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HYPOXIC-ISCHEMIC ENCEPHALOPATHY- DIAGNOSIS, HYPOTHERMIA TREATMENT AND OUTCOME

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à ma famille nombreuse.

ABSTRACT

Hypothermia treatment (HT) is now proven to be neuroprotective, is associated with favourable outcomes, and is considered as the standard of care for moderate to severe hypoxic ischemic encephalopathy (HIE). The treatment should be regionalized with a minimum of ten treated infants per year with regard to securing patient safety, staff training, development and future research. Still, many infants are in desperate need of additional therapies for neuronal rescue to reduce the risk of death or severe handicap. The implementation of a national HT register has revealed differences in the regional incidence of HT, indicating that infants that could benefit from HT do not receive this therapy.

All of the following main findings in this thesis have led to changes in clinical practice:

- Prevention of HIE by using fetal scalp blood lactate combined with fetal heart rate/cardiotocogram during labour is feasible. We consider fetal scalp blood lactate measurement at the cut-off level of 4.8 mmol/L (75th percentile) to be a better predictive marker for hypoxia-ischemia during labour than pH. The predictive capacity is higher and the sampling technique is easier, with a high success rate.
- Early induction of HT is feasible prior to transport. Earlier start of HT could mean that the neuroprotective effect is more beneficial. However, passive cooling results in a high risk of excessive cooling and should be used with caution, i.e. temperature should be monitored continuously and personnel should be trained in HT induction at all delivery units.
- Moderate HT alters the predictive value of amplitude integrated EEG (aEEG) in asphyxiated infants. These findings are of central value in the context of early prognosis and in decision making for withdrawal or continuation of intensive care treatment.
- The overwhelming majority of infants with moderate neonatal encephalopathy (NE) have major and/or cognitive disabilities at long-term follow-up. Most children with cerebral palsy (CP) also have cognitive dysfunctions. This is of great importance for early therapeutic interventions, allocation of habilitation resources and support for the educational system

In conclusion our findings give an additional diagnostic tool in prevention of HIE, give important information on implementation of hypothermia treatment and emphasize the necessity of long-term follow-up in encephalopathic infants.

LIST OF PUBLICATIONS

The thesis is based on the following original articles, listed in sequential order. Articles will be referred to by their Roman numerals.

- I. Kruger K, Hallberg B, Blennow M, Kublickas M, Westgren M.
Predictive value of fetal scalp blood lactate concentration and pH as markers of neurological disability.
American Journal of Obstetrics and Gynecology 1999; 181:1072-1078
- II. Hallberg B, Olson L, Bartocci M, Edqvist I, Blennow M
Passive induction of hypothermia during transport of asphyxiated infants -a risk of excessive cooling.
Acta Paediatrica 2009; 98:942-946.
- III. Hallberg B, Grossmann K, Bartocci M, Blennow M
The prognostic value of early aEEG in asphyxiated infants undergoing systemic hypothermia treatment
Acta Paediatrica 2010; 99:531-536.
- IV. Lindström K, Hallberg B, Blennow M, Fernell E, Wolf K, Westgren M
Moderate neonatal encephalopathy in term children; perinatal risk factors and a 15-19 years long term follow-up. Acta Obstetrica-Gynaecologica 2008; 87:503-509

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LIST OF ABBREVIATIONS

aEEG	Amplitude-integrated Electroencephalogram
AIMS	Alberta Infant Motor Scale
AS	Apgar Score
BGT	Basal ganglia-Thalamic
CP	Cerebral Paresis
CS	Caesarean Section
CTG	Cardiotocogram
DAMP	Deficit in attention, motor control and perception
DWI	Diffusion Weighted Imaging
EEG	Electroencephalogram
FSB	Fetal Scalp Blood Sampling
FHR	Fetal Heart Rate
GC	Glucocorticoids
HIE	Hypoxic-Ischemic Encephalopathy
HT	Hypothermia Treatment
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NE	Neonatal Encephalopathy
NICU	Neonatal Intensive Care Unit
PA	Perinatal Asphyxia
RCT	Randomized Controlled Trial
SGA	Small for Gestational Age
SHC	Selective head cooling
SMR	Severe Mental Retardation
VEP	Visual Evoked Potentials
WBC	Whole body cooling
WS	Watershed

1 INTRODUCTION

1.1 ASPHYXIA – HIE

The definition of perinatal birth asphyxia (=loss of pulse) is troublesome [1]. The Apgar score has been used as a marker for perinatal asphyxia but it has low sensitivity, specificity and predictive value for future outcome [1-5] [6, 7]. A shift to a multiple marker definition was made during the last 20 years using a combination of low Apgar scores, biochemical markers and the need for neonatal resuscitation. These markers reflect intrapartum fetal and neonatal distress [3, 8] [9-14], which can lead to a process of neurological cell injury and brain damage.

These studies address different aspects of hypoxic-ischemic encephalopathy (HIE) as a consequence of acute perinatal asphyxia. Using the criteria in Table 1 will help to identify infants suffering from acute perinatal asphyxia with a high risk of death or severe handicap.

These markers reflect disturbances in gas exchange, hypoxia, hypercarbia and acidosis. The infant has delayed onset of breathing and neurological signs of encephalopathy.

1.1.1 Incidence/prevalence/diagnosis

1.1.1.1 Incidence

HIE is a serious condition resulting in high morbidity and mortality rates in newborn infants all over the world [1, 13, 15-18]. The incidence of acute neurological symptoms within in the first hour of life – HIE – is 1-3 per 1000 live births [19] [20] [16]. In low socio-economic areas the rate is approximately 10 times higher [21], accounting for one million intrapartum-related deaths per year [15]. The incidence of HIE varies between countries and different studies depending on the inclusion criteria and study population. [1, 6, 15, 17, 22, 23].

AAP/ACOG (1996)[24]	International Cerebral Palsy Task Force (1999)[25]	AAP/ACOG (2002)[26]
pH < 7.0	pH < 7.0; BD ≥ 12 mmol/L (neonatal blood sampling)	pH < 7.0; BD ≥ 12 mmol/L (Umbilical or neonatal blood sampling)
APGAR ≤ 3@5 min	APGAR < 6@5 min	APGAR ≤ 3@5 min
	Sentinel event/Abrupt fetal heart rate change	Sentinel event/Abrupt fetal heart rate change
Neonatal Encephalopathy	Encephalopathy II-III	Encephalopathy II-III
Multi-organ dysfunction	Multi-organ failure	Multi-organ failure within 72 hours
	Cerebral Palsy	Cerebral Palsy and exclusion of other causes of brain injury
	Imaging evidence	Imaging evidence

Table 1. Criteria for diagnosing intrapartum asphyxia as a cause of brain injury.

1.1.1.2 Diagnosis

HIE is a clinical syndrome of disturbed neurological function manifested by difficulties with initiating and maintaining respiration, depression of tone and reflexes, subnormal level of consciousness and seizures [25]. Evidence of fetal distress and depression must also be present at birth. It is extremely important to distinguish the hypoxic-ischemic event from other causes of neonatal encephalopathy (NE) [27].

1.1.1.3 Classification

In clinical practice, the purpose of a classification system is to help diagnose, assess prognosis and be able to collect and compare research data [28]. Sarnat and Sarnat described their HIE grading system in 21 infants 1976 [12] in a study relating electroencephalographic findings to the clinical condition of the infants. Since then, it has been used by several authors and is now the basis for most modern evaluation schemes [13, 17, 29]. Amiel-Tison correlated the Sarnat scoring system to MRI, EEG and VEP and Miller to information about feeding difficulties [30, 31]. Dubowitz developed a revised system based on Amiel-Tison scoring with an optimality score [32] applicable during the first days of life. All classification systems have been evaluated with regard to long term prognosis. In Sweden, the classification system as modified by Levene is used for grading of HIE. The three stages – mild (I), moderate (II) and severe (III) – are based on clinical observation. During resuscitation, the infant is often hypotonic, apnoeic and lethargic. By assessing the consciousness, tone, posture, reflexes, autonomic functions and seizure activity of the infant during the first days of life, scoring is conducted as described in Table 2.

	Mild HIE (I)	Moderate HIE (II)	Severe HIE (III)
Level of consciousness	Hyperalert	Lethargic	Stuporose
Muscle tone	Normal	Mild hypotonia	Flaccid
Complex reflexes			
Suck	Normal/Weak	Weak/Absent	Absent
Moro	Strong	Weak/Incomplete	Absent
Seizures	Absent	Common	Frequent/difficult to control

Table 2. Hypoxic-ischemic encephalopathy (HIE). Classification modified from Sarnat and Sarnat (1976)

Infants with mild HIE (I) usually recover within 12-24 hours. They do not have seizure activity and present a normal aEEG/EEG pattern. The prognosis is uniformly good. Infants that deteriorate with altered levels of consciousness and clinical/subclinical seizure activity 12-24 hours after the hypoxic-ischemic event have developed moderate-severe HIE (II-III). Electrophysiology abnormalities are common, including seizure activity and abnormal background pattern on aEEG. Further deterioration to severe HIE leads to loss of reflex activity, respiratory failure and coma. These most severe cases usually die or survive with major handicap. It is important to recognize the change in severity since there is a good correlation between HIE and outcome. The Thompson score [33] is a composite grading of encephalopathy signs. At one year of age, it has a high predictive value: a peak score of 15 or higher has a positive

predictive value of 92% and a negative predictive value of 82% for abnormal outcome. This score has been widely introduced as it correlates well to neurological outcome already during the first hours of life in contrast to the Sarnat score, which is reliable only after 24 hours. Different outcome variables will be discussed in the following chapter.

1.1.1.4 Neonatal encephalopathy

NE is a wider entity and used mostly due to the fact that there are other underlying circumstances leading to encephalopathy and neurological impairment than HI. The term is used when the hypoxic-ischemic event is unknown or other risk factors associated with impaired motor outcome are identified. Cerebral palsy (CP) has been associated with infection [34], coagulopathy [35], metabolic disorders [29] and maternal disease [36]. Maternal fever during labour had the highest odds ratio for CP of all perinatal parameters in a large population based study [37]. NE is also associated with a high risk of death and long term neurological sequelae.

In this thesis, NE shown to be associated with a hypoxic-ischemic event is considered as HIE. Approximately 30% of infants with CP developed HIE in the neonatal period [38].

1.1.2 Pathogenesis

1.1.2.1 Timing of injury and prediction of outcome

Timing of injury and prediction are essential for several reasons. For children born after perinatal asphyxia, it is important to estimate the amount of injury and when it occurred. Based on this information, the clinician can counsel the parents with adequate information on short time prognosis and treatment opportunities. Other implications of the timing of the injury are the possibility for neuroprotection, for initiating hypothermia treatment (HT) and in severe cases for discussions regarding withdrawal of care due to assessed poor prognosis.

1.1.2.1.1 Clinical markers

Evidence published in recent years strongly supports the notion that brain injury may be closely related to birth. Prospective studies show a correlation between intrapartum hypoxic-ischemic events and neurological symptoms at birth. In two studies, conventional imaging techniques have reviewed the intrapartum events a) with ultrasound combined with autopsy [39], and b) MRI combined with scoring of HIE, predominant brain injury pattern and outcome [40, 41]. An MRI study of 360 full-term NE infants found that >90% of these infants had evidence of perinatally-acquired brain injury [42]. Taken together, there is strong evidence that intrapartum events contribute to the vast majority of brain injury in these infants.

1.1.2.1.2 Advanced Magnetic Resonance techniques

Studies with MR Spectroscopy (MRS) have defined a subgroup of infants with characteristic hypoxic-ischemic injury and have permitted non-invasive observation of brain metabolism after the insult [43, 44]. These studies showed a characteristic pattern of energy failure in infants with moderate-severe HIE scanned repeatedly with MRS and followed for 1-4 years. Immediately after the injury, normal intracerebral energy

storage was found, which declined within 6-15 hours, showing ATP depletion and a decrease in lactate [45]. This led to the concept of secondary energy failure, which has been shown to predict permanent brain injury and adverse outcome [45-48]. Animal work in rats, piglets and sheep later confirmed a dose-response relationship between the amount of secondary energy failure and severity of the brain injury [49-53].

Timing of brain injury can be estimated using MR techniques, as different patterns of injury are observed at different times after the insult. The accuracy and reproducibility are high [54] and MR is now considered the golden standard for imaging of the asphyxiated full-term infant [42, 55-58]. Diffusion-weighted imaging allows early detection of cytotoxic edema [59] already present during the first hours of life that normalizes towards the end of the first week. Another early marker of energy failure is the presence of lactate in the brain, which can be identified with proton/phosphorous-MRS [60]. In the basal ganglia and thalamus, the presence of lactate is always pathological and is correlated with adverse outcomes [61-63]. MRS is helpful when the cytotoxic edema is still evolving, during the first day of life, and before any structural changes have been established. The presence of lactate is always pathological and it is noteworthy that it can still be found when imaging is performed several months after the insult [46]. Cowan [42] found that 80% of studied infants with HIE had signs of acute injury on early DWI which was not established on later scans. During days 4-10, the injury evolves and two main patterns are found: a) basal-ganglia-thalamus (BGT) pattern [64] and b) watershed injury pattern [40]. This confirms that the damage is spread throughout the entire brain and that the patterns of injury are associated with the respective underlying causes: a) acute HI event or b) prolonged partial HI event. A BGT pattern with an abnormal signal in the posterior limb of internal capsule (PLIC) on T1W and T2W images is a robust marker for permanent injury and later sequelae. It has a high predictive value for adverse outcome [65]. Studies using serial MRI scans have revealed a robust evolution of the brain injury with acute changes during the first week of life, a pseudo-normalization during the second to third week and later newly affected areas of the brain showing permanent loss of tissue correlating to functional outcome [60].

1.1.2.1.3 Positron Emission Tomography (PET)

Positron emission tomography (PET) uses radio-labelled isotopes to measure in vivo biochemical and physiological changes in the brain. Using ¹⁸FDG-PET, an acute increase in glucose utilization has been shown in areas corresponding to the later diagnosed neurohandicap [66, 67]. This finding might reflect the on-going pathological processes where neurons are still viable. When scanning is postponed to 2-4 weeks after the insult, the same areas instead show a decrease in glucose utilization, probably reflecting permanent cell damage. PET remains a highly elaborate examination only available at a few research centres in the world, but has contributed important insights into the pathology of evolving brain damage.

1.1.2.1.4 Computer Tomography (CT)

CT is not widely used and is inferior in recognising the patterns described in 1.1.2.1.2. In a recent study, there was agreement in less than 2/3 of comparable images [68] on CT and MRI. Historically, CT has been used at 72 hours for predicting outcome [69].

1.1.2.1.5 Ultrasound and Doppler

Ultrasound imaging (US) is easily performed at the bedside and is therefore helpful [70]. An early scan to exclude antenatal injuries or malformations is of important value. US is usually normal for the first 6 hours after HI insult. BGT injury results in hyperechogenicity in the deep nuclei, and an abnormal reduction in echogenicity in PLIC has been correlated with abnormal PLIC signals on MRI [71]. Cerebral edema is easily visualized but has a low predictive value for future outcome. Doppler flow velocity measured through the anterior fontanel during the first days of life shows a low resistance index (RI; cerebral vasodilatation).[72], reflecting increased blood flow and decreased resistance, also called luxury perfusion [73]. There is a high correlation between low RI < 0.55 and untoward outcome [74].

1.1.2.1.6 Near Infrared Spectroscopy (NIRS)

Information about cerebral oxygen extraction can be obtained by using near infrared spectroscopy (NIRS), although this is still mainly a research tool. Increased cerebral oxygenation and low oxygen extraction is associated with death or severe disability [75].

1.1.2.1.7 Neurophysiology

Neurophysiological examinations reflect the functional integrity of the brain and have been shown to be useful even during the first days of life. High predictive values for later neurohandicaps following birth asphyxia have been reported for evoked potentials (somatosensory, visual and auditory) [76], full-band EEG [77] and amplitude integrated EEG (aEEG) [78]. The fact that aEEG has a very good early predictive value [79], together with the relative simplicity of recording and analyzing the traces has resulted in a widespread use of this method [80].

In two of the randomized controlled trials [81, 82] of induced HT following perinatal asphyxia, aEEG was used as inclusion criteria for starting HT, for monitoring brain function and seizures during treatment. The cortical activity has been shown to be influenced by many factors such as ventilatory management [83, 84], medication [85-88] and body temperature [89]. In ECMO-treated infants, Horan found that the background activity of the aEEG was not affected by lowering core temperature to 34 degrees centigrade [90].

1.1.2.1.8 Biomarkers

Several biomarkers have been evaluated for their ability to predict brain damage following birth asphyxia and HIE.

Brain-specific proteins and cytokines in cerebrospinal fluid and blood [91-97], intracellular enzymes [98], cord blood gases [99], and lactate in serum have all been found to be good early markers of the asphyxial event, but unfortunately their predictive values for later outcomes are not useful. For example, lactate in cord blood as a marker for 12-month developmental outcomes had a sensitivity of 12% [100] whereas when taken within 60 minutes after birth, 94% sensitivity and 67% [101] specificity for HIE II-III were shown.

1.1.2.2 Energy failure

Following the hypoxic-ischemic insult an initial phase of cell injury starts. Energy depletion of the nerve cells with consumption of glucose and anaerobic metabolism leads to loss of high-energy metabolites (ATP and CrP) [45, 48], depolarization of nerve cells with Ca^{2+} influx [102], cytotoxic edema, release of glutamate and other excitatory amino acids [103]. During this phase, depletion of glycogen stores and lactate production lead to metabolic acidosis.

Following resuscitation or intrauterine restoration of oxygenation and circulation, a latency phase follows, where energy metabolism and nerve cell function recovers.

The second phase is characterized by the production of free oxygen radicals [104, 105], high intracellular calcium [106], nitric oxide [107], mitochondrial dysfunction [52], pro-apoptotic pathway activation [108, 109], inflammatory response [110] and cell swelling. This cascade can lead to secondary nerve cell death and permanent brain injury [109]. In infants with HIE, the second phase is clinically associated with neurological deterioration, brain swelling and seizure activity. The second phase continues during several [49] days after the insult and seizures are often seen after 8-24 hours.

It is important to initiate brain protective strategies during the latency phase to disrupt the hazardous cascade of the second phase, and the effect of the therapy will be stronger when started as early as possible. The time between the initial phase and the secondary phase is often referred to as a “window of opportunity”, a short period 2-6 hours after the insult when nerve cells can still be rescued and future brain damage diminished.

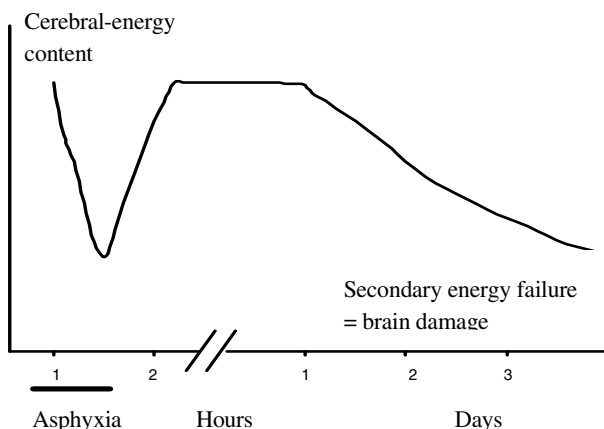


Figure 1. Cerebral high energy content and relationship to outcome.

1.1.2.3 Cell injury and neuroprotection

Pathophysiological pathways for brain injury are summarized in Table 3 together with possible neuroprotective strategies. Clinical management and treatment are described in detail in section 1.2.

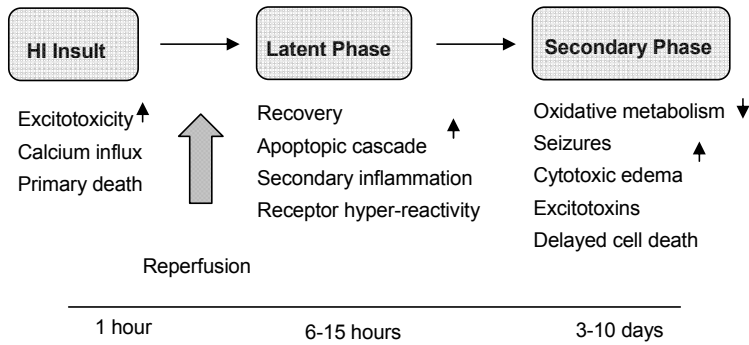


Figure 2. Diagram illustrating the phases of cerebral injury after hypoxic-ischemia. Neuroprotection such as HT must be initiated during the latent phase.

Table 3. Summary of clinical studies aiming for neuroprotection with mechanism of cell injury and neuroprotective effect.

Mechanism of cell injury	Pathway or condition influenced by treatment	Clinical studies	Protective effect
Programmed cell death, Apoptosis	Caspase activation	HT[81, 82, 111, 112]	+
Inflammatory second messenger activation	Cytokines e.g. interleukin ILs	HT [113] [81, 82, 112]	+
Oxidative stress Free radical release	O ₂ -> hypoxanthine release	Room air vs 100% ox[114, 115]	+
	NO	HT[116] [81, 82, 112]	+
	Xantine oxidase	Allopurinol[117] [118]	+/-/*
Excitatory amino acid release	Glutamate/ NMDA receptor inhibitor	HT [119] Animal exp[120] Magnesium Pilot[121] Magnesium RCT[122] [123]	+/- + +! -!/+
	HT induction	Xenon animal/RCT [124]	+/*
Ca ²⁺ influx -> cell membrane injury		Ca ²⁺ blocker[125] Bumetanide/NEMO[126, 127]	- */?
Ischemia/Reperfusion	Fe ²⁺ scavenger	Erythropoietin EPO[128]	+/*
Cytotoxic edema	Reduce cerebral edema	Mannitol [129]	-
		Dexamethasone [129, 130]	-
Seizures	Antiepileptic treatment AED	Barbiturates	
		Thiopental [131]	-
		Phenobarbitone [132]	-

(+)Significantly reduced neurological impairment, (-) Non significant or harmful, (!) Adverse event study stopped, (*) Ongoing study, (?) Study planned.

1.1.3 Prevention

Intrapartal asphyxia with significant metabolic acidosis is common and appears in 20-25/1000 births [133]. Of these, 1-3/1000 develops clinical symptoms of cerebral dysfunction, e.g. HIE [20]. The healthy fetus is prepared to sustain disturbances in blood flow, high energy consumption and hypoxia due to a high haemoglobin-concentration, high cardiac output, and the capacity to redistribute blood flow to prioritized organs [134].

Most infants will sustain the stress of being born and the hypoxic-ischemic situation that occurs in utero without complications. Anaerobic metabolism and therefore glucose consumption starts, and metabolic acidosis develops rapidly. However, in situations with prolonged hypoxia-ischemia below the individual infant's threshold [135], the cascade of brain injury described in the previous section takes place.

The fetus can be at even greater risk if it is compromised already at the start of labour, due to factors associated with poor outcome [36] such as growth restriction, placental compromise [136] and infections [137].

As many infants have their primary HI insult antenatally, the prevention of intrauterine asphyxia is important. The basic management measures are listed in Table 4.

Prevention of intrauterine asphyxia
Antepartum assessment and identification of high risk pregnancies
Intrapartal electronic fetal monitoring
Fetal scalp blood sampling
Intervention by instrumental delivery

Table 4. Basic management of intrauterine asphyxia.

1.1.3.1 High risk pregnancies

Fetuses at high risk can be identified by maternal factors (hypertension, chronic diseases) and fetal compromise (growth restriction, infections, decrease in amniotic fluid). The dyad of the mother and fetus will be in danger of complications higher than normal, and needs additional monitoring. Clinical risk scoring [138] and biophysical profiles both have a very low predictive value for adverse outcome and are not considered as tools for evaluating high risk pregnancies [139].

1.1.3.2 Fetal heart rate and scalp blood sampling

Fetal heart rate monitoring has been used for more than 30 years. The fetal heart rate is recorded continuously together with the uterine contractions and allows identification of developing hypoxia [140]. Cardiotocogram (CTG) alterations are recorded and interpreted by pattern recognition. An abnormal pattern of hypoxia during labour is recognized by alterations in basal heart rate – beat to beat variability correlated to uterine contractions. In deliveries with a reactive trace with adequate acceleration following fetal movements, an asphyxial event is very unlikely. Unfortunately, even in normal deliveries a very high rate of CTG changes is seen during labour – as high as fifty percent has been reported – resulting in a low specificity for diagnosing fetal

asphyxia [2]. The positive predictive value for fetal acidemia is also low. Thus, it is necessary to combine CTG with other methods to evaluate fetal wellbeing. Several randomized controlled trials (RCTs) with CTG have been conducted with over 33,000 women participating [141]. Unfortunately, only two of these have reported long-term follow up of the infants. In infants monitored by CTG, a reduction in neonatal seizures were found but no decrease in the risk for later CP could be detected [142]. Recently, a method of recording fetal electrocardiograms (ECG) has been introduced by automatically reading electrical changes in the fetal myocardium (ST waveform-analysis, STAN). STAN reflects ischemic processes in the myocardium. The published RCTs are contradictory about the predictive values for neonatal acidosis, low AS or Caesarean section rates. [143-145].

The poor specificity of CTG and STAN creates a need for additional diagnostic tool(s) with improved accuracy to avoid severe intrapartum asphyxia and HIE. Optimally, such methods should also have good specificity, to avoid unnecessary medical interventions. Fetal scalp blood (FSB) sampling for blood-gas analysis was first described by Saling in 1962 [146] and provides information of fetal acid-base status. It is used in many countries when an ominous CTG/FHR pattern is present. Fetal scalp blood sampling is invasive and 25-35 μ l is needed for blood-gas analysis. During significant HI, fetal acidemia progresses rapidly and can best be detected by repeated measurements in short intervals. Normal values for pH are established [147] and recommendations for interpretation are listed below.

Table 5. Fetal scalp pH and intervention [148]

7.25 normal	Observation
7.20 and 7.25 pre-acidosis	Repeat measure at 15- to 30-minute intervals
<7.20 acidosis	Indication for delivery

Perkins and Goodwin argued that the value of FSB was too low and that the procedure could be eliminated [149] without an increase in Caesarean section rates, low APGAR scores or asphyxia [150]. An correlation of < 7.25 between fetal acidosis and non-reassuring FHR is associated with a higher incidence of neurological abnormalities and disabilities [151]. Long-lasting pathological CTG tracing is associated with brain damage, and by combining FHR and FSB some cases could be prevented from deterioration [152].

Blood-gas analyses require a large amount of blood and many obstetricians find the sampling difficult to perform [153]. We have evaluated a simple point-of-care technique (5 μ L) to measure lactate with a result within 60 seconds after sampling [154] and found similar predictive values compared with APGAR scores [155], and a higher sampling success rate when compared to pH analyses [156].

Diagnosis of fetal asphyxia requires a blood gas and acid-base assessment. Recent studies support evidence that intrapartum fetal heart rate monitoring should be used as a diagnostic test in conjunction with appropriately scheduled FSB sampling [157]. The predictive capacity of FSB lactate is discussed further in paper I

1.2 MANAGEMENT

The management of a newborn infant who has sustained a hypoxic-ischemic insult aims at:

- Early identification of the infant at highest risk for evolving injury
- Supportive care to facilitate adequate perfusion and nutrients, especially to the brain.
- Intervention to stop the processes of ongoing brain injury.

1.2.1 Supportive care

HIE is associated with multi-organ dysfunction (MOD) [98, 158]. Most infants suffer both systemic MOD and neurological involvement [159]. In a recent study from Shah, 130 infants with HIE all had evidence of MOD (at least one organ dysfunction event in addition to HIE). Renal, cardiovascular, pulmonary, and hepatic dysfunction was present in 58-88% of infants with good outcome and 64-86% of infants with adverse outcome [160].

Supportive care of the asphyxiated infant with HIE is based on:

1.2.1.1 Ventilation

Pulmonary insufficiency is common and is caused both by cerebral depression and the risk of persistent pulmonary hypertension, meconium aspiration, and pneumonia. If mechanical ventilation is necessary, ventilation aims for a pO₂ of 7-9 kPa, pCO₂ 5.5-8 kPa and an oxygen-saturation of 90-95%. Hyperoxygenation could be toxic to the newborn brain [161] and hypocapnia should be avoided since it reduces cerebral blood flow and has been shown to affect outcome [162]. Special attention should be given to the temperature at which the blood gases are analyzed, since the pressure of the gases depends on temperature. During HT, the blood temperature will be 33-34°C and the thus the pCO₂ 10-15% lower than at 37°C. pCO₂ is also decreased by the reduction in metabolism during HT [163].

1.2.1.2 Cerebral perfusion

Hypotension often occurs in asphyxiated infants. Treatment is by inotropic support and volume expansion. The threshold for intervention is usually considered to be about 40 mm Hg [164]. The cardiovascular system is affected and the infant has pressure-passive circulation and transient myocardial ischemia leading to decreased ventricular contractility, diminished cardiac output and altered cerebral blood flow [165, 166]. Infants treated with HT will decrease their heart rate to 80-100 bpm but have the same incidence of hypotension as normothermic, asphyxiated infants [167]. Blood pressure, cardiac output and fluid balance should be monitored carefully. The clotting system is affected by both asphyxia and HT [168]. Capillary flow is slow when it is cold [169] and together with the high hematocrit of newborn infants [170], this results in an increased risk of microembolism and disseminated intravascular coagulation [171].

1.2.1.3 Blood glucose levels

Energy failure and mitochondrial function disorder was described in chapter 1.1.2. Animal data suggests that adequate glucose levels to be brain-protective [172] after HIE. Burns has shown that low glucose concentrations in the neonatal period are

associated with abnormal MRI findings in human infants [173]. After HI the glycogen and glucose stores are depleted. Glucose should be administered promptly intravenously at an infusion rate 4-6 mmol/kg/hour to maintain homeostasis.

1.2.1.4 Control of brain swelling and fluid restriction

Brain swelling is secondary to the cytotoxic edema [129] in HIE infants. Several intervention trials to counteract this edema have been performed using, for example, mannitol and glucocorticoids. None of these could show any benefit and are not considered to be of clinical use. In modern neonatal intensive care, the general practice is to avoid fluid overload and reduce fluids in infants that develop renal failure or have inappropriate excretion of antidiuretic hormone [174].

1.2.1.5 Seizure control

Seizures potentially increase the risk of permanent brain injury by accelerating the cerebral metabolic rate. In a recent study of term newborns with HIE, brain injury was independently associated with the severity of seizures [175]. Prolonged seizures cause progressive cerebral hypoxia and changes in cerebral blood flow [176]. These findings support the hypothesis based on data from animal models, that neonatal seizures are not only a manifestation of acute ischemic brain injury, but also exacerbate tissue damage [177] and provide evidence that effective treatment of neonatal seizures could attenuate acute brain injury. Most researchers and clinicians consider phenobarbitone to be the first line antiepileptic drug (AED) but this has not been properly tested in clinical trials. Prophylactic and interventional strategies of seizure treatment with barbiturates show no significant effect on death or severe disability [131, 132].

1.2.2 Other neuroprotective strategies

Several other active interventions have failed to prove benefits for the ultimate neurological outcome. Ca²⁺ blockers [125] have been abandoned after not being able to reproduce promising animal results. Magnesium sulphate, as an NMDA receptor blocker aimed at reducing excitotoxicity, failed to prove beneficial results in first clinical trials [121, 122] but in a small study with short term outcomes have proven to be advantageous [123]. Recently, a Dutch randomized study with allopurinol as a scavenger was published, after which Van Bel et al conducted a safety study [117]. They concluded that starting the treatment postnatally was too late and have now started a trial administering allopurinol to the mother during labour [118]. Most centres have already adapted the strategy of ventilation with room air to reduce the risk of free radical damage after the RCT studies by Saugstad [114] and Vento [115]. Neuroprotective strategies and interventions are presented in Table 3.

1.2.3 Hypothermia treatment (HT)

1.2.3.1 Background

Cooling treatment has been tentative for hundreds of years. Edwards and Gunn describes how clinicians have submerged allegedly dead infants in cold water for baptizing and how these babies then have recovered and come back to life [178]. The basic physiology during hypothermia was studied as early as the 1950s. Burnard showed how body temperature endogenously drops after HI with 2°C [179]. Westin reported on HT after birth asphyxia as a mode of resuscitation more than a method for neuroprotection. Six asphyxiated infants who did not respond to conventional resuscitation were placed in a bath of cold water (body temperature 23-30°C) and left there “until they screamed”. Five of these infants survived and only one infant died. [180, 181]. Miller found that outcome in HT infants was better than historical controls [182]. However, this therapy was discouraged by the fact that preterm babies have a higher oxygen consumption and a higher mortality when cold [183] and therefore further clinical studies did not follow at that time. In the 1990s HT after HI was proven to be effective in lamb, rodent and pig models [111, 119, 184].

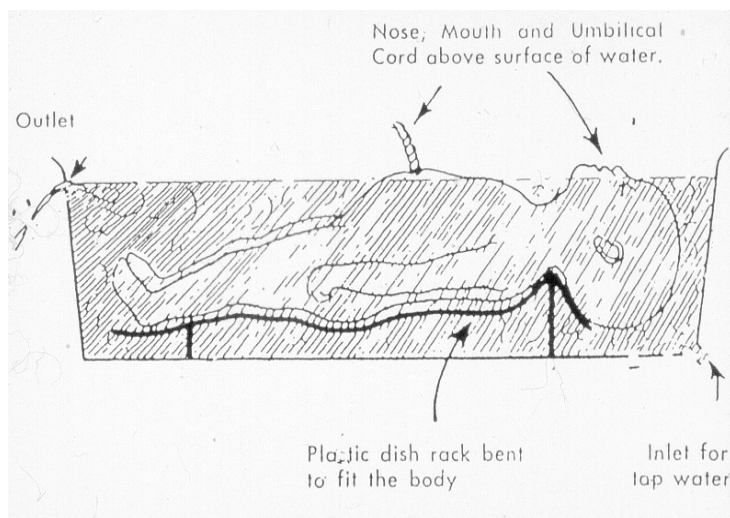


Figure 3. Facsimile from the seminal paper from Westin et al, describing hypothermia as a method for delivery room resuscitation[181].

1.2.3.2 Laboratory evidence

Several animal experiments support the neuroprotective mechanisms of hypothermia: a) HT reduces the excitotoxic cascade and inhibits the production of free radicals by lowering the release and increasing the uptake of glutamate [185], b) HT also reduces the metabolic need by lowering body temperature and thereby ameliorates the second energy failure [184], c) HT prevents apoptosis by suppressing the apoptotic pathways [111, 116], d) HT suppresses the secondary proinflammatory response and it is also proposed to have a protective effect by decreasing the release of cytokines and

interleukins [53, 116]. In animal models, HT has also been found to have sustainable long-term effects on functional outcomes [110, 186].

1.2.3.3 *Clinical evidence*

Several successful pilot studies have been conducted that focused on feasibility and safety. These studies were not powered to evaluate neurological outcome [167, 187, 188].

Three larger RCT studies with homogenous inclusion criteria consisting of a HI event, encephalopathy and outcome have been published. The CoolCap trial [81] was the first one. Infants with moderate-severe HIE assessed by clinical scoring and electrophysiology monitoring were randomized to mild selective head cooling (SHC) for 72 hours (n=116) or normothermia (n=118). Rectal temperature was kept at 34° to 35°C. Follow up at 18 months showed a borderline reduction in death or severe disabilities, (OR 0.57, 95% CI, 0.32-1.01, P=0.05). The National Institute of Child Health and Human Development (NICHD) trial [113] performed in the US at 12 centres examined whole body cooling (WBC) using a cooling blanket and a rectal temperature of 33° to 34°C, over 72 hours. Inclusion criteria were moderate to severe HIE examined by an experienced clinician. 208 infants were recruited (study group n=102). A significant reduction in death or disability at 18 months was found. 45% in the cooled group versus 62 % in the normothermic infants (RR 0.72, 95% CI, 0.55-0.93) The TOBY trial [82] is the largest RCT and included 325 infants and reports on neurological outcomes at 18 months of age. The inclusion criteria were the same as in CoolCap but a cooling mattress and WBC to a rectal temperature of 33°-34°C over 72 hours was used. No significant effect was found in reduction of the primary outcomes of death or disability (RR 0.86, 95% CI, 0.68-1.07) but an increased intact survival without neurological abnormality was found (RR 1.57, 95 % CI, 1.16-2.12, P=0.003). Finally, a small RCT using WBC in 65 infants with HIE has been reported. A slightly deeper temperature of 33°C over 48 hours was obtained and resulted in a reduction of the combined outcomes of death and severe disability at 12 months of age in the HT group (52% versus 84%, p=0.019) [188].

Several meta-analyses examining the published RCT studies have been performed. In a 2007 study of 506 infants, Jacobs found a significant benefit from HT on the primary outcome of death or disability (RR 0.75, CI 0.65-0.89) [189-191]. A recent meta-analysis of 767 infants from the TOBY, CoolCAP and NICHD trials found a reduced risk of death and disability at 18 months of age (RR 0.81, 95%CI 0.71-0.93); the analysis also found an increased rate of intact survival in the HT group (RR 1.53, 95%CI 1.22-1.93) and a number needed to treat (NNT) of eight. A summary of published RCT studies for HIE is given in Table 6. Two ongoing studies (ICE trial and neu.nEURO.network-trial) have not yet been reported.

A workshop organized by the NICHD in 2005 concluded that many questions were not yet answered at that time and warned against uncontrolled use [192]. In addition, the Swedish Health Assessment Bureau (SBU) published an alert in which they stated that hypothermia treatment was an evolving therapy [193]. In contrast to this, there is now sufficient data to conclude that HT is proven to be neuroprotective, is associated with favourable outcome and is considered as the standard of care for moderate-severe HIE.

Study	Cooled/Controls	Mode of cooling	Target temp.	Duration (hrs)	Follow-up
CoolCap 2005 [81]	116/118	Selective	34-35	72	18 months
NICHD 2005 [112]	102/106	Systemic	33.5	72	18 months
TOBY 2009 [82]	163/162	Systemic	33-34	72	18 months
Eicher 2005 [188]	32/33	Systemic	33	48	12 months
Robertson2008 [194]	21/15	Systemic	33-34	72	17 days
Lin 2006 [195]	32/30	Selective	34-35	72	7-10 days
Akisu 2003 [196]	11/10	Selective	36.5	72	4-10 days
Shankaran 2002 [197]	9/10	Systemic	34.5	72	Discharge

Table 6. Summary of published RCTs with moderate HT for HIE.

1.2.3.4 Prognostic markers and HT

The prognostic value of clinical scoring is altered after cooling, suggesting that infants with moderate encephalopathy have more favourable outcome than if non-cooled [198]. The TOBY trial reports a high sensitivity and specificity on conventional MRI for abnormal outcome or death at 18 months of age. The accuracy for predicting outcome were similar between groups (0.84 cooled; 0.81 non-cooled). These findings suggest that MRI is a robust proxy marker for outcome [56]. Paper III addresses the prognostic value of aEEG.

1.2.3.5 Systemic complication

Cooling infants using a standardized strict protocol during phase III studies has been proven to be safe in both pilot safety studies and performed RCT trials [81, 82, 112, 167, 187, 188, 199-201]. Significant adverse and reversible effects reported from meta-analyses are bradycardia (RR: 4.1) and thrombocytopenia (RR:1.3) [189, 190]. Drugs metabolized through the liver have been shown to have higher serum concentrations and morphine has a reduced clearance, resulting in potentially toxic concentrations if doses are not reduced [202]. Anuria and oliguria is common in HIE infants but the serum levels of gentamicin (during once-daily regime) are not affected by cooling but by reduced kidney function [203]. Hypothermia aggravates MOD and special attention is needed while treating HIE with a body temperature of 33°-34°C. The safety of deeper HT has not been examined except for a small pilot study reporting HT at a rectal temperature of 30°C to be safe [204].

1.2.3.6 Cooling strategies

HT should be induced within 6 hours after birth and maintained for 72 hours, after which re-warming is slowly started to avoid rebound effects. Two different modes of cooling, systemic (whole body cooling WBC, core temp 33.5°C) and selective (selective head cooling SHC, core temp. 34.5°C), have been tested in the RCTs. There is no difference in efficacy at 18 month follow up between WBC and SHC [191]. Further, no differences in side effects have been found when comparing the CoolCap trial versus the TOBY trial [205]. In one study, there was a difference in the incidence of cortical lesions but not in BGT lesions on MRI between selective versus systemic cooling. A lower incidence of cortical lesions in the SHC group but no difference was found in outcome at 18 months of age [206]. Long-term

follow up with cognitive function has not yet been reported but there might be a difference favouring the selective cooling strategy.

1.3 FOLLOW UP

1.3.1 Short term

The association between birth asphyxia and cerebral palsy (CP) is well recognized [27]. In the most recent Swedish epidemiological study, 35% of children born at term with subsequent CP were considered to have a perinatal origin for the injury [38].

Most outcome studies have focused on neurological functioning and severe deficits in young children under four years of age. Children with mild encephalopathy have a good chance of surviving without neurological impairments or have developed severe mental or motor retardation by preschool age [207].

In contrast, children with severe encephalopathy almost always die or develop severe impairments [16] such as CP, mental retardation, epilepsy and in some cases sensorineural hearing loss or cortical visual impairment [207]. Pin performed a meta-analysis of 13 studies meeting strict follow up criteria and found that mild NE was associated with favourable outcome, while 1/3 of moderate cases and almost all with severe NE had adverse outcome. These studies are retrospectively designed and the contribution of true HIE is not known. Children who have suffered moderate encephalopathy seem to form a more heterogeneous group [8]. In an 18-month follow up study of 17 children with moderate encephalopathy, [16] only about half of the children were found to develop normally whereas the other half developed cerebral palsy.

1.3.2 Follow up and MRI

The pattern of injury corresponds to the development of deficits, and new insights in the evolving brain injury have been contributed by MRI studies. BGT predominant pattern of injury in the deep grey nuclei and the periorlandic cortex [64, 65], is associated with dyskinetic CP or quadriplegia and follows acute near-total asphyxia. Infants with BGT injury can be so severely affected that they will not be able to perform in cognitive long-term follow up [38]. The severity of injury within the BGT has also been shown to correlate with the severity of cognitive impairments. [208]. Children with watershed (WS) predominant patterns usually present milder forms of CP or no motor impairment. They often have normal early outcomes at 12-18 months. Their MRI findings are considered to reflect “subacute partial asphyxia” [40]. Despite the normal short term motor outcome, Mercuri found that they later often have supoptimal head growth and cognitive deficits [209]. We and others have shown that cognitive dysfunction is associated both with structural damage on conventional MRI [31] [210] and disturbed white matter organization on advanced diffusion tensor imaging [211].

1.3.3 Other long-term follow up studies

Robertson and Finer [212] followed 145 children with mild, moderate and severe encephalopathy (56, 84 and five children, respectively) associated with birth asphyxia as term infants. Moster [3] studied a large population with low APGAR scores and early neonatal neurological symptoms, while Maneru [213] performed follow up on a cohort of infants with “moderate asphyxia”. Together with a study by Marlow at seven years of age [214] these studies provide evidence that infants who suffer a HI event at birth, will have a high risk of cognitive deficits without motor impairment when tested at school age. They are likely to be more than one grade level delayed, are at risk for physical and mental impairment and have reduced school performance. The deficits were reported especially in specific memory tasks, reading, spelling, arithmetic and attention. Children with mild encephalopathy had school performance scores similar to healthy controls.

In contrast, others [215, 216] have reported favourable neurological outcome in young adults who needed resuscitation at birth.

How perinatal risk factors are associated with long-term outcomes in children with NE is unknown.

2 AIMS OF THIS THESIS

The overall aim of this thesis is to help in understanding the devastating course of HIE, and to prevent, diagnose and treat infants at risk of severe brain injury.

2.1 STUDY I

Identify the predictive capacity of fetal scalp-blood lactate compared to pH for detecting outcome variables shown to have an impact on long-term outcomes. To establish cut-off values in fetal scalp-blood lactate for obstetric intervention.

2.2 STUDY II

Study the feasibility and safety of early induction of hypothermia by the practice of stopping active heating.

2.3 STUDY III

Investigate the predictive capacity of amplitude-integrated EEG in correlation with one-year motor outcome in HT-treated HIE infants.

2.4 STUDY IV

Describe pre- and perinatal data in a Swedish national cohort of infants born in 1985 with moderate encephalopathy (NE) at term in regard to long-term outcomes at 15-19 years of age.

3 METHODS

3.1 ETHICAL CONSIDERATIONS

Studies I and IV were approved by the local ethical committee at Huddinge University Hospital and Studies II and III by the ethical committee in Stockholm. Informed consent was obtained from the parents when needed.

3.2 PATIENTS AND METHODS STUDY I

We conducted a retrospective study of all patients that had fetal scalp blood sampling (FSB) due to an ominous fetal heart rate (FHR) at Huddinge University Hospital from October 1993 to October 1998. The department is a referral centre for high risk pregnancies. During this time period, annual birth rates were 3500 to 4000 deliveries, while perinatal mortality fluctuated and was 4-6 per 1000 live births. The rate of Caesarean section increased over time from 11.4% to 15.1%. The most common deviant FHRs were tachycardia (>160 beats/min), late or profound decelerations, decreased variability or silent patterns. Scalp blood sampling was performed by the attending obstetrician according to existing clinical protocols.

3.2.1 Fetal scalp blood sampling

FSB was carried out by the standard technique with the patient in supine position and the legs in stirrups. An amnioscope was used to avoid contamination with the amniotic fluid. The lactate value was analyzed immediately after sampling using an electro-chemical single-use strip method (Lactate Pro KDK Corporation, Kyoto, Japan) which requires only 5 µl of whole blood [154]. The pH and acid-base balance were analyzed with an acid-base meter (ABL 510, Radiometer, Copenhagen Denmark) and required 35 µl of blood for pH measurement.

3.2.2 Outcome variables

Analysed outcome variables included: pH <7.0, base deficit >16.0 mol/l in umbilical artery blood, AS < 7 at 1 minute, < 7 at five minutes, < 4 at five minutes, and HIE. HIE was defined according to the criteria by Sarnat & Sarnat [12], as mild, moderate or severe. The estimation of HIE was done retrospectively by Boubou Hallberg who was blinded for the pH and lactate values.

3.3 PATIENTS AND METHODS STUDIES II AND III

The Stockholm region has 5 obstetric departments, and neonatal services are provided at four of these hospitals, two of which are regional HT centres. Beginning in December 2006, after completion of recruitment for the TOBY trial, a joint protocol for therapeutic HT was accepted in the region (Table 7). Briefly, this stipulates that infants fulfilling the A-criteria of a hypoxic-ischemic event are evaluated for signs of hypoxic-ischemic encephalopathy (B-criteria) by the neonatologist in charge.

Systemic hypothermia treatment is considered for infants who fulfil following criteria.	
A <ul style="list-style-type: none"> • Apgar score ≤ 5 at 10 minutes after birth • Continued need for resuscitation including mask-ventilation at 10 minutes after birth • pH < 7.0 in cord blood or within 60 minutes after birth (arterial or capillary blood sampling). • Base-excess ≤ 16 within 60 minutes after birth 	If any of the criteria are fulfilled, infant neurology is assessed according to criterion B.
B <ul style="list-style-type: none"> • Seizures or moderate-severe HIE defined as the combination of <ul style="list-style-type: none"> ○ Altered level of consciousness ○ Hypotonia or opisthotonus ○ Abnormal primitive reflexes 	Assessment is done continuously during the first 60 minutes.
If criteria A and B are fulfilled, treatment is started before 6 hours of life. Amplitude-integrated EEG is not mandatory to start hypothermia treatment.	

Table 7. Swedish national guidelines for induced hypothermia in newborn infants ≥ 36 gestational weeks following perinatal asphyxia (www.blf.net).

For infants born in the two hypothermia centres, active hypothermia is started as soon as possible using a water-filled hypothermia mattress (Tecotherm®, TecCom, Germany). In infants born in the other three hospitals, active warming procedures are stopped, monitoring of rectal temperature is started and preparations for immediate transport to one of the hypothermia centres are instituted.

Systemic HT to a target temperature of 33.0-34.0°C is maintained for 72 hours, followed by a slow rewarming period (maximum temperature increase 0.5°C/hour). Rectal temperatures are collected every hour during hypothermia and for 24 hours following the start of rewarming. aEEG registration is immediately started and it is continued throughout the 72 hours of HT treatment and during rewarming.

3.3.1 Data collection (Studies II and III)

Data on all newborn infants treated with HT at the neonatal units in Stockholm are collected according to a preset protocol and entered in a national perinatal database (PNQ) [217]. This database contains an add-on module for hypothermia treatment that has been available since December 2006. In addition to the basic parameters of neonatal care, the module collects information on inclusion criteria, mode of hypothermia treatment, rectal and environmental temperatures before and during treatment, treatment complications, and follow-up data.

3.3.2 Passive induction of HT (Study II)

In this study (December 2006-May 2008), rectal temperatures at the time of decision to start hypothermia treatment, at the start of transport and on arrival at the hypothermia centre were extracted from the hypothermia module. For the purpose of this report, rectal temperatures $>37.0^{\circ}\text{C}$ before and during transport are considered as

hyperthermia, 35.0-36.9°C as normothermia, 33.0-34.9°C as therapeutic hypothermia and <33.0°C as sub-therapeutic.

Ambient outdoor temperatures and the time on the days of all transports were collected from the Swedish Meteorological and Hydrological Institute (SMHI).

3.3.3 Prognostic value of aEEG (Study III)

We prospectively collected and digitally stored aEEG recordings using the Nervus[®] monitor (Viasys, Nicole Biomedical, Madison, Wisconsin, USA). The traces were blindly assessed by two independent researchers (BH and KRG). The scoring system suggested by Westas et al [218] (Table 8) was used and the predominant pattern assessed at 6, 12, 24, 36, 48 and 72 hours after birth. In all recordings there was full congruity between the two readers with regard to the background pattern, sleep-wake cycling and presence/absence of ictal activity.

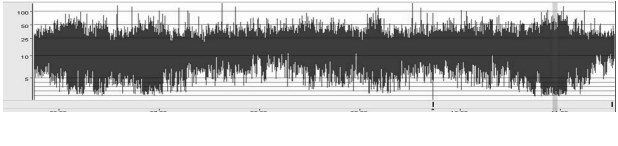
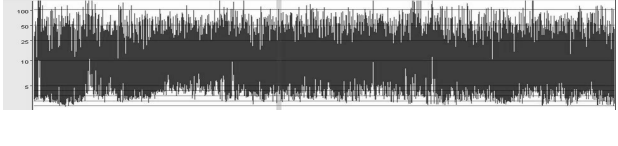
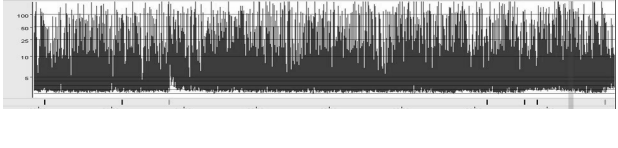
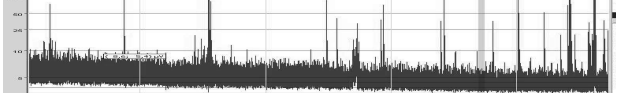
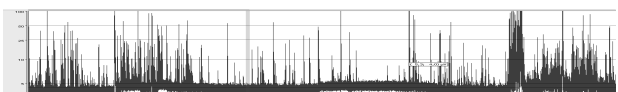
<p>Continuous Normal Voltage CNV: continuous background activity with minimal amplitude 5-10 μV and maximal amplitude 10-50 μV.</p>	
<p>Discontinuous Normal Voltage DNV: discontinuous background activity with varying amplitude minimum below 5 μV and maximum amplitude > 10 μV.</p>	
<p>Burst suppression BS: Discontinuous background with lowest amplitude without variability at 0-2 μV intermixed with burst of higher amplitude.</p>	
<p>Low Voltage LW: Continuous background with very low voltage around or below 5 μV.</p>	
<p>Inactive, flat trace FT: Isoelectric background activity < 5 μV.</p>	

Table 8. Definition of aEEG pattern adapted from L. Hellström-Westas [216].

At 4 months of age, a neuromotor developmental assessment using the Alberta Infant Motor Scale (AIMS) was performed. AIMS was administered by a physiotherapist and it examines the gross-motor capacities of the infant in the supine, prone, sitting and standing positions. The results are reported as a percentile in relation to data validated in a Canadian cohort of healthy full-term infants [219].

Mortality, neurological examinations with overt signs of spasticity and an AIMS score below the 5th percentile were considered as abnormal outcomes.

3.4 PATIENTS AND METHODS STUDY IV

We retrospectively studied all infants born in Sweden 1985 with an AS < 7 at 5 minutes. In the Swedish Medical Birth Register from 1996, 684 infants were identified, out of 96,478 live-borns. The Swedish population was 8.4 million this year. The medical records were evaluated for 560 infants, delineated in Figure 4. Children with major malformations, chromosomal aberrations, severe perinatal infections, and opioid induced neonatal-depression were excluded.

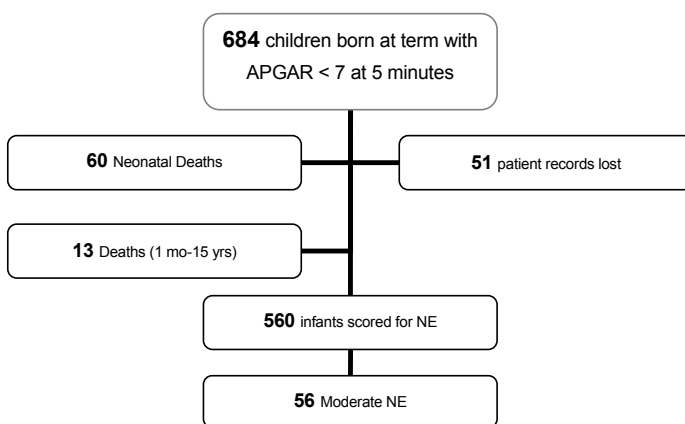


Figure 4. Flowchart of the patients from the 1985 birth cohort.

Neonatal encephalopathy was independently scored according to Sarnat & Sarnat [12] by a neuropediatrician (KL) and a neonatologist (BH,MB). 54 infants with moderate NE and two with borderline moderate-severe NE were included in the further analysis of the study. Thirteen cases were excluded due to parental refusal to participate, giving a final study group consisting of 43 children (male:female 26:17).

3.4.1 Obstetric and Neonatal data

The maternity records – antenatal and intrapartum care – were evaluated by two independent obstetricians (MW, KW), to assess obstetric risks and management. The following antenatal characteristics were recorded; diabetes, IUGR (according to ultrasound), hypertension, preeclampsia, renal disease, maternal bleedings after 20 weeks gestation, oligo- and polyhydramnios, prolonged rupture of membranes (> 24 hours), multiple pregnancies. The data were compared with the general statistics for Sweden that year (Swedish Medical Birth Register).

All available fetal heart-rate tracings were reviewed. The cardiotocogram (CTG) was considered to be abnormal if episodes of late or severe variable decelerations and/or decreased variability with absence of accelerations, and/or tachycardia (>160 bpm) occurred for periods of more than 30 minutes. A general evaluation of the standard of obstetric care was performed and classified as normal, questionable or suboptimal.

For all included patients, the neonatal records were collected and evaluated to determine whether the infants had an AS of < 7 at 10 minutes, were born small for

gestational age (SGA), needed mechanical ventilation, had seizures and/or needed prolonged periods of neonatal care.

3.4.2 Neurological assessment

For children with diagnosed CP or other major neuro-impairments, patient charts were collected from their neuropediatric and habilitation departments. These documents were evaluated to determine the type of CP [220] and the neuro-psychological assessment when present. Infants without CP were recalled and tested for cognitive function through interviews using specific rating scales and questionnaires presented in a separate paper [221].

3.5 STATISTICAL ANALYSIS

Study I: All data were entered into a computerized database (Perinatal Database, MedSciNet, Stockholm) and analyzed with the MedCalc statistical package (© 1993-1999 MedCalc Software, Mariakerke, Belgium) Receiver operating characteristic (ROC) curves for pH and lactate were calculated in relation to the outcome variables. If both pH and lactate had been analyzed in the same patient, the areas under the two ROC curves were compared [222] and the negative (NPV) and the positive (PPV) predictive values were calculated.

Study II and III: Descriptive data and linear regression analysis was performed using Statistica® 7.0 (Statsoft, Tulsa, USA).

Study IV: A chi² –test was used for calculating p-values for the differences in proportions across groups (Statistica 6.0, Statsoft, Tulsa, USA).

The significance level was set at $p < 0.05$ for all tests.

4 RESULTS

4.1 RESULTS STUDY I

During the study period, 1709 fetal scalp-blood samples were performed. pH was analyzed in 1221 and lactate in 814. The values with highest accuracy for predicting different outcomes were calculated. Lactate values varied from 4.3-6.5 mmol/L and pH from 7.20-7.26.

		Sensitivity and 90% confidence interval		Specificity and 90% confidence interval	
	Outcome variable	Lactate	pH	Lactate	pH
75 th percentile lactate > 4.8	Apgar < 4 at 5 min	58.3 (27.8-84.7)	30.0 (9.36-61.4)	75.9 (72.8-78.9)	73.4 (70.8-75.8)
25 th percentile pH < 7.21	HIE II	66.7 (22.7-94.7)	50.0 (12.4-87.6)	75.7 (72.6-78.7)	73.4 (70.9-75.9)
90 th percentile lactate > 6.1	Apgar < 4 at 5 min	41.7 (15.3-72.2)	0	91.0 (88.8-92.9)	89.4 (87.6-91.1)
10 th percentile pH < 7.15	HIE II	66.7 (22.7-94.7)	16.7 (2.8-63.9)	91.0 (88.8-92.8)	89.9 (87.7-91.2)

Table 9. Sensitivity and specificity in relationship to Apgar<4 at 5 min. and HIE II-II

No.	Antenatal course	FHR/CTG	Lactate mmol/L	pH	Time to, mode of delivery	Birth Weight (GA)	Agar 1,5,10 min.	Outcome HIE
1	Pre-eclampsia Induction	Decreased variability, late decelerations	7.3	7.28	3 h 15 min VE	2700 (40)	1 1 1	Dead at 20 min.
2	Uneventful	Late decelerations	7.8	7.32	1 h 10 min VE->CS	3608 (39)	0 3 6	Moderate
3	Uneventful	Pronounced variable Decelerations	6.6	7.20	25 min VE->CS	3855 (41)	1 3 4	Moderate
4	Uneventful	Decreased variability	4.6	-	20 min PN	3290 (40)	1 4 4	Moderate
5	Pre-eclampsia Induction	Variable decelerations	-	7.29	3 h 20 min PN	3680 (40)	2 3 5	Severe
6	Uneventful	Decreased variability, late decelerations	10.4	7.21	15 min CS	2610 (40)	5 8 8	Moderate

Table 10. Descriptive data on patients with HIE II-III. VE; Vacuum extraction, CS; Caesarean section, PN; Partus normalis, GA; Gestational age

The 75th and 90th percentiles for the lactate concentrations were 4.8 and 6.1 mmol/L respectively. The 25th and 10th percentiles for pH were 7.21 and 7.15. Table 9 shows the sensitivity and specificity for these percentiles in regard to AS < 4 at 5 minutes and moderate HIE. There was generally higher sensitivity and specificity in the lactate group in relation to the outcome variables. This was most pronounced for AS < 4 at 5 minutes and to moderate HIE.

Moderate to severe HIE occurred in 6 cases. Detailed data on these patients is given in table 10. One patient in the HIE II-III group died, one experienced a shoulder dystocia and one developed a group B streptococcus infection. Five of these patients had lactate measurements and for four of these the concentration exceeded the 75th percentile (4.8 mmol/L).

4.1.1 pH vs Lactate

In 326 patients, both lactate and pH measurements were performed concomitantly permitting a comparison between the methods. The area under the ROC curve was significantly better for lactate than for pH values in predicting AS < 4 at 5 minutes ($p=0.033$) and moderate to severe HIE ($p=0.015$).

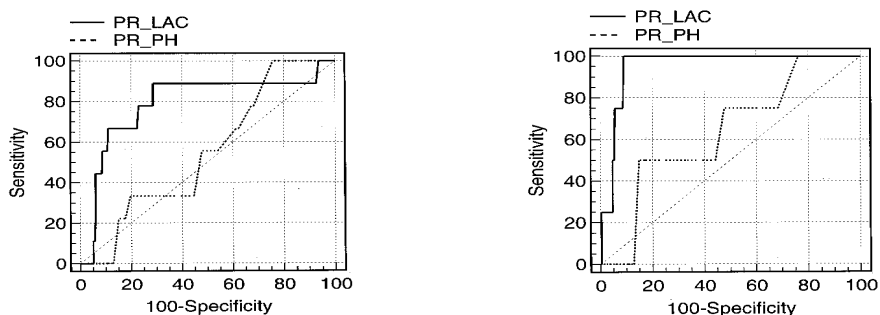


Figure 5. ROC curves for lactate/pH in relation to AS <4 at 5 minutes and moderate/severe HIE.

The predictive value for the 75th percentile for lactate and 25th percentile for pH was similar but there was a tendency for better predictive capacity for lactate in regard to AS < 4 at 5 minutes (PPV 8.3 vs. 3.8; NPV 99.1 vs 97.2) and HIE II-III (PPV 3.6 vs 2.8; NPV 100 vs. 99.4) than for pH.

4.2 RESULTS STUDIES II AND III

During Study III (1 Dec 2006-31 Dec 2007), 86 infants had a significant hypoxic-ischemic event fulfilling the A-criteria for hypothermia treatment and were admitted to the neonatal intensive care units for treatment and evaluation. During the same period, a total of 28,837 infants were born in the region, resulting in an incidence of the A-criteria in 2.98/1000 of live-born full term infants. During the study periods, the incidence for fulfilling both the A and the B treatment criteria was 0.9/1000. Three of the infants were not treated with therapeutic hypothermia; for two of these resuscitation and stabilization was still on-going at 6 hours of age, and in one case the infant was transferred to the pediatric intensive care unit (due to possible need for extracorporeal membrane oxygenation) where hypothermia treatment was not available. Study II (Dec 2006-May 2008) included 34 infants, and Study III 23 infants. Baseline data are presented in Tables 11 and 12.

4.2.1 Passive induction Study II

Eighteen (53%) of the 34 infants were outborn. This group did not differ from the inborn infants with regard to baseline parameters. At the timepoint when transport started, rectal temperatures varied from 33.0-36.4°C. No infant was recorded to be in the hyperthermic or sub-therapeutic intervals.

	Inborn	Outborn	All
N	16	18*	34*
Gestational age (weeks)	40+3/7	40+3/7	40+3/7
Birth Weight (kg)	3.6	3.6	3.6
Sex ♂/♀	7/9	12/6	19/15
Apgar 5 min/10 min (median)	3/5	3/4	3/4
Umbilical artery pH	6.8	6.9	6.9
HIE			
Mild/Moderate/severe	1/14/1	4/10/4	5/24/5
Referred	0	18	18
Dead	1	3	4

Table 11. Basal clinical data in Study II

* Three infants who fulfilled the treatment criteria were not treated.

On arrival at the referral centre, temperatures ranged from 31.0-36.5°C. At this time, six out of 18 infants had rectal temperatures in the sub-therapeutic range. In two cases, a marginal increase in rectal temperature was noted after transport, while nine infants had decreased rectal temperatures, the most pronounced loss being 2.6°C.

In our group of transported infants, there was a strong correlation between the postnatal age at which all warming sources were turned off and the temperature at the start of active cooling (Figure 6, $r=0.81$, $p<0.001$).

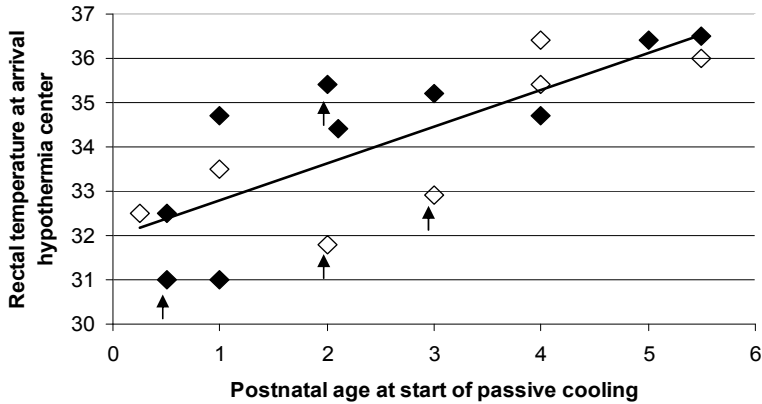


Figure 6. Correlation between post-natal age at which all warming resources were turned off and the temperature at start of active cooling.

Six infants were intubated and mechanically ventilated during the transport. Four of these infants were hypothermic (31.0-32.9°C) on arrival at the hypothermia treatment centre. In the whole cohort, 4/34 died and three of these were transported for treatment. Two of these infants were hypothermic on arrival (31.8°C and 32.9°C respectively).

4.2.2 aEEG predictive capacity Study III

The interpretation of the aEEG at 6, 24, 36, 48 and 72 hours of age are presented in Figure 7. Continuous (CNV) or discontinuous (DNV) normal voltage patterns were found in five infants. CNV and DNV at 6 hours of age were always associated with a favourable outcome.

Baseline patient data. N=23		
GA Weeks + days (mean range)	40+1	36+5 - 41+6
BW kg (mean, range)	3.56	2.45 - 4.99
Apgar 5' (median, range)	2	0 - 6
Apgar 10' (median, range)	4	1 - 6
Sex Boys/Girls	8/15	
Inborn/Outborn	12/11	
pH umb (mean range)	6.92	6.57 - 7.30
BE umb (mean range)	-16.3	-24 - -4.5
pH infant < 1h (mean range)	6.89	6.6 - 7.08
BE infant < 1h (mean range)	-20.6	-25 - -10
Age at HT start h (mean range)	3.15	0.5 - 10
HIE mild/moderate/severe	3/16/4	
AIMS at 4-6 months		
<5, 5-25, 25-74, >75	3/1/12/4	
Neurological exam. 12 months		
CP /Delayed/ Normal	4/1/15	
Mortality	2	

Table 12. Baseline patient data study III.

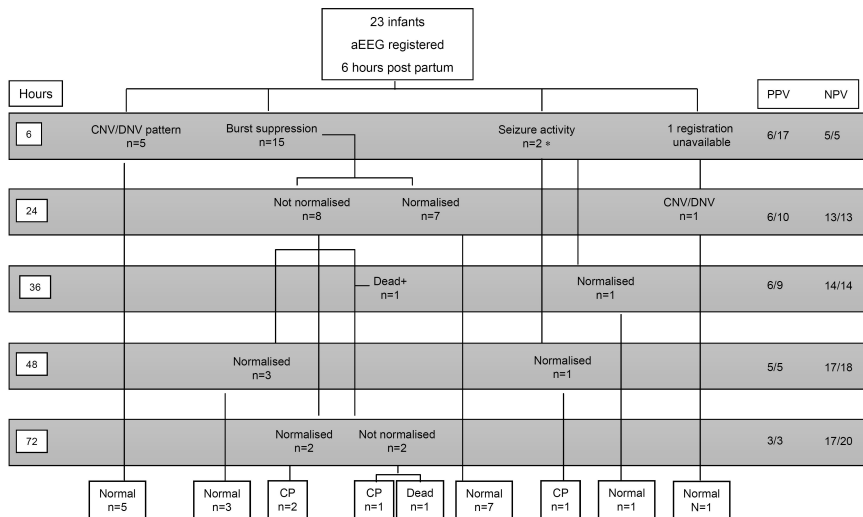


Figure 7. Predominant aEEG pattern in HT infants at 6-72 h in relationship to predictive values and neurological outcome at 12 months.

Fifteen infants had a severely abnormal burst-suppression (BS) pattern at 6 hours of age. In two infants, ongoing seizure activity obscured the possibility to analyze the background pattern at this time point. All 17 infants had aEEG seizure activity at some point during the registration period. In seven of these infants, a normalization of aEEG was observed within the first 24 hours of life. In all these infants, AIMS at four months of age was above the 25th percentile with a normal examination result at one year of life. Ten infants had persistent burst-suppression or seizure activity beyond 24 hours after birth. Adverse outcome was found in six of the infants with late normalization (36 hours or later). Out of these six children, two died during the neonatal period and four have cerebral palsy at 12 months of age. In three infants with initial burst-suppression, a normalization was observed before 48 hours of life. These infants also had AIMS > 50th percentile at four months of age.

All four infants with AIMS <10th percentile at four months of age had an abnormal motor outcome at 12 months of age. No infant with AIMS > 25th percentile developed CP at 12 months; one infant has slightly delayed gross-motor function.

4.3 RESULTS STUDY IV

Of the 43 children with moderate NE, 13 (30%) had cerebral palsy (CP), 22 (51%) had cognitive dysfunctions without CP and only 8 (19%) had no obvious impairments. (Figure 8) Gender distribution is presented in Table 13.

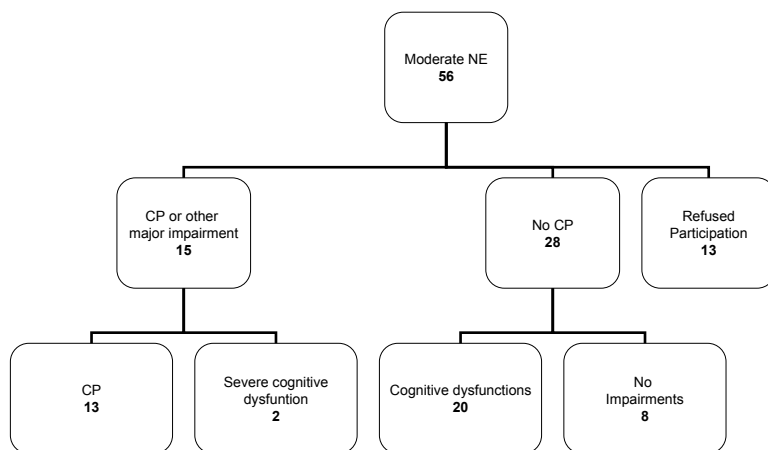


Figure 8. Long-term outcome after moderate encephalopathy

Gender	Male	Female
CP	6	7
Cognitive dysfunction; No CP	13	9
No impairments	7	1
Total encephalopathy	26	17

Table 13. Gender distribution and long-term outcome after moderate encephalopathy.

4.3.1 Cerebral Paresis (CP)

All forms of CP were found; dyskinetic (5), spastic (5) hemi-, di-, tetraplegia and ataxic (3) forms. The majority of these 13 children had additional impairments and 10 also had definite cognitive dysfunctions. Eight children were mentally retarded, three of them severely. Eight of these children had epilepsy and hearing impairment was diagnosed in three.

4.3.2 Cognitive dysfunction

The group comprised 22 children, 20 children with cognitive dysfunctions without CP and another two with early identified major cognitive impairments (one with severe mental retardation (SMR) and one with deficits in attention, motor control and perception (DAMP/ autism spectrum disorder).

The twenty children with cognitive dysfunctions without CP had problems ranging from learning disabilities to problems with executive functions including attention,

planning, short-term memory, motor control, time-perception and social functioning [221]. In another report, we described 11 children with corresponding disturbances seen in cerebral white matter organization when examined with advanced MRI diffusion tensor imaging. [223]

4.3.3 Without impairments

Eight children had no impairment.

4.3.4 Obstetric and Neonatal data

The mean age of the mothers was 30 years (range 21-40 years). Post-term pregnancies (>41 weeks) were more common, with eight of the 42 births being post-term (19%), which is significantly higher than the post-term incidence in Sweden that year (8 %: $p < 0.01$).

Caesarean section was more common than in the general population (17/42=40.5% vs 11.8%), as were instrumental delivery (11/42=26.2% vs 6,5%, $p < 0.001$) and breech presentation (5/42=11.9% vs 3%, $p < 0.001$). Ominous FHR patterns were common and 33/38 demonstrated such a pattern over a period of 30 minutes or more before delivery, with 14/38 showing such a pattern over a period of at least 90 minutes. The standard of obstetric care is rated in Table 14. Suboptimal and questionable care was common in these cases (55 %).

Obstetric care	CP (n=12)	Cognitive dysfunction; No (CP n=22)	No impairment (n=8)	Total (n=42)
Normal	3	14	2	19
Questionable	6	2	1	9
Suboptimal	3	6	5	14
Ominous FHR				
> 30 min	10	17	6	33
> 60 min	9	9	6	24
> 90 min	6	6	2	14

Table 14. Evaluation of standard of obstetrical care and occurrence of ominous FHR pattern .

Children in all outcome groups had prolonged depressed AS (below 7) for at least 10 minutes. Only two infants were small for gestational age (SGA). All three groups contained children who needed mechanical ventilation in the NICU and children hospitalized for more than 14 days. All children with CP had seizures in the neonatal period, but children with seizures were also found in the other outcome groups.

5 DISCUSSION

In *Study I* we examined the predictive capacity of lactate in regard to pH in fetal scalp blood sampling. We chose the 75th percentile for lactate 4.8 mmol/L as a cut-off value. The 75th percentile seems to yield a reasonable number of abnormal test results and predictive values. FBS is used as an additional test for ominous fetal heart rate on CTG. It is most important that this test does not yield a large number of false negatives. This study was designed to find those who are at high risk for adverse outcomes and still have an acceptable level of instrumental deliveries. We previously used the 99th percentile (4.2 mmol/L) from a population of healthy patients with normal FHR. This population were not at risk and sampling was done to evaluate the method. We found that this tentative cut-off level in that population gave too many false positives [224]. Evaluating a prognostic test is complicated when the test result affects outcomes such as death or severe morbidity. During the study period we performed observational studies (1993-1994), prospective randomized studies (1995-1997) and introduced lactate as a diagnostic tool (1997-present). This study is retrospectively performed but the number of patients included is still one of the largest studies performed to date. A recent RCT study used the 75th percentile for lactate (4.8 mmol/L) and the 25th percentile for pH (<7.20) as the cut-off level [157] and concluded that there were no significant differences in rate of acidemia at birth using the methods for determining hypoxia during birth. It is also known that lactate normally rises during the second stage of labour [225]. Our results are in agreement with that of others [226], i.e. lactate is a more sensitive tool for predicting outcomes associated with severe neurodisabilities in a population at risk [1, 10, 227].

Scalp-blood pH has been found to be poor predictor of s in a review of 1547 samples [228]. The reason why lactate should be a better predictor than pH for neonatal morbidity is not completely clear: in a rat model, lactate concentrations in subcutaneous tissue preceded a decrease in brain pH and a decrease in survival [50]. In infants suffering from HIE, urinary lactate levels are associated with death or moderate/severe neurodevelopmental disability [229]. Similar results have been found using proton spectroscopy measurements of lactate [43, 230]. These data support our findings that determination of scalp-lactate could be a more sensitive tool than pH in the detection and prevention of significant perinatal asphyxia. In our study, several infants with HIE had normal pH values but increased lactate concentration. However, it must be remembered that during the study lactate was not used as a tool for intervention.

FBS is one of several interventions used in the clinical evaluation and management of a patient with abnormal CTG. In our unit, FBS was performed in 8% of all deliveries and the Caesarean section rate is still reasonable (15%). We consider lactate measurement to be a better predictive marker than pH in fetal monitoring due to both its predictive capacity and its feasibility for sampling. The only way to properly address the predictive capacity is to perform a prospective randomized trial with long term outcomes.

Studies II and III

Hypothermia treatment is now proven to be neuroprotective and associated with an improved outcome [191]. Today, many regional- and local hospital NICUs that did not participate in any of the randomized controlled trials are offering cooling, and HT is rapidly becoming the standard of care for HIE treatment [231]. In Sweden, a national HT programme was introduced in 2007, and our group has established a national HT register. In total, 191 infants were included in the register until 31 Dec 2009 by 10 reporting sites. This gives an incidence of 0.5/1000 cooled infants. In the population-based Stockholm cohort described in paper III the incidence was 0.9/1000. Safety data are based on the administration of cooling in NICUs, with strict cooling protocols and often at centres with considerable expertise with cooling. However, it is not proven that HT can be applied with maintained patient safety and effective neuroprotection outside these protocols. Establishing an HT programme takes a long time and requires multidisciplinary collaboration [232].

In prospective *Study II*, we show that the initiation of passive cooling before and during transport is possible and that it effectively reduces the rectal temperature of the infants born outside hypothermia centres during transport. However, when hypothermia is induced passively, a significant number of infants arrive at the treatment centre with a body temperature below the therapeutic range. We also found that excessive cooling covariates with morbidity, mortality and is more frequent in infants where passive cooling was started early. In excessively cooled infants, prolonged periods with unstable temperatures were noted, even resulting in some infants needing mattress temperatures of 35-38°C during the initial day of treatment. This could lead to periods of rapid rewarming which is known to be associated with cardiovascular instability, recurrences of seizures and neurological side effects [233, 234]. The possibility of reducing the body temperature by turning off the active warming devices has been shown in a recent case report [235]. Fairchild and colleagues transported during active cooling and found that overcooling to <32°C occurred in 34% of patients and at least one recorded temperature was below < 30°C [236]. Their findings and ours highlight the risks of overcooling when passive induction of HT is started early by inexperienced staff and continuous core temperature monitoring is inadequate. In fact, in all six infants with subtherapeutic temperatures on arrival, passive induction was started before three hours of age, and in four of these already during the first hour of life.

It has long been known that asphyxiated babies lose body heat at a higher rate than non-asphyxiated ones [179], and this might be more pronounced in severely affected infants. Two out of three of the infants that died arrived with a temperature of less than 33.0°C. This could indicate that the most severely affected infants had the highest rate of temperature loss. An alternative explanation could be that the excessive hypothermia *per se* worsens prognosis. In a recent retrospective study, Compagnoni et al compared asphyxiated infants treated at normothermia (n= 11) to a group treated with moderate hypothermia (32-34°C, n=11) and a group treated with deep hypothermia (30.0-33.0°C, n=18) [204]. They found no differences in serious side effects between the mild and the deep hypothermia group, and both hypothermia treated groups had better outcomes than the controls. The conclusions of that study are of course hampered by the retrospective study design and, as in our study, by the small number of infants studied.

We have shown that early induction of HT is feasible prior to transport. Thus the neuroprotective effect could theoretically be more effective [116]. Using continuous core temperature monitoring and water-filled mattress with a set temperature in the range of 33-34°C or, alternatively, a mattress with a phase-changing material [237] could reduce the risk of excessive hypothermia in these infants.

In *Study III*, we investigated the predictive capacity of aEEG during hypothermia treatment. We found that a considerable proportion of infants changing from a pathological to a normal aEEG beyond 24 hours of age had normal outcomes at one-year of age. In 1995, Hellström-Westas showed that infants with CNV/DNV within the first 6 hours of life were likely to survive without sequelae with a predictive value of 96%. In contrast, infants with BS or worse pattern at 6 hours were at a risk of death or neurological sequelae with a predictive value of 86% [78]. These results were supported by the study of Toet et al, who found a positive predictive value (PPV) of 78% and 86% at 3 and 6 hours respectively; the corresponding negative predictive values were 84% and 91% [238]. Infants with BS at 6 hours of age, but who recovered before 24 hours, were reported to have a risk of later death or severe handicap (PPV) of 6/12 [239]. Later, Ter Horst and coworkers reported that the sooner abnormalities on aEEG disappeared during the first three days of life, the better the prognosis. The likelihood ratio of BS or worse for adverse outcome was 2.7 between 0 and 6 h, increasing to a highest value of 19 between 24 and 36 h [240]. When using a proposed voltage classification the results were confirmed with a positive predictive value of 85% and a negative predictive value of 100%, studied in 24 infants within 12 hours after birth [79]. In our study, in infants treated with HT, all five infants with CNV/DNV at 6 hours had a normal one-year outcome, supporting the data from these previous trials which had an overall sensitivity of 91% (95% CI 87-95) and a negative likelihood ratio of 0.09 (95% CI .06-.15) to accurately predict poor outcome [80]. However, 10/15 of our infants with BS or flat traces and 1/2 with SZ at 6 hours had a normal AIMS at 4 months and normal neurological examination at one , giving a positive predictive value of only 32%. Further, at 24 hours of life, BS/FT/SZ were seen in 10 infants and for 4 of these, where aEEG normalized between 36 and 48 hours, outcomes were favourable (PPV 60%). Thus, for infants normalizing after 24 hours, our data seems to be as good as those mentioned above, normalizing after 6 but before 24 hours [239] The predictive capacity in the pre-HT era is summarized in Table 15.

Study	Sensitivity	Specificity	NPV	PPV
Hellström-Westas 1995 (n=47) [78]	95%	89%	86%	96%
Eken 1995 (n=34) [76]	94%	79%	84%	92%
Toet 1999 (n=68) [238]	91%	86%	86%	96%
al Naqeeb 1999 (n=56) [79]	93%	70%	77%	90%
van Rooij 2005 (n=160)[239]	83%	85%	88%	91%
Shany 2006 (n=39) [241]	100%	87%	69%	100%
Thoresen 2010 (n=31)[242]	86%	72%	84%	72%

Table 15. Predictive capacity of aEEG for abnormal outcome.

Several explanations, with the exception of a change in the predictive capacity of aEEG, might explain our findings. The populations might differ in the severity of illness. Our baseline data indicates that this is not the case and if there was a true difference in the severity of the insult, this should most probably have been reflected in less pronounced aEEG abnormalities. Other plausible explanations could be that the hypothermia *per se* affects the background pattern of the aEEG. A study with HT during ECMO treatment did not find any changes on aEEG voltage [90] and Rundgren found that aEEG pattern was predictive for outcome in cardiac arrest hypothermia treated adult patients [243]. Another explanation could be that medication administered alters aEEG voltage or that the effect of HT on pharmacodynamics has this effect. This study was not designed to address these questions. Our observation of short-lasting effects on the aEEG in the few infants with excessive HT and at phenobarbital administration give some support to this explanation, but further studies are needed to explore this in more detail.

This is the first report on an altered predictive value of aEEG in asphyxiated infants treated with moderate hypothermia. Thoresen et al [242] report on 74 infants (HT=31 NT = 43) fulfilling the cooling criteria. They found that the positive predictive value of an abnormal aEEG pattern at the age of 3-6h was 86% for NT whereas it was 59% for HT. Time to normalization of background pattern was the strongest predictor of outcome at 18 months of age and was found to be associated with good outcome at 48 hours of age in infants treated with HT. We think that these findings are of central value in the context of early prognosis and in decision making for withdrawal or continuation of intensive care treatment. In the pre-HT era, neurophysiological abnormalities have had a high impact on discussions for discontinuation of intensive care [80]. Our findings during the implementation of HT into clinical practice challenges this, as several of our infants with normal outcomes had abnormalities that persisted beyond 24 hours of age. Furthermore, they also show that the time point for making early prognosis based on aEEG is delayed during HT and our group of infants is too small for analyzing the optimal time point for prognosis. Combining our data with the data from Thoresen gives 66 HT infants and suggests that the optimal time point could be at 48 hours. It is still uncertain which predictive method is the most valid. Neurological scoring has to wait until 72 hours before significant conclusions can be drawn [198]. Therefore, considerable caution and thorough clinical investigation combining HIE grading, neurophysiology and advanced imaging techniques should be used before discontinuing intensive care in HT-treated infants.

A limitation with our Studies III and III is the small number of infants studied, related to the low incidence of infants fulfilling the HT treatment criteria. However, during the two years that HT has been implemented in our units as the standard of care, we have only missed three infants for treatment and one infant to follow-up and this indicates that our data are regionally valid. The Stockholm region has an annual birth rate of approximately 28,000 deliveries, thus our region is responsible for 30% of the annual births in Sweden. Another problem is our hitherto short follow up time, only 12 months, which obviously does not allow us to draw conclusions on minor delays in motor skills, or later development of cerebral palsy or cognitive dysfunction.

In Study IV we examined the long-term outcome after perinatal asphyxia and subsequent moderate NE. In these patients, we found an unexpectedly high rate of cognitive dysfunction (81%) with (30%) or without (51%) CP. This means that only 20% of the patients with moderate/severe encephalopathy survived without impairment.

Most mothers had an uneventful pregnancy and few presented with severe risk factors before parturition. Consequently, most of the mothers experienced a spontaneous onset of labour. However, post-term start of labour was common and lack of initiation of parturition could be regarded as a subtle sign of poor coordination between the mother and the fetoplacental unit. Children born post-term have previously been shown to be at risk of developmental deviations [244]. Likewise, fetuses in breech presentation are known to be at an increased risk for long-term neurological morbidity [245, 246] and breech presentation was more common amongst those included in this investigation than in the population as a whole.

Several patients demonstrated ominous FHR patterns for sustained periods of time. At the time when the children of the present study were born the perceived reasonable Caesarean section rate among obstetricians in Sweden was 10%. This attitude is well illustrated in the present study where it is obvious that the obstetrician often preferred vaginal deliveries despite poor progress and ominous FHR patterns. The risk for abnormal outcome when an intrapart event occurs is well known [247] and neurological signs after repeated prolonged asphyxia is associated with impaired neurological function [29]. 80% of the infants in our material with disabilities had an intrapart event. As described in paper I, FHR monitoring can be predictive and used as a diagnostic tool for prevention of HIE [248], although it will not prevent all cases of moderate and severe HIE. Since 1985, obstetric care has changed dramatically and today it is routine in Sweden to induce labour or perform CS in pregnancies > 41+6 gestational age, deliver abdominally for breech presentations and use FHR/FBS sampling during delivery. Thus, might reduce the risk for long-term handicap.

In this cohort we found that the standard management for infants suffering from HIE was a brain-oriented intensive care treatment with hypocarbia ventilation, glucocorticoids (GC) and seizure control with barbiturates [130]. None of these regimes is proven to be neuroprotective and, even worse, there is evidence that this strategy could be harmful to the brain. Hypocapnea reduces cerebral blood flow and is now known to be associated with worse neurological outcome in full term infants [249]. GC in early life restricts brain growth, development and increase disability [250]. A meta-analysis of barbiturate administration versus control after severe perinatal asphyxia including 77 patients showed no beneficial effect on death or severe disability [251]. One can only speculate if the regime at that time contributed to the severe neurological impairment in our patients and especially in regard to cognitive outcome. The most severely affected children had seizures, an increased need for assisted ventilation, and longer duration of neonatal hospitalization. We could not perform a valid statistical comparison between outcome and perinatal prognostic risk factors due to the retrospective nature of the study and the consequent lack of information in the material. In 1985, few children had proper neuroimaging, neurophysiological investigations or measurement of biochemical markers for brain injury. The study group all had AS <7 at 5 min and moderate encephalopathy. The vast majority also (76%) had seizures. Ellenberg described a cluster of perinatal events associated with poor outcomes and found that 50% had motor disabilities and 70% died or had disabilities when combining low AS, SZ and neurological signs [10]. However, they did not report on cognitive dysfunction. All types of CP were present among our 13 children with motor impairment. This is in agreement with the assumption that the vulnerability in the brain differs between individuals and the duration of the HI event. BGT lesions are

associated with motor impairment and WS injuries with cognitive function [57, 64]. We have shown that in a subgroup of infants without CP the fractional anisotropy values are lower in white matter regions including corpus callosum and the internal capsule [211]. We lack information about the severity of the asphyxial event and therefore some of the cases could be due to other underlying causes such as metabolic disease, infection, genetic causes and coagulopathy [27] and the term NE is more appropriate if the HI event is not absolutely known [27].

The study is population based on a well-defined birth cohort of children with AS < 7 at 5 minutes, born at term ($GA \geq 37$ weeks) in Sweden in 1985. 7.1% of the records from the Swedish medical birth register were retrieved and we assume that if records were not found, the infants were not treated in neonatal units. Classification of the children into NE groups was retrospective, implying that the data had to be interpreted from clinical files not primarily intended for research. However, the three clinicians involved in this process found the data to be adequate for this task. We have no evidence that the children whose mothers declined participation differed from the group of children included in the investigation. On the other hand, the material covers a well defined birth cohort from an entire country and relates to a follow-up 15-19 years after NE. The prevalence of AS < 7 at 5 minutes was 7/1000 (684/97468) which is similar to the 1995 year birth cohort in Sweden (6.9/1000) [16]. The prevalence of moderate NE of 0.57/1000 seems reasonable considering that in the Stockholm region the incidence for HT in 2007-2009 was 0.9/1000 including some mild and severe HIE cases. Thornberg et al found an incidence of 0.4/1000 but mentioned that they had a number of “non-determined” cases.

In conclusion and in contrast to common belief, we have shown that the overwhelming majority of infants with moderate NE have major and/or cognitive disabilities in a long-term follow up. Most children with CP also have cognitive dysfunctions. This is considered in the new proposed definition of CP [220].

Impaired cognitive function needs to be addressed to monitor the long lasting neuroprotective and reparative effects in ongoing and planned interventional studies after HIE.

6 CONCLUSION

Hypothermia treatment is now proven to be neuroprotective, is associated with favourable outcome and is considered as the standard of care for moderate-severe HIE. The treatment should be regionalized with a minimum of ten treated infants per year in regard to secure patient safety, staff training, development and future research. Still, many infants are in desperate need for additional therapies for neuronal rescue to reduce the risk for death or severe handicap. Implementation of a national HT register has revealed a difference in the regional incidence of HT, indicating that infants that could benefit from HT do not receive this therapy.

All of the following main findings in *Studies I-IV* have led to changes in clinical practice:

- Prevention of HIE by using FBS-lactate combined with FHR during labour is feasible. We consider fetal scalp blood lactate measurement at the cut-off level at of 4.8 mmol/L (75th percentile) to be a better predictive marker for hypoxia-ischemia during labour than pH. The predictive capacity is higher and the sampling technique is easier with a high success rate.
- Early induction of HT is feasible prior to transport. Earlier start of HT could mean that the neuroprotective effect is more beneficial. However, passive cooling results in a high risk of excessive cooling and should be used with caution, i.e. temperature monitored continuously and personnel trained in induction of HT in all delivery units.
- Moderate HT alters the predictive value of aEEG in asphyxiated infants. These findings are of central value in the context of early prognosis and in decision making for withdrawal or continuation of intensive care treatment.
- The overwhelming majority of infants with moderate NE have major and/or cognitive disabilities at long-term follow up. Most children with CP also have cognitive dysfunctions. This is of great importance for early therapeutic interventions, allocation of habilitation resources and support for the educational system.

In conclusion our findings gives an additional diagnostic tool in prevention of HIE, gives important information on implementation of hypothermia treatment and emphasis the necessity of long-term follow-up in encephalopathic infants.

7 CLOSING REMARKS AND FUTURE PERSPECTIVES

Several challenges persist and will be or are currently under investigation. The implementation of HT is ongoing and reports from these phase IV studies are essential to improve the therapy. The long-lasting effect of HT is not proven and therefore long-term follow up is awaited. Several groups are investigating cooling and additional therapies to enhance neuronal rescue with different agents such as Xenon, AEDs, and growth factors. In the near future, stem cell therapy could be an alternative approach. A critical dilemma is the lack of good proxy biomarkers for both identifying infants that are in need for neuroprotection and for early assessment of the effect of such therapies to predict long-term outcomes. Future research is needed in this area, and hopefully with early electrophysiological surveillance, advanced MR sequences, and biochemical markers these answers will be found.

Future research should also focus on:

- What is the optimal time period and depth of hypothermia?
- How can cooling be facilitated in a low resource setting and during transport?
- Is cooling effective when started after 6 hours or given to infants < 36 weeks?
- Do infants with mild HIE have impaired cognitive functions and would they benefit from HT?

Finally, prevention of HIE must be the ultimate goal;

But most of these infants have an uneventful fetal course after which they experience a hypoxic-ischemic event sometimes associated with an antenatal factor such as an infection that compromises the fetus. There is enough evidence to support the notion that a significant number of these infants were healthy days or hours prior to birth. Better methods for understanding the evolving brain injury, identifying infants at high risk and helping those that develop neurological symptoms are challenges for future research.

8 SWEDISH SUMMARY

Årligen dör 1 miljon barn i världen till följd av syrebrist vid förlossningen, och flerdubbelt fler ådrar sig livslånga neurologiska handikapp. Akut och svår syrebrist (asfyxi) i samband med födelsen drabbar i västvärlden 1-4 barn av 1000 födda. För 1-2/1000 av dessa är asfyxin av sådan svårighetsgrad att de utvecklar en måttlig till svår hjärnfunktionspåverkan (hypoxisk-ischemisk encefalopati, HIE) med stor risk för livslånga handikapp. Tidigare har behandlingen av dessa barn endast utgjorts av ett stödjande av de vitala funktionerna med sedvanlig neonatal intensivvård.

Inducerad kylbehandling under 72 timmar till svårt asfyktiska barn efter syrebrist i samband med förlossningen har visat sig reducera risken för hjärnskada eller död vid 18-22 månaders ålder. Effekten är god med en ökad chans till frisk överlevnad med ca 50% och NNT 8.

Sedan 2006 finns nationella riktlinjer för hypotermi behandling i Sverige.

Behandlingen innebär att barnens kroppstemperatur sänks till 33,5 grader C i 72 timmar, varefter en långsam uppvärmning sker. Terapin anses idag vara ”Standard of care” och skall erbjudas på Neonatal intensivvårdsd avdelning med full intensivvård (level III). Sedan introduktionen av HT i Sverige i början av 2007 har cirka 200 barn i landet behandlats med metoden. Vår genomgång av behandlingens genomslag visar på kraftigt varierande incidens i landets regioner. Detta tyder på att behandlingen inte erbjuds till samtliga barn som skulle kunna ha nytta av den.

I denna avhandling presenteras 4 studier som samtliga förändrat klinisk handläggning av asfyktiska barn;

I studie I visar vi att Laktat taget i skalp blod hos barn med ökad risk för syrebrist under förlossningen är en bättre markör än pH för allvarlig påverkan av barnet i form av låg Apgar poäng eller måttlig-svår hjärnpåverkan under förlossningen. Vi föreslår en cut-off gräns på 4.8 mmol/L.

I studie II visar vi att metoden att stänga av yttre värmekällor före och under transport till HT-center är förenat med allvarliga risker för excessiv nedkylning. Det är därför av stor vikt att samtliga förlossningsenheter skaffar rutiner för passiv nedkylning och att dedikerade transport team utför transport till HT-center.

I studie III visar vi för första gången att aEEGs prediktiva värde hos kylbehandlade barn förändras och att ett gravt patologiskt aEEG först vid 48 timmars ålder förutsäger en dålig prognos för barnet. Detta ska jämföras med data före HT då ett patologiskt aEEG redan vid 6 timmars ålder med ett positivt prediktivt värde på 88% förutsade dålig prognos. Denna information är av yttersta vikt framför allt vid diskussion om avbrytande av livsuppehållande behandling.

Långtidsprognosen för barn med måttlig-svår hjärnpåverkan har tidigare framförallt redovisats i form av motorisk funktionsnedsättning (CP). Vi visar i *studie IV* att endast 19 % av dessa barn är utan funktionsnedsättning vid 15-19 års ålder. Majoriteten har kognitiva svårigheter och uppföljnings program för att identifiera dessa svårigheter implementeras nu på ett flertal centra.

Sammanfattningsvis erbjuder våra studier ett diagnostiskt verktyg för att förebygga syrebrist relaterade hjärnskador, klarlägger viktig information under införande av kylbehandling och understryker behovet av långtidsuppföljning hos asfyktiska barn.

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