset sepsis which is higher number compared to the study of Jacobs et al. [25].

Our study has several limitations. First, it had a retrospective design, and some clinical data were not reported in much detail. Furthermore, some units did not perform routine surface cultures, thereby limiting the detection of the causative organism in infants with clinical sepsis. The effect of TH on CRP levels is controversial [26, 27]. Nevertheless, by using very high cutoff values for CRP (>50 mg/L), twice the upper level as those mentioned by others [28, 29], and a clinical picture of early-onset sepsis, we considered the risk of false positives to be low. Second, follow-up was not performed uniformly, which may have led to somewhat diverse outcome data. By using cutoff values of the separate tests, we were able to identify infants with an adverse outcome. Third, the numbers of sepsis cases were too small to provide detailed information on the outcome of Gram-positive versus Gram-negative neonatal sepsis, but the sample size was large enough to demonstrate that neuroprotection by TH was retained in infants with perinatal asphyxia and early-onset sepsis. Furthermore, no lumbar puncture was performed in most infants because of the severity of illness, and the presence of accompanying meningitis is unknown.

Conclusion

A good outcome was reported in more than 60% of infants with perinatal asphyxia, sepsis, and therapeutic hypothermia. Therapeutic hypothermia should not be withheld from infants with perinatal asphyxia, neonatal encephalopathy, and early-onset sepsis.

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Statement of Ethics

For this observational study analyzing and reporting a large set of anonymized data a waiver of informed consent was obtained according to European legislation.

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