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OPTIMIZING THE INTENSIVE CARE OF ASPHYXIATED NEONATES

PhD thesis

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Abbreviations

ACA: anterior cerebral artery

ADC: apparent diffusion coefficient

aEEG: amplitude integrated electroencephalography

ANOVA: analysis of variance

ATP: adenosine triphosphate

BBB: blood– brain barrier

BD: base deficit

BRS: behavioral rating scale

BS: burst suppression

Ca^{2+}: calcium

CBF: cerebral blood flow

CBFV: cerebral blood flow velocity

CI: confidence interval

CNV: continuous normal voltage

CO_2: carbon- dioxide

$CrSO_2$: cerebral oxygen saturation

DGM: deep gray matter

DNA: deoxyribonucleic acid

DNV: discontinuous normal voltage

DWI: diffusion-weighted imaging

ECMO: extracorporeal membrane oxygenation

Epo: erythropoietin

EPP: exchangeable phosphate pool

FiO_2: fraction of inspired oxygen

FT: flat trace

GABA: gamma aminobutyric acid

HENRIC: hypoxic-ischemic encephalopathy therapy optimization in neonates for better neuroprotection with inhalative CO_2

HI: hypoxia-ischemia

HIE: hypoxic-ischemic encephalopathy

H-MRS: proton magnetic resonance spectroscopy

HR: heart rate

ICC: intra-class correlation coefficient

iNO: inhaled nitric oxide

IVH: intraventricular hemorrhage

IQR: interquartile range

MABP: mean arterial blood pressure

MAS: meconium aspiration syndrome

MCA: middle cerebral artery

MDI: mental developmental index

MRI: magnetic resonance imaging

MRS: magnetic resonance spectroscopy

Na^+: sodium

NAA: N-acetylaspartate

Na^+/K^+ ATPase: sodium-potassium adenosine triphosphates

NE: neonatal encephalopathy

NETS- PCA: Neonatal Emergency &Transport Services of the Peter Cerny Foundation

NICHD: National Institute of Child Health and Human Development

NICU: neonatal intensive care unit

NIRS: near infrared spectroscopy

NMDA: N-methyl-D-aspartate

NO: nitric-oxide

NO synthase: nitric-oxide synthase

NTP: nucleotide tri-phosphate

O_2: oxygen

OR: odds ratio

$PaCO_2$: arterial partial pressure of carbon-dioxide

PaO_2: arterial partial pressure of oxygen

PCO_2: partial pressure of carbon-dioxide

PCr: phosphocreatine

PDI: psychomotor developmental index

PEEP: positive end expiratory pressure

Pi: inorganic phosphate

PI: pulsatility index

PIP: peak inspiratory pressure

P_{max}: peak inspiratory pressure limit

P-MRS: phosphorus magnetic resonance spectroscopy

PPHN: persistent pulmonary hypertension

PVL: periventricular leukomalacia

RDS: respiratory distress syndrome

RI: resistance index

ROSC: return of spontaneous circulation

RR: respiratory rate

SAH: subarachnoid hemorrhage

SD: standard deviations

SDH: subdural hemorrhage

SpO_2: peripheral oxygen saturation

SWI: susceptibility-weighted imaging

TCD: transcranial doppler ultrasonography

TH: therapeutic hypothermia

TOBY: Total Body Hypothermia for Neonatal Encephalopathy

Vd: diastolic peak- flow velocity

Vs: systolic peak- flow velocity

WM: white matter

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1. Introduction

Perinatal asphyxia is a devastating condition that may lead to hypoxic-ischemic encephalopathy (HIE) and permanent neurological deficit in the adulthood. HIE affects up to 3 infants per 1000 live birth every year in developed countries, placing a huge burden on families and the health care system (1). The magnitude of the problem is clear from both a personal and an economic perspective. To date, only therapeutic hypothermia (TH) has been shown to reduce mortality and adverse neurodevelopmental outcome in patients with moderate to severe HIE (2). The neuroprotective effect of TH continues to childhood (3, 4). Even with hypothermia, nearly 40% of infants affected either die or develop severe disabilities such as cerebral palsy, mental retardation and learning difficulties (2). Over the last decade, several pharmacological agents have been tested in order to augment the hypothermic neuroprotection. To date none of the adjunctive therapies have been proven to be beneficial in the treatment of HIE (5). The optimization of TH and the provision of intensive care support to these infants have become the center of attention lately. First, it is well established that TH must be initiated as soon as possible after the hypoxic-ischemic insult to enhance the neuroprotective effect of cooling (6, 7). Further, the optimal respiratory and cardiovascular management during TH has the ability to prevent the secondary progression of brain injury leading to improved outcomes. The consistent findings of the association between hypocapnia and adverse neurological outcome suggest that the avoidance of hypocapnia may be a reasonable approach to optimize long term outcomes in this high risk population (8).

In the present work our aim was twofold, first, we evaluated the safety and feasibility of active TH during neonatal transport in order to achieve the target temperature of TH as soon as possible after the hypoxic- ischemic insult. Second, we described the incidence of hypocapnia in actively cooled infants and conducted an interventional pilot study to correct hypocapnia in infants treated with TH for HIE.

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1.1. Perinatal asphyxia and hypoxic-ischemic encephalopathy

Perinatal asphyxia is defined as the impairment in the exchange of respiratory gases leading to progressive hypercapnia, hypoxia and metabolic acidosis depending on the extent and the duration of interrupted placental blood flow (9, 10). Asphyxia at birth account for 23% of deaths in the neonatal period (11) and 8% of deaths in children younger than 5 years of age (12). Perinatal asphyxia can lead to multiorgan failure, affecting the heart, liver, kidneys and the central nervous system (Table 1) (13). The complex neurological syndrome with distinct neuropathological and clinical features that develops due to perinatal asphyxia is termed as HIE (10). Depending on the severity of the asphyxial insult, HIE is further categorized into mild, moderate or severe disease states. The overall incidence of HIE is estimated to be 1.5 (95% CI 1.3; 1.7) per 1000 live birth in developed countries (1). In Hungary the estimated number of infants born with HIE is 150-200 (1.7-2.2/1000 live birth) in each year. Between 2013 and 2015, 204 neonates with perinatal asphyxia were admitted to the tertiary level Neonatal Intensive Care Unit (NICU) of the 1st Department of Pediatrics, Semmelweis University. Of those, 142 infants fulfilled the criteria of moderate or severe HIE (14).

HIE is a subset of neonatal encephalopathy (NE), which is clinically defined as a disturbance in neurologic function demonstrated by altered level of consciousness, hypotonia, depressed or absent primitive reflexes, difficulties with initiating and maintaining respiration and often by accompanied seizures (9). NE is an “umbrella” term, that does not specify the etiology. Infection, metabolic and genetic disorders as well as hypoxic-ischemic insult can lead to NE (15). Of note, the appropriate terminology for HIE and NE has been a subject of controversy for 20 years and the terms have been used interchangeably in the literature (16). However, the discussion of this debate is beyond the scope of the present work, and the HIE terminology will be used throughout the thesis to describe the encephalopathy in neonates who suffered perinatal asphyxia.

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Table 1. Systemic consequences of perinatal asphyxia.

System	Effects
Central nervous system	Hypoxic-ischemic encephalopathy, Intracranial hemorrhage, Seizures, Cerebral Edema, Infarction, Apnea
Cardiovascular	Myocardial ischemia, Poor contractility, Cardiac stunning, Tricuspid insufficiency, Hypotension
Pulmonary	Pulmonary hypertension, Pulmonary hemorrhage, Respiratory Distress Syndrome (RDS), Meconium aspiration syndrome (MAS)
Renal	Acute tubular or cortical necrosis
Adrenal	Adrenal hemorrhage
Gastrointestinal	Perforation, Necrosis
Metabolic	Inappropriate secretion of antidiuretic hormone, Hyponatremia, Hypoglycemia, Hypocalcemia, Myoglobinuria
Hematology	Coagulopathy, Disseminated Intravascular Coagulation, Thrombocytopenia

The American College of Obstetricians and the American Academy of Pediatrics recommended a multidimensional assessment of the neonatal status and the protentional contributing factors to establish the diagnosis of HIE (17). Caregivers should assess if the following signs are present: 1) Apgar Score of less than 5 at 5 minutes and 10 minutes; 2) fetal umbilical artery acidemia (pH<7.0 or Base Deficit (BD) ≥ 12 mmol/L); 3) neuroimaging evidence of acute brain injury seen on brain Magnetic Resonance Imaging (MRI) or Magnetic Resonance Spectroscopy (MRS) consistent with hypoxia–ischemia and excluding other causes; 4) presence of multisystem organ failure, including renal, hepatic, gastrointestinal injury, cardiac and hematologic abnormalities; and 5) excluding other significant risk factors such as maternal infection, neonatal sepsis, and chronic placental lesions.

Contributing factors to a hypoxic-ischemic event can be: 1) a sentinel hypoxic or ischemic event occurring before or during labor and delivery such as uterus rupture, placental abruption, maternal hypotension and hypoxemia or cardiovascular collapse, and massive fetomaternal hemorrhage; 2) abnormal fetal heart rate tracing.

1.2. Pathophysiology

Clinical and experimental studies have shown that the neuronal death occurs in 2 phases following the hypoxic-ischemic insult. During the primary energy failure, the lack of oxygen (O_2) results in anaerobic acidosis and consequently high energy metabolites (adenosine triphosphate (ATP) and Phosphocreatine (PCr)) decrease and lactic acid accumulates. The failure of the membrane ion pumps (sodium-potassium adenosine triphosphatase (Na^+/K^+ ATPase) and sodium- calcium exchanger) results in the accumulation of sodium (Na^+), calcium (Ca^{2+}) and water leading to cytotoxic edema. The membrane depolarization due to the Ca^{2+} and Na^+ influx initiates the extracellular release of excitatory amino acids such as glutamate. The intracellular Ca^{2+} accumulation induces the production of nitric-oxide (NO) and free radicals through the activation of xanthine oxidase (Figure 1). The primary energy failure is followed by a short period of latent phase from about 6 hours after birth. The latent phase is characterized by the initial recovery of cerebral oxidative metabolism. The second decline in the high energy phosphates occurs after the latent phase in the next 24 hours and evolves in the following hours to days. The mechanisms of the secondary energy failure involve reperfusion injury with an extensive oxidative stress and mitochondrial dysfunction due to the extended reaction from the primary phase. Inflammation may also contribute to the delayed cell death (18-20). These mechanisms lead to neuronal cell death via the passive process of necrosis and the programmed cell death of apoptosis. Severe injury often results in necrosis, whereas apoptosis can be associated with milder insult and the activation of genetic programs (19, 21).

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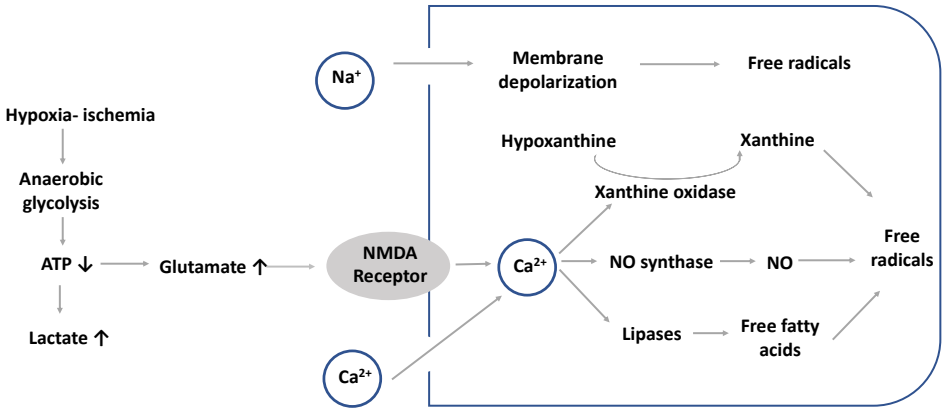

Figure 1. Cascade of events lead to neuronal cell death after hypoxia-ischemia Abbreviations: ATP, adenosine triphosphate; NMDA, N-methyl-D-aspartate; NO, nitric-oxide; NO synthase, nitric-oxide synthase. Adapted from Douglas-Escobar, M. et al. and Perlman, J.M (19, 22). See text for details.

The phases of the HIE have been confirmed with phosphorus (P-MRS) and proton MRS (H-MRS) in preclinical (23) and clinical studies (24-26). After the insult there is an increase in cerebral lactate and decrease in high energy phosphates. In the latent phase, the high energy phosphates return to the baseline, lactate levels also improve but does not recover completely. The latent phase is followed by a second decline in high-energy phosphate and a rise in cerebral lactate (27) (Figure 2). Severely affected infants who did not show recovery and ATP and PCr were absent on MRS died or had poor neurodevelopmental outcomes at 1 year of age (24). In addition, the experimental studies using repeated MRS measurements showed that TH prevented the secondary energy depletion and reduced the lactate peak (23). These results directly led to the randomized trials of hypothermia treatment.

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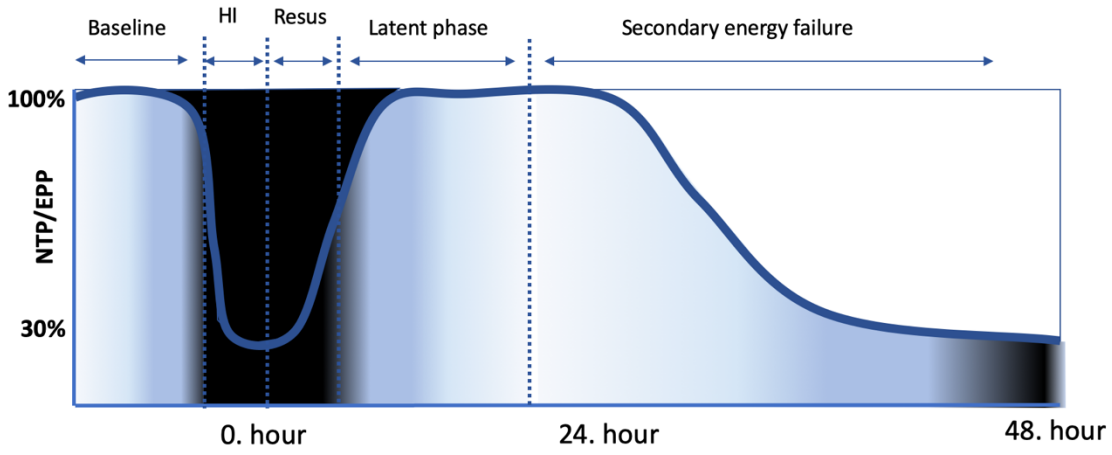

Figure 2. The biphasic pattern of energy failure detected by P-MRS.
Abbreviations: HI, hypoxia-ischemia; EPP, exchangeable phosphate pool (EPP=Pi+PCr+NTP); NTP, nucleotide tri-phosphate; PCr, phosphocreatine; Pi, inorganic phosphate. Adapted from O'Brien, F.E., et al (25). See text for details.

1.3. Clinical presentation

HIE is a well-recognized clinical syndrome with variable clinical symptoms that depend on the severity of brain injury and evolve over time (10). The most widely used classification of HIE is the Sarnat staging system, which evaluates the level of consciousness, muscle tone, reflexes and automatic functions of the infants after initital stabilization. The Sarnat staging divide infants into 3 categories: stage I (mild), stage II (moderate), and stage III (severe) category (28). Recently, clinical trials have started adopting the Thompson encephalopathy score to assess a set of clinical signs associated with neurological dysfunction (Table 2) (29). The Thompson score was found to be highly predictive for adverse outcomes (30-32).

In the long term, HIE can lead to cerebral palsy, developmental delay, intellectual impairment, blindness, sensorineural deafness requiring amplification, and epilepsy (33). Forty- seven percent of survival infants who received TH had an IQ score below 70, whereas the incidence of cerebral palsy was 17% (4). Moreover, 20% of affected children even with normal IQ received special educational support services.

Learning difficulties were mediated through motor control problems, attention-deficit hyperactivity disorder, impairment of episodic memory and behavioral concerns (34, 35). Although it is clear that TH reduces the combined outcome of mortality or major neurodevelopmental disability, still a significant number of infants live with long-term neurological impairment (2).

Table 2. Thompson encephalopathy score.
The final score sums the individual points and is interpreted as follows: mild HIE 0-10 points, moderate HIE: 11-14 points, severe HIE: 15-22 points (29).

Sign	**0 point**	**1 point**	**2 point**	**3 point**
Tone	normal	hypertonia	hypotonia	flaccid
Level of consciousness	normal	Hyperalert, stare	lethargic	comatose
Fits	none	< 3 per day	> 2 per day	
Posture	normal	fisting, cycling	strong distal flexion	decerebrate
Moro reflex	normal	partial	absent	
Grasp	normal	poor	absent	
Sucking reflex	normal	poor	absent ± bites	
Respiration	normal	hyperventilation	brief apnea	apnea
Fontanelle	normal	not full tense	tense	

1.4. Neuromonitoring

Non-invasive bedside monitoring tools, including amplitude integrated electroencephalography (aEEG), Transcranial Doppler Ultrasonography (TCD) and Near Infrared Spectroscopy (NIRS) which allow for the continuous monitoring of pathophysiological changes during the evolving hypoxic- ischemic injury (36).

Amplitude integrated electroencephalography

The aEEG is a simplified neurophysiological monitor, which is suitable for assessing electrical activity of the brain and screen for seizures. The predictive value of neonatal aEEG for long-term neurological outcome has been well described in infants with HIE. Abnormal background activity within the first 6 hours of life in normothermic infants can be useful for the selection of those infants with significant brain injury who would need neuroprotective therapy.

However, among infants treated with hypothermia, the positive predictive value of aEEG is lower in the first 6 hours compared to normothermic infants (37). On the other hand, abnormal aEEG traces beyond the second day of life is a strong predictor of death or moderate/severe disabilities even during hypothermia treatment (37, 38).

Transcranial doppler ultrasonography

The TCD is able to measure changes in systolic, diastolic, and mean cerebral blood flow velocity (CBFV) in the basal cerebral arteries including the middle cerebral artery (MCA), anterior cerebral artery (ACA) and the posterior cerebral artery (36). After the hypoxic ischemic insult, the initially low cerebral blood flow is followed by a period of hyperperfusion related to the high lactate levels and other vasodilatators accumulation during the secondary energy failure (39). It is well known, that vasoparalysis and the lack of autoregulation are typically present in extensive forms of brain injury (40, 41). Thoresen et al reported that low resistance index (RI) is less predictive of poor outcome during hypothermia than normothermia. The positive predictive value of RI $\leq$ 0.55 for death and severe disabilities was only 60%, whereas the negative predictive value was 78% in cooled asphyxiated infants (42). In conclusion, the results of TCD has to be interpreted with caution when predicting the prognosis of asphyxiated infants.

Near infrared spectroscopy

Close monitoring of cerebral oxygen saturation ($CrSO_2$= oxygenated hemoglobin/ total hemoglobin) determined by NIRS, may contribute to improved neurocritical care of this patient population (43). Several studies have reported an association between higher $CrSO_2$ and adverse outcomes in infants receiving TH for HIE (44, 45). The increased $CrSO_2$ values in infants with severe brain injury may reflect the combination of higher cerebral blood flow and a decreased extraction of oxygen associated with neuronal cell death. Studies have shown that a combined score of NIRS and aEEG has a better predictive value when compared to assessments of NIRS data and aEEG background alone (45, 46).

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Magnetic resonance imaging

The MRI and the H-MRS are the preferred imaging tools to quantify the extent of brain injury and to predict long-term neurodevelopmental outcomes in this patient population (47, 48). Diffusion-weighted imaging (DWI) refers to a specific MRI sequence that measures the random Brownian motion of water molecules within a voxel of tissue. DWI is the most reliable sequence to assess hypoxic-ischemic injury earlier than conventional MRI sequences. However, it may underestimate the extent of brain injury during the first 24 hours (49). The sensitivity of DWI can be further improved by quantification of the apparent diffusion coefficient (ADC), which is performed by voxel-wise analysis of the information contained within diffusion-weighted imaging (49, 50).

Abnormal signal intensity is commonly detected in the basal ganglia and thalami, corticospinal tract, white matter, and cortex. Basal ganglia and thalami lesions are usually accompanied by the presence of abnormal signal in the posterior limb of the internal capsule (51). These major MRI abnormalities are strongly predictive of death or disabilities at 18 months of age (48).

The MRS enables in-vivo quantitative analysis of cerebral metabolites. A wide range of H-MRS derived metabolites including the elevation of choline relative to creatine, decreased N-acetylaspartate (NAA), and the presence of a lactate peak were suggested to be a bridging biomarker for long term disabilities (22, 51). A meta-analysis concluded that deep gray matter Lactate/NAA peak area ratio was the most accurate predictor of adverse outcome before the hypothermic era (52). A multicentric cohort study recruiting neonates who received TH for HIE confirmed the predictive value of Lactate/NAA ratio with sensitivity of 88% (95% CI 70–98) and specificity of 90% (84–95). In this study of Lally, P.J., et al. the thalamic NAA concentration had the highest prognostic accuracy and was associated with cognitive, language, and motor performance at 2 years of age (53).

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1.5. Hypothermia treatment

After 20 years of preclinical and clinical trials mild total body hypothermia to a depth of 33 to 34 °C is now the gold standard therapy for infants with HIE in high-income countries. Ideally, TH must be started before the secondary energy failure and continued for 72 hours to improve cell survival (54). TH initiated beyond 6 hours after the insult had no benefit (55). Moreover, both preclinical and clinical studies suggested that to be more effective, TH should be started as soon as possible after the hypoxic-ischemic insult (6, 7). Selective head cooling initiated 90 minutes after HI insult was associated with a significant reduction in the extent of cortical neuronal loss and greater residual electroencephalogram activity in lambs (6). Similar results were detected in rat models after moderate HIE (56). Notably, initiating TH within 3 h of age significantly improved the motor outcome in asphyxiated infants compared to starting TH between 3 and 6 h of age (7).

The depth of hypothermia is also an important question. Animal studies in lambs (6) and term piglets (57) demonstrated that target temperature of 34 °C prevented neuronal death. Deeper cooling was associated with reduced neuroprotection and increased risk of adverse effects (58, 59). In agreement with this, deeper (32 °C) and longer cooling (120 h) and the combination of both in clinical trial showed no benefit and a trend toward higher risk of death. Furthermore, deeper cooling was associated with increased risk of inhaled nitric oxide (iNO) therapy and extracorporeal membrane oxygenation (ECMO), whereas longer cooling was related to more arrhythmia, anuria and longer hospital stay (60).

The potential mechanisms of hypothermic neuroprotection include reduction in cerebral metabolism by about 5% per Celsius degree with parallel decrease in O_2 consumption and CO_2 production (61). In addition, TH reduces the loss of high energy phosphates and consequently attenuates the release and accumulation of excitatory amino acids (62). Hypothermia prevents many of these acute events, however it provides a permanent neuroprotection with altering intrinsic and extrinsic pathways leading to apoptotic cell death (63).

Further, the inhibition of inflammatory responses by cooling can have a beneficial effect on cell survival (64). In the longer term, TH may affect the repair mechanisms including the differentiation of precursor cells, and the enhancement angiogenesis and neuronal connectivity (63).

A meta-analyses of 11 randomized trials including 1505 infants proved that hypothermia reduced significantly the combined outcome of death and major neurodevelopmental disabilities at 18 months of age (typical Risk Ratio 0.75 (95% CI 0.68 to 0.83)), the number needed to treat for beneficial outcome was 7 (95% CI 5 to 10). Major neurodevelopmental disabilities were defined as the following: cerebral paresis, Bayley test or Griffith assessment more than two standard deviations (SD) below the mean or intellectual impairment (intelligence quotient more than two SD below mean), blindness (vision < 6/60 in both eyes), sensorineural deafness requiring amplification (2). The neuroprotective effect of TH continues to childhood (3, 4, 65).

1.6. Therapy optimization

It is clear that TH reduces adverse outcomes after hypoxic-ischemic insult, however roughly 40% of the treated infants die or live with long term disabilities (2). Additional interventions are warranted to further improve the outcomes of such infants. Over the last decade several pharmacologic agents have been tested in order to augment hypothermic neuroprotection. Protentional agents include xenon, erythropoietin (epo), melatonin, and stem cell therapy (5).

Xenon is used as an inhaled anesthetic without any neurotoxic or adverse cardiovascular effects. The neuroprotective effect of inhaled Xenon is based on the inhibition of excitatory amino-acids and apoptotic pathways. In combination with TH, Xenon was able to reduce neuronal injuries in animal models (66). Although inhaled Xenon was safe and feasible in infants with HIE, the treatment did not have additional neuroprotective effects (67).

Epo is a hematopoietic cytokine with an anti-inflammatory and anti-apoptotic effects and its receptors are widely expressed in the central nervous system. A randomized control trial of 50 infants found that 1000 U/kg intravenous Epo given on days 1, 2, 3, 5, and 7 reduced MRI-detected brain injury and improved the short term motor outcome in the interventional group compared to the controls (68).

Melatonin increases the endogenous protective mechanisms in response to oxidative stress that is associated with the hypoxic- ischemic insult. Acting via an anti-oxidant, anti-apoptotic and anti- inflammatory process, melatonin is a protentional neuroprotective agent. A randomized control pilot study showed that melatonin with TH might be clinically efficacious, however, larger randomized trials are needed to confirm this beneficial effect (69).

The administration of autologous umbilical cord blood stem cells was safe and feasible in neonatal HIE (70, 71). In order to optimize the potential of this treatment, systematic clinical studies of optimal dosing, timing and type of stem cells are needed.

In conclusion, neither of the recently tested pharmacological agents have been proven to be beneficial in the treatment of neonatal HIE. Although there are some promising adjunctive therapies, further studies are needed to determine the effectiveness of these drugs in combination with TH. Based on the neuroprotective effect of TH, 750 infants would be needed in a trial to detect further 10% improvement in neurodevelopmental outcomes with additional therapies (60). Only those treatments that showed promise at small proof-of concept trials are candidate for multicentric randomized trials due to the high costs associated with such large trials.

In contrast, the optimization of intensive care related to therapeutic hypothermia such as respiratory management and cardiovascular support may prevent further damages and improve neurodevelopmental outcomes in a cost effective manner compared to the currently tested pharmacological agents (72, 73).

1.7. Time is brain, hypothermia during neonatal transport

The "time is brain" phrase emphasizes the time-dependent effect of TH on the central nervous system. Both preclinical and clinical studies suggest that TH must be initiated as soon as possible after HI insult to enhance the neuroprotective effect of cooling (6, 7). However, more than 50% of the infants who meet the criteria of TH born out of neonatal intensive care units that provide TH treatment (74). These neonates require neonatal transport during the first hours of life. Ideally, TH must be started after the initial stabilization at the referring hospital and maintained during transport in order to achieve the target temperature shortly after the asphyxial insult. There are two main modalities of TH during transport: 1) passive cooling: allowing the baby to naturally cool down by removal of external heating devices; 2) active cooling with water bottles and cool packs in low resource settings or preferably with high-tech cooling devices such as manual or servo-controlled cooling mattresses (75).

Passive cooling is a low-cost alternative to cooling devices however active controlled TH provides improved temperature control with decreased incidence of overcooling (74, 76-78). Overcooling can be associated with cardiovascular instability, cardiac arrhythmia, thrombocytopenia, electrolyte disturbance and increased risk of sepsis (79, 80). In addition, with active TH during transport significantly more infants can reach the target temperature prior to the arrival to the hypothermia center compared to the method of passive cooling (74, 76-78). To summarize, this intervention is extremely important considering the time-dependent neuroprotective effect of TH.

1.8. Respiratory management

1.8.1. Effect of perinatal asphyxia and hypothermia on pulmonary function

Respiratory management of infants with HIE is challenging because both hypothermia and hypoxic-ischemic insult have an impact on respiratory functions (Figure 3). Severe hypoxia at birth can abolish the initiation of spontaneous breathing and without prompt physical intervention the primary apnea is followed by gasping, terminal respiration (18, 81). Direct injury to respiratory centers and seizure burden also can lead to respiratory depression.

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Hypoxia is a significant risk factor for pulmonary hypertension at birth. Deprived O_2 supply leads to increased endothelin production followed by impaired NO- synthesis and consequently vasoconstriction occurs (82). The frequently observed MAS in association with perinatal asphyxia can further increase the pulmonary vascular tone and release inflammatory cytokines (83, 84). Beside pulmonary hypertension, perinatal asphyxia is often complicated by RDS and pulmonary hemorrhage (13, 85) .

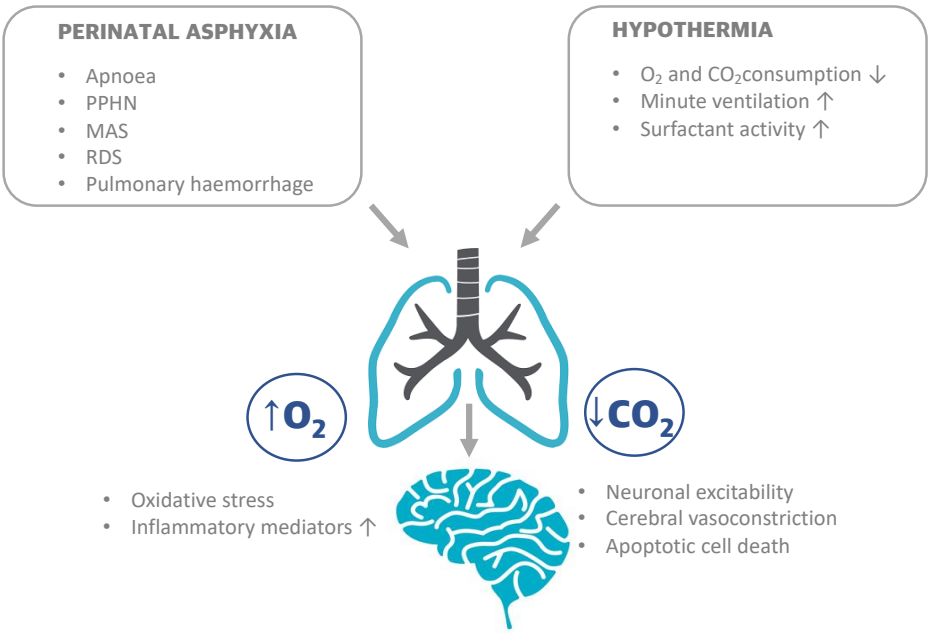

Figure 3. Effect of perinatal asphyxia and hypothermia on respiratory and central nervous system.
Abbreviations: CO_2, carbon-dioxide; MAS, meconium aspiration syndrome; O_2, oxygen; PPHN, persistent pulmonary hypertension; RDS, respiratory distress syndrome. Adapted from Szakmar E. et. al (8). See text for details.

Hypothermia can also affect respiratory functions via multiple mechanisms. First, lower body temperature can induce pulmonary vasoconstriction mainly through neuronal mechanisms (86).

In line with this, TH may increase the risk of persistent pulmonary hypertension (PPHN) in asphyxiated infants (86); however, the meta-analysis of randomized hypothermia trials did not show an elevated risk of PPHN and use of iNO in infants receiving TH compared to controls (2). In contrast, underlying pulmonary pathology such as MAS, pulmonary hemorrhage, maternal age, outborn status and other comorbidities were associated with the presence of PPHN (87, 88).

Second, hypothermia decreases O_2 release due to the leftward shift of hemoglobin dissociation curve, requiring a lower arterial oxygen tension (PaO_2) to achieve the same peripheral oxygen saturation (SpO_2) (89, 90).

Third, as temperature decreases, the solubility of gases in blood or any other fluid increases. To address this problem, pH-stat method is used widely in the intensive care of asphyxiated infants. During pH-stat management blood gas values are corrected to the actual body temperature of the patients. For example a PCO_2 of 40 mmHg will decrease to 34 mmHg and a pH of 7.4 will rise to 7.5, if the actual body temperature is reduced to 33 °C during hypothermia treatment (91).

In addition, recent observational trials reported that, TH appears to have a beneficial effect on ventilation performance including oxygenation (92, 93). According to Dassios et al minute ventilation as well as PaO_2 tended to increase during TH compared to pre-cooling and rewarming phase (93). Clearly, further trials are needed to evaluate the effects of TH on lung mechanics.

Clinical practice in ventilatory management might differ between cooling centers. In some units all infants are sedated and receive mechanical ventilation routinely during TH. However, today there is an increasing trend that infants especially with mild HIE are not ventilated routinely for cooling (94, 95).

Based on the Neonatal Encephalopathy Registry of Vermont Oxford Network, 65% of the infants with moderate- severe HIE receive invasive mechanical ventilation (94) due to underlying respiratory morbidities, altered level of consciousness or seizure burden. In comparison, the rate of mechanical ventilation was 50% in infants with mild HIE in a large Canadian cohort (96).

The detrimental effects of hyperoxia and hypocapnia are well-recognized on the previously injured brain, that is why using the lowest fraction of inspired oxygen (FiO_2) possible, and using modest ventilatory settings are required to maintain a recommended PaO_2 level of 50-100 mmHg and arterial CO_2 tension ($PaCO_2$) level of 40-50 mmHg (97, 98).

1.8.2. Oxygen management

Hyperoxia through oxidative stress and inflammatory mediators can increase the risk of secondary brain injury after asphyxial insult (99-101). The oxygen management in such infants has two distinct phases in clinical practice. First, the resuscitation in the delivery room, which has been studied extensively in randomized clinical trials and second a return of spontaneous circulation (ROSC) phase followed by TH. In the recent years several clinical trials established that resuscitation of asphyxiated infants with room air is as effective as resuscitation with 100% oxygen. Resuscitation with 21% FiO_2 showed a 31% reduction in neonatal mortality and a trend toward a reduction in the incidence of moderate (Sarnat stage 2) and severe HIE (Sarnat stage 3) when compared to resuscitation with 100% FiO_2 (102); however the long-term follow up at 18–24 months of age found no significant difference between the 2 groups in neurodevelopmental outcomes (103).

Regarding ROSC period, Sabir et al. found an association between high inspired oxygen concentration during the first 6 h of life and poor neurodevelopmental outcomes; however high PaO_2 was not associated with adverse outcome (104). In contrast, in the retrospective study of Klinger et al. PaO_2>200 mmHg during the first 20-120 minutes was an independent risk factor of adverse outcome. In addition, infants with the combination of severe hyperoxia and hypocapnia had three time higher odds to develop adverse outcome (105).

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1.8.3. Carbon-dioxide management

Partial pressure of CO_2 (PCO_2) can fluctuate over a wide range in infants with HIE. During the sentinel event hypercapnia is common, but subsequently hypocapnia may develop in the early hours of postnatal life. In the secondary analysis of the NICHD (National Institute of Child Health and Human Development) randomized trial of hypothermia treatment, it was found that 88.7% (181/204) of the infants had at least one PCO_2 below 35 mmHg and 49.0% (100/204) of the participants had at least one PCO_2 below 25 mmHg within the first 12 hours of life (106). In line with this, in a retrospective analysis of 52 asphyxiated infants with mild HIE found that only 6 out of 52 infants maintained normocapnia throughout the first 72 hours of life (107).

There are several physiologically plausible explanations for the frequently observed hypocapnia in asphyxiated infants: 1) severe brain injury may result in decreased CO_2 production; 2) compensatory hyperventilation due to severe metabolic acidosis may result in hypocapnia (108); 3) TH causes a reduction in metabolic rate and a parallel decrease in CO_2 production (63); 4) the solubility of gases within fluids increases with a decrease in temperature, indicating a lower gas tension during TH (91); 5) the intensity of resuscitation, as well as the parameters of mechanical ventilation may have an impact on the CO_2 clearance.

Hypocapnia may exacerbate brain injury via multiple mechanisms. First, it is well-established that CO_2 is one of the most potent regulators of cerebral blood flow (CBF). Hypocapnia may cause further cerebral hypoperfusion in the injured brain via hypocapnia-induced vasoconstriction. Overall, there is a 4% change in CBF per 1 mmHg change in PCO_2 under normal conditions, thus reducing $PaCO_2$ to 20 to 25 mmHg decreases CBF by 40 to 50% (109, 110). Second, hypocapnia may worsen brain ischemia by decreasing O_2 supply due to the leftward shift of oxyhemoglobin curve and increasing O_2 demand via increased neuronal excitability throughout glutamate transmission and suppressing gamma aminobutyric acid (GABA) inhibition. In addition, the increased level of excitatory amino acids may trigger seizure activity and further increase the O_2 demand (108, 109).

In addition, hypocapnia was associated with nuclear deoxyribonucleic acid (DNA) fragmentation in the cerebral cortex, membrane lipid peroxidation, and apoptotic cell death in animal models (111, 112).

The most popular hypothesis is that the effect of $PaCO_2$ on the central nervous system is mediated through alteration of the intracerebral pH (113). However, based on animal and human studies CO_2 seems to have more profound effect on central nervous system than pH (114-116), because the blood– brain barrier (BBB) is permeable to CO_2 and relatively impermeable to bicarbonate ions, causing a more rapid crossing of CO_2 over the BBB than hydrogen ions (117).

It is well- established that disturbance in CBF related to the changes in PCO_2 may predispose preterm infants to brain injury (110), including intraventricular hemorrhage (IVH) (118), cerebellar hemorrhage (119) and periventricular leukomalacia (PVL) and consequently adverse long term outcomes such as cerebral palsy and cognitive deficits may occur (120). Although the definition of hypocapnia is not uniform in the literature, there is also an increasing evidence on the association between hypocapnia and adverse outcome in resuscitated adults (121, 122) and children following cardiac arrest (123). Patients with normocapnia or mild hypercapnia had better neurological outcomes compared to those with hypocapnia.

This notion is further supported by the results from infants with moderate to severe HIE. It has been established that there is a dose-dependent effect between hypocapnia and unfavorable outcome at 2 years of age. Before the hypothermic era, Klinger et al. reported that infants with HIE who were hypocapnic within the first 2 h of life had a 2.34 higher odds for develop adverse neurological outcome than infants without hypocapnic episodes (105). Similarly, the secondary analysis of the NICHD randomized, multicentric hypothermia trial found that both minimum and cumulative exposure to PCO_2 less than 35 mmHg within the first 12 h of life increased the risk of death and adverse neurodevelopmental outcome (106). A post-hoc analysis of the CoolCap hypothermia trial also demonstrated in 196 infants with moderate to severe HIE that the probability of unfavorable outcome raised dose-dependently with decreasing PCO_2 within the 72-hours of life (124).

Recently, a retrospective study showed an association between hypocapnia over the first 4 days of life and brain injury on post-rewarming MRI (125). Although there is an increasing evidence on the association between hypocapnia and unfavorable outcomes, in the lack of a randomized trial of controlled normocapnia it remains unclear whether hypocapnia is a biomarker of poor outcome or a modifiable risk factor

Currently, clinicians have limited options to lower the risk of hypocapnia in spontaneously breathing asphyxiated infants. With deep sedation or muscle relaxation, control of ventilation is feasible; however, these approaches add the adverse effects of paralytics, sedatives, and analgesics which can accumulate and reach a potentially toxic level in hypothermic infants (126, 127). Inhalation of low concentration carbon dioxide could be a reasonable approach to avoid hypocapnia in this patient population.

Inhaled CO_2 has been tested in several indications in pediatric and neonatal patients (128-132). A Canadian research group conducted three interventional trials to find the optimal concentration and duration of inhalative CO_2 of 0.5-1.5% via nasal prongs to prevent premature apnea. They consequently found mild but tolerable increase in minute ventilation without any respiratory discomfort and improved oxygenation (128-130). They conclude that the inhalation of low concentration CO_2 was feasible and safe without any significant side effect, but in long-term it was less effective than theophylline (130).

The anticonvulsant properties of inhalative CO_2 has been tested in detail in both preclinical and clinical models. It has been well described, that in febrile seizure the disproportionally increased respiratory rate leads to respiratory alkalosis and the elevation of intracellular pH, thus an increased excitability in cortical neurons (133, 134). Preclinical studies and case reports demonstrated that inhalation of 5% CO_2 was able to stop febrile seizures promptly (134). These observations lead to a placebo controlled randomized study which has been investigated the safety and efficacy of 5% CO_2 and 95% air gas mixture, administered by parents at home in children with febrile seizure. The results of the CARDIF-study are still awaited (132).

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2. Aims

The general objective of the present PhD thesis was to study the therapy optimization for hypoxic-ischemic encephalopathy specifically the intensive care during therapeutic hypothermia. The specific aims were the following:

2.1. To evaluate the safety and feasibility of controlled active hypothermia compared to standard intensive care alone during neonatal transport.

Since timing of TH has a key role in its neuroprotective effect, implementation of active hypothermia during neonatal transport is crucial. We hypothesized that controlled active TH during neonatal transport is safe and feasible. First, to confirm the safety we investigated the critical care needs of infants receiving active TH during neonatal transport compared to a historical control group with standard intensive care alone.

For safety analysis, we compared acid-base status and vital signs (heart rate (HR), blood pressure) before and after transport in the two groups. We also compared the need for cardiac support and the rate of overcooling. In addition, we closely monitored for three adverse events: 1) severe hypotension (mean arterial blood pressure (MABP) < 25 mmHg) despite full inotrope support and volume replacement. 2) Persistent hypoxemia (SpO_2 < 90%) despite adequate ventilation, and 3) cardiac arrhythmias leading to circulatory failure. In addition, we compared the in-hospital mortality rates (occurring after 12 h of postnatal life but before discharge).

Second, the feasibility of active TH during transport was assessed by comparing the temperature profile between the actively cooled group and historical controls: 1) the time when hypothermia treatment was initiated, and 2) the time when the upper limit of target temperature (34°C) was achieved , and 3) the rectal temperature at admission to the cooling center. Moreover, we compared the length of stabilization (defined as the time that was spent at the referring hospital by the transport team to prepare the patient for transport).

Finally, we categorized the infants in the actively cooled group based on the temperature ranges on admission (< 33°C, ≤ 34°C and ≥ 33°C, and > 34°C) and compared the disease severity and the transport characteristics between the 3 groups.

2.2. To investigate the incidence of hypocapnia in mechanically ventilated infants receiving hypothermia for HIE and to evaluate the association between hypothermia and developing hypocapnia.

Based on the physiological phenomenon that TH reduces the metabolic rate, we hypothesized that the incidence of hypocapnia is higher during active TH compared to asphyxiated infants receiving standard care. Hypocapnia was defined as a temperature corrected PCO_2 that decreased below 35 mmHg during transport. Furthermore, low core temperature can add to the risk of developing hypocapnia during the early hours of postnatal life. To test our hypothesis, we compared infants undergoing active TH during neonatal transport to historical controls receiving intensive care alone.

2.3. To test the safety and feasibility of adding low concentration CO_2 (5% CO_2 + 95% air) to the inhaled gas mixture in mechanically ventilated asphyxiated infants to achieve a desired range of PCO_2 of 40–60 mmHg.

There is an increasing evidence in the literature that there is a dose- dependent association between hypocapnia and adverse neurodevelopmental outcome at 2 years of age. However, there are limited options to avoid the hypocapnia in asphyxiated infants. We hypothesized that adding 5% CO_2 to the inhaled gas is a reasonable approach to correct hypocapnia and maintain temperature corrected $PaCO_2$ level between 40 and 60 mmHg.

The primary outcome was the percentage of time spent in the desired temperature corrected PCO_2 range of 40–60 mmHg during CO_2 inhalation.

The secondary outcomes were defined as: 1) hours of life until BD decreased < 5 mmol/L; 2) hours of life until pH increased > 7.25; 3) severe hypotension (MABP < 25 mmHg), despite administration of more than one inotropic agent and volume replacement within the 72 h of life; 4) number of seizures, either detected clinically or by aEEG monitoring within the 72 h of life; and 5) intracranial hemorrhage detected by MRI within the first week of life.

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3. Methods

We enrolled term infants with the diagnosis of moderate to severe HIE in accordance with the Total Body Hypothermia for Neonatal Encephalopathy (TOBY) trial protocol (Table 3) (80). All infants were outborn and admitted to the Level-III NICU of the 1st Department of Pediatrics, Semmelweis University. The transport was provided by the Neonatal Emergency &Transport Services of the Peter Cerny Foundation (NETS-PCA). Active TH has been adopted by the transport team since 2009 October. In general, infants were excluded if they had congenital disorders, presented with mild asphyxia not fulfilling the criteria for TH or died within 12 hours of life.

Ethical permission for the studies was obtained from the Scientific and Medical Research Council Ethics Committee of Hungary (11790–2/2016/EKU and 5705-1/2016/EKU).

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Table 3. Inclusion criteria for TH based on TOBY trial (80).

The infants were assessed sequentially by criteria A, B and C, as listed below
A. Infants ≥ 36 completed weeks gestation admitted to the NICU with at least one of the following: • Apgar score of ≤ 5 at 10 minutes after birth • Continued need for resuscitation, including endotracheal or mask ventilation, at 10 minutes after birth • Acidosis within 60 minutes of birth (defined as any occurrence of umbilical cord, arterial or capillary pH < 7.00) • Base Deficit ≥ 16 mmol/L in umbilical cord or any blood sample (arterial, venous or capillary) within 60 minutes of birth Infants that meet criteria A would be assessed for whether they meet the neurological abnormality entry criteria (B) by trained personnel:
B. Moderate to severe encephalopathy, consisting of altered state of consciousness (lethargy, stupor or coma) AND at least one of the following: • hypotonia • abnormal reflexes including oculomotor or pupillary abnormalities • absent or weak suck • clinical seizures Infants that meet criteria A & B will be assessed by aEEG:
C. At least 30 minutes duration of aEEG recording that shows abnormal background aEEG activity or seizures. There must be one of the following: • normal background with some seizure activity • moderately abnormal activity • suppressed activity • continuous seizure activity

3.1. Patients

3.1.1. Safety and feasibility of controlled active hypothermia during neonatal transport

Medical records of 214 asphyxiated infants treated with TH between 2005 and 2015 were retrospectively reviewed. Of those, 136 infants who received active TH during transport between 2009 October and 2015 were compared to a historical control group of 78 infants born between January 2005 and September 2009. Control patients received standard intensive care alone without any type of cooling methods during transport and TH was initiated only after admission to the Level-III NICU.

3.1.2. Risk of hypocapnia in asphyxiated infants treated with hypothermia

A total of 126 infants, who received TH between 2007 and 2011 in our unit, were assessed retrospectively for study purposes. Five infants were excluded due to death within 12 hours of life related to severe asphyxia, 2 neonates presented with mild asphyxia and 2 patients had major congenital anomalies. Altogether, 117 patients were included in this cohort study. Patients who were cooled actively during neonatal transport (n=71) were compared to a historical control group (n=46) who received active TH only on arrival at the cooling center.

3.1.3. Hypoxic-Ischemic Encephalopathy Therapy Optimization in Neonates for Better Neuroprotection with Inhalative CO_2: the HENRIC feasibility and safety trial

Between February 2016 and June 2017, 62 term infants with moderate or severe HIE were assessed for eligibility and a total of ten infants were enrolled in this open-label, single center interventional trial (Figure 4). Inclusion criteria were: 1) fulfilling criteria for TH based on the TOBY trial; 2) temperature corrected arterial $PCO_2 \leq 40$ mmHg at any time within 6 h of life; 3) presence of spontaneous respiratory efforts while being intubated and ventilated; and 4) presence of an indwelling arterial line; 5) written informed parental consent.

Exclusion criteria were the following: 1) MAS or FiO_2 > 40%; 2) severe metabolic acidosis (pH < 6.8 and/or lactate levels > 15 mmol/L) on admission; 3) cardiovascular compromise requiring more than one inotropic agent; 4) anemia (hematocrit < 35%); and 5) >1 mmol/kg bicarbonate administration during initial stabilization; and 6) major birth defects. The study protocol was registered with ClinicalTrials.gov number NCT02700854.

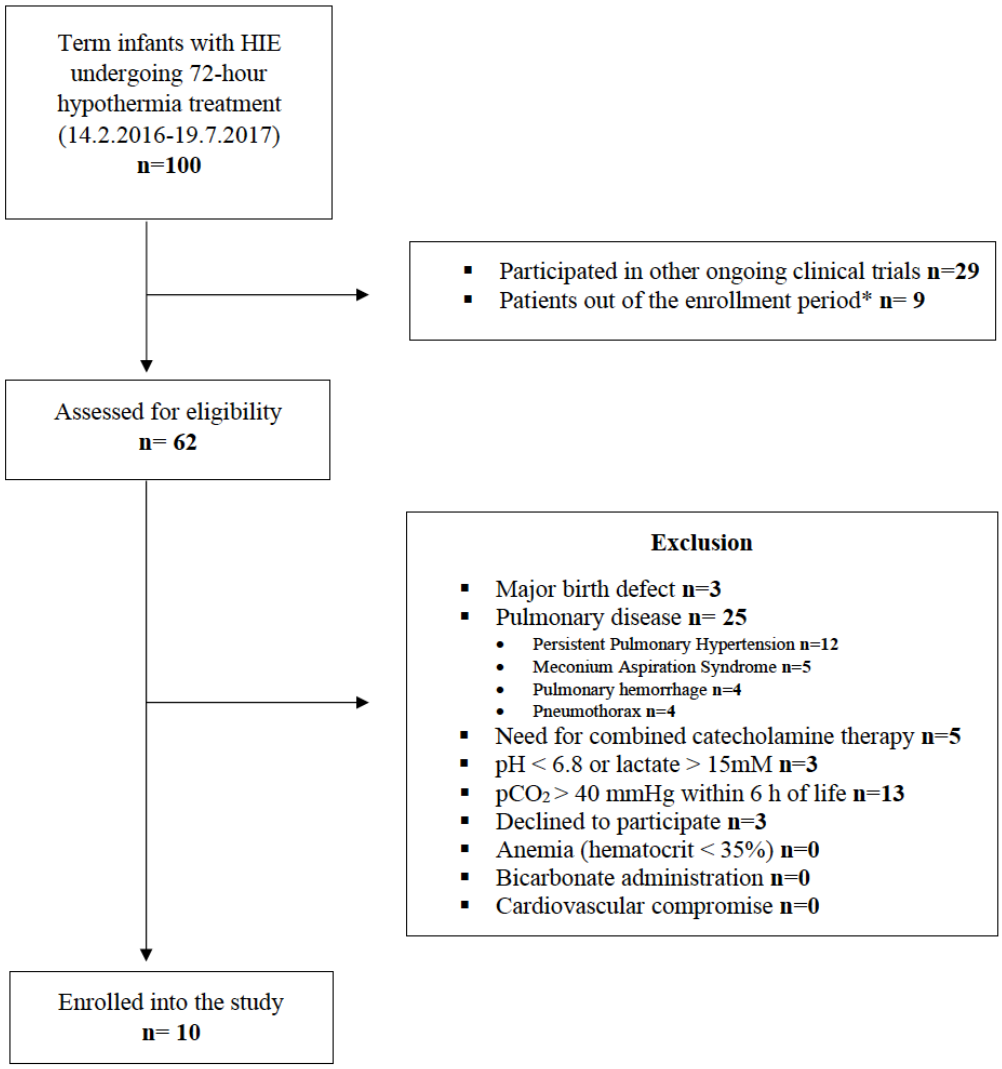

Figure 4. Patient enrollment into the HENRIC study.
* Enrollment was suspended briefly after each patient, while four independent neonatologists reviewed the data of the latest study participant and permitted the continuation of the study.

In addition, an external Data and Safety Monitoring Committee consisting of four independent neonatologists reviewed the data after each patient enrolment and permitted the continuation of the study.

3.2. Clinical management during neonatal transport

Active TH has been adopted by our neonatal transport team since 2009 October using a manually regulated cooling device (Tecotherm Ts med 200 N; Inspiration Healthcare, Leicester, United Kingdom). Before this date TH, was started only upon the arrival to the cooling center. Rectal temperature was monitored continuously to secure temperature stability. As per clinical guideline, all patients were intubated and ventilated while receiving TH. The ventilator settings during transport recommended the use of the lowest possible FiO_2 to achieve SpO_2 greater than 90%, using the lowest possible mean airway pressure, and a target PCO_2 of 40-70 mmHg. If warranted, fentanyl (2–5 μg/kg) was given for analgesia and sedation. Actively cooled infants had at least two blood gas values recorded: one before transport, to confirm the indication of TH, and another one after arrival to the NICU using the Abbott i-STAT1 blood gas analyzer (Abbott Diagnostics, Princeton, NJ, USA). Additional blood gas samples were taken as needed.

3.3. Clinical management and study protocol during the HENRIC trial

Active hypothermia has been initiated within 6 hours of life and continued for 72 hours followed by a 6-hour rewarming period with a rewarming rate of 0.5 ^{o}C using a servo controlled cooling device, Tecotherm Neo (Inspiration Healthcare, East Midlands, UK). Per clinical practice guideline, morphine was used for sedation during TH with a loading dose of 50 μg/kg followed by 10 μg/kg/h for maintenance. According to our local protocol, all patients are intubated and ventilated throughout TH and rewarming. Initial mode and parameters of mechanical ventilation were set based on our guideline: synchronized intermittent mandatory ventilation with volume guarantee mode was used with target tidal volume of 5 ml/kg, respiratory rate (RR) of 20/min, positive end expiratory pressure of 5 cm H_2O.

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Peak inspiratory pressure (PIP) limit (P_{max}) was set 5 cmH_2O above the "working" PIP, inspiratory time was 0.35–0.4 s, with an inspiratory and expiratory circuit flow of 7–8 L/min. FiO_2 was adjusted to maintain SpO_2 between 90% and 96%.

If any time within 6 hours of life arterial PCO_2 dropped below 40 mmHg, inhalation of low concentration CO_2 (5% CO_2 +95% air) (N-carbogen, Messer Hungarogaz Kft, Budapest, Hungary) was started. The gas mixture was administrated into the inspiratory arm of the ventilatory circuit. For safety reasons a CO_2 sampling line was built into the inhalation circuit to monitor CO_2 delivery continuously. The ventilator (Fabian, Acutronic Medical System, Hirzel, Switzerland) displayed the partial pressure of the inhaled 5% CO_2, which was equal to 36 mmHg at atmospheric pressure. In addition, arterial blood gas samples were taken initially every 30 minutes followed by a 2 hourly sampling rate after the stabilization of arterial PCO_2.

According to the study protocol, CO_2 inhalation was discontinued after 12 h or earlier, if the base deficit decreased to < 5 mmol/L. During the CO_2 exposition the targeted PCO_2 range was between 40 and 60 mmHg. The initial settings of the ventilation were changed if the $PaCO_2$ decreased below 35 mmHg or increased above 65 mmHg. PCO_2 administration had to be stopped if $PaCO_2$ raised above 85 mmHg.

Heart rate, MABP, RR and SpO_2 were monitored routinely and recorded every 30 minutes before, during the CO_2 administration and for up to 6 hours after stopping the CO_2 inhalation. For treatment of hypotension our protocol recommended single or double isotonic saline boluses (10-20 mL/kg), followed by the infusion of dopamine (5-20 μg/kg/min).

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3.4. Neurointensive monitoring during the HENRIC trial

Single-channel biparietal aEEG (Olympic CFM 6000 Monitor; Olympic Medical, Seattle, WA, USA) was used for continuous neurointensive monitoring and for seizure detection. The aEEG recordings were subsequently analysed by a trained neonatologist (Dr. Ünőke Méder) until the end of the rewarming, the reader was blinded to the time and duration of CO_2 exposition. Phenobarbitone with a loading dose of 20 mg/kg was administrated as a first line drug in the presence of clinical and/ or electrophysiological seizures.

Transcranial Doppler ultrasound measurements of CBFV in the anterior and medial cerebral arteries were performed before initiation of inhalative 5% CO_2 to record a baseline, during the CO_2 exposition every second hour and after the offset of 5% CO_2, using a HD11XE ultrasound machine with intracavity probe C8-4V (4-8MHz) (Philips, Andover, MA). Systolic peak-flow velocity (Vs), end diastolic peak- flow velocity (Vd) were measured by the pulse wave Doppler technique. Resistance index and pulsatility index (PI) were calculated using the following formulas: RI= (Vs-Vd)/Vs and PI=(Vs-Vd)/mean velocity.

Brain MRIs were carried out within the first week of life on a 3 Tesla Philips Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands). Images were obtained according to a standard protocol that included proton MR spectroscopy, T1-, T2-, diffusion- and susceptibility-weighted imaging (SWI) and analyzed by a pediatric radiologist (Dr. Andrea Lakatos) blinded to the clinical data.

Bayley Scales of Infant Development II examination was performed at 18–22 months of age by trained examiners. Moderate disability was defined as mental developmental index (MDI) and/or psychomotor developmental index (PDI) score 1 SD below the mean (70–84). Severe disability was defined as any of the following: severe cerebral palsy, hearing impairment, bilateral cortical visual impairment, MDI and/or PDI > 2 SD below the mean (<70).

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3.5. Statistical analysis

Data are presented as mean (standard deviation), or median (interquartile ranges), or number (percentages), as appropriate. Differences were assessed by using Mann-Whitney U and Kruskal-Wallis test for nonparametric and Student's t test or one-way analysis of variance (ANOVA) for parametric variables. Paired t test and Wilcoxon signed-rank test were used to account for repeated measures when appropriate. For categorical variables, differences were assessed using Chi-squared or Fisher's exact test.

In the retrospective analysis of active TH during transport a two-way mixed ANOVA procedure was used to test for interaction between time (before-after transport) and intervention (hypothermia or control treatment).

To investigate the association between TH and incident hypocapnia multivariable logistic regression model was built. Five predictor variables were included in the analysis: hypothermia treatment, use of sedation, respiratory rate of the mechanical ventilator, base deficit and hours of age on admission to the NICU.

In the HENRIC trial we calculated the mean values for cardiorespiratory and CBFV values for each infant during the 3 time epochs of the study: before, during, and after the administration of 5% CO_2 and compared the means between the 3 epochs by Friedman test. Linear interpolation was used to estimate the time spent in the desired PCO_2 range throughout the CO_2 inhalation. Regression modeling was performed to predict the changes of BD, pH, and lactate over time during the CO_2 inhalation, using a repeated-measures linear mixed-effect model with first-order autoregressive within-group correlation structure fitted by maximizing the restricted log-likelihood. The following variables were considered to have fixed effects: blood gas value at the beginning of inhalation as baseline, time in hours since the beginning of inhalation, and Thompson encephalopathy score predicting adverse neurological outcome (low as 0, medium as 1, high as 2). Intra-reader reproducibility of Vs and Vd measurement in ACA was evaluated on seven patients' images before the study commencement by calculating the intra-class correlation coefficient (ICC).

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Matched control patients were selected from our cooling database (14). Patients were matched for HIE severity according to the Thompson encephalopathy score, $PCO_2 \leq 40$ mmHg any time within the first 6 h of postnatal life, and for birth weight (<3000 g; ≥ 3000 g and <4000 g; ≥ 4000 g). We have analyzed the blood gas values from the first hypocapnic value (≤ 40mmHg) measured up to 16 h in the control group similarly to the intervention group. Similar regression modeling was employed using all patients to compare the changes of BD, pH, and lactate over time between the intervention and control groups. A "treatment" term (1 = CO_2 inhalation, 0 = none) was used to assess the significance of the intervention. Baseline values, treatment, time elapse in hours, and treatment–time interaction were considered to have fixed effects.

We used SPSS version 22 (IBM Corp., Armonk, NY), R Statistical Software 3.4.4 and GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA) with significance set at p value of less than 0.05 to analyze and plot the data.

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4. Results

4.1. Safety and feasibility of active hypothermia during neonatal transport

The baseline patient characteristics of the asphyxiated infants in the actively cooled group (n = 136) and control group (n = 78) were comparable, with the exception of the 5- and 10-minute Apgar scores that were significantly lower in the control group. However, there was no difference in the severity of acidosis at referral between the study groups. The pH, PCO_2, and BD values were similar in both groups before and after transport, suggesting that active cooling did not affect acidosis recovery during the transport period (Table 4).

Mean arterial blood pressure was similar in the actively cooled and control groups before and after transport. Heart rate was comparable before transport but decreased significantly among neonates who were cooled during transport, as expected due to the lower body temperature and metabolic rate. Interestingly, less neonates received inotropic support in the actively cooled group compared with the control group (23.5% vs 37.2%; p=0.033). The median of the maximum dose of dopamine and dobutamine administered and volume therapy were similar in both groups.

Newborns with active cooling were more likely to receive conventional mechanical ventilation and sedation/ analgesia treatment during transport, as the current protocol of the transport team and our NICU recommend respiratory support in patients with hypothermia treatment (Table 4).

Importantly, we did not notice any adverse events leading to pulmonary or circulatory failure during the transport period in the entire study population.

In-hospital death rate (occurred after 12 h of life) was found to be eventually higher in the control group (Table 4). Deaths occurred on a median 4.3 [1.7–7.6] postnatal day in the actively cooled group and on 2.4 [0.9–3.9] postnatal day in the control group (p= 0.104). In seven of 14 cases (50.0%) in the actively cooled group and nine of 18 (50.0%) in the control group, the cause of death was severe hypoxic-ischemic encephalopathy.

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In further three cases in the actively cooled group and six cases in the control group, the severe encephalopathy was associated with multiple organ failure, predominantly with acute renal failure. In another three cases (n = 2 in the cooled group and n = 1 in the control group), the encephalopathy was complicated with septicemia. In the remaining four cases, the cause of death was disseminated intravascular coagulation combined with bilateral pneumothorax, twin-to-twin transfusion syndrome in the control group, and aspiration pneumonia and fetomaternal transfusion syndrome in the actively cooled group.

Table 4. Baseline neonatal and transport characteristics. Blood gas values, vital signs, cardiac support and the use of sedation and mechanical ventilation during the transport period. Data are shown as median [IQR] or mean ± SD. See text for details. [1]Before transport at referring hospital. [2]After transport at the NICU. [§]n=48 in the control group. [*] n=75 in the control group.

Baseline characteristics	**Actively cooled group n=136**	**Control group n=78**	**p values**
Neonatal characteristics			
Gestational age (weeks)	39 [38; 40]	39 [37; 40]	0.719
Birth weight (g)	3205 ± 522	3095 ± 543	0.150
Male, n (%)	74 (54.4%)	44 (56.4%)	0.777
Outborn/ Transported, n (%)	136 (100%)	78 (100%)	1.000
Apgar scores			
1 min	2 [1; 3]	2 [0; 4]	0.916
5 min	5 [3; 6]	4 [3; 6]	0.038
10 min	6 [4; 7]	5 [4; 6]	0.013
Transport characteristics			
Age when hypothermia was initiated (h of life)	1.42 [0.83; 2.07]	4.00 [2.08; 5.79]	< 0.0001
Age when target temperature (34°C) was achieved (h of life)	2.42 [1.58; 3.63]	4.25 [2.42; 6.08]	< 0.0001
Stabilization time (h)	0.99 [0.82; 1.25]	0.71 [0.55; 0.96]	< 0.0001
Duration of transport (h)	1.67 [1.33; 2.17]	1.13 [0.89; 1.46]	< 0.0001
Distance of transport (km)	10 [5.0; 58.8]	8 [5.0; 44.5]	0.238
Age when arrived at the NICU (h of life)	2.46 [1.92; 3.35]	1.85 [1.31; 3.18]	< 0.0001

	Actively cooled group n=136	Control group n=78	p values
Blood gas values			
pH [1]	7.1 [6.91; 7.19]	7.1 [6.98; 7.25]§	0.092
pH [2]	7.2 [7.01; 7.31]	7.2 [7.07; 7.29]*	0.244
pCO_2 [1] (mmHg)	44 [36; 60]	41 [32; 62]§	0.241
pCO_2 [2] (mmHg)	36 [27; 49]	38 [30; 51]*	0.433
Base deficit [1] (mmol/L)	15.8 ± 5.9	14.2 ± 7.6 §	0.152
Base deficit [2] (mmol/L)	11.5 ± 8.6	12.6 ±6.2*	0.335
Vital signs			
Mean Arterial Pressure [1] (mmHg)	43 [36; 49]	45 [38; 53]	0.210
Mean Arterial Pressure [2] (mmHg)	47 [39; 54]	45 [39; 53]	0.493
Heart Rate [1] (/min)	130 [119; 142]	130 [120; 140]	0.417
Heart Rate [2] (/min)	115 [100; 130]	134 [122; 146]	< 0.0001
Cardiac support/ Medication			
Inotropic support, n (%)	32 (23.5%)	29 (37.2%)	0.033
Dopamine (μg/kg/min)	5 [3; 6]	4 [3; 5]	0.411
Dobutamine (μg/kg/min)	8 [5; 9]	6 [5; 8]	0.314
Volume therapy (ml)	32 [25; 50]	30 [20; 40]	0.283
Sedation, n (%)	96 (70.6%)	25 (32.0%)	< 0.0001
Conventional mechanical ventilation, n (%)	125 (91.9%)	60 (76.9%)	0.002
Death, n (%)	14 (10.3%)	18 (23.0%)	0.012

Hypothermia treatment was initiated a median 2.58 hours earlier in the actively cooled group compared with the control group, and the upper limit of the target temperature of TH (34°C) was also achieved a median 1.83 hours earlier in the cooled group ($p < 0.0001$ for both comparisons, see details in Table 4). Rectal temperature at admission to the NICU was 33.8°C ± 0.81°C in the actively cooled group, indicating the provision of appropriate active hypothermia treatment during the transport. In contrast, rectal temperature did not change during the transport period and remained 35.3°C ± 1.4°C in the control group ($p < 0.0001$).

In addition, there was a statistically significant interaction between the time elapsed during transport and the intervention (active cooling or standard care) on rectal temperature (two-way mixed ANOVA model, $F [1,209] = 103.3$; $p < 0.001$; effect size $\eta 2 = 0.331$). Figure 5 shows the rectal temperature values before and after transport in the two groups.

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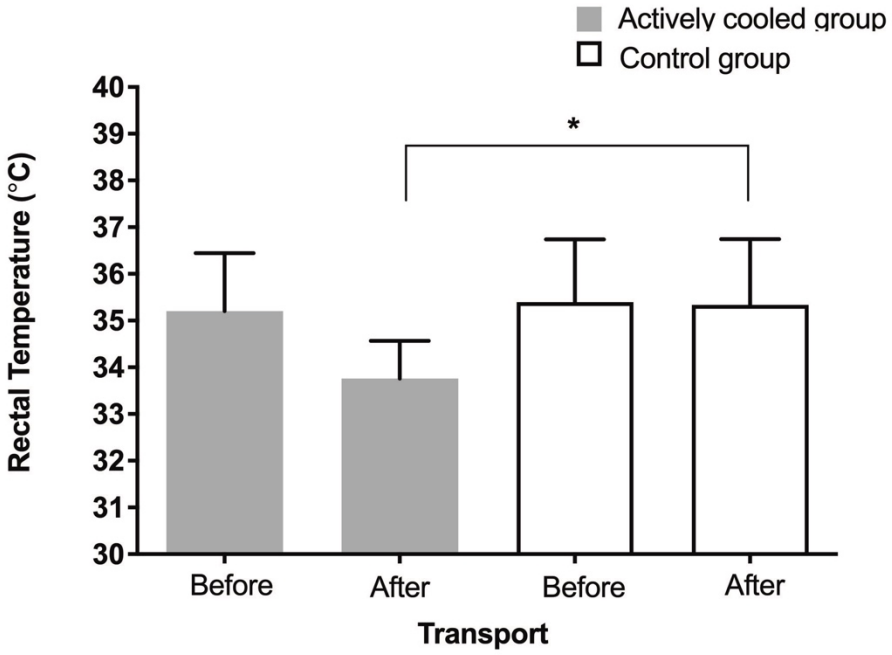

Figure 5. Bar graph for rectal temperature measurements before and after transport in the actively cooled and the control groups.
Before transport, rectal temperature was 35.2°C ± 1.24°C in the actively cooled group and 35.4°C ± 1.34°C in the control group. After transport, on admission to the NICU, the rectal temperature was 33.8°C ± 0.81°C in the actively cooled group and 35.3°C ± 1.4°C in the control group ($p < 0.0001$). There was a statistically significant interaction between the intervention (actively cooled vs control group) and time on rectal temperature (two-way mixed analysis of variance model, $F [1,209] = 103.3$, $p < 0.001$, effect size $\eta 2 = 0.331$). Data are presented as mean ± SD. *$p < 0.0001$ (Student's t test).

Temperature profiles were similar before transport; however, the proportion of infants in the target temperature range (33–34°C) was higher in the actively cooled group after transport compared with controls (58.0% vs 10.3%, respectively; $p < 0.0001$). The rate of overcooling (< 33°C) did not differ significantly between the two groups (11.8% vs 5.1%; $p = 0.144$) (Table 5).

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Table 5. Temperature profiles in the actively cooled and control groups.
Data are shown as mean ± SD. See text for details.

Temperature profile	Actively cooled group n=136	Control group n=78	p values
Rectal temperature before transport (^{0}C)	35.2 ± 1.24	35.4 ± 1.34	0.288
Rectal temperature after transport (^{0}C)	33.8 ± 0.81	35.3 ± 1.40	< 0.0001
Temperature range before transport (^{0}C)			
<33 ^{0}C	6 (4.4%)	2 (2.6%)	0.493
≤34 ^{0}C and ≥33 ^{0}C	17 (12.5%)	8 (10.3%)	0.623
>34 ^{0}C	113 (83.1%)	68 (87.2%)	0.425
Temperature range after transport (^{0}C)			
<33 ^{0}C	16 (11.8%)	4 (5.1%)	0.144
≤34 ^{0}C and ≥33 ^{0}C	79 (58.0%)	8 (10.3%)	< 0.0001
>34 ^{0}C	41 (30.1%)	66 (84.6%)	< 0.0001

The stabilization time at the referring hospital was longer in the actively cooled group by a median 16 minutes. Accordingly, the duration of transport, calculated as the time from the arrival of the transport team to the referring hospital until their arrival to the NICU (= stabilization time + transit time), was a median 32 minutes longer in the actively cooled group (1.67 [1.33–2.17] vs 1.13 [0.89–1.46] h; $p < 0.0001$). Consequently, infants in the actively cooled group were older when admitted to the NICU compared with controls (Table 4); however, NICU admission still occurred before 3 hours of life in 69.16% of all patients (66.91% of actively cooled neonates and 73.07% of controls).

Finally, to further examine the transport characteristics in the actively cooled group, we analyzed some variables of interest according to the temperature profiles on arrival to the NICU. Among the overcooled infants (rectal temperature < 33°C on admission to the NICU; n = 16) Apgar, pH, base deficit, and eventual death rate (7/16; 43.8%) indicated more severe disease, suggesting that patients with more profound HIE have poor temperature control. On the other hand, patients who remained warmer than the target temperature (rectal temperature > 34°C on admission to the NICU; n = 41) had a shorter distance and duration of the transport than those who reached the target temperature range (Table 6).

Table 6. **Patients of the actively cooled group divided according to temperature ranges achieved by the time of admission to NICU.** Apgar and neurological scores, rectal temperature and acid-base parameters shown were registered before the transport, at the referring hospital. The neurological score was determined based on the criteria described in the TOBY trial (Reference: https://www.npeu.ox.ac.uk/toby/protocol on 22/06/2017). Data are shown as median [IQR] or mean ± SD. See text for details.

Temperature range after transport (^{0}C)	**<33 ^{0}C n=16**	**≤34 ^{0}C and ≥33 ^{0}C n= 79**	**>34 ^{0}C n= 41**	**p values**
Apgar 1 min	1 [0; 3]	2 [1; 3]	2 [1; 4]	0.049
Apgar 5 min	5 [2; 6]	4 [3; 6]	5 [4; 7]	0.201
Apgar 10 min	3 [2; 7]	6 [4; 7]	7 [6; 8]	0.006
Neurological score	16 [11; 20]	14 [8; 17]	13 [8; 18]	0.185
Rectal temperature before transport (^{0}C)	34.0 ± 1.62	35.1 ±1.16	35.8 ± 0.81	<0.0001
pH	6.9 [6.73; 7.09]	7.1 [6.92; 7.21]	7.1 [6.94; 7.18]	0.035
Base deficit (mmol/L)	18.2 [25.2; 16.2]	15.3 [22; 12]	14.9 [18.8; 12.6]	0.012
Duration of transport (h)	1.66 [1.18; 2.38]	1.70 [1.40; 2.33]	1.43 [1.10; 1.79]	0.021
Distance of transport (km)	8 [1; 56]	18 [9; 81]	8 [5; 12]	0.000
Death, n (%)	7 (43. 8%)	4 (5.06%)	3 (7.32%)	<0.0001

4.2. Risk of hypocapnia in asphyxiated infants treated with hypothermia

The baseline characteristics of the 117 neonates with moderate-to-severe HIE that were included in this retrospective cohort study are summarized in Table 7. All of the 117 infants were transported from the referring hospitals to the regional center by the same neonatal transport system detailed above. As expected from the study design, controlled active cooling was initiated at a median of 3.59 hours earlier in infants who were actively cooled during transport than in controls, who only received therapeutic hypothermia when they arrived at the cooling center.

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Cooling was commenced 1.24 hours after birth in the actively cooled transport group, with an interquartile range of 0.7–1.8, versus 4.83 [3.1–6.7] hours after birth for the control group ($p < 0.0001$). In the actively cooled transport group, the median rectal temperature was 33.6 [33.1–36.2]°C on arrival at the cooling center indicating the provision of appropriate hypothermia treatment during transport. In contrast, rectal temperature did not change in the control group during transport (Table 7). Although this was not an aim of this study, we did note that 10 (14%) babies in the actively cooled transport group were overcooled (rectal temperature below 33°C) on admission.

Table 7. Baseline clinical characteristics of the 117 infants included in the second retrospective cohort. Data are shown as median [IQR]. See text for details. [1]Before transport at referring hospital. [2]After transport at the NICU.

	Actively cooled transport group n= 71	**Control group n= 46**	**p values**
Gestational age (weeks)	39 [38-40]	39 [38-40]	0.708
Birth weight (g)	3150 [2850-3490]	3200 [2800-3500]	0.562
Male, n (%)	37 (52.1%)	27 (58.7%)	0.485
Apgar 1 min	2 [1-3]	2 [1-3]	0.969
Apgar 5 min	5 [4-6]	4 [2-6]	0.078
Apgar 10 min	6 [4-7]	6 [4-6]	0.111
Age when hypothermia was initiated (h of life)	1.24 [0.7-1.8]	4.83 [3.1-6.7]	<0.0001
Age on admission to NICU (h of life)	2.25 [1.9-3.5]	2.69 [1.7-3.8]	0.928
Rectal temperature[1] (°C)	35.2 [34.5-36.2]	35.6 [34.6-36.4]	0.237
Rectal temperature[2] (°C)	33.6 [33.1-36.2]	35.5 [34.9-36.2]	<0.0001
Death, n (%)	10 (14.1%)	10 (21.7%)	0.283

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The heart rate decreased among infants in the actively cooled transport group during the transport period due to their lower rectal temperature, and their mean arterial pressure was lower before the transport, compared to the controls, but still remained in the normal range. Furthermore, the use of inotropic support and the median dose of dopamine and dobutamine administrated were similar in both groups (Table 8). In accordance with our protocol more neonates in the actively cooled transport group were intubated and ventilated compared to the control infants (88.7% versus 73.9%, p = 0.038, respectively). The ventilator settings were similar in the two groups. Consistent with this finding, neonates in the actively cooled transport group were more likely to receive analgesia and, or, sedation (69.0% versus 39.1%, p = 0.002) (Table 8). Adverse events leading to pulmonary or circulatory failure were not recorded in either group during the transport period. Blood gas values were evaluated before transport, at the referring hospital and after transport, on admission to the cooling center in both groups. The initial pH in the actively cooled transport group was statistically significantly lower, but the difference was not clinically remarkable at 7.02 [6.90–7.18] versus 7.10 [6.89–7.26], respectively, (p = 0.019). Furthermore, the base deficit was comparable between the two groups (Table 8).

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Table 8. Blood gas values, vital signs, ventilatory parameters and use of medication during transport. Data are shown as median [IQR]. See text for details. [1]Before transport at referring hospital. [2]After transport at the NICU. Abbreviation: BD, Base deficit; ΔP, delta pressure; PIP, peak inspiratory pressure; PEEP, positive end expiratory pressure; Respiratory Rate is shown for patients on mechanical ventilator.

	Actively cooled transport group n=71	**Control group n=46**	**p values**
Vital signs			
Heart Rate[1] (/min)	130 [116-142]	135 [122-150]	0.314
Heart Rate[2] (/min)	117 [105-132]	135 [127-146]	<0.0001
Mean Arterial Pressure[1] (mmHg)	42 [34-49]	46 [40-54]	0.045
Mean Arterial Pressure[2] (mmHg)	44 [36-52]	46 [40-53]	0.487
Inotropic support, n (%)	23 (32.4%)	19 (41.3%)	0.326
Dopamine (μg/kg/min)	5 [3-6]	4 [3-5]	0.533
Dobutamine (μg/kg/min)	7 [5-9]	6 [5-8]	0.624
Mechanical ventilation			
Conventional mechanical ventilation, n (%)	63 (88.7%)	34 (73.9%)	0.038
Respiratory Rate setting (/min)	34 [27-49]	41 [22-51]	0.469
Mean Airway Pressure (H_2Ocm)	10 [9-11]	9 [8-10]	0.358
Delta Pressure (mmHg) (ΔP = PIP-PEEP)	15 [14-17]	16 [14-18]	0.344
FiO_2 (%)	40 [21-83]	30 [21-83]	0.559
Analgesia, n (%)	49 (69.0%)	18 (39.1%)	0.002
Anticonvulsive treatment, n (%)	12 (16.9%)	3 (6.5%)	0.156
Blood gas values			
pH[1]	7.02 [6.90-7.18]	7.10 [6.89-7.26]	0.019
pH[2]	7.21 [7.11-7.31]	7.26 [7.12-7.29]	0.921
PCO_2[1] (mmHg)	45 [36-68]	42 [32-63]	0.182
PCO_2 [2] (mmHg)	35 [26-48]	41 [29-52]	0.184
BD[1] (mmol/L)	15.5 [12.9-21.3]	14.6 [10.1-18.9]	0.105
BD[2] (mmol/L)	11.4 [7.6-15.6]	12.0 [4.9-15.4]	0.434

We evaluated the PCO_2 levels in each group during transport and found that 54% of the total cohort had at least one PCO_2 value below 35 mmHg during the study period. The rate of incident hypocapnia during transport, defined as a PCO_2 value of > 35 mmHg before and ≤ 35 mmHg after transport, was significantly higher in the actively cooled transport group (26 of 71, 36.6%) compared to the control group (8 of 46, 17.4%, p = 0.025). In addition, in the actively cooled transport group, PCO_2 decreased from the normal range, with a median of 45 [36–68] mmHg, to 35 [26–48] mmHg (p < 0.0001) during transport. In contrast, PCO_2 did not change significantly during transport in the control group, from 42 [32–63] to 41 [29–52] mmHg (p = 0.322). There were similar ventilatory parameters in both groups. We did not find any significant differences in the ventilator settings between the group of incident hypocapnic versus normocapnic and hypercapnic patients (Table 9). Figure 6 shows the temperature corrected PCO_2 values before and after transport in the actively cooled transport group and control group.

Table 9. Parameters of mechanical ventilation in hypocapnic and normocapnic, and hypercapnic group. Data are shown as median [IQR]. See text for details. Abbreviation: ΔP, delta pressure; PIP, peak inspiratory pressure; PEEP, positive end expiratory pressure; FiO2, Fraction of inspired oxygen

	Incident hypocapnia n= 34	**Normo- or hypercapnia n= 83**	**p values**
Hypothermia treatment, n (%)	26 (76.5%)	45 (54.2%)	0.025
Conventional mechanical ventilation, n (%)	28 (82.4%)	69 (83.1%)	0.919
Respiratory rate setting (/min)	28 [25-45]	38 [26-50]	0.236
Peak inspiratory Pressure (cm H_2O)	19 [18-22]	20 [18-22]	0.668
Positive end expiratory pressure (cm H_2O)	4 [4-4]	4 [3-4]	0.426
Delta pressure (cm H_2O) (ΔP = PIP-PEEP)	15 [14-17]	16 [14-17]	0.335
Inspiratory Time (sec)	0.35 [0.33-0.35]	0.35 [0.33-0.35]	0.968
F_iO_2 (%)	40 [21-60]	40 [21-100]	0.267

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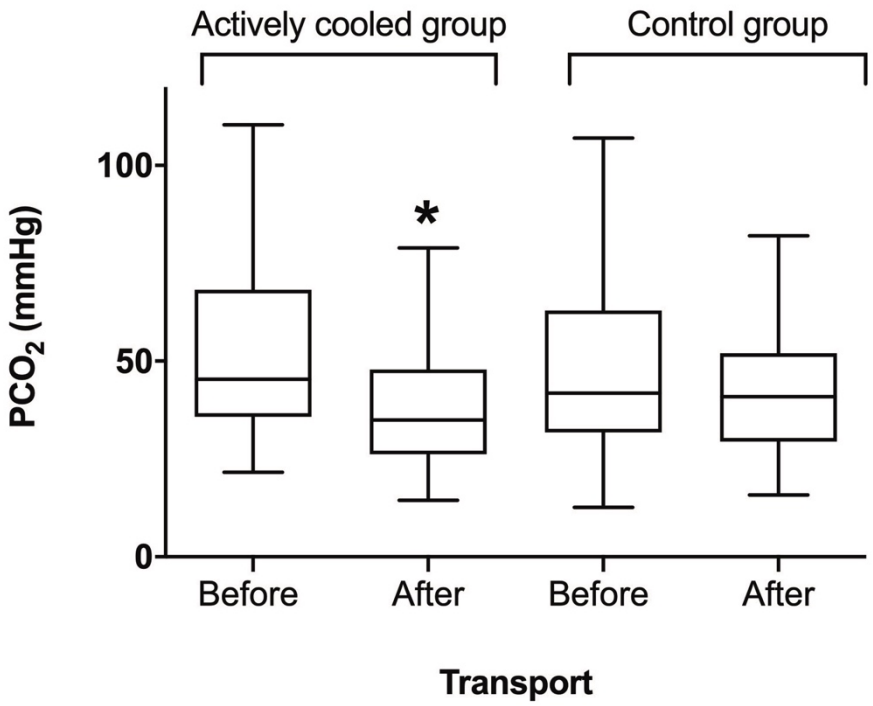

Figure 6. Box plot for temperature corrected PCO_2 values before and after transport in the actively cooled and the control groups.
In the actively cooled group, median PCO_2 decreased from the normal range to hypocapnia (45 [IQR 36-68] versus 35 [IQR 26-48] mmHg; $p<0.001$). In the control group, PCO_2 did not change during transport (42 [IQR 32-63] versus 41 [IQR 29-52] mmHg; $p = 0.322$). Data are presented as median with interquartiles (boxes) and ranges (whiskers). * = $p < 0.001$

Multivariable logistic regression analysis was used to ascertain the effect of active therapeutic hypothermia (33– 34°C), on the likelihood that patients would develop incident hypocapnia, adjusting for other clinically relevant parameters, including sedation, the respiratory rate setting of the mechanical ventilator, base deficit and age of hours on admission to the NICU. Sedation is likely to decrease hypocapnia by reducing spontaneous respiratory activity. In contrast, base deficit reflects the degree of metabolic acidosis, which can increase the risk of hypocapnia by compensatory hyperventilation. Similarly, high respiratory rate settings on the mechanical ventilator can also lead to hypocapnia. The hours of age on admission to the NICU corresponding to the time of the second blood gas sampling was included in the model to control for the time-dependent acidosis recovery in asphyxiated infants.

Table 10 demonstrates the adjusted and unadjusted odds ratios for each variable. The model correctly classified 78.1% of cases. Of the five predictor variables, hypothermia and sedation were statistically significant. Infants who underwent hypothermia treatment during transport had 4.23 times higher odds (95% CI 1.30–13.79) of developing hypocapnia than patients in the control group, who received standard intensive care without cooling during transport. More frequent use of sedation was associated with a reduction in the likelihood of incident hypocapnia with an odds ratio (OR) of 0.35 (95% CI 0.12–0.98).

Based on these results, we can show that hypocapnia was common in asphyxiated infants and therapeutic hypothermia seemed to be an independent risk factor for developing hypocapnia in neonates with HIE.

Table 10. Logistic regression predicting likelihood of hypocapnia.
Logistic regression model predicting likelihood of incident hypocapnia based on hypothermia treatment (ref. category: none), use of sedation (ref. category: none), respiratory rate setting on mechanical ventilator, base deficit and hours of age on admission to NICU**.**
Abbreviation: aOR, adjusted odds ratio; OR, odds ratio; CI: confidence interval

	Unadjusted odds ratios			**Adjusted odds ratios**		
	OR	**95% CI**	**p values**	**aOR**	**95% CI**	**p values**
Hypothermia	2.74	1.11-6.77	0.028	4.23	1.30-13.79	0.017
Use of sedation	0.66	0.27-1.47	0.311	0.35	0.12-0.98	0.046
Respiratory Rate setting (/min)	0.99	0.96-1.01	0.312	0.98	0.95-1.02	0.305
Base deficit (mmol/L)	1.06	0.97-1.06	0.522	1.02	0.96-1.07	0.580
Age on admission to NICU (h)	1.04	0.82-1.34	0.734	0.98	0.69-1.39	0.915

4.3. The HENRIC feasibility and safety trial

Sixty-two term infants with moderate or severe HIE were assessed for eligibility, and a total of ten patients were enrolled prospectively into the trial (Figure 4). Baseline clinical characteristics of the ten infants are summarized in Table 11. The median PCO_2 was 33 mmHg (range 26–40 mmHg) at the start of 5% CO_2 administration; which was commenced at a median 5.5 h of life (range 3.9–6.6). The CO_2 inhalation was stopped at a median 12.9 h of life (range 5.4–18.6), after a median duration of 7.7 h (range 0.6–12.0). During the CO_2 administration, a total of 50 arterial blood gases were taken from the 10 patients. The temperature corrected arterial PCO_2 was between the targeted 40 and 60 mmHg in 96% (48/50) of the samples. All PCO_2 values were >40 mmHg, and the highest PCO_2 value detected was 64 mmHg (infant no. 6). Calculating with a linear interpolation between the blood gas measurements, patients spent a median 95.1% (range 44.6–98.5%) of time in the desired PCO_2 range during the 5% CO_2 inhalation (Table 11 and Figure 7a). In comparison, the patients in the control group spent significantly less time (median 45.3% ((range 0–91.7%), $p = 0.002$) in the target range (Figure 8). The 5% CO_2 exposure was continued for the predefined maximum of 12 h in 4 cases, while in 6 cases, the BD decreased <5mmol/L earlier (Table 11) and CO_2 administration was stopped accordingly. It is noteworthy that the latter 6 infants' BD normalized within 13 h of life, while in the former 4 cases the BD recovery was longer and normalized between 23.0 and 59.8 h of life (Table 11). The end point of acidosis (defined as pH > 7.25) was median 9.1 h of life with a wide range of 1.5–59.8 h. In 3/10 cases, the pH was >7.25 initially, before the inhalation was initiated and pH did not decrease below this threshold during the exposition (Figure 7c). The statistical modeling for trends of BD, pH, and lactate during the study period (Figure 7a–d) revealed that baseline value at the beginning of inhalation had a significant effect on the changes of blood gas values over time.

The regression equation predicted that BD decreased by 0.61 mmol/L and lactate decreased by 0.55 mmol/L per hour after the beginning of inhalation, whereas pH remained stable over time. Interestingly, the Thompson encephalopathy score, measured on a three-step scale of low, medium, and high showed significant association only with BD trends (Table 12).

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Table 11. Patient characteristics and details of 5% CO_2 inhalation.

Infant No.	Gest. age (weeks)	Birth weight (gram)	Apgar 10'	Blood gas values on admission		Admission (h of life)	Age to target temp. 33.5 C (h of life)	Age at intub. (h of life)	5% CO_2 inhalation		Blood gas values at the start of 5% CO_2 inhalation				H of life until BD< 5 mM	H of life until pH> 7.25	Percent of time spent in the target $PaCO_2$ range
				PCO_2 (mmHg)	pH				Start (h of life)	Duration (h)	PCO_2 (mmHg)	Lactate (mmol/L)	BD (mmol/L)	pH			
1	40	2840	8	27	7.31	4.0	3.3	2.3	5.6	7.7	26	11	12.7	7.34	13.0	8.1	96.5
2	40	2900	8	26	7.25	2.6	4.8	3.2	5.4	0.6	30	8	6.9	7.38	6.0	3.9	44.6
3	38	3850	8	35	7.33	3.2	3.2	2.5	4.9	7.6	30	5	6.4	7.40	12.3	3.2	95.2
4	40	3690	6	28	7.10	2.1	1.6	0.2	4.9	12.0	27	12	15.6	7.25	53.6	18.0	95.5
5	39	4050	6	32	7.35	2.4	3.4	1.6	3.9	1.5	33	8	6.9	7.35	5.1	1.5	63.2
6	39	3300	-	21	7.33	1.8	4.6	0.1	6.0	12.0	33	6	9.6	7.31	59.8	59.8	81.1
7	41	3210	6	34	7.26	3.1	3.8	1.8	4.7	4.6	39	7	9.9	7.25	9.2	15.4	98.7
8	38	3230	3	29	7-30	4.0	3.7	0.2	6.1	12.0	38	5	8.7	7.28	29.9	20.3	97.9
9	40	2490	4	42	7.30	3.7	2.8	1.5	6.0	6.0	33	13	16.6	7.17	11.9	10.1	92.3
10	37	3100	5	34	7.31	3.4	4.2	0.2	6.6	12.0	40	5	6.9	7.30	23.0	6.9	95.0
Median (range)	40 (37-41)	3220 (2490-4050)	6 (3-8)	31 (21- 42)	7.31 (7.10-7.35)	3.2 (1.8-4.0)	3.5 (1.6-4.8)	1.6 (0.1-3.2)	5.5 (3.9-6.6)	7.7 (0.6-12.0)	33 (26-40)	8 (5-13)	9.2 (6.4-16.6)	7.31 (7.17-7.40)	12.6 (5.1-59.8)	9.1 (1.5-59.8)	95.1 (44.6-98.5)

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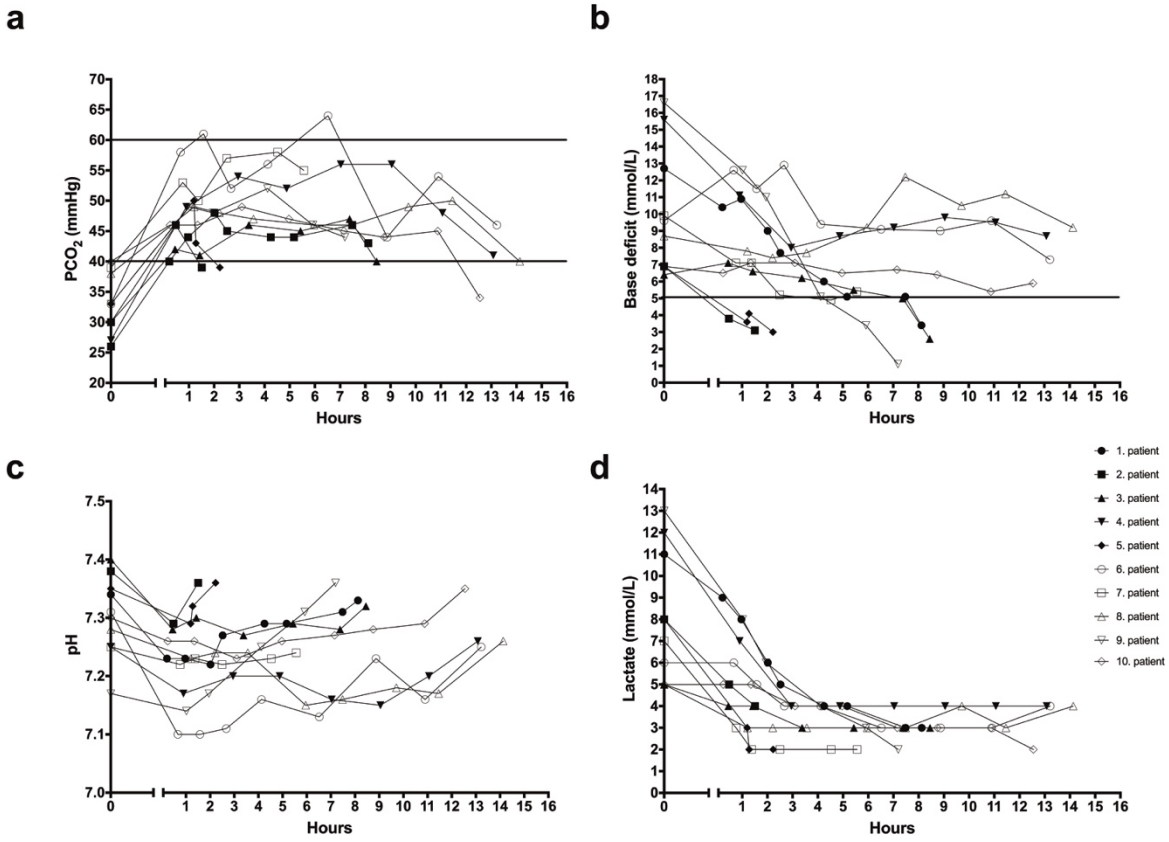

Figure 7. The trends of temperature corrected arterial blood gas values (PCO_2, Base deficit, pH) and lactate during CO_2 administration. Each symbol represents one patient. Baseline values (0-point) correspond to the last measured value prior to the start of CO_2 inhalation. The last data point on the graphs correspond to values measured after the offset of CO_2 administration. The x-axis displays the time in hours since the start of CO_2 inhalation.
a. PCO_2 trends for each patient during the study. Patients spent 95.1% of time (range: 44.6-98.5%) in the desired PCO_2 range (40-60 mmHg) during the 5% CO_2 administration, calculated by linear interpolation between the blood gas measurements. All PCO_2 values were above 40 mmHg, the lower value of the target range.
b. **Base deficit trends for each patient during the study.** The same model predicted that base deficit decreased by 0.61 mmol/L per hour throughout the CO_2 inhalation period.
c. pH trends for each patient during the study. A repeated-measures linear mixed-effect model predicted that pH remained stable over time during the CO_2 administration. Baseline value, time in hours since the beginning of inhalation and Thompson encephalopathy score were considered to have fixed effects.
d. Lactate trends for each patient during the study. The same model predicted that lactate levels decreased by 0.55 mmol/L per hour throughout the CO_2 inhalation period.

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Table 12. Trend analysis of blood gas values over time during the 5% CO_2 inhalation.
A repeated-measures linear mixed-effect model was run for trend analysis of base deficit, lactate and pH during the 5% CO_2 inhalation. The baseline corresponds to the last measured value prior to the start of CO_2 inhalation. The time in hours since the start of CO_2 inhalation baseline blood gas values and Thompson encephalopathy score (low as 0, medium as 1, high as 2) were included as variables with fixed effects. See text for details.
Mathematical modeling yielded the following equations:

- Base deficit (mmol/L) = 0.64 + (Baseline * 0.74) - (Time elapse in hours * 0.61) + (Thompson encephalopathy score * 1.40)
- Lactate (mmol/L) = 1.14 + (Baseline * 0.58) - (Time elapse in hours * 0.55) + (Thompson encephalopathy score * 0.88)
- pH = 2.73 + (Baseline * 0.62) - (Time elapse in hours * 0.00) - (Thompson encephalopathy score * 0.03)

Outcome	Variables	Regression coefficient (β)	95% CI	p values
Base deficit	Intercept	0.64	-2.15; 3.43	<0.0001
	Baseline	0.74	0.47; 0.99	0.001
	Time elapse (h)	-0.61	-1.11; -0.11	0.045
	Thompson encephalopathy score	1.40	0.28; 2.53	0.021
Lactate	Intercept	1.14	-2.14; 4.42	<0.0001
	Baseline	0.58	0.21; 0.94	0.010
	Time elapse (h)	-0.55	-0.84; -0.26	0.001
	Thompson encephalopathy score	0.88	-0.49-2.24	0.172
pH	Intercept	2.73	-0.04; 5.51	<0.0001
	Baseline	0.62	0.18; 1.07	0.016
	Time elapse (h)	-0.00	-0.01; 0.00	0.260
	Thompson encephalopathy score	-0.03	-0.06; 0.01	0.135

The matched controls showed a similar tendency in recovery from acidosis, except for the changes in pH. The pH showed a slower recovery in the intervention group by a 0.01/h (Table 13 and Figure 8).

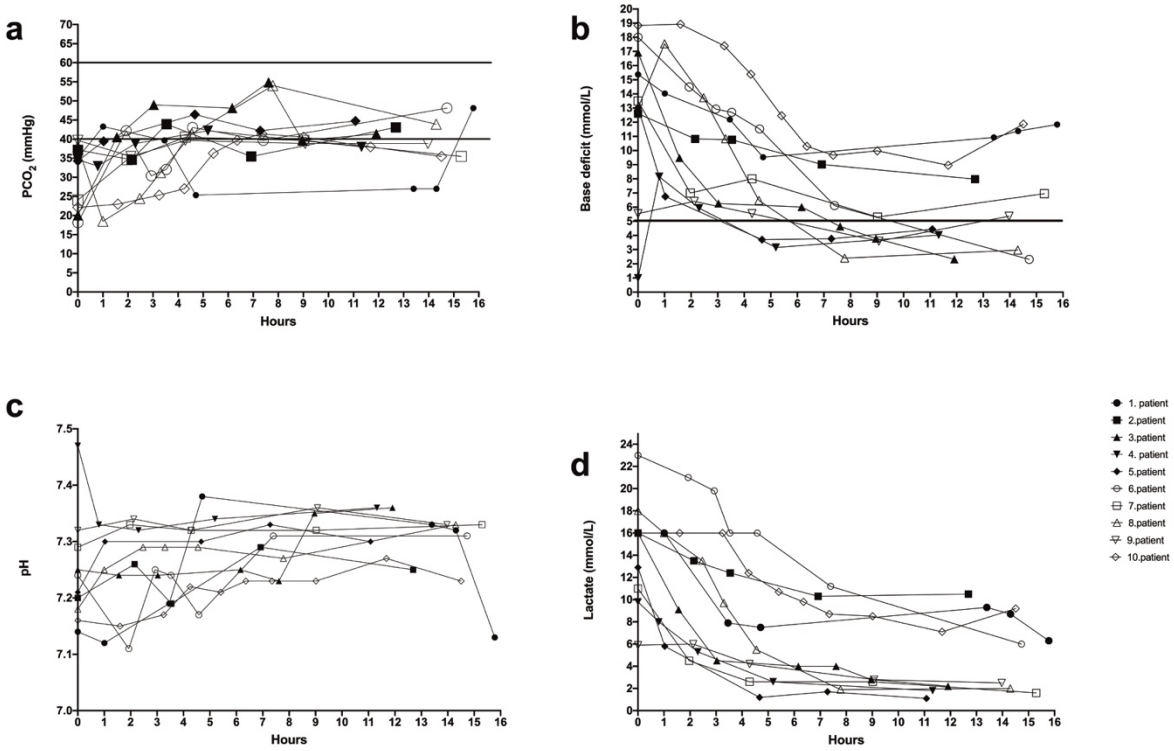

Figure 8. The trends of temperature corrected arterial blood gas values (PCO_2, Base deficit, pH) and lactate over time in the matched controls.
Each symbol represents one patient. Baseline values (0-point) correspond to the first measured hypocapnic value (≤ 40 mmHg). The x-axis displays the time in hours since the first hypocapnic value measured up to 16 hours. Patients spent 45.3% of time (range 0-91.7%) in the desired PCO_2 range (40-60 mmHg), calculated by linear interpolation between the blood gas measurements.

Table 13. Trend analysis of blood gas values to compare the 2 groups over time.
A repeated-measures linear mixed-effect model was run using all patients to compare the changes of BD, pH and lactate over time between the intervention and control groups. A "treatment" term (1= CO_2 inhalation, 0= none) was used to assess the significance of the intervention. The baseline corresponds to the first hypocapnic (≤ 40 mmHg) value measured in the control group and the last measured value prior to the start of CO_2 inhalation in the intervention group. Baseline values, treatment, time elapse in hours and treatment-time interaction were considered to have fixed effects.
The regression modeling predicted that the intervention was associated with a 0.5 mmol/L lower BD (β= -0.53) compared to the matched controls (p= 0.011); and 1 mmol/L lower (β = -1.12) lactate levels (p< 0.0001). Importantly, the treatment-time interactions were not significant, indicating that cases and controls showed similar tendency in recovery from acidosis, except for the changes in pH. The pH showed a minimally slower recovery in the intervention group by a 0.01/hour (p= 0.013).

Outcome	Variables	Regression coefficient (β)	95% CI	p values
Base deficit	Intercept	2.98	0.15; 5.82	<0.0001
	Baseline	0.67	0.46; 0.87	<0.0001
	Treatment	-0.53	-2.60; 1.54	0.011
	Time elapse (h)	-0.43	-0.72; -0.14	0.000
	Treatment*Time	0.00	-0.48; 0.48	0.996
Lactate	Intercept	2.10	-1.89; 6.10	<0.0001
	Baseline	0.69	0.43; 0.96	<0.0001
	Treatment	-1.12	-4.06; 1.81	<0.0001
	Time elapse (h)	-0.55	-0.77; -0.36	<0.0001
	Treatment*Time	0.10	-0.25; 0.46	0.565
pH	Intercept	3.28	1.85; 4.71	<0.0001
	Baseline	0.55	0.34; 0.76	<0.0001
	Treatment	-0.02	-0.06; 0.03	0.178
	Time elapse (h)	0.00	0.00; 0.01	0.354
	Treatment*Time	-0.01	-0.01; -0.00	0.013

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Cardiovascular and respiratory parameters of the study population were analyzed at three time epochs: pre-inhalation, during, and post-inhalation. We detected a statistically significant decrease in heart rate ($p = 0.007$) during the study, which is a physiological response to reduced body temperature. Mean arterial blood pressure and peripheral oxygen saturation did not change (Figure 9a). Severe hypotension or cardiovascular collapse did not occur. The maximum dose of dopamine was 10 μg/kg/min within the first day of life in our study population and a median 13.5 ml/kg volume was administrated during the CO_2 exposition as fluid boluses, including blood products for the correction of anemia or coagulopathy. Regarding respiratory parameters, the RR reduced significantly in the post-inhalation epoch of the study compared to the RR before and during CO_2 administration ($p = 0.008$, Friedman test). Furthermore, we detected an increased tidal volume ($p = 0.002$, Skillings–Mack test) during the 5% CO_2 inhalation, reflecting a physiological response to the intervention (Figure 9b).

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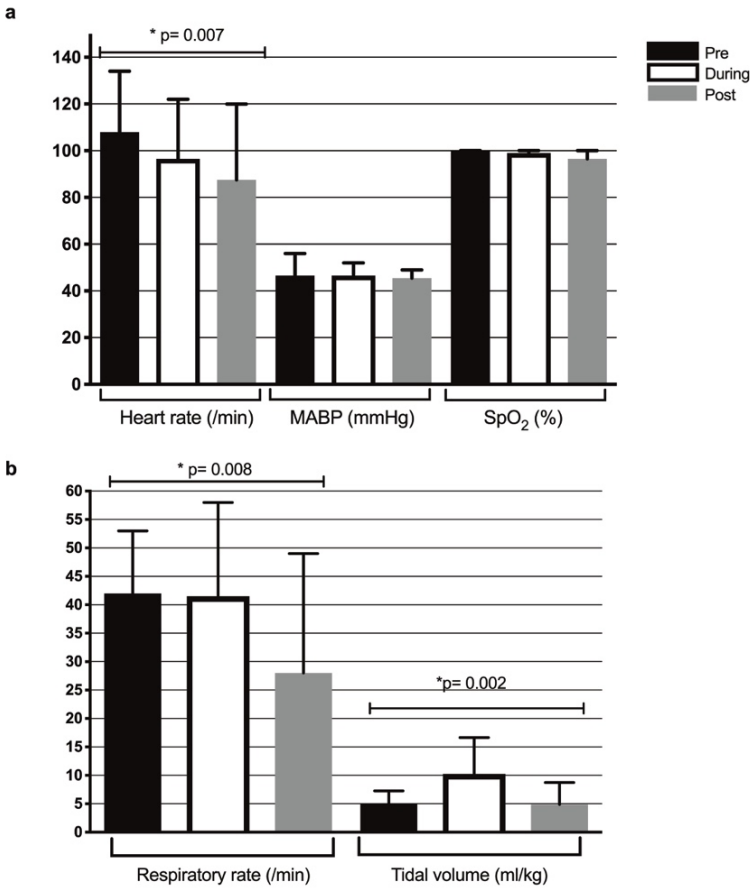

Figure 9. Cardiovascular and respiratory parameters during the study are shown in 3 time epochs: before, during and a 6-hour period after the CO_2 inhalation.
Data are presented as medians with ranges in the entire cohort.

a. Heart rate (HR) decreased from a median 108/min (87-134) to 97/min (82-122) during CO_2 inhalation. After the CO_2 administration, the HR reduced further to 88/min (75-120); (p= 0.007, Friedman test). The mean arterial blood pressure (MABP) and peripheral oxygen saturation (SpO_2) did not change, and remained in the physiological range throughout the 3 time epochs. (MABP medians: pre-study: 47 mmHg (44-56); during: 47 mmHg (40-52); post-study: 46 mmHg (40-49); (p= 0.07, Friedman test). SpO_2 medians: pre-study: 100% (98-100); during: 99% (97-100); post-study: 97% (93-100); (p=0.07; Friedman test).

b. The respiratory rate and tidal volumes changed significantly over the three study epochs. Respiratory rate was 42/min (24-53) and 42/min (25-58) before and during the CO_2 inhalation, respectively, and reduced to 28/min (19-49) in the post-study period (p=0.008; Friedman test). Peak tidal volumes were the following: pre-study: 5.0 mL/kg (2.8-7.3); during: 10.3 mL/kg (5.3-16.6); and post-study: 4.9 mL/kg (3.9-8.7); (p=0.002; Skillings-Mack test).

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The temperature corrected arterial PO_2 was >100 mmHg in 40% (20/50) of the samples in 7 patients during the 5% CO_2 exposition while the oxygen requirement was 21% in all cases. The highest PO_2 was 141 mmHg. Importantly, we did not observe any case of pulmonary or circulatory failure during the study period.

Continuous aEEG monitoring detected electrophysiological seizures in 3 patients during the 5% CO_2 exposition, 2 of them already had seizure activity before, and all 3 had seizures after the inhalation, resulting in permanent anticonvulsive treatment as per decision of the clinical team (Table 14). Brain MRI scans were carried out at a median of 3.5 (range 2–8) days of life. Diffusion-weighted imaging showed the presence of hypoxic–ischemic injury in 6/10 patients. Deep gray matter and white matter involvement, including corpus callosum, was noted as the most frequent type of brain injury. Six neonates were noted to have intracranial hemorrhage in subdural, subarachnoid, and intraventricular (grade I) location, but none of them developed intraparenchymal bleeding (Table 14). It is important to note that the neurological findings and the incidence of intracranial bleeding did not show significant difference between the intervention and the control group (Table 15).

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Table 14. Neurological function, MRI findings and neurodevelopmental outcomes.
Bayley Scales of Infant Development II examination was performed at 18 to 22 months of age. The mental developmental index (MDI) and the psychomotor developmental index (PDI) are classified as moderate disability if values are between 70-84 and as severe disability if <70. Abbreviation: aEEG, amplitude-integrated electroencephalography; BRS, behavioral rating scale; BS, burst suppression; CNV, continuous normal voltage; DGM, deep gray matter; DNV, discontinuous normal voltage; FT, flat trace; susp, clinically suspected seizure, not confirmed by aEEG; IVH, intraventricular hemorrhage; MDI, mental developmental index; MRI, magnetic resonance imaging; PDI, psychomotor developmental index; PVL, periventricular leukomalacia; SAH, subarachnoid hemorrhage; SDH: subdural hemorrhage; WM: white matter.

Infant No.	Thompson encephalopathy score	aEEG pattern on admission	Time to CNV (h of life)	Seizure			MRI findings			Outcome (Bayley II at 18–22 months)	
				Pre	During	Post	Age at MRI (d of life)	Intracranial hemorrhage	Diffusion abnormalities	MDI	PDI
1	11	DNV	4	no	no	no	2	SAH	-	≥ 85	70-84
2	10	DNV	3	no	no	no	3	-	-	70-84	<70
3	11	FT	15	susp.	no	no	3	SDH	WM, DGM	≥ 85	≥ 85
4	13	FT	-	no	no	yes	4	-	WM, cortex, corpus callosum	<70	<70
5	10	DNV	19	no	no	no	8	SDH	-	≥ 85	≥ 85
6	15	BS	-	yes	yes	yes	2	-	WM, DGM, corpus callosum	died	
7	10	BS	37	susp.	no	no	4	SAH	corpus callosum	Behavioral problems, total score of BRS: 88 (6 pc)	
8	17	FT	-	susp.	yes	yes	5	SDH, IVH grade I	WM, DGM, corpus callosum	<70	<70
9	7	DNV	4	no	no	no	3	-	-	≥ 85	70-84
10	16	FT	62	no	yes	yes	7	SDH	WM, corpus callosum	≥ 85	≥ 85

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Table 15. Clinical characteristics of the study population and matched control patients. Data are presented as median (ranges) or number (percentages). # MRI brain scans were carried out only in 9 patients in the control group due technical reasons. Abbreviation: aEEG, amplitude-integrated electroencephalography; BRS, behavioral rating scale CNV: continuous normal voltage; MRI, magnetic resonance imaging.

	Patients with CO_2 inhalation n=10	**Control patients n=10**	**p values**
Gestational age (week)	40 (37-41)	40 (36-41)	0.994
Birth weight (gram)	3220 (2490-4050)	3290 (2450-4200)	0.957
Apgar 1 min	3 (0-5)	2 (0-8)	0.399
Apgar 5 min	5 (1-7)	4 (0-9)	0.639
Apgar 10 min	6 (3-8)	6 (1-7)	0.683
Admission (h of life)	3.2 (1.8-4.0)	3.2 (1.3-5.9)	0.494
Age to target temperature (h of life)	3.5 (1.6-4.8)	5.4 (2.6-8.7)	0.019
Neurology, MRI findings #			
Thompson encephalopathy score	11 (5-17)	12 (5-21)	0.926
Time to CNV (h of life)	15 (3-62)	18 (4- 42)	0.710
Seizures on aEEG within 84 h of life, n (%)	4 (40%)	5 (50%)	>0.999
Age at MRI (days of life)	3.5 (2-8)	5 (3-9)	0.143
Intraventricular bleeding on MRI, n (%)	1/10 (10%)	3/9 (33.3%)	0.303
Intraparenchymal bleeding on MRI, n (%)	0/10 (0%)	0 /9 (0%)	>0.999
Neurodevelopmental outcome			
Normal	3 (30 %)	4 (40%)	>0.999
Moderate disability	2 (20 %)	2 (20%)	>0.999
Severe disability	3 (30%)	2 (20%)	>0.999
Non-optimal test behavior (BRS <10pc)	1 (10%)	0 (0 %)	>0.999
Loss to follow up	0 (0%)	1 (10%)	>0.999
Death, n (%)	1 (10%)	1 (10%)	>0.999

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Transcranial Doppler ultrasonography measurements of CBFV were performed by the same physician. Intra-reader reliability revealed excellent reproducibility for Vs (ICC = 0.899, 95% CI 0.64–0.98) and Vd (ICC = 0.860, 95% CI 0.44–0.97). CBFV was measured in the ACA and MCA before the study commencement (at median 5.6 h of life (range 3.6–6.3)), every 2 h during CO_2 exposition, and shortly after the cessation of CO_2 (at median 17.9 h of life (range 8.2–19.2)). We present the values measured in the ACA of 7 patients (infant nos. 4–10) who were investigated at each time epoch (Figure 10). We could not find differences in CBFV values when comparing the three epochs of the study. The MCA blood flow velocities showed similar tendencies (data are not shown).

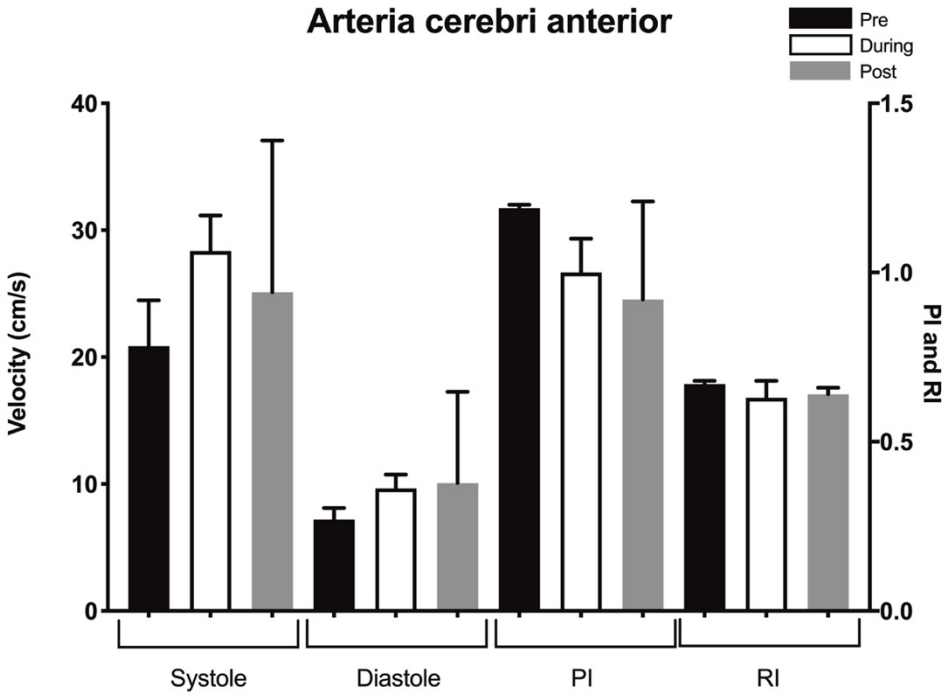

Figure 10. Cerebral blood flow velocities in the anterior cerebral artery.
Median values with ranges are shown for systolic peak-flow velocity (Vs), end diastolic peak- flow velocity (Vd), resistance index (RI) and pulsatility index (PI) of the anterior cerebral artery in the 3 epochs of the study. RI and PI were calculated using the following formulas: RI= (Vs-Vd)/Vs and PI=(Vs-Vd)/mean velocity. We could not find differences in CBFV values when comparing the 3 epochs of the study. See the text for details.

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No death occurred during the perinatal period in our study population. One infant (no. 6) with severe HIE subsequently died in another hospital due to aspiration pneumonia. Severe disability (PDI and/or MDI <70) occurred in three cases; two infants were classified as moderate (PDI and/or MDI 70–84) on Bayley II scales at 18–22 months of age. One child (no. 7) had a total score of 88 (=6 percentile) on the behavior rating scale indicating a nonoptimal test behavior. Three infants had normal outcome (both PDI and MDI ≥85). The rate of severe and moderate disabilities did not differ between the treated and control groups (Table 15).

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5. Discussion

5.1. Safety and feasibility of active hypothermia during neonatal transport

Although the provision of active TH during neonatal transport has been studied extensively (74, 75, 78, 135, 136), our retrospective study provided a detailed analysis on physiologic parameters and intensive care needs of transported neonates in one of the largest cohort suggesting the safety and feasibility of TH during transport even in relatively short transport distances. It is well established that timing has an outstanding role in enhancing the neuroprotective effect of hypothermia treatment based on both animal and human studies (6, 7, 56). Since most of the asphyxiated infants born out of Level-III NICUs, which provide TH for infants with HIE, an increasing number of neonatal transport teams have started to adopt hypothermia treatment during transportation.

Early attempts on a small patient cohort with passive and active cooling with gel packs were less successful because many patients, as high as 34% of asphyxiated newborns, had core body temperatures below the target range of 33–34°C, predisposing them to the detrimental effects of overcooling (135). Hallberg et al (136) published similar results regarding overcooling with passive initiation of hypothermia. However, Kendall et al (137) reported that 67% of the infants who were cooled passively were in the target temperature range on arrival at the cooling center, and only 11% of the newborns were below 33°C. Recent retrospective and prospective randomized trials have shown that servo-controlled active cooling provides more temperature stability, and a similar approach was used in the present study. Chaudhary et al (78) reported that 100%, whereas Akula et al (74) found that 80% of actively cooled asphyxiated newborns have reached the target temperature during transport using a servo-regulated cooling device. In these trials, most of the infants had cooling initiated prior to arrival of the transport team. We observed that 58.1% of our cooled infants were in the target temperature range at admission to the NICU, and the rate of overcooling was 11.8%. The significantly lower success rate in our study compared with the other reports is explained by the relatively short transport period among the infants who did not reach the target temperature until the admission to the NICU.

In addition, the lack of any kind of cooling method before the arrival of the transport team may also contribute to the relatively high percentage of patients remaining above the target temperature by the end of the transport.

It has been known for over 50 years that asphyxiated infants lose temperature at a higher rate than healthy newborns, and this might be more pronounced in severe asphyxia (138). This presumption is consistent with our findings. The overcooled infants had deeper acidosis, lower Apgar scores, and a tendency for lower neurologic scores, all of which could indicate a more severe asphyxial insult. There are multiple risks for overcooling in this subgroup of patients with greater perinatal injury: 1) they experience an extended period of being unwrapped or lying on wet surfaces during resuscitation, 2) they have decreased metabolism and heat production due to the severe asphyxial insult, 3) they may have seizures prompting treatment with anticonvulsants or muscle relaxation for intubation that counteracts natural shivering and causes loss of muscle tone, and 4) addition of active cooling without adequate temperature control (98, 139). Therefore, a special attention is needed to ensure the proper temperature control in neonates with the most severe brain injury, including the use of overhead heater during resuscitation, and addition of active cooling only after stabilization, continuous rectal temperature monitoring, and the use of servo-controlled cooling devices. The stabilization time of our transport team varied between 16.2 minutes and 3.08 hours, and it was a median 16 minutes longer in the actively cooled group compared with the controls. The longer stabilization time occurred probably due to the longer decision-making on hypothermia treatment, the procedural time for the premedication, intubation and initiation of mechanical ventilation, and equipment assembly for cooling. We believe that the somewhat extended stabilization time has relatively small clinical importance, outweighed by the advantage of early initiation of hypothermia. The overall stabilization time of 1 hour is still considerably shorter than the average time of 2.5 hours reported in the literature (78). Finally, and importantly, no serious adverse events were observed, underscoring the safety and feasibility of providing active cooling during transport. It is noteworthy that in-hospital mortality was higher in the control group than in those who received active cooling during transport, even though both groups received 72-hour standard hypothermia treatment after neonatal transport.

One explanation might be that with increasing clinical experience with therapeutic hypothermia, it is likely that less severe cases have become routinely referred and transported throughout the study period, resulting in less mortality in recent years. In addition, it has been shown previously that hypothermia itself reduces death (140); and based on our results, the hypothermia treatment which started 2.58 hours earlier in the actively cooled group during transport may provide additional survival benefit. However, this finding should be interpreted with caution, because control neonates were born with a median 1 point lower Apgar scores, and needed more inotropic support. Apgar score has its own limitation, and 1 point difference might not carry a huge clinical impact; however, these differences may imply that perhaps more severe cases were selected for 72-hour hypothermia treatment in the past when control neonates were born. On the other hand, both groups had comparable acid-base status and vital signs; therefore, markedly different clinical conditions between actively cooled and control neonates were unlikely.

Our study has limitations that should be taken into consideration. First, our study is a retrospective analysis, with its inherent disadvantages. However, this is one of the largest patient cohort with controlled active cooling during neonatal transport. Second, as the promising results of the large hypothermia trials were published around 2010, patient selection for transport and hypothermia treatment might have slightly changed, and neonates with less severe asphyxial insult also have received cooling therapy. Importantly, the dedicated neonatal transport team was the same throughout the study period, which may be considered as a strength of our analysis. Finally, we could not report on patient neurodevelopmental outcomes; however, it was not the main objective of the present study to assess long-term effects of active cooling. We believe that cooling during transport is necessary to provide the best possible treatment for outborn infants who suffered perinatal asphyxia and present with moderate-to-severe HIE because neonatal transport is a time-consuming procedure and the efficacy of hypothermia is time dependent. The method of cooling and the experience of the transport team may influence temperature stability during transport. More importantly, the time of hypothermia initiation could affect neurologic outcome, and subsequent studies are needed to confirm that early cooling could indeed provide additional benefit.

5.2. Risk of hypocapnia in asphyxiated infants treated with hypothermia

As a second step, we investigated the development of hypocapnia in the early hours of life among neonates with moderate-to-severe HIE receiving active hypothermia treatment during neonatal transport. The introduction of active cooling during transport in late 2009 made it possible to compare groups before and after this procedure was used over a two-year period. We found that infants who were cooled during transport were more likely to develop hypocapnia than the historical control group of asphyxiated infants who only received hypothermia treatment after they arrived at the cooling center. Even after adjusting for clinically relevant factors, hypothermia remained a strong independent predictor of hypocapnia with an OR of 4.23 (95% CI 1.30–13.79). This finding lends further support, albeit indirect, to the speculation that increased incidence of hypocapnia might be caused, at least partly, by the lower body temperature (63). As we discussed before, there are several physiologically plausible explanations for the frequently observed hypocapnia in this patient population including the compensatory hyperventilation secondary to metabolic acidosis, the reduction in metabolic rate due to hypothermia and inappropriate ventilator settings (63, 108). Hence, we adjusted our statistical model for this factor as well. On the other hand, sedation or muscle relaxation can diminish patients' respiratory efforts, thereby reducing the risk of hypocapnia due to spontaneous hyperventilation. In accordance with this notion, we found that sedation protected against incident hypocapnia in our study population when we used multivariable regression modelling.

Our study had several limitations that should be taken into consideration. First, this was a retrospective cohort study with the inherent disadvantages associated with that study design. Second, neonates with HIE were transported from different referring hospitals with possibly different resuscitation practices that could have influenced the initial patient characteristics. However, the neonatal transport team and the treatment protocol used for providing intensive care were the same throughout the study period, except for the hypothermia treatment during transport. This may be considered as a strength of our analysis. Third, our transport team used a manually controlled cooling device for hypothermia provision, instead of a servo-controlled method, and it is possible that this could have provided tighter temperature control.

We note that 10 (14%) babies in the actively cooled transport group were overcooled on admission. However, it is unlikely that the observed relatively low rate of overcooling played a significant role in the high rate of hypocapnia among the infants actively cooled during transport. Fourth, continuous carbon-dioxide monitoring using transcutaneous or end-tidal CO_2, may have yielded a better real-time measurement of PCO_2 trends. However, the technical limitations and lack of experience in asphyxiated infants made these techniques problematic and unattainable in our cohort. Fifth, a major limitation of our study is that we could not include the severity of encephalopathy as a predictor of interest in our models. In the control group, neurological scoring was not performed routinely before transport, as the transport team did not have to evaluate the severity of the encephalopathy for decision-making on cooling. Finally, we have not reported on patient outcomes, although this was not the objective of the present study.

5.3. The HENRIC feasibility and safety trial

We speculate that the advantage of initiation of hypothermia treatment shortly after delivery might be further enhanced by avoiding frequently observed hypocapnia. However, clinicians have limited options to lower the risk of hypocapnia in asphyxiated infants, who tend to spontaneously hyperventilate due to severe metabolic acidosis (141). Beside muscle relaxation, which has well-known side effects (127), inhalation of low concentration carbon dioxide can be a reasonable approach to avoid hypocapnia in this patient population. Therefore, we conducted a safety and feasibility trial of 5% CO_2 and 95% air inhalation to correct hypocapnia in mechanically ventilated asphyxiated infants undergoing therapeutic hypothermia. Based on our small, single-center trial adding CO_2 to the inhaled gas mixture in low concentration was able to correct hypocapnia in asphyxiated, cooled infants with spontaneous hyperventilation as the temperature corrected PCO_2 was within the target range (40–60 mmHg) in 95.1% of time throughout the inhalation period, and no PCO_2 values were <40 mmHg. The regression modeling predicted that BD and lactate had a tendency to decrease, whereas pH remained stable during the CO_2 exposition.

Matched, control patients with HIE spent significantly less time in the target PCO_2 range and showed a similar tendency in recovery from acidosis. We consider the minimally slower pH recovery (0.01/h) clinically insignificant. Importantly, serious adverse events were not registered during the study period and the cardiovascular and respiratory status of the neonates remained stable.

There were several safety concerns that we tried to address in our study. First, we considered the fluctuation in PCO_2 as a risk factor for developing intraparenchymal hemorrhage, because hypercapnia has been described to increase its risk in preterm infants (142), and both birth asphyxia and HT treatment could cause impaired coagulation (143, 144). We performed MRI scans in all patients and found subdural, subarachnoid, and intraventricular hemorrhage (grade I) in six infants who are likely to be associated with birth trauma and found no intraparenchymal hemorrhage. In addition, PCO_2 is one of the most potent regulators of CBF with 4% increase in CBF per 1 mmHg under normal conditions (110). In our study, ultrasound assessment of CBFVs revealed no differences in the ACA and MCA before, during, and after the intervention in the seven patients who had measurements in all three time epochs. The first three patients had no measurements before the initiation of CO_2 inhalation due to technical reasons. In general, the lack of CO_2 reactivity present in infants with extensive brain injury (41). However, the lack of change in CBFV in our patients is more likely related to the fact that our study was conducted in the early hours of life. In patients with HIE, vascular reactivity may be transiently absent before the physiologic CO_2 reactivity of cerebral vasculature appear (145-147).

Second, it has been described that CO_2 inhalation elicits a physiological response of increased minute ventilation mainly due to the increase in tidal volume (148-151). In line with this, a Canadian research group using inhaled CO_2 of 0.5–1.5% via nasal prongs to prevent apnea in preterm infants noted a mild but tolerable increase in minute ventilation without any respiratory discomfort (128-130). Similarly, we also found an increased tidal volume during CO_2 inhalation and a reduction in RR after the offset of CO_2 administration. The increase in the depth of respiration was clearly related to the CO_2 exposition.

As all patients received sedato-analgesia (10 μg/kg/h morphine infusion) during the study period; we were unable to assess the possible discomfort caused by our intervention, which would be an important subject for future studies. In addition, the significance of the increased PO_2 during the intervention requires further investigation.

Third, we closely monitored brain background and seizure activity of our patients. We could not find a direct relationship between CO_2 inhalation and electrophysiological brain activity in our small clinical trial, although our study was not designed and lacked power to systematically assess seizure activity.

The rate of normal outcome and moderate disabilities in both groups were similar to the rate reported in the literature (152). Our study has several limitations that should be taken into consideration. First, this was a small pilot study using a historical control group, which limits powerful efficacy analysis. Also, p value should be interpreted carefully within the context of the small sample size. Second, continuous monitoring of PCO_2 was not feasible during the study because of current technical limitations. However, we performed frequent arterial blood gas sampling. Transcutaneous CO_2 monitoring has not been tested systematically under HT treatment. Although end-tidal CO_2 monitoring has become available recently, its precision is in question (153). Furthermore, owing to the gas flow turbulence in patient circuits, the exhaled gas can be diluted with the CO_2- enriched inhaled gas mixture prior to reaching the sampling collected manually throughout the study period, allowing for safety analysis of respiratory parameters. Of note, the manual data collection that we used could lead to an observational bias. This pilot trial was an initial step to explore a novel intervention and identify the modifications that are needed for a larger randomized trial to test the efficacy of controlled normocapnia on long-term neurodevelopmental outcomes.

Despite the limitations of the present trial, we suggest that controlled normocapnia with the inhalation of 5% CO_2 could be a reasonable approach to enhance hypothermic neuroprotection.

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6. Conclusions

1. Hypothermia treatment during transport is safe and feasible, allowing for the initiation of TH and achievement of the upper limit of target temperature of cooling (34°C) significantly earlier in infants who received active cooling during transport compared with infants who received intensive care alone.

2. Overcooled infants (rectal temperature below 33°C) on admission to the hypothermia center had deeper acidosis, lower Apgar scores, and a tendency for lower neurologic scores indicating that they had suffered a more severe asphyxial insult, that was associated with poor temperature control in this subgroup of patients.

3. Incident hypocapnia is more frequent in asphyxiated infants who received TH for HIE during neonatal transport.

4. Hypothermia is an independent predictor of hypocapnia even after adjusting for potential confounding factors including provision of sedation, ventilatory settings and acidosis severity.

5. Adding CO_2 to the inhaled gas mixture in low (5%) concentration is a physiologically plausible, safe and feasible intervention for correcting hypocapnia in mechanically ventilated asphyxiated infants undergoing therapeutic hypothermia.

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7. Summary

Optimization of intensive care of infants with HIE might have the potential to prevent injury progression and further improve neurodevelopmental outcomes in this patient population. Therefore, our first aim was to describe the safety and feasibility of hypothermia during neonatal transport in order to achieve and maintain target temperature as soon as possible after the asphyxial insult to provide the benefit of early cooling for neonates with HIE. Second, we studied the incidence of hypocapnia among asphyxiated infants receiving TH and the association between hypothermia treatment and developing hypocapnia, which is known to influence neurological outcomes negatively. Since clinicians have limited options to lower the risk of hypocapnia in this patient population, we conducted a safety and feasibility trial to correct hypocapnia by adding CO_2 to the inhaled gas mixture in low concentration.

First, we established that therapeutic hypothermia during transport is feasible and safe, allowing for significantly earlier initiation and achievement of target temperature, possibly providing the maximum possible effect of neuroprotection. Second, we reported that the infants undergoing TH develop hypocapnia more frequently. Moreover, active cooling increases the risk of developing hypocapnia even after controlling for potential confounding factors including provision of sedation, ventilatory settings and acidosis severity. We speculated that the advantage of initiation of hypothermia treatment shortly after delivery might be further enhanced by avoiding frequently observed hypocapnia. However, we need to point out that in the lack of randomized trials of controlled normocapnia it remains unclear whether hypocapnia is a biomarker or a modifiable risk factor of unfavorable outcome. The results of our interventional trial suggested that inhaled 5% CO_2 administration is a physiologically plausible, safe and straightforward intervention for correcting hypocapnia in mechanically ventilated, spontaneously breathing infants. Further studies are warranted to test the efficacy of CO_2 inhalation in providing better neurodevelopmental outcome in asphyxiated, hypocapnic neonates treated with HT. This pilot study could pave the way for such randomized trials investigating the efficacy of controlled normocapnia through 5% CO_2 administration.

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8. Összefoglalás

Az intenzív terápia optimalizálása szerepet játszhat a további károsodások megelőzésében és az idegrendszeri kimenetel javításában a hypoxiás-ischaemiás encephalopathiával kezelt újszülöttek körében. Első célkitűzésünk a transzport alatti hypothermia kivitelezhetőségének és biztonságosságának vizsgálata volt, hogy a terápiás célhőtartományt az inzultust követően minél hamarabb elérjük, ezzel maximalizálva a hypothermia kedvező hatásait. Ezt követően a bizonyítottan káros hypocapnia gyakoriságát vizsgáltuk hűtött újszülöttek körében, valamint a hypothermiás kezelés és a hypocapnia kialakulásának összefüggésére kerestünk választ. Jelenleg a klinikusoknak kevés eszköz áll rendelkezésükre a hypocapnia rizikójának csökkentésére, ezért egy intervenciós vizsgálatban alacsony dózisú CO_2 belélegeztetését teszteltük hypocapnia korrekciójára.

Megállapítottuk, hogy az aktív hypothermiás kezelés transzport alatt kivitelezhető és biztonságos, lehetővé téve ezzel a hypothermiás kezelés szignifikánsan korábbi indítását és a célhőmérséklet hamarabbi elérését, valószínűleg növelve ezzel a hypothermia neuroprotektív hatását. Második lépésként kimutattuk, hogy a hypocapnia gyakrabban fordul elő hypothermiás kezelésben részesülő asphyxiás újszülöttekben. Ezenfelül a hypothermiás kezelés növeli az alacsony CO_2 szint kialakulásának rizikóját. A hypothermia és a hypocapnia kialakulása között fennálló asszociáció szignifikánsnak mutatkozott egyéb befolyásoló tényezőkre történő korrekciót követően is, mint a szedáció alkalmazása, lélegeztetőgép beállításai vagy az acidózis súlyossága. Feltételezhető, hogy a hypocapnia elkerülésével a korai hypothermia neuroprotektív hatása tovább növelhető. Azonban randomizált vizsgálat hiányában nem megítélhető, hogy a hypocapnia biomarkere vagy módosítható rizikófaktora a késői kedvezőtlen kimenetelnek. Intervenciós vizsgálatunk eredményei szerint alacsony koncentrációjú, 5%-os CO_2 belélegeztetése kézenfekvő és biztonságosan kivitelezhető intervenció az alacsony CO_2 szintek korrigálására, megtartott spontán légzés mellett invazívan lélegeztetett asphyxiás újszülöttekben. Későbbiekben nagy esetszámú vizsgálatok szükségesek az 5%-os CO_2 belélegeztetés neurológiai kimenetelre kifejtett hatásának tesztelésére. Eredményeink mindezek alapján kontrollált normocapnia hatását vizsgáló randomizált klinikai vizsgálatok alapjául szolgálhatnak.

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10. Bibliography of the candidate's publications

10.1. Publications related to the thesis:

Szakmar E, Kovacs K, Meder U, Nagy A, Szell A, Bundzsity B, Somogyvari Z, Szabo AJ, Szabo M, Jermendy A. (2017) Feasibility and Safety of Controlled Active Hypothermia Treatment During Transport in Neonates With Hypoxic-Ischemic Encephalopathy. Pediatr Crit Care Med, 18: 1159-1165.
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10.2. Publications not related to the thesis:

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