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LONG-TERM OUTCOME AFTER HYPOTHERMIA-TREATED HYPOXIC-ISCHAEMIC ENCEPHALOPATHY

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Karolinska Institutet

Stockholm 2022

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2022

ISBN 978-91-8016-608-9
Cover illustration: Andreas Hurme Lundin and Matilda Grossmann, 2022

Long-term outcome after hypothermia-treated hypoxic-ischaemic encephalopathy
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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The thesis will be defended in public at Karolinska Institutet, Huddinge, 2022-06-03.

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Under snötäcket
vilar trädens spegelbild
på sjöns blanka is

Mid-pandemic haiku by Katarina Robertsson Grossmann, January 2021

POPULAR SCIENCE SUMMARY OF THE THESIS

Caused by interrupted or impaired blood flow or oxygen supply to the foetus during labour and delivery, perinatal asphyxia accounts for nearly a quarter of all deaths in new-born infants worldwide. Approximately 1-6/1000 live-born infants exhibit clinical signs of brain injury following perinatal asphyxia. This condition is called hypoxic-ischaemic encephalopathy (HIE) and is characterised by altered level of consciousness, muscle tone, reflexes, heart rate and pupillary reaction. HIE is potentially life-threatening, and survivors are at risk of life-long impairments including cerebral palsy (CP), epilepsy, intellectual disability, sensory disruption, executive difficulties and behavioural problems. HIE is commonly stratified into three degrees of severity: mild (grade 1), moderate (grade 2), and severe (grade 3). In infants with moderate or severe HIE, seizures are common. Seizures in new-born infants are known to be harmful, particularly following perinatal asphyxia. Besides treatment for seizures and supporting the body's vital functions, therapeutic hypothermia (TH) is the only treatment proven to reduce the risk of death or severe disability in infants with moderate or severe HIE. The infant is wrapped either in a cooling blanket or placed on a cooling mattress and for a duration of 72 hours, core body temperature is reduced to 33.5°C. This is then followed by slow rewarming to 36.5°C. The treatment is time-sensitive - a shorter time between the hypoxic-ischaemic insult to initiation of TH has been demonstrated to improve outcome. Plenty of research efforts have therefore been made with the aim to find biomarkers that may help identify infants who would benefit from TH as early as possible. To date, reports on outcomes after hypothermia-treated HIE extend into early school-age, but outcomes in adolescence or adulthood are not known. Historically, the prognosis in infants with mild HIE was perceived to be good as early studies reported that these infants survived and did not develop major neuromotor disability. As a result, infants with mild HIE were not purposely included in the randomised controlled trials of TH. More recently, there has been a therapeutic drift with some centres now routinely admitting also infants with mild HIE for treatment with TH. Recent evidence indicates that outcomes following mild HIE are not uniformly good.

Perinatal asphyxia affects not only the brain but many other of the body's organs as well. The kidneys are particularly sensitive to hypoxia-ischaemia. In studies done prior to the introduction of TH, up to 72% of infants with HIE also suffered acute kidney injury (AKI). The field of neonatal AKI has seen important progress in the last decade, with experts agreeing on a definition modified specifically for use in new-born infants that allows for staging of the kidney injury. Historically, studies on neonatal AKI have been small, single-centre reports focusing on specific patient populations and have used many various definitions for AKI. Recently, large multi-centre collaborative efforts have shed light on AKI being a common occurrence among patients treated in neonatal intensive care units. AKI is associated with increased mortality and prolonged hospital stay. There is evidence that neonatal AKI is underrecognised during hospital stay and at the time of discharge. Contrary to previous beliefs, there is now evidence that patients who suffer AKI are at increased risk for chronic kidney disease (CKD). To date, the knowledge about long-term kidney-related

outcomes in neonatal patient populations remains limited. It is not yet clear if TH may also have a protective effect in the kidneys.

In study I, we investigated the prognostic value of early amplitude-integrated EEG (aEEG) in relation to outcome at 1 year of age among infants treated with TH. Poor outcome was only seen in infants who had a persisting severely abnormal pattern on aEEG at 24 hours of age and beyond.

In study II, we studied long-term neuromotor, neurologic, cognitive and behavioural outcomes in children with a history of hypothermia-treated HIE. In our cohort, 17% of children developed CP. Most children with CP also had other neurologic comorbidities. On a group level, children without major neuromotor disability had cognitive abilities within normal range. In early adolescence, 26% of children with previously good outcome had new deficits affecting everyday life. Executive difficulties appear to be more common in this patient population.

In study III, we studied the incidence and severity of neonatal AKI in infants with hypothermia-treated HIE. Using a staged definition of AKI for which there is international consensus, we found that 45% of infants in our cohort suffered neonatal AKI. Severe AKI was rare. Monitoring of creatinine and urinary output is important for the identification infants with AKI. As reported in several other studies, AKI was associated with increased mortality. At 10-12 years of age, 21% of children had decreased glomerular filtration rate (GFR) estimated from creatinine.

In study IV, we investigated renal functions in greater detail at age 10-12 years following perinatal asphyxia and TH. The children were examined with blood and urine samples, blood pressure measurement and magnetic resonance imaging of the kidneys. We found that renal impairments were rare in early adolescence in this patient population.

ABSTRACT

Hypoxic-ischaemic encephalopathy (HIE) is a major cause of acquired brain injury in new-born infants. It is a potentially life-threatening condition that leaves survivors at substantial risk of life-long debilitating sequelae including cerebral palsy, epilepsy, intellectual disability, sensory disruption, behavioural issues, executive difficulties and autism spectrum disorder. More subtle cognitive impairments are common among survivors free of major neuromotor disability. Therapeutic hypothermia (TH) reduces the risk of death and disability in near-term/term new-born infants with moderate and severe HIE. Outcomes in adolescence and adulthood following HIE treated with TH are not yet known.

The majority of infants with HIE also suffer multi-organ dysfunction resulting from the hypoxic-ischaemic insult. The kidneys are particularly sensitive to hypoxia-ischaemia, with up to 72% of asphyxiated infants suffering acute kidney injury (AKI) prior to the advent of TH. Evidence point to AKI being independently associated with increased neonatal morbidity and mortality. To date, very little is known about long-term renal consequences following neonatal AKI in asphyxiated infants treated with TH.

The overall aim of this thesis was to contribute to the improved treatment and care of infants with HIE by means of increased knowledge about the predictive value of early aEEG, neonatal AKI, and long-term outcomes in the era of TH.

In a small population-based cohort, the predictive value of early amplitude-integrated EEG (aEEG) was demonstrated to be altered in infants treated with TH due to HIE. Poor outcome at the age of 1 year was only seen among infants with a persisting abnormal aEEG background pattern at and beyond 24 hours of age.

In a population-based, prospective, longitudinal study including all children treated with TH between 2007 and 2009 in Stockholm, Sweden, we assessed neuromotor, neurologic, cognitive and behavioural outcomes at 6-8 and 10-12 years of age. Seventeen per cent of survivors developed CP. Survivors free of major neuromotor impairment had cognitive abilities within normal range. Repeated assessment in early adolescence revealed new deficits in 26% of children with previously favourable outcome. The proportion of children with executive difficulties in this patient population appears to be higher than in the general population. Outcomes in children with a history of moderate HIE remain heterogenous also in the era of TH.

In a population-based cohort of all children treated with TH between 2007 and 2009 in Stockholm, Sweden, 45% suffered neonatal AKI. Severe AKI necessitating kidney support therapy was rare. Among infants with AKI, 20% fulfilled only the urinary output criterion of the neonatal modified KDIGO (Kidney Disease Improving Global Outcomes) definition. Mortality was higher among infants with AKI. At 10-12 years of age, 21% of children had decreased glomerular filtration rate (GFR) estimated from creatinine with the Schwartz-Lyon equation.

A more in-depth assessment of renal functions in the above-mentioned population-based cohort demonstrated that renal sequelae (defined as decreased GFR, albuminuria, hypertension or normal high blood pressure, reduced renal volume on magnetic resonance imaging, or elevated Fibroblast Growth Factor 23) were rare at 10-12 years of age following perinatal asphyxia and TH. The Schwarz-Lyon equation appears to underestimate GFR in this patient population.

LIST OF SCIENTIFIC PAPERS

I. Hallberg B, Grossmann K, Bartocci M, Blennow M. The prognostic value of early aEEG in infants undergoing systemic hypothermia treatment. ACTA Paediatrica, 2010; 99:531-536

II. Robertsson Grossmann K, Eriksson Westblad M, Blennow M, Lindström K. Outcome at early school age and adolescence after hypothermia-treated hypoxic-ischaemic encephalopathy.
Submitted manuscript

III. Robertsson Grossmann K, Bárány P, Blennow M, Chromek M. Acute kidney injury in infants with hypothermia-treated hypoxic-ischaemic encephalopathy: An observational population-based study. ACTA Paediatrica, 2022; 111:86-92

IV. Robertsson Grossmann K, Vishnevskaya L, Ruiz Diaz S, Kublickiene K, Bárány P, Blennow M, Chromek M. Renal outcomes in early adolescence following perinatal asphyxia and therapeutic hypothermia.
Submitted manuscript

CONTENTS

LIST OF ABBREVIATIONS

AAP	American Academy of Pediatrics
ACOG	American College of Obstetricians and Gynecologists
aEEG	Amplitude-Integrated Electroencephalography
AIMS	Alberta Infant Motor Scales
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
ABPM	Ambulatory Blood Pressure Measurement
ASD	Autism Spectrum Disorder
CI	Confidence Interval
CKD	Chronic Kidney Disease
CRRT	Continuous Renal Replacement Therapy
CNS	Central Nervous System
CP	Cerebral Palsy
DCD	Developmental Coordination Disorder
GCP	Good Clinical Practice
EEG	Electroencephalography
eGFR	Estimated Glomerular Filtration Rate
FGF 23	Fibroblast Growth Factor 23
FSIQ	Full-Scale Intelligence Quotient
FTF	Five-to-Fifteen questionnaire
GFR	Glomerular Filtration Rate
HIE	Hypoxic-Ischaemic Encephalopathy
ILAE	International League Against Epilepsy
IQR	Inter-Quartile Range
KST	Kidney Support Therapy
LMICs	Low- and Middle-Income Countries

LOS	Length of Stay
NE	Neonatal Encephalopathy
NGAL	Neutrophil Gelatinase Associated Lipocalin
NICU	Neonatal Intensive Care Unit
NO	Nitric Oxide
MABC-2	Movement Assessment Battery for Children, 2nd Edition
MBD	Mineral Bone Disorder
MRI	Magnetic Resonance Imaging
PD	Peritoneal Dialysis
PPHN	Persistent Pulmonary Hypertension in the New-born
pRIFLE	Paediatric Risk, Injury, Failure, Loss, End stage renal disease
RCT	Randomised Controlled Trial
RD	Risk Difference
RIFLE	Risk, Injury, Failure, Loss, End stage renal disease
RR	Relative Risk

1 INTRODUCTION AND LITERATURE REVIEW

1.1 PERINATAL ASPHYXIA IN THE NEAR-TERM OR FULL-TERM INFANT

1.1.1 Epidemiology

The World Health Organization (WHO) first introduced the term birth asphyxia in 1997 to describe infants who "fail to initiate and sustain breathing at birth" [1]. Worldwide, perinatal asphyxia contributes substantially to neonatal mortality, causing approximately 24% of all neonatal deaths and approximately 11% of all under-five mortality [2]. The vast majority (98%) of neonatal deaths related to perinatal asphyxia occur within the first week of life [3]. Approximately 1-6/1000 live-born infants go on to develop clinical signs of hypoxic-ischaemic encephalopathy following perinatal asphyxia [4-7]. HIE is a leading cause of acquired brain injury in new-born infants, and survivors are at risk for life-long debilitating sequelae including cerebral palsy (CP), intellectual impairment, epilepsy, deafness, blindness, executive difficulties and behavioural problems [8-11].

1.1.2 Causes

A wide range of risk factors have been demonstrated to be associated with perinatal asphyxia. Known risk factors include post-date pregnancy, primiparity, chronic maternal illness, low or advanced maternal age, oligohydramnios, infrequent antenatal care visits, low maternal socio-economic status, malpresentation, epidural analgesia, oxytocin use, obstructed labour, male foetal sex, and being born at night [5, 7, 12, 13]. Table 1 lists selected intrauterine, perinatal and neonatal causes of perinatal asphyxia.

Intrauterine causes	• Affected cardiac output in the foetus
	  o Congenital heart defects
	  o Foetal arrhythmia
	  o Twin-to-twin transfusion syndrome
	• Foetal anaemia
	  o Foeto-maternal transfusion
	  o Immunisation
	  o Infection
	  o Twin-to-twin transfusion syndrome
	• Infection
	• Maternal hypoxaemia
	• Maternal environmental factors
	  o Smoking
	  o Substance abuse
	• Placental insufficiency
	  o Diabetes mellitus
	  o Chronic disease
	  o Hypertension
	  o Pre-eclampsia

Perinatal causes	• Dystocia
	• Haemorrhage
	  o Foeto-maternal haemorrhage
	  o Vasa praevia
	  o Velamentous insertion
	• Infection
	  o Premature rupture of membranes
	• Placental abruption
	• Umbilical cord compression
	  o Cord knot
	  o Cord prolapse
	  o Nuchal cord
	• Uterine hyperstimulation

Neonatal causes	• Airway abnormalities • Severe cardio-respiratory disease • Severe circulatory compromise, including haemorrhage • Neurologic disorder, including medication effects • Infection

Table 1. Selected known risk factors for and causes of perinatal asphyxia.

1.1.3 Mechanisms of Brain Injury in HIE

Because of a high haemoglobin concentration, high cardiac output and the ability to redistribute blood flow to the more vital organs (brain, heart, adrenal glands), the healthy foetus is well equipped to sustain transient disturbances in blood flow, substrate provision and hypoxia [14]. In perinatal asphyxia, foetal gas exchange is affected, causing hypoxia, hypercarbia and acidosis [14]. To a degree, the foetal brain has resistance to asphyxia and the presence of foetal acidaemia alone is not a sufficient marker of cerebral injury [6, 15, 16]. When foetal compensatory mechanisms are no longer sufficient, the foetal brain is affected by hypoxaemia (reduced oxygen level in the blood) and ischaemia (insufficient blood supply) [17]. A moderate hypoxic-ischaemic insult leads to shunting of blood flow from the anterior to the posterior circulation to preserve sufficient perfusion in the brain stem, basal ganglia and cerebellum. This reaction restricts damage to the cortex and parasagittal watershed areas of the cerebral hemispheres. More prolonged and severe hypoxic-ischaemic insults cause diffuse cortical and basal ganglia-thalamic injury. Cerebral blood flow autoregulation may be

insufficient when the hypoxic-ischaemic insult is more acute or abrupt, leading to injuries in the basal ganglia and brain stem [14, 17, 18].

In the acute phase of hypoxic-ischaemic injury, supply of oxygen and glucose to the brain is reduced due to decreased cerebral blood flow. Anaerobic metabolism ensues, resulting in decreased production of adenosine triphosphate. Production of lactic acid contributes to metabolic acidosis. Adenosine triphosphate is depleted and the resulting reduction of transcellular transport causes sodium, water and calcium to accumulate intracellularly [19]. Depolarisation of the cell membrane triggers release of glutamate, an excitatory amino acid. Calcium then flows into the cell through the N-methyl-D-aspartate-gated channels. In turn, this induces lipid peroxidation, production of nitric oxide (NO) and oxidative stress causing primary cell death and apoptotic cascade activation. This initial glycogen storage depletion and failure of oxidative metabolism is frequently thought of as the *primary insult*. Following this cascade of events, referred to as excitotoxicity, a partial recovery is usually observed upon delivery and resuscitative interventions. This transient phase of partial recovery is frequently referred to as the *latent phase* of injury and is characterised by a recovery of oxidative metabolism. However, secondary inflammatory processes and activated apoptotic cascades continue [14]. During this phase, neural metabolism and activity is suppressed [20]. The latent phase, referred to as the "therapeutic window", lasts up to 6 hours before the *secondary phase* of neuronal injury ensues [21]. This secondary phase of deterioration is characterised by excitotoxicity, cytotoxic oedema, near-complete mitochondrial dysfunction causing secondary energy failure, ultimately resulting in apoptosis [20, 22-29]. The secondary phase is frequently associated with deterioration of the infant's clinical condition and encephalopathy due to cerebral oedema and seizures [28, 30]. The clinical condition of encephalopathy following an hypoxic-ischaemic insult around the time of birth is commonly referred to as hypoxic-ischaemic encephalopathy [6, 17].

Findings in both animal and clinical studies suggest that hypoxia-ischaemia triggers a neuro-inflammatory process that lasts long beyond the neonatal period [31]. During months after the initial insult, a *tertiary phase* occurs. During this phase, over-activation of microglia and astrocytes, release of chemokines and cytokines, and epigenetic changes are thought to be part of active processes preventing regeneration or even exacerbating injury [20, 23]. Elevated peripheral cytokines have been demonstrated in children with CP born preterm many years after the initial neonatal insult, suggesting ongoing cerebral inflammation [32, 33]. It has been suggested that treatment of these tertiary mechanisms of injury may be possible by inhibition or modulation of persistent inflammation [23].

1.1.4 Diagnosis and Staging of HIE

The umbrella term neonatal encephalopathy (NE) is used to describe a heterogenous disorder of abnormal neurological function in the late preterm and term new-born infant. NE is characterised by difficulty initiating and maintaining adequate respiration, decreased level of consciousness and activity, reduced muscle tone, persistence of primitive reflexes, and seizures. The term NE covers various causes of the condition, while the term HIE is reserved

for infants with encephalopathy occurring after a presumed perinatal hypoxic-ischaemic insult [6, 17]. Table 2 lists American College of Obstetricians and Gynecologists' (ACOG) and American Academy of Pediatrics' (AAP) criteria for determining whether or not NE is likely to be the result of peri- or intrapartum hypoxia-ischaemia.

ACOG/AAP criteria to determine if NE is caused by peri- or intrapartum hypoxia-ischaemia
1. Does the baby meet the definition for NE? 35+ weeks gestational age, abnormal consciousness ± difficulty initiating and maintaining respiration, seizures, abnormal tone, abnormal primitive reflexes
2. What is the likelihood that the major contributor to NE was an acute peri- or intrapartum event? Neonatal signs: • Apgar score < 5 at 10 minutes of life • Foetal umbilical artery acidaemia: pH < 7 or Base Deficit ≥ 12 • Distinct basal-ganglia-thalamus, watershed or near-total cortical injury pattern on MRI of the brain obtained at age 24 – 96 hours and up to postnatal day 10 • Presence of multi-organ failure (can include cardiac, gastrointestinal, haematologic, hepatic, metabolic and renal dysfunction) Type and timing of contributing factors consistent with an acute or peripartum event: • Sentinel hypoxic or ischaemic event immediately before or during labour/delivery • Foetal heart rate pattern that deteriorated to absent variability with: recurrent or late variable decelerations, bradycardia, or a sinusoidal patterns for ≥ 20 min • Distinct basal-ganglia-thalamus or watershed pattern injury on MRI of the brain obtained between age 24 – 96 hours and up to postnatal day 10 • No evidence of other proximal or distal factors that could contribute substantially or indicate other underlying patho-biology e.g., abnormal foetal growth, congenital microcephaly, maternal infection, neonatal sepsis Developmental outcome is spastic quadriplegic or dyskinetic cerebral palsy (CP): • Other subtypes of CP are less likely to be associated with an acute peri- or intrapartum event and, spastic quadriplegia and dyskinesia can also have other causes • Other developmental disabilities may occur, but are not specific to acute peri- or intrapartum event and may rise from a variety of causes

Table 2. ACOG/AAP criteria to determine whether NE is caused by peri- or intrapartum hypoxia-ischaemia. Adapted from American Academy of Pediatrics. Neonatal encephalopathy and neurologic outcome, second edition, Report of the American College of Obstetricians and gynecologists' Task Force on neonatal encephalopathy. Pediatrics 2014 [34].

The Sarnat and Sarnat score for staging of HIE stems from the seminal 1976 publication of clinical symptoms in 21 term infants with an Apgar score <5 at five minutes of age following perinatal asphyxia. Sarnat and Sarnat described three clinical stages of encephalopathy, as

seen in Table 3 [35]. These stages were later defined as mild, moderate and severe. To date, the Sarnat scoring system remains the most widely used.

	Stage 1 (mild)	**Stage 2 (moderate)**	**Stage 2 (severe)**
Level of consciousness	Hyperalert	Lethargic or obtunded	Stuporous
Neuromuscular control			
Muscle tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch reflexes	Overactive	Overactive	Decreased or absent
Segmental myoclonus	Present	Present	Absent
Complex reflexes			
Suck	Weak	Weak or absent	Absent
Moro	Strong, low threshold	Weak, incomplete high threshold	Absent
Oculovestibular	Normal	Overactive	Weak or absent
Tonic neck	Slight	Strong	Absent
Autonomic function			
	Generalised sympathetic	Generalised parasympathetic	Both systems depressed
Pupils	Mydriasis	Miosis	Variable, often unequal, poor light reflex
Heart rate	Tachycardia	Bradycardia	Variable
Bronchial and salivary secretions	Sparse	Profuse	Variable
Gastrointestinal motility	Normal or decreased	Increased, diarrhoea	Variable
Seizures	None	Common, focal or multifocal	Uncommon (excluding decerebration)
Electroencephalogram findings	Normal (awake)	Early: low-voltage, continuous delta and theta. Later: periodic pattern (awake) Seizures: focal 1- to $1^1/_2$ Hz spike and wave	Early: periodic pattern with isopotential phases Later: totally isopotential
Duration	Less than 24 hours	Two to 14 days	Hours to weeks

Table 3. Sarnat scoring system, adapted from Sarnat and Sarnat, Neonatal encephalopathy following fetal distress: A clinical and electroencephalographic study, Arch Neurol, 1997 [35].

Thompson et al later constructed a scoring system for prediction of outcome in infants with HIE [36], as seen in Table 4. The positive and negative predictive value of a peak score ≥ 15 for abnormal outcome at 1 year of age was 92% and 82%, respectively, with a sensitivity of 71% and a specificity of 96% [36]. The Thompson score excludes evaluation of spontaneous

activity, heart rate and pupils. Somewhat simpler than the Sarnat score, it is not as commonly used to grade encephalopathy. With the advent of therapeutic hypothermia (TH) for infants with Sarnat stage 2 (moderate) and 3 (severe) HIE, grading of HIE severity became increasingly important to determine eligibility for treatment. Within each stage of HIE severity is thought to be a continuum, and the addition of numerical values to each abnormal category in the Sarnat examination to produce a total Sarnat score has been proposed by Chalak et al [37]. Furthermore, HIE severity exhibits variation over time. It is thus possible that early grading of HIE, performed within six hours after birth, may subsequently progress [35, 38, 39].

Sign	Score 0	Score 1	Score 2	Score 3
Tone	Normal	Hyper	Hypo	Flaccid
Level of consciousness	Normal	Hyper-alert, stare	Lethargic	Comatose
Fits	None	Infrequent, < 3/day	Frequent, > 2/day	
Posture	Normal	Fisting, cycling	Strong, distal flexion	Decerebrate
Moro	Normal	Partial	Absent	
Grasp	Normal	Poor	Absent	
Suck	Normal	Poor	Strong ± bites	
Respiration	Normal	Hyperventilation	Brief apnoea	IPPV (apnoea)
Fontanel	Normal	Full, not tense	Tense	

Table 4. Thompson score, adapted from Thompson et al, The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome, ACTA Paediatrica, 1997 [36]. Abbreviations: IPPV, Intermittent positive pressure ventilation

Historically, the prognosis for infants with mild HIE has been thought to be uniformly favourable, as studies from the pre-hypothermia era did not report mortality or major disability in this group [40, 41]. The probability of death or neurodevelopmental disability was 75-100% among infants with severe HIE. Outcomes among infants with moderate HIE were more heterogeneous. In this group, 60-75% were demonstrated to have good outcomes at toddler and early school age [41, 42]. Extended follow-up in this group, however, revealed that more subtle disabilities were common among survivors free of major neuromotor disability [43, 44]. In a study by Lindström et al, cognitive disabilities were seen in 71% of teenagers without CP [45].

1.1.5 Neurophysiology

1.1.5.1 Neonatal seizures

Approximately 1-3/1000 new-born infants develop seizures [46]. HIE is the most frequent underlying aetiology of neonatal seizures, but many other conditions/disorders including hypoglycaemia, sepsis/meningitis, stroke, intracranial haemorrhage, inborn errors of metabolism and genetic disorders are also known to cause seizures [47-49]. Thirty to 60% of infants with moderate or severe HIE develop seizures [49], typically starting 12-24 hours after the hypoxic-ischaemic insult [49]. An earlier onset of seizures may be indicative of an antepartum insult [50]. In 1989, Volpe proposed a clinical classification of neonatal seizures, categorising them as “multifocal clonic”, “focal clonic”,”tonic”, “myoclonic”, or “subtle” seizures [51]. Recently, the International League Against Epilepsy (ILAE) suggested a new

classification. In the ILAE classification, all seizures are considered to be focal at onset. Therefore, division of seizures into focal and generalised is thought to be redundant. As per the ILAE classification, seizures may occur also without clinical manifestations, and thus a new class of electrographic-only seizures has been added [52]. Even among experienced providers, using only clinical evaluation to differentiate between seizure-related and non-seizure movements has been demonstrated to have poor sensitivity and specificity [53]. Among infants with HIE, 50-80% of seizures have been reported to be electrographic only [54]. Monitoring with electroencephalography (EEG) is therefore of utmost importance in the diagnosis of neonatal seizures. Most seizures in new-born infants result from underlying neurologic conditions [55], which themselves are the main contributor to later adverse outcomes. Evidence, however, points to neonatal seizures being harmful also on their own, particularly following perinatal asphyxia [56-58]. In a study involving 472 near-term/term infants requiring EEG-monitoring, infants treated with anticonvulsants within one hour of seizure onset had significantly lower seizure burden compared to infants who received treatment after more than one hour ($p = 0.029$), indicating that the treatment of neonatal seizures may be time-critical [59]. To date, there are only few randomised trials evaluating initial treatment strategies for neonatal seizures. Painter et al randomised 59 infants with seizures to receive either intravenous phenobarbital or phenytoin, and infants whose seizures could not be controlled with the assigned drug subsequently received both medications. The authors concluded that the treatments were equally but incompletely effective in new-born infants [60]. More recently, Sharpe et al investigated the efficacy and safety of levetiracetam in comparison to phenobarbital as a first-line treatment for neonatal seizures. Phenobarbital was found to be more effective than levetiracetam, however, the rates of adverse events was higher among infants treated with phenobarbital [61]. A few studies have investigated second-line treatment of neonatal seizures. In a cohort of 413 infants (preterm and term), Weeke et al investigated seizure response rate following administration of lidocaine as a second- or third-line treatment for neonatal seizures. In term infants, lidocaine was demonstrated to have a significantly better response rate than midazolam as a second-line treatment (21.4% versus 12.7%, $p = 0.049$). In a study involving 45 infants with electrographically confirmed seizures, Castro Conde et al reported that midazolam effectively controlled seizure in infants who had not responded to phenobarbital or phenytoin [62]. The NEMO trial investigating bumetanide as an add-on treatment in infants with HIE and seizures not responding to phenobarbital was terminated early due to serious adverse reactions and limited improved seizure control [63]. A systematic review of neonatal seizure management strategies by Hellström-Westas et al demonstrated that while phenobarbital is the first drug of choice, there is no consensus concerning the optimal treatment of seizures in the neonatal period [64].

1.1.5.2 Neuromonitoring using EEG and amplitude-integrated EEG

Full-band EEG has been demonstrated to have high predictive value for later neurodisability in infants with perinatal asphyxia [9]. It is, however, a method that requires expertise to apply appropriately and interpret correctly [9]. In 1969, Maynard and Prior introduced a novel

technique for measuring continuous cerebral function [65]. While the device called cerebral function monitor (CFM) was initially created with the purpose of monitoring brain function in adult patients undergoing intensive care treatment, it was introduced in the NICU a little over a decade later [66]. Amplitude-integrated EEG (aEEG) is based on the registration of one or two channels of EEG from bi-parietal electrodes or from frontal-central/parietal electrodes. The EEG-signals are filtered and the frequencies below 2 Hz and above 15 Hz are reduced. The rectified and compressed signal is then displayed on a semi-logarithmic scale. This enables monitoring of changes and trends in the electrocortical background activity in the brain over longer periods of time [67]. The background pattern is the dominating type of electrocortical activity seen on the aEEG-registration. Hellström-Westas et al established a classification for the background pattern into five categories [68]:

- "Continuous Normal Voltage (CNV): a continuous background activity with a minimal amplitude of 5-10 μV and a maximal amplitude of 10-50 μV"
- "Discontinuous Normal Voltage (DNV): a discontinuous background activity with a varying minimal amplitude below 5 μV and a maximal amplitude above 10 μV"
- "Burst-Suppression (BS): a discontinuous background activity with a non-variable minimal amplitude between 0-2 μV and bursts with amplitudes above 25 μV"
- "Low Voltage (LV): a continuous background activity with very low voltage around or below 5 μV, sometimes referred to as iso-electric or flat trace"

Sinusoidal variations in mainly the minimal amplitude of the trend characterise sleep-wake cycling (SWC). A wider bandwidth as a sign of discontinuous background activity is seen during times of calm sleep and a narrower bandwidth as a sign of more continuous background activity is seen during times of active sleep or being awake [67]. Figure 1 provides examples of aEEG background patterns.

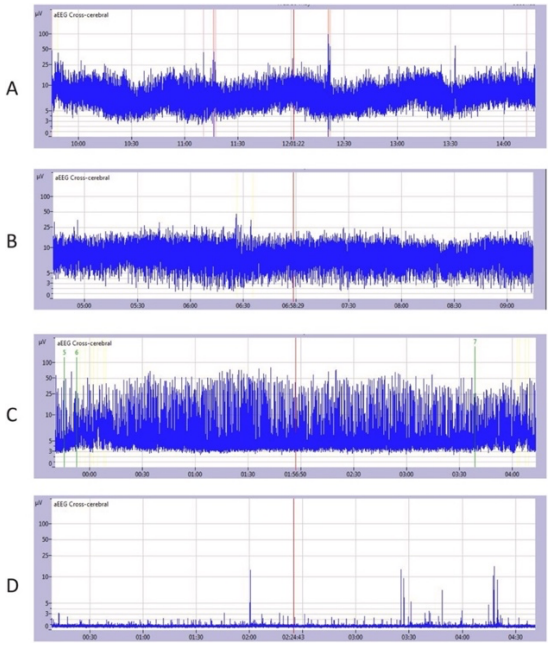

Figure 1. Examples of aEEG background patterns. (A) Continuous normal voltage with sleep-wake cycling (B) Discontinuous normal voltage (C) Burst-suppression (D) Flat trace or isoelectric aEEG. Reprint from Chalak et al, Bedside and laboratory neuromonitoring in neonatal encephalopathy, Seminars in Fetal and Neonatal Medicine, 2021, with permission from Elsevier [69].

Compared to full-band EEG, aEEG is much simpler to apply and correct interpretation does not require expertise in neurophysiology. It is often used in NICUs to monitor background activity and detect seizures. Prior to the introduction of TH, the aEEG background pattern was demonstrated to have high predictive value for adverse neurodevelopmental outcome as early as before 6 hours post-partum in infants with perinatal asphyxia [70, 71]. In asphyxiated infants treated with TH, however, the predictive value of early aEEG is lower [72, 73]. A recent meta-analysis demonstrated that a persistently abnormal background pattern on aEEG at 48 hours of age or beyond was associated with adverse neurodevelopmental outcome (defined as death or moderate/severe disability at 1 year of age) [74]. The time to recovery of the background pattern on aEEG has been proposed as an alternative prognostic marker in hypothermia-treated new-born infants [72, 73]. aEEG has been demonstrated to have poor sensitivity for seizure detection in new-born infants when the seizure prevalence is low, particularly if the individual interpreting the trace lacks experience [75]. For the detection of seizures in new-born infants, full-band EEG therefore remains the gold standard [52]. Continuous video-EEG has been demonstrated to be associated with improved electrographic detection of seizures. Furthermore, it was associated with reduced phenobarbitone burden as well as antiseizure medication use at the time of discharge from the NICU [76].

1.2 TREATMENT OF HIE

1.2.1 Therapeutic hypothermia

Support of the body's vital functions forms the basis of care for new-born infants with HIE, but such measures have little impact on the underlying process of the hypoxic-ischaemic

injury. Supportive measures aim to prevent further deterioration, thus reducing the risk of further cerebral injury. Pyrexia in conjunction with hypoxic-ischaemic events has been demonstrated to contribute to worsened injury in both animal and observational studies [77-79]. Hypothermia, on the other hand, has been shown to reduce secondary energy failure following a hypoxic-ischaemic insult [27]. Within the temperature range from 28°C to 41°C, the relationship between cerebral energy utilisation and temperature is linear [80]. The concept of TH is not new. Records of physicians submerging new-born infants in cold water as a resuscitative measure date back over 100 years [81, 82]. Already in the 1950's, Swedish physician Björn Westin published reports on "pre-viable" human foetuses surviving approximately twice as long when body temperature was reduced to 25ºC compared to normothermia. Approaching hypothermia more as a means of resuscitation rather than a mode of neuroprotection, he placed asphyxiated new-born infants in a home-made, primitive cooling contraption consisting of a small bath-tub filled with cold water. In order to keep the infants' airways and umbilicus above the water surface, the infants were positioned onto a modified dish-rack. He then left them there "until they screamed" [83]. However, around the same time, Silverman et al published a report that demonstrated improved survival in premature infants if kept warm. Following these findings, no new-born infants regardless of gestational age were allowed to get cold. The use of TH continued in the former Soviet Union, but did not spread to the West due to both language and political barriers [81]. Approximately two decades ago, the concept of delayed cerebral energy failure and a possible therapeutic window [29] sparked renewed interest in hypothermia for neuroprotection [81].

The latest Cochrane review included 11 randomised controlled trials (RCTs) of TH for the treatment of moderate-severe HIE [84]. The CoolCap trial was the largest study investigating selective head cooling [85], while the NICHD trial and the TOBY trial were the largest studies investigating whole-body cooling [86-94]. Depending on the study, infants were treated with induced hypothermia initiated within 5.5-6 hours after birth, reducing body temperature to 33.0°C to 36.5°C for 48-72 hours. This meta-analysis including 1505 infants with encephalopathy concluded that the risk of death or neurodevelopmental disability at 18-24 months of age is reduced by 25% in infants treated with TH. A composite outcome of "death or disability" was used as the primary outcome in all larger clinical trials of TH, albeit with some variation between definitions of disability between trials [95]. Number-needed-to-treat to avoid one death was 5-10 [84]. Importantly, despite the substantial reduction of the risk for the composite outcome of death or disability, survivors are still at high risk for neurodevelopmental disability [95]. TH is a time-sensitive treatment – early initiation of TH has been demonstrated to be associated with better motor outcome [96]. Deeper and longer cooling has been investigated in a randomised trial by Shankaran et al without any difference in the probability of adverse outcome (defined as death or disability at 18 months of age) compared to "standard" TH with a temperature reduction to 33.5°C for 72 hours [97]. In observational studies, TH has been used outside of the clinical trial protocols to treat, among others, infants who suffered post-natal collapse, infants born late preterm, infants with

surgical/metabolic/chromosomal diagnoses in addition to perinatal asphyxia, and infants for whom cooling was initiated after more than 6 hours following the hypoxic-ischaemic event. With the exception of infants with ongoing haemorrhage, TH appears to be feasible and possibly beneficial also in these patient groups [98]. To date, TH remains the only therapeutic intervention with proven neuroprotective effects in neonatal HIE. Several trials investigating potential adjuvant therapies, including allopurinol, xenon, sildenafil, high-dose erythropoietin, allogenic mesenchymal stromal cells, and combination therapy with erythropoietin and magnesium sulphate are ongoing [99].

1.2.2 Biomarkers in HIE

Due to the time-sensitive nature of TH, it is important that infants who may benefit from the treatment are identified as quickly as possible. Much research has been dedicated to investigating possible biomarkers that may be useful in infants who suffer perinatal asphyxia [100]. Many small studies have reported promising results for several different inflammatory and brain-specific biomarkers, but to date no such biomarker is recommended in regular clinical practice [101]. Ideally, biomarkers would aid clinicians not only in the identification, but also in the timing and monitoring of a hypoxic-ischaemic insult [101]. Clinically, acid-base balance in cord blood or a blood sample taken within the first 60 minutes after birth and Apgar score are still used as biomarkers following perinatal asphyxia.

1.2.2.1 Apgar score

An infant's 5 and 10 minute Apgar score has a strong correlation with adverse neonatal outcomes [102] and is still commonly used as a predictor of neurologic outcome following perinatal asphyxia although its sensitivity and positive predictive value are low [103]. Another inherent problem with a low Apgar score is substantial inter-observer variability [104]. The association between a low Apgar score and an increased risk for CP, epilepsy, cognitive developmental delay and death has been demonstrated [102]. Every gained Apgar point at 10 minutes of age confers significant reduction of the risk for adverse outcomes. However, even with an Apgar score of 0 at 10 minutes of age, nearly 21% of term infants survive without disability at school age [105].

1.2.2.2 pH in cord blood

A low pH in cord blood has been demonstrated to be strongly associated with neonatal mortality, HIE and cerebral palsy [106]. The sensitivity as well as the positive predictive value for adverse neonatal outcomes, however, are poor [103]. For a cord pH > 7.00, the association between neonatal acidaemia and adverse outcomes is weak [107].

1.2.2.3 Scalp lactate

In a study by Hallberg et al, the sensitivity and specificity of a lactate concentration of 4.8 mmol/L in foetal scalp blood for moderate-severe HIE were 66.7% and 75.7%, respectively. It was therefore suggested as a suitable cut-off limit as an indicator of perinatal asphyxia [108]. Murray et al, however, did not find that HIE severity could be predicted by initial level

of serum lactate. The authors found that the time to normalisation of serum lactate was associated with EEG grade of encephalopathy and electroencephalographic seizure burden [109].

1.2.2.4 Magnetic Resonance Imaging

Despite being resource demanding, time consuming and the images requiring expertise to interpret, magnetic resonance imaging (MRI) is still the gold standard for predicting injury severity and future outcome in new-born infants with HIE [22, 110]. Several scoring systems have been developed. Scoring systems by Barkovich et al, Rutherford et al and Shankaran et al are not item based-but instead share the common feature of grouping patterns of injury together [111-113]. A novel scoring system that includes diffusion-weighted imaging as well as assessment of deep grey matter, white matter and cerebellum has been proposed by Weeke et al [114]. Motor or cognitive deficits are generally uncommon in infants with a normal MRI of the brain following HIE [115]. Motor outcomes are poor among infants with basal ganglia and/or thalamic injuries. Injuries to the basal ganglia and/or thalamus and the posterior limb of the internal capsule have a strong association with later motor impairment [42, 116]. Basal ganglia injury is also associated with impairments involving speech/language development, feeding and vision. Injuries extending to the brainstem in addition to the basal ganglia and thalamus, as seen after severe asphyxia, are associated with mortality rates up to 35% and disability rates among survivors > 90% [116]. Infants with lesions mainly in the watershed regions, on the other hand, more commonly exhibit cognitive deficits [42, 115, 117]. Absence of brain lesions on MRI performed in the neonatal period, however, does not rule out later deficits. Rollins et al reported that among infants without brain lesions on MRI, 20% had moderate developmental delays and 6% had severe developmental delays when examined with the Bayley Scales of Infant and Toddler Development, 3rd edition (BSID-III) at an age of 22±7 months. Among infants with only focal grey or white matter injury, 65% had moderate developmental delays, and 6% had severe developmental delays [118]. A meta-analysis comprising 17 articles and 799 patients concluded that brain MRI in infants with HIE is highly prognostic of neurodevelopmental outcomes, with the strongest prognostic value for infants with moderate or severe HIE [119]. Furthermore, the authors concluded that TH has not significantly influenced the value of brain MRI obtained in the neonatal period as a biomarker of neurodevelopmental outcomes [119].

1.3 OUTCOME AFTER HIE

1.3.1 Assessments of neurodevelopmental outcome

1.3.1.1 Alberta Infant Motor Scale

The Alberta Infant Motor Scale (AIMS) is an observational assessment scale. It is used to assess maturation of gross motor functions in infants, from birth until the child walks independently. Organised into the four positions prone, supine, sitting, and standing, the assessment contains 58 items. Every item describes weight-bearing, posture, and anti-gravity movements. The raw score is then converted into an age-based percentile rank. AIMS has

been normed on 2200 Canadian infants between the ages of 1 week to 18 months, and further validated in a Canadian cohort of 506 infants [120]. The Canadian norms are applied virtually everywhere across the globe, however, the validity of Canadian norms for non-Canadian infants has been debated. Recently, a study involving 1697 Dutch infants reported that 73% scored < the 50th percentile, 38% < the 10th percentile and 28% below the 5th percentile when compared to Canadian norms [121]. The authors suggested new norms for Dutch infants. Similar findings have been reported also in a small Belgian study population [122] and in a larger cohort of Brazilian infants [123]. Studies done in South African and Greek infants born at term, however, have reported AIMS scores comparable to those of the Canadian sample [124-126].

1.3.1.2 Bayley Scales of Infant and Toddler Development, 3rd edition

The Bayley Scales of Infant and Toddler Development, 3rd edition (BSID-III) is widely used both in clinical settings and in studies investigating neuroprotective strategies, and is considered the gold standard for assessment of neurodevelopmental outcomes in children up to the age of 42 months [127]. The latest revision changed the structure of the instrument substantially as the former Mental Developmental Index was divided into the Cognitive Index and the Language Index. The BSID-III was standardised in a heterogenous sample including 10% with, or at risk of, developmental difficulties to be representative of the general U.S. population. Concerns have been raised the BSID-III can be too forgiving and thus underestimate neurodevelopmental delay as a result of the inclusion of children with developmental deficiencies in the normative sample [128-132]. The ability of the BSID-III to predict later cognitive outcome has been investigated both in studies on preterm children [133, 134] and in healthy full-term children [135] with conflicting results. In a critical review of the available literature in 2017, Anderson and Burnett suggested a need for new BSID-III norms, or even a new edition of the Bayley Scales [136].

1.3.1.3 Wechsler Scales of Intelligence in Children, 4th and 5th edition

The Wechsler Scales of Intelligence in Children (WISC) are commonly used to assess cognitive ability in children between the ages 6 to 16 years. The age range for which the test is suitable is the same for both the WISC, 4th edition (WISC-IV) and the WISC, 5th edition (WISC-V). The WISC-IV comprises four indices measuring specific intellectual domains: Verbal comprehension Index, Perceptual reasoning Index, Working memory Index, and Processing speed Index. Norms in the Swedish version of WISC-IV are based on Swedish children [137]. The newer version, WISC-V, comprises five primary indices: Verbal comprehension Index, Visual-spatial Index, Fluid reasoning Index, Working memory Index, and Processing speed Index. Norms in the Swedish version of WISC-V are based on Scandinavian children [138]. Both versions yield a full-scale intelligence quotient (FSIQ) representing a child's general intellectual ability. Scores range between 40 to 160 points. All results are expressed as an age-standardised score, with a mean of 100 and a standard deviation of 15. Arguments have been made against the usage of FSIQ to predict academic performance in individuals exhibiting significant differences between WISC indices [139].

An FSIQ < 70 (equal to -2 SD or less) constitutes one of the diagnostic criteria for intellectual disability according to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) [140]. An FSIQ between 70 and 84/85 is considered borderline intellectual functioning (BIF). BIF is characterised by "heterogenous cognitive difficulties and a failure to meet the developmental and socio-cultural standards for person independence and social responsibility that affects daily activity"[140]. Compared to the general population, cognitive and academic difficulties are common in this population. Children with BIF may exhibit pervasive working memory and executive deficits [141, 142] and are at risk for poor school performance [143]. Furthermore, children and adolescents with BIF commonly struggle with regards to social functioning [144]. It is not uncommon for BIF and autism spectrum disorder (ASD) to coexist. Among individuals with Attention Deficit and Hyperactivity Disorder (ADHD), BIF is common. However, children with BIF may also exhibit attention problems due to non-adapted academic demands [144].

1.3.1.4 Movement Assessment Battery for Children, 2nd edition

The Movement Assessment Battery for Children, 2nd edition (MABC-2) is a test of motor impairments. The test is divided into three age bands: 3-7, 7-10, and 11-16 years. It consists of the three subscales Manual dexterity, Aiming and catching, and Balance tasks. Each domain gets a standard score and percentile equivalents and is then compared to a British normative sample. A total test score ≤ 5th percentile (for the age) indicates significant motor difficulty, and a total test score between the 6th and 15th percentile indicates a child at risk of motor difficulty. A total test score above the 15th percentile is considered normal [145]. Hayes et al investigated neurodevelopmental outcomes in survivors of HIE free of CP. Sixty-four children not treated with TH were assessed with the MABC-2. In the middle age band, children were significantly more likely to have a score ≤ 5th percentile, however, in the younger age band children exhibited significant deficits only in the Manual dexterity domain [146]. In a recent study, Jary et al reported MABC-2 scored indicating motor impairment in 38% of survivors without CP at school age following HIE treated with TH [147].

1.3.1.5 Five-to-Fifteen questionnaire

The Five-to-Fifteen questionnaire (FTF/FTF revised form) was developed in the Nordic countries to screen for developmental and behavioural disorders [148]. It has been widely used both in clinical practice and for research purposes in the Nordic countries. The FTF consists of 181 items. These are divided into eight domains: Motor skills, Executive functions, Perception, Memory, Language, Learning, Social skills, and Emotional/behavioural problems. For each statement, three response options are possible: does not apply (score 0), applies sometimes/to some extent (score 1), or definitely applies (score 2). The FTF has been normed for Swedish children aged 5-15 years and has good reliability and validity [149-152]. It has been used in a stratified sample of Swedish children from the general population and means, 75th, 90th, and 98th percentiles for domain scores for boys and girls have been published [150, 153]. The 90th percentile is used as a cut-off for obvious difficulties and the 98th percentile for severe/major difficulties.

1.3.2 Moderate and severe HIE

1.3.2.1 Mortality

The seminal 1976 study by Sarnat and Sarnat involved 21 infants with encephalopathy, of whom four died. Infants with signs of moderate HIE lasting < 5 days had normal outcome at 1 year of age. Poor outcome was seen in infants with symptoms of moderate HIE persisting > 7 days, and in infants with persisting EEG abnormalities [35]. In 1989, Robertson et al described school age outcomes following moderate or severe HIE. Among children with moderate and severe HIE, the mortality rate during the first eight years of life were 5% and 82%, respectively [154]. Following the advent of TH, mortality rates among infants randomised to treatment with TH have varied between 17 and 38% in the randomised trials [87-91, 93, 94, 155-158]. In the most recent Cochrane review of trials of TH, meta-analysis of all 11 trials showed a significant reduction in mortality among patients randomised to TH (typical RR 0.75, 95% CI 0.64 – 0.88 and typical RD -0.09, 95% CI -0.13 – -0.04). Meta-analysis of the five trials that employed selective head cooling did not show any significant effect of TH on mortality. Meta-analysis of the six trials that employed whole-body cooling, however, demonstrated a significant reduction in mortality among patients randomised to TH (typical RR 0.73, 95% CI 0.61 – 0.89 and typical RD -0.10, 95% CI -0.16 – -0.04).

1.3.2.2 Neuromotor and other neurodevelopmental disability

Neurodevelopmental disabilities are common among survivors of severe HIE [154]. Adverse outcomes include CP, cerebral/cortical visual impairment (CVI), sensorineural deafness, blindness, cognitive delay, problems with feeding and speech, and seizures [159]. Outcomes following moderate HIE have been demonstrated to be more heterogenous [158, 159]. Robertson et al were the first to report long-term outcomes among survivors at 5.5 and 8 years of age. In their cohort, survivors of moderate NE free of disability had delays in reading, spelling, and arithmetic. Furthermore, they were more likely to have been held back at least one grade in school compared to survivors of mild NE [154]. Marlow et al reported outcomes at 7 years in 65 children with a history of NE. Disability was seen in 6% of children who had suffered moderate NE and 42% of children who had suffered severe NE. More subtle impairments were more common following severe NE [160]. Lindström et al followed survivors of moderate HIE without major neuro-impairment up to the age of 15-19 years, reporting that 71% had definitive cognitive dysfunctions interfering with everyday life [45]. Cognitive impairments continue to be a concern in children who suffered HIE also in the era of TH [161].

Although TH has improved survival and reduced the risk for disability at the age of 18-24 months, approximately 40% of infants with hypothermia-treated HIE die or have adverse outcomes in early childhood [85-87, 162]. In the most recent Cochrane review of trials of TH, 23% of infants treated with TH later developed CP [84]. Children with CP often exhibit other functional impairments in addition to the motor impairment [163, 164]. These include epilepsy, learning disabilities, behavioural issues, visual impairment among others [164-166].

Motor impairment can also be evident in the absence of CP. Developmental coordination disorder (DCD) is a neuromotor disability that interferes with daily activities or academic achievement [140]. The difficulties experienced must not be explained by other impairments such as delayed cognitive development [140, 167]. DCD is a heterogenous disorder, sometimes affecting only gross motor skills, sometimes only fine motor skills, and sometimes both. Children with DCD may struggle to learn "typical" childhood skills, such as riding a bicycle or tying shoelaces [168, 169]. DCD has been associated not only with learning difficulties and lower cognitive function, but also mental health issues and problems with psychosocial adjustment. In populations of typically developing individuals, the prevalence of DCD is reported to be 5-6% and is more common among boys (2:1) [170]. A higher incidence of DCD has been described among children with a history of prenatal or perinatal complications [171].

Long-term outcomes following HIE treated with TH are not well studied. To date, much of what is known about long-term outcomes among survivors of HIE stems from the pre-hypothermia era. Three of the major trials of TH have reported outcomes at early school age. In the NICHD study, 91% of survivors were assessed at 6-7 years of age. Incidence of their primary outcome death or IQ < 70 was 47% among infants treated with TH compared to 62% among infants in the normothermia group (adjusted RR 0.78, 95% CI 0.61 – 1.01). While they observed a significant reduction in mortality as well as the composite outcome of death or CP among children treated with TH, there was no difference in incidence of IQ < 70, CP or moderate/severe disability [172]. The TOBY study also assessed outcomes among survivors at 6-7 years of age. In contrast with findings in the NICHD study, they reported no difference in mortality between children treated with TH and those treated with normothermia. At early school age, 85% of survivors were evaluated. Survival with a FSIQ ≥ 85 occurred more frequently among children treated with TH (RR 1.31, 95% CI 1.01 – 1.71). The incidence of mild/moderate/severe disability was lower among children treated with TH. In the hypothermia group, 21% developed CP compared to 36% among children treated with normothermia [173]. Both the NICHD and the TOBY study evaluated neuropsychological outcomes using items from A Developmental NEuroPSYchological Assessment (NEPSY) 2nd edition. The NICHD study reported similar proportions of participants with standardised scores < 70 on the scales attention/executive and visuospatial function in the two groups [172]. The TOBY study reported IQ and NEPSY subscale scores; while the direction of effect for both was in favour of TH, the difference between the two groups was not significant. Results on the Strengths and Difficulties questionnaire and the duPaul RS4 rating scale screening for attention deficit disorder (ADD) were not significantly better among children treated with TH. Children in the TH group required special educational resources less frequently compared to the control group (8.2% versus 26.9%, $p < 0.01$) [173]. The CoolCap study evaluated outcomes in 46% of survivors at age 7-8 years, administering the parent-report WeeFIM ratings scale. While disability status at 18 months of age was reported to strongly correlate with WeeFIM, no effect of TH was detected [174].

Recently, a few studies have reported early school age outcomes in survivors of hypothermia-treated HIE free of CP. Jary et al reported that a third of children without CP had MABC-2 scores ≤ the 15th percentile, thus indicating motor impairment, that was not identified upon assessment with the BSID-III at 18 months of age. Low MABC-2 score was associated with lower FSIQ ($p = 0.045$), lower Working memory Index score ($p = 0.03$), lower Perceptual reasoning Index score ($p = 0.005$) on WISC-IV and need for extra support in school ($p = 0.01$) [147]. A case-control study by Lee-Kelland et al investigated cognitive, motor and behavioural performances in 29 survivors free of CP after hypothermia-treated HIE in comparison with matched control group of non-HIE children at early school age. Children were assessed using the WISC-IV, MABC-2 and the Strengths and Difficulties Questionnaire. Children with a history of hypothermia-treated HIE had significantly lower mean FSIQ and total MABC-2 scores compared to controls. In the case group, the reported median total and emotional behavioural difficulties were significantly higher compared to controls [175]. The same research group also investigated dorsal-stream function at age 6-8 years in 29 children with a history of HIE treated with TH. Measures of attention and visuo-spatial function on the Conner's Performance Test (CPT) 2nd edition were significantly worse among children with a history of hypothermia-treated HIE compared to matched controls [176]. In a study of 31 children with a history of hypothermia-treated HIE and 20 typically developing children, Edmonds et al compared school readiness at around five years of age. Children with a history of HIE had general cognitive abilities within normal range, yet their scores on fine motor skills, executive functions, memory and language were significantly lower compared to controls. The authors suggested that more focussed assessments are needed to detect more subtle difficulties in this patient population [177]. In a sample of 40 children without major impairments following hypothermia-treated HIE, Cainelli et al assessed cognitive performance and neuropsychological abilities at early school age. Thirty-three healthy peers served as controls. Although still within normal range, mean FSIQ was significantly lower among children with a history of HIE treated with TH. These children also performed significantly lower on tests examining visuomotor skill, executive function, as well as attention [178].

1.3.3 Mild HIE

Historically, the prognosis following Sarnat stage 1 (mild) HIE was perceived to be uniformly good as earlier studies reported neither death nor major disability in this patient group [154, 179]. Infants suffering mild HIE were therefore not intentionally enrolled in any of the RCTs of TH [4, 39, 84, 154, 155, 179]. The recent systematic review on long-term developmental outcomes following mild HIE comprised 20 studies and a total of 304 patients. Apart from four RCTs [89, 90, 180, 181], most trials were prospective cohort studies. By the age of two years, approximately 25% of children with a history of mild HIE had mild to moderate disability. The proportion of children experiencing difficulties in one or more areas increased to 35% by five years of age [182]. In the Prospective Research in Infants with Mild Encephalopathy (PRIME) study, abnormal outcome (defined as early aEEG, MRI or neurological exam) at the time of discharge from the hospital was reported in

52% of children with a history of mild HIE. At 18-22 months of age, 68% of children were assessed using the BSID-III. Among children who completed assessment, 40% scored ≤ 85 on any subscale. Disability (defined as "cognitive score of 70-84 alone" or "a cognitive score ≥ 85 and GFMCS level 1 or 2" or "seizure disorder" or "hearing deficit with ability to follow commands without amplification") occurred in 16% of children [78]. The PRIME study did not enrol a control group for comparison, and there was substantial loss to follow-up. Finder et al demonstrated that children with a history of mild HIE had BSID-III cognitive composite scores that were not significantly different to those seen in survivors of moderate HIE treated with TH [183].

To date, there is no evidence that TH is beneficial in infants with mild HIE. Still, there has been a therapeutic drift in several centres to routinely admit infants with mild HIE for treatment with TH [38, 39, 184, 185].

1.4 ACUTE KIDNEY INJURY IN THE NEAR-TERM/TERM NEW-BORN INFANT

1.4.1 Neonatal AKI – epidemiology and current definition

Acute kidney injury (AKI) is an umbrella term, describing a rapid loss of the kidney's excretory function. This results in reduced ability of the kidneys to eliminate waste products and disturbed capacity to maintain fluid, electrolyte and acid-base homeostasis [186]. Traditionally, causes of AKI are divided into three categories: pre-renal, renal/intrinsic, and post-renal/obstructive [187]. The aetiology of AKI can, however, sometimes be multifactorial [188]. Historically, studies on neonatal AKI have been small, single-centre studies in select neonatal patient populations. Also, various arbitrary definitions of AKI have been used. This lack of a standardized definition for AKI in both clinical care and research has hampered comparisons across populations and between studies. The paediatric "Risk, Injury, Failure, Loss of function, End-stage kidney disease" (pRIFLE) criteria was modified from the original RIFLE score on two accounts to better align with the paediatric low changes in serum creatinine (SCr) levels and different urinary output (UO) flows [189]. The authors of this study adapted GFR decline criteria from the original adult RIFLE definition but kept the same UO criteria. A neonatal population was not included in this study. Later, the pRIFLE score was argued to have UO criteria insufficient for neonatal patients [190]. Subsequently, modifications were proposed in the neonatal RIFLE (nRIFLE) classification [191]. Jetton and Askenazi first suggested modifications to the Acute Kidney Injury Network (AKIN) definition [192] for AKI in 2012 [193]. One year later at the National Institutes of Health workshop, there was consensus for this modification. It is now referred to as the neonatal modified Kidney Disease: Improving Global Outcomes (KDIGO) definition of AKI [194, 195], see Table 5. For pragmatic reasons, UO is frequently measured in 24-hour increments.

AKI stage	Serum creatinine (SCr) criteria	Urine output (UO) criteria (hourly rate)
1	SCr rise ≥ 26.5 µmol/L within 48 hours or SCr rise ≥ 1.5-1.9 x baseline SCr within 7 days	≤ 1 ml/kg/h for 24 hours
2	SCr rise ≥2.0-2.9 x baseline SCr within 7 days	≤ 0.5 ml/kg/h for 24 hours
3	SCr rise ≥ 3 x baseline SCr within 7 days or SCr ≥ 221 µmol/L or need for kidney support therapy	≤ 0.3 ml/kg/h for 24 hours

Table 5. KDIGO definition of AKI modified for use in neonatal patients. Baseline SCr defined as the lowest previous SCr value. SCr value of 221 µmol/L represents glomerular filtration rate of < 10 ml/min/1.73m^2. Adapted from Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Workgroup. KDIGO clinical practice guideline for acute kidney injury [195].

Similar to definitions utilised in older patient populations, this definition of AKI is staged and is based on both SCr changes and UO. Staging allows for improved description of the spectrum of injury occurring in patients with AKI. The neonatal modified KDIGO definition has since been validated in the Assessment of Worldwide Acute Kidney Injury Epidemiology in Neonates (AWAKEN) study, an international multicentre trial [196]. This is the largest study of neonatal AKI to date, including 2022 new-born infants admitted to 24 different neonatal intensive care units (NICUs) over a 3-month long period of time. Although the agreement upon and utilisation of the neonatal modified KDIGO definition constitutes a very important step towards harmonizing both recognition and staging of AKI, it has become evident that there are limitations also to the current definition [197]. In a study of 106 new-born infants with HIE, Gupta et al have suggested that the failure of serum creatinine to decrease as expected over the course of the first postnatal week might also constitute AKI [198]. The authors reported that a failure of SCr to decrease by 50% or fall below 53 µmol/L within the first postnatal week was associated with adverse outcomes. As per the current neonatal modified KDIGO definition, this does not constitute AKI. Furthermore, the ideal definition of neonatal AKI may differ depending on gestational age, as shown in a secondary analysis of the AWAKEN study published in 2019 [197]. Allegaert et al have suggested that assay-specific centile SCr values used to plot individual SCr observations or trends over time may better allow for precision medicine in the NICU [199].

The AWAKEN study reported an overall incidence of AKI of 30%. Upon stratification for different gestational ages, there was variation in the incidence of AKI from 48% among infants born ≥ 22 to < 29 weeks' gestation, to 18% among infants born at ≥ 29 to < 36 weeks' gestation, and 37% among infants born at > 36 weeks' gestation. The proportion of infants needing some form of dialysis was 4.1%. After adjusting for 16 variables, AKI was found to be independently associated with increased mortality and length of stay [196].

Although AKI is altogether common among NICU-patients, certain patient populations have been identified to be at particularly high-risk. Near-term and term new-born infants needing cardiac surgery or extra-corporeal life support, exposed to nephrotoxic medication, or suffering from hypoxic-ischaemic encephalopathy are known to be at high risk for AKI. The recent Neonatal and Pediatric Heart and Renal Outcomes Network study is the first multicentre study of cardiac-surgery associated AKI, including 22,040 new-born infants who

underwent major cardiac surgery. They reported an incidence of cardiac-surgery associated AKI in 53.8% of patients, the majority suffering stage 1 AKI. Interestingly, the rates of cardiac-surgery associated AKI varied substantially (27-86%) between the 22 participating centres. Only stage 3 AKI was found to be independently associated with hospital mortality [200].

Exposure to nephrotoxic medications is frequent among critically ill new-born infants in the NICU. In a study by Rhone et al on 107 very low birth-weight infants, 87% of the included infants were treated with at least one nephrotoxic medication. In this study, infants were treated with nephrotoxic medication for a median of 8 days (IQR 3-21). Infants who suffered AKI were exposed to nephrotoxic medication significantly longer than infants who did not (23.9 days compared to 9.9 days, $p < 0.001$) [201]. Further studies have since evaluated exposure to nephrotoxic medications in new-born infants. In a recent publication evaluating the impact of combinations of nephrotoxic medications in 8,269 infants born at 22 to 36 weeks' gestation across 268 NICUs, Salerno et al reported an AKI incidence of 17%. Increased duration of exposure to nephrotoxic medication was associated with increased risk of AKI [202]. Published in 2019, the study "Baby Nephrotoxic Injury Negated by Just-in-time Action (NINJA)" sought to investigate if a systematic surveillance program could prevent AKI among NICU patients and help identify infants at high risk of AKI caused by exposure to nephrotoxic medication. Infants in a single-centre level IV NICU were screened for "high-risk nephrotoxic medication exposure" (defined as 3 or more nephrotoxic medications within a time frame of 24 hours, alternatively four or more calendar days of intravenous aminoglycoside). In infants meeting these criteria, SCr was measured daily until 2 days after exposure or resolved AKI. This quality improvement project was demonstrated to reduce high nephrotoxic medication exposures (16.4 versus 9.6/1000 patient-days, $p = 0.03$), as well as percentage of AKI resulting from nephrotoxic medication exposure (30.9% versus 11%, $p < 0.001$). There was also a significant reduction in the number of AKI days per 100 susceptible patient-days (9.1 versus 2.9 per 100 susceptible patient-days, $p < 0.001$) [203].

Among new-born infants needing extra-corporeal life support, the incidence of AKI has been reported to be as high as 70% [204, 205]. In the retrospective, single-centre study by Zwiers et al, the incidence of AKI among 242 new-born infants treated with extra-corporeal life support was 64%. They found that the most severe stage of AKI was associated with increased mortality [204]. The multicentre Kidney Interventions During Membrane Oxygenation study group recently reported an overall AKI incidence of 51% among 446 new-born infants needing extra-corporeal life support treatment. The AKI incidence varied depending on the underlying condition; infants with cardiac disease had a higher incidence of AKI (68%) compared to infants with congenital diaphragmatic hernia (38%) or respiratory disease (38%) [206]. Furthermore, the association between AKI and outcomes was found to vary depending on the underlying condition of the infant.

New-born infants with HIE after perinatal asphyxia frequently suffer multi-organ dysfunction [207, 208]. In fact, one of the AAP/ACOG criteria to help distinguish if NE is likely to have been caused by perinatal asphyxia is the presence of multi-organ dysfunction [34]. Prior to the advent of TH, the incidence of AKI among new-born infants with HIE ranged between 30 to 72% [207-211]. Since the introduction of TH, there has been a small number or reports on AKI in infants with HIE. In a single-centre study on 96 hypothermia-treated infants, Selewski et al reported an AKI incidence of 38%. AKI was found to independently predict prolonged duration of mechanical ventilation and LOS in the NICU, but was not associated with increased mortality [212]. Another recent single-centre study by Bozkurt et al evaluated the incidence of AKI in a cohort of 166 infants treated with TH. In this study, 29.5% of infants suffered AKI and the mortality rate was significantly higher among infants with AKI compared to those without (41% vs 5%, $p < 0.001$). Furthermore, the authors found that initial and 12-hour lactate concentrations (> 15 mmol/L and > 6 mmol/L, respectively) could be used for early prediction of AKI [213]. These two studies both used a modified AKIN definition of AKI, essentially the predecessor of the neonatal modified KDIGO definition.

Within the AWAKEN initiative, one sub-study focused on infants born at ≥ 34 weeks' gestation with HIE [214]. Among the 113 infants with HIE, 41.6% developed AKI. The SCr criterion of the neonatal modified KDIGO definition was fulfilled by 27.7%, whereas 46.8% fulfilled only the UO criterium. Both SCr and UO criteria were fulfilled in 25.5% of infants with AKI. Intrauterine growth restriction, being out-born and needing transfer to a tertiary level NICU, and presence of meconium-stained amniotic fluids were perinatal factors conferring increased odds for AKI. Out-born infants with AKI also had significantly lower body-temperature at the time of admission to the NICU (34.5 ± 2.2°C) compared to infants who did not suffer AKI (35.6 ± 1.8°C), ($p = 0.04$). The authors also compared the demographic characteristics of all near-term/term infants with AKI, with and without NE. Infants with both AKI and NE were more often born at ≥ 37 weeks' gestation, born outside of the admitting hospital, needed more advanced resuscitation in the delivery room and had lower Apgar scores. Intrapartum complications were more common among infants with AKI and NE than among infants with only AKI. Furthermore, infants suffering both AKI and NE were more often exposed to fluid overload and nephrotoxic medications. In contrast to many other studies on infants with AKI, the authors found no difference in mortality rates between infants with and without AKI. After controlling for several confounders, infants who suffered AKI stayed 8.5 days longer in the hospital than infants without AKI (95% CI 0.79 – 16.2, $p = 0.03$). In this study, however, information on certain important variables such as Sarnat stage of HIE and proportion of infants treated with TH were not reported [214]. The therapeutic drift in some centres towards routinely admitting infants with Sarnat stage 1 (mild) HIE for treatment with TH [215] may to some extent explain the lack of difference in mortality rates as a NE patient population comprising also infants with mild HIE is likely to be less critically ill.

A prospective single-centre study by Alaro et al from Nairobi investigated AKI in a cohort of 60 term infants with HIE not treated with TH, defining AKI as SCr > 133 µmol/L on day

three of life. In this study, the prevalence of AKI was 11.7%, possibly due to the high SCr threshold value chosen. The overall mortality in this cohort was reported to be 17%. Infants with AKI had a 24-fold increase in mortality (71.4%) compared to infants without AKI (p = 0.001) [216].

A recent systematic review and meta-analysis investigating the effect of TH on both myocardial and renal function in (near) term asphyxiated infants included nine RCTs published between 1996 and 2015 [217]. While all included studies examined the incidence of AKI, the studies used heterogenous definitions of AKI, with some studies choosing an arbitrary SCr cut-off level or only the presence of oliguria/anuria. The RCT by Tanigasalam et al on 120 term-born infants was the only included study using a staged definition of AKI (AKIN definition) [218]. In the case of one included study, the definition of AKI used, or renal parameters assessed were not disclosed. Four studies included only infants with Sarnat stage 2 or 3 HIE, four studies also included infants with Sarnat stage 1 HIE. One study did not disclose the ratios of included patients with various stages of HIE. In total, 504 infants underwent treatment with TH and 501 infants received standard supportive treatment. The rate of AKI observed in infants treated with TH was significantly lower than in the control group (RR = 0.81, 95% CI 0.67 – 0.98, p = 0.03). The RD was -0.09 (95% CI -0.16 – -0.01, p = 0.02). Subsequent subgroup analysis revealed a significant effect only in the studies using whole-body hypothermia treatment. Despite considerable heterogeneity in AKI definitions used, the authors of this meta-analysis concluded that TH confers also a reno-protective effect not only in the acute setting, but also in a more long-term perspective.

In contrast, authors of the 2013 Cochrane review reached a different conclusion. This meta-analysis included 11 RCTs, six of which reported the effect of TH on a diagnosis of “renal impairment” or “acute renal failure”. Out of 667 infants, 279 developed “renal impairment” or “acute renal failure”. Rates of renal impairment did not differ significantly between infants treated with TH and controls (typical RR 0.87, 95% CI 0.74 – 1.02, typical RD -0.06, 95% CI -0.12 – 0.01). Six of the included RCTs reported the effect of TH on UO. Out of 865 infants, 201 were oliguric with UO < 1 ml/kg/hour. The rate of oliguria in infants treated with TH did not differ significantly compared to controls (typical RR 0.95, 95% CI 0.76 – 1.19, typical RD -0.01, 95% CI -0.06 – 0.04).

Despite defined diagnostic criteria for neonatal AKI, the condition is frequently overlooked in the NICU, possibly due to the already complicated medical course in many patients. Several studies have revealed that neonatal AKI is underreported in the NICU discharge records [219-221]. Reluctance to measure SCr frequently based on concern for blood loss may further contribute to underestimation of the true incidence of AKI in the NICU [222]. Studies have also highlighted the association between increased nephrology integration in the NICU and reduced incidence of AKI [223, 224]. Lack of a formal AKI-diagnosis may result in new-born infants not being identified for needed long-term follow-up [225]. This in turn limits the opportunity for early recognition of CKD and interventions to slow disease progression. In 2020, a publication by Starr et al investigated the impact of clinical practice changes to

increase nephrology integration in the NICU on incidence and recognition of AKI and referral for follow-up [223]. After clinical practice changes, AKI occurred less frequently. Furthermore, reporting of AKI by neonatology providers increased from 9% to 23% between the two cohorts ($p < 0.001$). While the rate of inpatient nephrology consultations remained unchanged, the rate of referral to the outpatient nephrology clinic increased. Similar findings are described by Vincent et al [224]. In a recent publication, Starr et al compared different standardised approaches used in three academic NICUs with the aim to improve identification of neonatal AKI, the impact of these interventions on AKI identification and incidence, as well as nephrology consultation and subsequent referral at discharge from the NICU [225]. This study highlights that various strategies may help improve neonatal AKI identification and increase nephrology involvement.

1.4.2 Pathophysiology of AKI following hypoxia-ischaemia in new-born infants

Nephrogenesis begins at 5 weeks' gestation and continues until 34-36 weeks' gestation [226]. Approximately 60% of nephrons are formed in the third and last trimester [227]. At birth, the number of nephrons can range between 200 000 and 2.3 million [228]. Many factors can influence nephron endowment, among them prematurity. Birth weight has been shown to be a strong determinant for total glomerular number [228]. As the result of changes in the renin-angiotensin-system and prostaglandins, renal blood flow changes significantly after birth in the term new-born infant, renal blood flow changes significantly after birth. Renal blood flow increases from approximately 3 to 4% of cardiac output to 6% by 24 hours and subsequently to 10% by 1 week of age [229].

The kidneys in a new-born infant may be particularly susceptible to ischaemic injury due to limited blood flow, dependency on the above mentioned postnatal physiological changes and their high metabolic activity [230]. Renal hypoperfusion or hypoxaemia leads to insufficient oxygen delivery in the tissues. This, in turn, results in ATP depletion, causing injury of the renal tubules, glomeruli and vasculature [231]. Lower oxygen conditions at baseline and a lower capacity for anaerobic metabolism renders the straight proximal tubule segment especially vulnerable to ischaemic injury [232]. Pathophysiological processes caused by ischaemic-reperfusion injury are complex. Loss of cytoskeletal integrity and polarity in tubular epithelial cells causes membrane proteins to lose their function [232]. Following necrosis or apoptosis, necrotic debris accumulates in the tubular lumen [233]. Glomerular filtrate leaks into the interstitium as epithelial barrier function is lost [234]. Reperfusion then causes production of reactive oxygen species, causing protein carbonylation and lipid peroxidation which in turn exacerbates damage to cell membranes, cytoskeleton as well as DNA. Immune response become activated, promoting cytokine production. Vasoactive agents are released, further affecting renal blood flow and microcirculation [235]. Microvascular dysfunction ensues as the result of ischaemia inducing a prothrombotic state. Macrophages become chronically activated during repair processes following AKI, potentially contributing to deposition of extracellular matrix and fibrosis [232].

1.4.3 Biomarkers in neonatal AKI – Serum creatinine and urinary output

Assessment of kidney function is crucial for adequate planning of fluid administration, nutritional and electrolyte support, as well as for adjustment of medication dosage. An ideal biomarker of kidney function in this patient population should enable early detection of kidney dysfunction. Furthermore, they would provide guidance both for the management of fluids and medications, and ultimately predict both short- and long-term kidney outcomes. While the implementation of a standardized AKI definition also for neonatal patients constitutes a major progress for the studying of AKI in various neonatal patient populations, there are shortcomings to the current AKI diagnostic criteria. Increased SCr and decreased UO is not necessarily seen until 25-50% of kidney function is lost [236]. Serum creatinine is not a biomarker for kidney injury. Rather, it is a biomarker of kidney function. Interpretation of SCr in new-born infants is further complicated by the initial presence of maternal SCr. New-born infants with NE are frequently born to mothers with conditions that may result in elevated creatinine, such as maternal diabetes mellitus, hypertension, or pre-eclampsia [237]. Estimation of kidney function and recognition of AKI based on the new-born infant's initial SCr values are therefore especially challenging. In term new-born infants with normal kidney function, SCr levels decrease gradually during the first post-natal week. Nadir is usually reached around post-natal day 5 [238]. Gupta et al have suggested an alternative approach to identifying early onset neonatal abnormal kidney function, based on a lack of normal decrease of SCr during the first week of life, also in the absence of a rise in SCr [198].

Similarly, using UO to diagnose AKI in new-born infants presents challenges. Oliguria or anuria is not always a feature of kidney dysfunction in new-born infants [193, 211]. When a urinary catheter is not used, measuring UO is difficult when urine is mixed with stool. Nevertheless, it is important to try to monitor UO. There is growing evidence of poorer outcomes among patients with AKI defined by decreased UO, possibly secondary to the adverse effects of fluid overload (FO) [239]. Insight into UO enables precise provision of fluids and reduced effects of FO. Evaluation of urine sediment is helpful in establishing a diagnosis of acute tubular necrosis [240].

1.4.4 Neutrophil Gelatinase Associated Lipocalin as an early biomarker of AKI

The search for novel serum and urinary biomarkers has been a major target of AKI research in the past decade. An ideal biomarker of AKI should be upregulated shortly after kidney injury and, importantly, be independent of GFR. Neutrophil Gelatinase Associated Lipocalin (NGAL), a lipocalin protein, is expressed in the kidney as well as many other tissues. NGAL is widely considered to be one of the most promising novel biomarkers of renal dysfunction [188] Following kidney injury, NGAL is released into both urine and blood [241]. In a prospective study on 43 asphyxiated infants and 30 healthy controls, Baumert et al measured serum NGAL, copeptin, creatinine and osmolality in cord blood and at 24 hours of age. The authors reported that among the 18.6% of asphyxiated infants who suffered AKI (all stage 1 as per the AKIN definition), serum NGAL levels were significantly higher in both cord blood

and at 24 hours of age. In contrast, there was no difference in copeptin, creatinine or osmolality between groups. NGAL levels > 140.7 ng/mL predicted AKI with 88.9% sensitivity and 95% specificity. No infants in this study were treated with TH [242]. A prospective cohort study by Essajee et al evaluated serum creatinine and urine NGAL in 108 term infants with perinatal asphyxia on days 1 and 3 or life. Infants with AKI had significantly higher urinary NGAL compared to infants without AKI [243]. In a study involving 120 term infants with perinatal asphyxia, Tanigasalam et al collected urine samples within the first 6 hours of life to estimate urine NGAL levels. Infants with Sarnat stage 2 (moderate) or stage 3 (severe) HIE were treated with TH. Forty-six per cent of infants suffered AKI. Infants with AKI had significantly higher median NGAL level compared to infants without AKI (165 ng/mL versus 59 ng/mL, $p = 0.001$). Furthermore, NGAL levels increased significantly with increasing AKI severity ($p = 0.001$) [244]. A meta-analysis and systematic review from 2018 including nine NGAL studies in this patient population concluded that NGAL levels appear to be elevated in asphyxiated infants with AKI and that NGAL may therefore serve as a biomarker. Urinary NGAL levels had greater sensitivity (89.7%), although serum NGAL levels were more specific (specificity 87%) for identification of AKI [245].

1.5 TREATMENT OF NEONATAL AKI

Many unanswered questions remain regarding optimal management of fluid and electrolytes in new-born infants with AKI resulting from perinatal hypoxic-ischaemic injury to the kidneys. Further studies are needed to elucidate ideal strategies for timely detection of AKI, as well as means to minimise renal dysfunction. To date, there are no specific therapies or interventions for new-born infants suffering AKI following perinatal asphyxia [188]. Treatment aims to limit further renal damage, ameliorate AKI and aiding recovery.

New-born infants with HIE and AKI are at high risk of fluid overload (FO). In the six trials on TH that reported UO data, oliguria (defined as UO < 1 ml/kg/hour) occurred in approximately 25% of infants [84]. The effect of TH on rate of oliguria in these studies was inconsistent. In patients with non-oliguric AKI, glomerular filtration rate may be maintained, but tubular dysfunction may result in loss of water and electrolytes [188]. The recommendations for fluid restriction in new-born infants with HIE were extrapolated from treatment practices in older children and adults with traumatic brain injury, placing them at risk for cerebral oedema [246]. Recommendations also took into consideration effects of induced hypothermia on urinary, respiratory and evaporative water losses [247]. Tanigasalam et al randomised 80 infants with HIE to be given a normal fluid intake of 60 ml/kg/day on day 1 of life followed by a daily increase of 20 ml/kg/day or a restricted fluid intake (2/3 of normal fluid intake) during the first 4 days of life. The more restrictive fluid approach did not reduce adverse outcome (defined as a composite outcome of death or adverse neurodevelopment) compared to the normal fluid regimen. Experts therefore recommend that new-born infants with HIE be given an initial fluid intake of 60-70 ml/kg/day on the first day of life, emphasizing the need of individualised fluid management depending on the medical

situation [188]. Fluid balance should be monitored carefully, tracking bodyweight, UO, fluid intake and calculating fluid balance. The use of a Foley catheter allows for more precise monitoring of UO. As most infants subjected to TH receive narcotic sedatives to reduce shivering and discomfort, urinary retention may occur. Later during the course of AKI, UO can be high. This also warrants careful monitoring of fluid balance. In infants with poor UO due to hypovolaemia, a bolus of fluid or blood products may be necessary. Poor cardiac output and hypotension can be treated with inotropic support. Diuretics may be useful for the mobilisation of oedema after rewarming to normothermia. The majority of infants treated with TH receive only trophic feeding as a result of clinical instability and concern for intestinal ischaemia. Thus, early nutrition will consist of intravenous fluid administration.

Many factors contribute to the high risk of hyponatraemia in infants with HIE, among them AKI, fluid overload, sepsis, syndrome of inappropriate anti-diuretic hormone, and reduced reabsorption of sodium in the renal tubules [248]. Among infants with HIE, hyponatraemia is mostly caused by free water excess rather than sodium depletion and restricting water intake to insensible losses plus UO may be required. A recent retrospective study by La Haye-Caty et al demonstrated that a restricted sodium intake was associated with lower serum sodium concentrations and increased AKI ($p = 0.02$) [249]. Expert recommendations therefore include avoiding the use of systematic sodium restriction in this patient population [188]. In the 2013 Cochrane review, approximately 42% of infants with HIE developed hypokalaemia. No significant difference was seen in the proportions of infants with hypokalaemia when comparing infants treated with TH to controls, even though hypothermia is known to cause a shift of potassium to the intracellular space [84, 250]. Conversely, infants with AKI are at risk for hyperkalaemia as potassium shifts back to the extracellular space during rewarming. In infants with non-oliguric AKI, urine potassium losses can be substantial. Experts recommend careful monitoring and replacement of potassium during TH, rewarming and until AKI is resolved [188]. Hypocalcaemia is also a common feature in infants with HIE, likely caused by influx of calcium to the intracellular space resulting from Na+/K+-ATP-ase failure and depolarisation on the cellular membrane. A retrospective analysis by Prempunpong et al reported that the incidence of hypocalcaemia was lower in infants treated with TH but instead that the risk of hypercalcaemia was increased [251]. In the 2013 Cochrane review, on the other hand, no effect of TH on hypocalcaemia could be identified [84]. Experts suggest reducing the initial calcium provision to 50% of normal parenteral intake in infants who receive early parenteral nutrition [188].

In 2012, KDIGO issued the recommendation that “a single dose of theophylline may be given in neonates with severe neonatal encephalopathy, who are at high risk of AKI” [195]. These recommendations are based on studies done in the pre-hypothermia era. Recently, a systematic review of six studies investigating the efficacy of theophylline or aminophylline compared to standard treatment for the prevention of AKI in new-born infants with HIE demonstrated that a single dose of theophylline (5 mg/kg or 8 mg/kg) administered within the first hour of life following perinatal asphyxia reduced the incidence of AKI by 60% (RR 0.40, 95% CI 0.3 – 0.54). Furthermore, administration of theophylline resulted in improved fluid

balance. It was not associated with an increased risk of seizures or death [252]. All studies included in this systematic review and meta-analysis were single-centre studies and did not include infants treated with TH. In a recent publication, Chock et al investigated the renoprotective effects of aminophylline in asphyxiated infants treated with TH [253]. Sixteen infants with low UO and/or rising SCr were given a loading dose of aminophylline (5 mg/kg) starting at 25±14 hours of life, followed by a maintenance dose (1.8 mg/kg every 6 hours). Infants who received aminophylline had increased UO compared to infants in the control group, while rates of SCr decline were similar between groups. In a recent study by Maleki-Sadeghi, term new-born infants with Sarnat stage 2 (moderate) or stage 3 (severe) HIE were randomised to receive either a dose of aminophylline 5mg/kg or placebo within 3 hours after birth. Urinary NGAL levels were measured on day 1 and 4 of life. While there was no difference on day 1 of life, mean urinary NGAL level was significantly lower in the aminophylline group compared to controls on day 4 of life ($p = 0.001$) [254]. Further and sufficiently powered studies are needed to investigate if aminophylline or theophylline can prevent or ameliorate AKI in new-born infants with HIE treated with TH.

Uraemia with accompanying extra-renal organ dysfunction, electrolyte and acid-base disturbances not responding to conservative treatment, inborn errors of metabolism, inability to provide adequate nutrition, and FO are indications for kidney support therapy (KST) in new-born infants [188]. Choice of KST modality will depend on the patient's underlying clinical condition, goals of therapy, centre experience and other factors. In new-born infants, peritoneal dialysis (PD) is often preferred modality as large vascular access catheters and large fluid shifts can be avoided [255]. Historically, the large fluid volume necessary to prime the filter/tubing relative to the total blood volume of the patient has constituted a major challenge in KST in the new-born population. A recent study by Nishimi et al described hypotension at onset in 56% of continuous renal replacement therapy (CRRT) sessions in new-born infants [256]. During the last decade, novel machines have been developed with the younger and smaller patient in mind. The "Aquadex Flexflow" machine, designed for patients weighing > 20 kg, has been used "off-label" to provide continuous veno-venous haemofiltration in new-born infants [257, 258]. Colleagues in Italy have developed the "Cardio-Renal Pediatric Dialysis Emergency Machine (CARPEDIEM)", a device designed for new-born and young infants with a body weight of 2.5-10.0 kg. The first report of a neonatal patient treated with the CARPEDIEM device was published in 2014 [259]. In the U.S.A., the FDA approved its use for paediatric patients in 2020. The first report on new-born patients treated with the "Newcastle infant dialysis and ultrafiltration system" (NIDUS) machine was also published in 2014 [260]. The extracorporeal volume used by the NIDUS machine is < 10 ml. A multi-centre study of the NIDUS machine is currently ongoing in the United Kingdom.

1.6 BRAIN AND KIDNEY CROSS-TALK

AKI leads to an inflammatory reaction that may result in remote organ dysfunction [261]. Uraemic toxins, pro-inflammatory molecules, oxidative stress, electrolyte imbalances, and

drug accumulation may lead to endothelial dysfunction, disruption of the blood-brain-barrier (BBB) which in turn may result in infiltration of inflammatory molecules into the CNS [262]. The association between neonatal AKI and adverse outcomes, including longer hospital stay, prolonged need for mechanical ventilation, and increased mortality has been demonstrated in several studies [212, 213, 218, 263-265].

Prior to the advent of TH, studies in asphyxiated new-born infants have reported AKI to be associated both with worsening HIE as well as adverse long-term neurodevelopmental outcomes [210, 266, 267]. In a study on 88 new-born infants treated with TH due to HIE, Sarkar et al reported that 39% of patients suffered AKI (any stage). Brain MRI abnormalities related to hypoxia-ischaemia were seen in 59% of study participants. MRI abnormalities were more common among infants with AKI compared to infants without AKI (73% versus 46%, $p = 0.012$). Multivariate analysis confirmed an independent association between AKI and presence of hypoxic-ischaemic lesions on brain MRI, OR 2.9 (95% CI 1.1-7.6) [268]. In 2019, a prospective study by Cavallin et al involving 101 near-term/term infants with hypothermia-treated HIE demonstrated that AKI was associated with unfavourable outcome defined as death or disability according to the Griffiths Mental Development Scales at 12 to 24 months of age (100% among infants with a history of AKI compared to 59% among those without AKI, $p = 0.01$). Neonatal AKI was reported to have a high positive predictive value (1.00, 95% CI 0.71 – 1.00) as well as specificity (1.00, 95% CI 0.88 – 1.00). The negative predictive value and sensitivity, however, were low (0.41, 95% CI 0.30 – 0.52 and 0.19, 95% CI 0.11 -0.32, respectively). Using both the creatinine and UO criteria of the neonatal modified KDIGO definition, this study only reported an AKI incidence of 10%, possibly explained in part by a lower proportion of study participants with Sarnat stage 3 (severe) HIE.

1.7 OUTCOMES AFTER NEONATAL AKI ASSOCIATED WITH HYPOXIA-ISCHAEMIA

Previously thought of as a completely reversible condition, there is now convincing evidence that AKI is likely to result in permanent damage and is associated with later development of chronic kidney disease (CKD) in both adult and paediatric patients [269-271]. As per the 2012 KDIGO guidelines, CKD is defined as:

- "GFR < 60 ml/min/1.73m^2 body surface area *and/or*
- Presence of markers of kidney damage, including albuminuria (urine-albumin/creatinine ratio ≥ 3 mg/mmol), urinary sediment abnormalities, markers of tubular dysfunction or imaging abnormalities persisting for > 3 months" [272].

The phenotype of CKD is variable; chronic kidney impairment may manifest in the form of decreased GFR or hyperfiltration, proteinuria, tubular dysfunction, and/or hypertension [222].

Several small studies on near-term/term infants have investigated long-term consequences of an episode of AKI in infancy or childhood [273]. Anand et al were among the first to describe outcomes among survivors of HIE and AKI [273]. Nine survivors were followed until the age of 23-57 months. Among them, 44% had reduced GFR and 33% had hypertension. Children

with decreased GFR were found to have cortical scarring and atrophy upon examination with excretory urogram. Urine concentrating ability was measured in four of the study participants and was demonstrated to be impaired for all, indicating possible tubulo-interstitial injury. A small cohort study by Polito et al followed six full-term infants with AKI after perinatal asphyxia for a duration of at least five years [274], reporting hypertension in one child and reduced eGFR in five (83%). One child progressed to kidney failure, needing KST by six years of age. This study reported reduced urinary concentrating ability in two of the participants (33%). More recently, a prospective cohort study on 169 infants treated with extra-corporeal membrane oxygenation (ECMO) reported outcomes at a median age of 8.2 years [275]. Thirty-two per cent of survivors were demonstrated to have at least one sign of CKD and/or hypertension. Decreased eGFR occurred in 5% and proteinuria in 12% of children, whereas 19% had hypertension. Only a history of AKI had a significant association with the development of CKD. In a two-centre prospective, longitudinal study, Huynh et al reported outcomes at a median age of six years in 58 children who had undergone corrective heart surgery in the neonatal period [276]. Among them, 58% had suffered cardiac surgery-associated AKI. Decreased eGFR occurred in 17% and hypertension in 30% of children. Interestingly, cardiac surgery-associated AKI was not associated with decreased eGFR or hypertension. Only post-operative cyanosis was found to independently predict decreased eGFR. Longer duration of hospital stay was associated with hypertension at the follow-up assessment. Akkoc et al recently evaluated renal outcomes at a mean age of 6.8±2.9 years of age in a cohort of 72 children with heterogeneous causes of neonatal AKI. Renal functions were investigated using a multi-modal approach including estimation of GFR, urine sample for assessment of albuminuria, ambulatory 24-hour BP-monitoring (ABPM) and kidney ultrasonography. At least one marker of long-term renal dysfunction was observed in one third of the cohort. Notably, 50% of children had abnormal results on ABPM [277].

The heterogeneity of the studies that have hitherto reported long-term renal outcomes following neonatal AKI makes proper assessment of the relationship between neonatal AKI and future CKD development difficult. The studies have used various approaches both to define AKI and to assess markers of chronic kidney injury. Whereas most studies have estimated GFR using the Schwartz equation [186, 271, 275], some have instead used more accurate techniques to measure GFR. Similarly, quantification of proteinuria has varied between spot urine measurements, urine electrophoresis and timed urinary total protein excretion. Hypertension was mostly determined by office BP readings, only rarely were 24-hours ambulatory BP measurements used for confirmation of hypertension. Furthermore, few studies have included also a control group for comparison. Because of the small sample sizes and retrospective cohort design in most of the studies, attrition or loss-to-follow-up bias may result in under- or overestimation of the incidence of CKD.

Current clinical practice guidelines recommend evaluation after 3-6 months after an AKI-insult to assess for CKD for all patients who suffer AKI [195]. Despite these recommendations, neonatal AKI remains an underrecognised and underreported entity in NICUs with studies demonstrating that only 10-30% of neonates with AKI are formally

diagnosed with such [220, 223, 224]. Experts on neonatal AKI have argued that further screening for hypertension and albuminuria on an annual basis is indicated in all children with a history of neonatal AKI [222]. The same experts also recommend assessments of growth and BMI at each follow-up visit, emphasising also the importance of education on healthy lifestyle habits. In children with a history of stage 2 or stage 3 AKI or other additional CKD risk factors such as significant prematurity, low birth weight, IUGR or structural abnormalities of the kidney and urinary tract, it is recommended that renal function is evaluated using SCr and/or cystatin C. Segar et al have suggested assessment of BP, urine protein and SCr on a yearly basis for all children with a history of neonatal AKI [188].

Figure 2 illustrates various causes of neonatal AKI and factors that may contribute to progression to CKD following an AKI-insult.

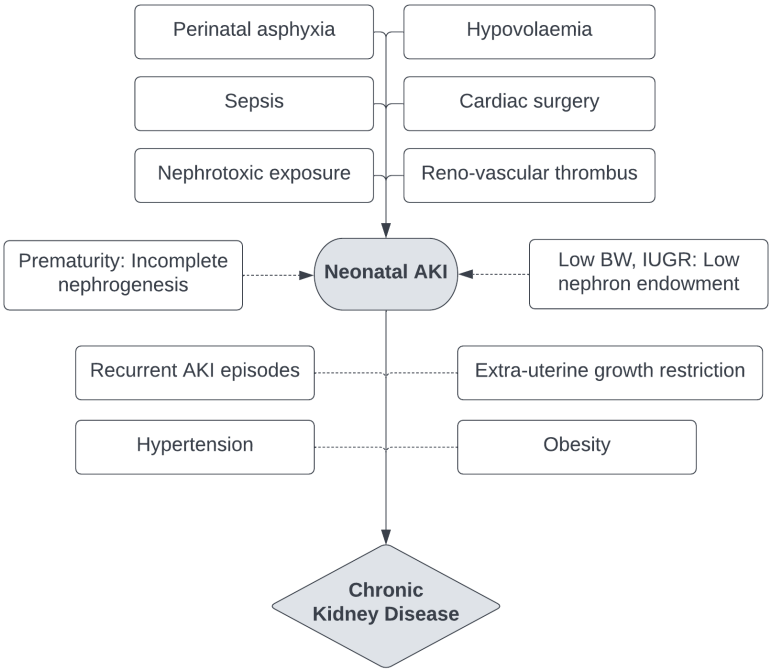

Figure 2. Schematic diagram illustrating multiple causes of neonatal AKI and factors that may contribute to the development of CKD following an episode of AKI. Adapted from Chaturvedi et al. The path to chronic kidney disease following acute kidney injury: a neonatal perspective, Pediatric Nephrology, 2016 [222].

1.7.1 Estimation and measurement of GFR

Before the age of two years, body surface area-adjusted GFR values are not expected to be comparable to those of an adult. For children below the age of two years, age appropriate GFR and SCr values can be found in references [278, 279]. Measured GFR techniques are resource demanding and relatively expensive. The use of GFR-estimating equations has therefore been recommended instead, reserving measured GFR examinations for situations where SCr may be inaccurate or in children with suspected hyperfiltration [222].

1.7.2 Albuminuria

In order to minimise orthostatic effects, a first morning urine sample is recommended to assess albuminuria. A comprehensive review by Rademacher et al from 2009 provides normal urine-albumin/creatinine ratios and albumin excretion rates in children of various ages [280]. In the KDIGO guidelines for CKD, albuminuria is categorised as follows:

- "(A1) Normal to mildly increased: < 3mg/mmol"
- "(A2) Moderately increased: 3-30 mg/mmol"
- "(A3) Severely increased: > 30 mg/mmol"

In the event of an abnormal test result, the test should be repeated to confirm. Albuminuria is a potentially modifiable risk factor of CKD progression [281, 282]. It is not only a marker of kidney damage; the leakage of protein also causes progressive kidney injury. Urinary protein levels increase as the result of either damage to the capillary wall of the glomeruli or a decrease in tubular reabsorption of protein [283]. Tubular exposure to urinary proteins has been shown to cause inflammation and subsequent fibrosis and proximal tubule cell apoptosis [284]. A large study of North American children with non-diabetes related CKD assessed the role of albuminuria as an indicator for CKD progression. The authors concluded that urine-protein/creatinine-ratio, urine-albumin/creatinine-ratio and urine-nonalbumin/creatinine-ratio had similar ability to characterise disease progression [285]. In studies on adult populations, the renoprotective effects of renin-angiotensin-system antagonists have been demonstrated [286, 287].

1.7.3 Hypertension and elevated blood pressure

Obesity, CKD, sleep disorders/sleep apnoea, prematurity and/or low birth weight, diabetes mellitus, exposure to certain medications, and organ transplantation are factors demonstrated to be associated with an increased risk of hypertension [288, 289]. Swedish national guidelines are based on the revised European recommendations from 2016 [290]. Swedish normative values for casual oscillometric blood pressure (BP) measurements in children age 6-16 years were published in 2015 [291]. In children and adolescents aged 0-15 years, hypertension is defined as "a systolic and/or diastolic BP ≥ the 95th percentile for age, sex and height". High normal BP is defined as "a systolic and/or diastolic BP ≥ the 90th but < the 95th percentile for age, sex and height". Swedish national guidelines recommend a 24-hour ABPM to confirm a suspected diagnosis of hypertension (www.nefro.barnlakarforeningen.se).

Most children with CKD are hypertensive [292], with one longitudinal study of children with CKD reporting the incidence of left ventricular hypertrophy increasing from 19% to 39% over the course of two years [293]. Increased carotid intima media thickness, a well-established surrogate marker for atherosclerosis in adults, has been observed among hypertensive children and adolescents [294, 295]. Cardiovascular disease is recognised as the leading cause of morbidity and mortality among adult individuals with childhood-onset CKD, reported to account for 35 to > 50% of all deaths in this patient population [296-298]. In

2009, a study by the ESCAPE Trial Group evaluated the effect of strict BP control on CKD progression in children aged 3-18 years. Over the study period of five years, intensified BP control conferred a significantly lower risk of a 50% decline in GFR or progression to kidney failure. Furthermore, reaching BP targets (mean arterial BP < the 50th percentile) and reduced proteinuria were identified as independent predictors of delayed disease progression [299].

1.7.4 Renal volume

Kidney size is an important indicator of adequate kidney growth as well as an indicator for evaluation of kidney disease in children. A strong correlation between renal volume, body height and body weight in healthy children has been demonstrated in several large cohort studies in healthy children [300-303]. There is still much debate regarding the usefulness of renal volume as a marker of renal function [304]. Several studies have argued that relative total renal volume can be used as a non-invasive marker of renal function in both adult and paediatric populations [305-307]. Renal length on ultrasonography is widely used to approximate renal size. Measuring renal volume, however, is difficult using this modality. Due to the kidneys' complex shape, renal volume may be a more sensitive index of renal size than simply measuring renal length. Current kidney size nomograms are still based on renal lengths [308]. Historically, calculation of renal volume by ellipsoid formula method on ultrasound has been considered the gold standard [300, 304, 308]. When compared to computer tomography (CT) or MRI segmentation, however, this method has been demonstrated to underestimate renal volume [309]. Presently, the use of MRI to measure renal size and volume is not common practice. Applying a segmentation volumetry method provides a more accurate estimation of renal volume compared to the ellipsoid formula calculation without being overly time-consuming [310, 311]. Currently, there are no normal ranges of renal volume based on CT or MRI segmentation methods in paediatric populations. There is growing interest for developing reliable reference values for renal volume in relation to body size parameters. A study by Park et al from 2017 investigated the relationship between anthropometric indices and renal length and volume as measured on CT in 272 Korean children without renal disease. The authors demonstrated that body surface had the strongest correlation with renal volume. The authors suggested that their results may serve as normative standards for assessment of renal growth, as they determined mean renal size for each age and height group [312].

1.7.5 Fibroblast Growth Factor 23 and Klotho as early biomarkers of CKD

There is frequently a delay in the diagnosis as CKD is initially asymptomatic [313]. Delayed identification of CKD in its early stages is associated with more rapid disease progression and premature death in patients with kidney failure [313]. CKD-associated mineral bone disorder (MBD) is known to be an important contributor for the increased risk of cardiovascular disease in this patient population [314]. Fibroblast Growth Factor 23 (FGF 23) is a new marker of CKD-MBD and is associated with hypertension in paediatric and adult patients [315]. Produced mainly in osteocytes and osteoblasts, FGF 23 is involved in the regulation of phosphate and vitamin D homeostasis, promoting urinary phosphate excretion, inhibiting

production of active vitamin D and augmenting active vitamin D catabolism [316, 317]. Tranœus Lindblad et al reported that logFGF 23 levels increased significantly at a GFR of 45 to 38 ml/min/1.73m^2 in CKD and transplanted CKD patients [318]. In comparison, Portale et al found that that GFG23 levels increased significantly at GFR 60 to 69 ml/min/1.73m^2 and below [319]. Paediatric reference values for FGF 23 have been published [320, 321]. The transmembrane protein Klotho is expressed mainly in the cells of the proximal and distal tubule, acting as a co-receptor for FGF 23 [322]. Even in patients with CKD stage 1 and 2, Klotho levels have been demonstrated to be reduced [323]. Klotho deficiency has been shown to be positively correlated to kidney function decline [323, 324]. A recent meta-analysis reported a significant correlation between levels of soluble Klotho and eGFR in patients with CKD [325]. Research investigating the potential of restoring Klotho levels with the aim of improving renal function and mitigating complications in patients with CKD is ongoing [322].

2 RESEARCH AIMS

The overall aim of this thesis was to contribute to the improved treatment and care of infants with HIE by means of increased knowledge about the predictive value of early aEEG, neonatal AKI, and long-term outcomes in the era of TH.

2.1.1 Study I

The aim of Study I was to investigate the predictive value of aEEG in infants with HIE treated with therapeutic hypothermia in relation to outcome at 4 and 12 months of age.

2.1.2 Study II

The aim of Study II was to assess long-term outcomes in children and young adolescents with a history of hypothermia-treated HIE.

2.1.3 Study III

The aim of Study III was to investigate the incidence, severity and consequences both short- and long-term of AKI in infants with hypothermia-treated HIE.

2.1.4 Study IV

The aim of Study IV was to in greater detail investigate renal functions in early adolescence following perinatal asphyxia and TH.

3 MATERIALS AND METHODS

3.1 STUDY POPULATIONS AND CLINICAL DATA

3.1.1 Study I

The Karolinska University Hospital took part in the multi-centre TOBY-trial, an RCT. Upon completion of recruitment for the TOBY-trial at the beginning of December 2006, a protocol for TH was accepted in the region and TH was implemented in regular clinical practice starting January 2007. The cohort described in Study I comprises all 23 infants born at > 34 weeks' gestation treated with TH in Stockholm between December 2006 and December 2007. The recruitment criteria were the following: Apgar score ≤ 5 at 10 minutes of age, continued need for resuscitation at 10 minutes of age, pH < 7.0 in either cord blood or within 60 minutes after birth (arterial or capillary blood sampling) and/or base excess (BE) ≤ -16 within 60 minutes after birth (A-criteria). Infants fulfilling any of these A-criteria for TH were then continuously assessed during the first 60 minutes of life with the aim of identifying seizures or signs of moderate/severe HIE defined as the combination of altered level of consciousness, hypotonia or opisthotonus or abnormal primitive reflexes (B-criteria). For infants fulfilling both A- and B-criteria, TH was initiated within 6 hours after birth. aEEG was not mandatory to start TH, however, registration was commenced as soon as possible upon admission to the NICU. Exclusion criteria were any known genetic disorders and/or inborn errors of metabolism and/or expected need for surgical intervention within the first 3 days of life. Perinatal data were collected prospectively from the time of birth.

3.1.2 NeoCool cohort (Study II, III and IV))

The NeoCool cohort is a population-based cohort comprising all infants born at >34 weeks' gestation treated with TH in Stockholm, Sweden between January 2007 and December 2009. The recruitment criteria as well as exclusion criteria were identical to those described above for Study I. Perinatal data was prospectively recorded from the time of birth and has subsequently been retrospectively validated January to May 2021. Figure 3 outlines follow-up procedures at 6-8 and 10-12 years of age.

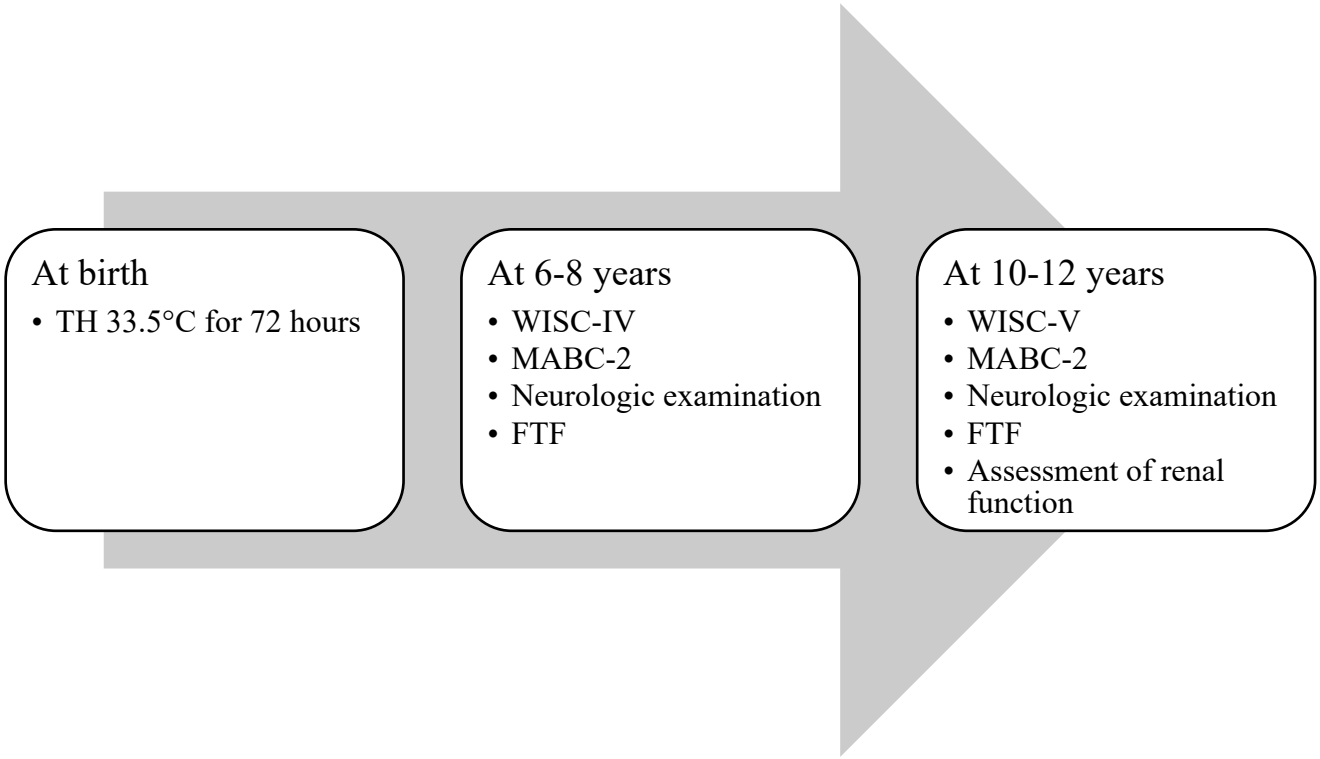

Figure 3. Overview of follow-up procedures at 6-8 and 10-12 years of age in the NeoCool cohort. Abbreviations: TH, therapeutic hypothermia; WISC-IV, Wechsler Intelligence Scales for Children, 4th edition; MABC-2, Movement Assessment battery for Children, 2nd edition, FTF, Five-to-Fifteen questionnaire; WISC-V, Wechsler Intelligence Scales for Children, 5th edition.

3.2 EXPOSURES, OUTCOMES AND STATISTICAL ANALYSIS

3.2.1 Study I

3.2.1.1 Exposure – aEEG during HIE treated with TH

Applying the Nervus® monitor (Viasys, Nicole Biomedical, Madison, WI, USA), aEEG recordings were collected starting immediately once the decision to commence TH had been made. aEEG registration continued during the 72 hours of TH and slow rewarming to normothermia. The recordings were then stored digitally. Two researchers blindly assessed the aEEG traces using the scoring system suggested by Hellström-Westas et al [68]. The predominant aEEG pattern was assessed at 6, 12, 24, 36, 48 and 72 hours of age with full agreement between the two researchers with regards to both the dominating background pattern, sleep-wake cycling as well as presence or absence of seizure activity. Any aEEG registrations recorded before 6 hours of age were not included.

From the add-on TH-module in the national perinatal database, we collected information on HIE severity, administered medications (with special focus on anticonvulsants, inotropes and analgesics) and mortality. Furthermore, we assessed temperature stability (measured rectally) throughout the TH treatment and the rewarming period, paying special attention to the rectal temperature at the time-points when the aEEG traces were assessed.

3.2.1.2 Outcome – Neurodevelopment at 4 and 12 months of age

The Alberta Infant Motor Scale (AIMS), administered by a physiotherapist, was used to assess neuromotor development at 4 months of age [40, 326]. At 12 months of age, a standardised neurologic examination was performed by a neonatologist in our out-patient clinic as part of follow-up protocol. Abnormal outcome was defined as overt signs of spasticity upon neurologic examination, an AIMS score below the 5th percentile, or death.

3.2.1.3 Statistical analysis

Descriptive data analysis in this paper was done by co-author Boubou Hallberg and Katarina Robertsson Grossmann, using Statistica® 7.0 (Statsoft, Tulsa, USA).

3.2.2 Study II, III and IV

3.2.2.1 Exposure - TH

All study participants in the NeoCool cohort underwent treatment with TH in addition to conventional intensive care. Whole-body cooling was the only modality used in these studies. Using either a Thecoterm® mattress (TecCom) or a CritiCool® suit (Mennen Medical Ltd), core body temperature was reduced to 33.5ºC for a duration of 72 hours, which was then followed by slow rewarming, increasing core body temperature by 0.5 ºC per hour.

3.2.2.2 Outcome Study II – Neurologic, motor, cognitive and behavioural outcome at early school age and in early adolescence

At age 6-8 years and 10-12 years, survivors still residing in Sweden were invited for a comprehensive assessment. After written parental consent had been obtained, assessments were performed in our outpatient clinic. If a child had undergone psychometric testing at 6-8 or 10-12 years, we did not re-administer the test but reviewed the results after obtaining parental permission. An experienced paediatric neurologist performed a structured neurologic examination to detect signs of CP or more minor neurologic dysfunction. Level of CP was categorised using the Gross Motor Function Classification System (GMFCS). Scores range from 1 to 5. Higher scores indicate greater impairment [327]. Motor abilities were further assessed with the Movement Assessment Battery for Children, 2nd Edition (MABC-2) [145], administered by a paediatric physiotherapist. Cognitive abilities were assessed using the Wechsler Intelligence Scales for Children, 4th or 5th edition (WISC-IV, WISC-V). Five-to-Fifteen-questionnaire response was requested from the parents [148].

The primary outcome in our study was survival without neurologic abnormalities (defined as an FSIQ ≥ 85, a normal neurologic examination, normal hearing, normal vision, no autism spectrum disorder (ASD) [140], no attention deficit disorder without/without hyperactivity (ADD/ADHD) [140], no developmental coordination disorder (DCD) [328], and no epilepsy). We also investigated the incidence of CP, ADD/ADHD, ASD, and DCD.

3.2.2.3 Statistical analysis Study II

Statistical analysis was performed by the author in collaboration with co-authors Mimmi Eriksson Westblad and Katarina Lindström. Analyses were done using GraphPad Prism version 9.3.1 for MacOS (GraphPad Software, San Diego, California, USA, www.graphpad.com) or SPSS version 27.0 for Windows (IBM Corp., Armonk, New York, USA). For normally distributed continuous variables, means and standard deviations (SD) with 95% confidence intervals (CI) are presented. A two-sided t-test was used to compare WISC scores between two subgroups. When comparing our cohort with norms, we used a binomial test and subsequently a McNemar test to investigate any difference between two assessment occasions. A p-value < 0.05 was considered statistically significant for all analyses.

3.2.2.4 Outcome Study III – Neonatal AKI and its association with mortality, morbidity and eGFR at 10-12 years of age

The primary outcome measure in Study III was the development of neonatal AKI as per the neonatal modified KDIGO definition. Secondary outcome measures were mortality, length of hospital stay (in days), need for cardiac compressions and/or intubation during initial resuscitation, need for and duration of mechanical ventilation, a diagnosis of persistent pulmonary hypertension (PPHN), need for inhaled nitric oxide (NO), need for inotropic support, need for transfusion of blood products and need for kidney support therapy (KST) in the neonatal period. At 10-12 years of age, we studied creatinine-based eGFR using the Schwartz-Lyon equation among survivors still residing in Sweden.

3.2.2.5 Statistical analysis Study III

Statistical analysis was performed by the author with support from co-author and co-supervisor Dr Milan Chromek using GraphPad Prism version 8.0.1 for MacOS (San Diego, CA). Data are presented as dot plots with individual values, and as medians and ranges. Fischer's exact test was used to compare dichotomous variables between two groups, whereas Mann-Whitney test was used to compare continuous variables that were not normally distributed. For comparison of more than two groups, Kruskal-Wallis test was used. A p-value < 0.05 was considered statistically significant for all analyses.

3.2.2.6 Outcome Study IV – Measures of renal function at 10-12 years of age

In Study IV, the children in our cohort were invited for a multi-modal assessment of renal functions at 10-12 years of age. Height and weight were measured and plotted on Swedish national growth charts. Oscillometric BP-measurements were performed three times after 10 minutes of rest in a supine position using a size-appropriate cuff. We then compared the average systolic and diastolic BPs with age-, height- and sex-specific BP nomograms in accordance with Swedish national guidelines for management of hypertension in children and adolescents published in 2020 (www.nefro.barnlakarforeningen.se). These are, in turn, based on the 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents [329]. Children with elevated office BP were further

examined with 24-hour ambulatory BP-measurement SpaceLabs, U.S.A.). Blood samples were drawn in a standardised manner. Cystatin C was measured by turbidimetry. Estimated GFR (cyst C eGFR) was calculated using the CAPA formula [330]. Children with decreased cyst C eGFR were further examined using iohexol clearance. A bolus-dose of intravenous iohexol was given and a first sample taken immediately thereafter. The concentration-time curves at 180, 210, 240, and 270 minutes following bolus injection were analysed. Iohexol concentration was measured using ultra high-performance liquid chromatography and photometry.

Urine-albumin and creatinine were measured in a morning spot urine sample. Urine-albumine was measured using an immune-turbidimetric method, and urine-creatinine using an enzymatic photometric method. In children with elevated urine-albumin/creatinine ratio, a second sample was requested approximately three months later.

FGF 23 (pg/ml) was analysed by second-generation human sandwich enzyme-linked immunosorbent assay (ELISA, Quidel, Ireland).

MRI of the kidneys was acquired using a Sigma 3.0 Tesla MR scanner (Discovery MR750, General Electric Healthcare, U.S.A.). T2-weighted, fat suppressed images with enhanced reconstruction (PROPELLER) were acquired with 20 slices per sequence (slice thickness 3.0 mm, TE 84.8 ms, flip angle 140 degrees, TR 11.639 sec, FOV 30 cm) using a head and neck CTL spine coil. The semi-automatic segmentation method based on manual contour delineation with contour interpolation was used within the MM Reading protocol of Syngo.Via software (Siemens, Germany) [310, 311]. Sinus fat and renal pelvis were excluded from the segmented area of parenchyma. Renal length and volume were compared to sonographic growth charts based on measurements of renal length and use of ellipsoid formula method to calculate renal volume [308]. We calculated individual age-specific Z-scores for renal volume.

Primary outcomes were decreased GFR, hypertension, and albuminuria. Decreased GFR was defined as GFR < 90 ml/min/1.73m^2 as estimated by cystatin C (cyst C eGFR) or measured by iohexol clearance. Category of albuminuria was classified according to the KDIGO definition as follows: normal to mildly increases (A1) defined as a urine-albumin/creatinine ratio < 3 mg/mmol, moderately increased (A2) defined as a urine-albumin/creatinine-ratio 3-30 mg/mmol, and severely increased (A3) as a urine-albumin/creatinine ratio > 30 mg/mmol [272]. Hypertension was defined as a systolic and/or diastolic BP ≥ the 95th percentile for age, height and sex, or a history of hypertension currently being treated with antihypertensive medication. High normal BP was defined as a systolic and/or diastolic BP > the 90th but < the 95th percentile for age, sex and height. Secondary outcome measures were renal volume on MRI and levels of FGF 23.

3.2.2.7 Statistical analysis Study IV

Statistical analysis was performed by the author with support from co-author and co-supervisor Dr Milan Chromek using GraphPad Prism version 9.3.1 for MacOS (San Diego,

CA). Variables were tested for normality using the Kolmogorov-Smirnoff test. Data are presented as means with standard deviations (SD) and 95% confidence intervals (CI) for normally distributed numerical variables, and as medians with ranges for numerical variables that are not normally distributed. Univariate analysis with Fischer's exact test was used to compare dichotomous, nominal variables between two groups. A t-test was used to compare normally distributed, numerical variables between two groups. The Mann-Whitney test was used for comparison of non-normally distributed continuous numerical variables between two groups. A p-value < 0.05 was considered statistically significant for all analyses.

3.3 ETHICS CONSIDERATIONS

Any research involving infants or young children demands considerable ethics considerations as the individuals are unable to consent to participate in the research but are instead represented by their parents/legal guardians.

At the time of hospitalisation in the NICU, the studies included in this thesis did not involve any extra invasive testing of any sort. The study participants were not subjected to any blood or urine samples that were not part of routine care after perinatal asphyxia. Any differences in number or frequency of blood samples were at the discretion of the treating physician.

In Study II, III and IV, the children had reached an age of 10-12 years at the time of the latest follow-up assessment. For both ethical reasons as well as for the integrity of the data, it was integral that any assessment, procedure or exam performed was carried out with both the active consent and participation of the child in question. The children received age-appropriate written information about the study beforehand and were given the opportunity to ask any questions they might have prior to the assessments. Parents received detailed written information and were also given the opportunity to ask questions. Written consent was collected from the parents. Parents and children could choose to refrain from any part of the assessments, if they so desired. All children were offered local anaesthetic cream prior to taking blood samples. No blood or urine samples were biobanked. Children who agreed to MRI examination could do a "test drive" prior to the actual examination to become accustomed to the machine. They were informed that the examination could be stopped at any time should they experience claustrophobia or discomfort of any kind. The MRI examinations were performed without any sedation. There was initially concern that the long-term follow up of the NeoCool cohort could cause parents harm by bringing up painful memories from the time of their child's birth and NICU hospitalisation. Instead, parents expressed appreciation for the extended follow-up and many of them also expressed a wish to talk about their past experiences both with other parents and with the research staff involved in these studies. Our research group has also focused specifically on the parental experience of treatment with TH [331, 332]. Any assessment of cognitive or motor performance done in older children or adolescents is a delicate task, as the children are certainly old enough to understand that their performance is being evaluated. For parents, it may be hard to see their child be exposed to testing, particularly if the child is not reaching expectations for his/her age. At the same time, it is of great importance that any difficulties or health issues that a

child might experience as a result from perinatal asphyxia are detected to ensure that adequate measures can be offered in a timely manner. Results from the assessments were conveyed to the parents, offering referral for neuropsychiatric testing, further follow-up at the department of paediatric neurology or nephrology or elsewhere, and/or psychological support if deemed necessary.

The study protocols for study I and the follow-up assessment at age 6-8 years of study II were approved by the Regional Ethical Review Board in Stockholm. This department was later replaced by the Swedish Ethical Review Authority in 2019. The study protocols for the assessments at age 10-12 years in studies II, III and IV were approved by the Swedish Ethics Review Authority.

4 RESULTS

4.1 STUDY I

The aim of this study was to investigate the prognostic value of early aEEG in infants with HIE undergoing TH. A total of 23 infants treated with TH at Karolinska University Hospital between December 2006 and December 2007 were included in this study.

4.1.1 Dominating background pattern on aEEG and association to outcome at four and twelve months of age

Five infants were found to have continuous normal voltage (CNV) or discontinuous normal voltage (DNV) pattern. All infants with CNV or DNV at 6 hours of age had favourable outcome. At 6 hours of age, a severely abnormal burst-suppression pattern was seen in 15 infants. In the case of two infants, ongoing ictal activity rendered analysis of the background pattern impossible at this time point. A normalisation of the dominating aEEG background pattern was seen within the first 24 hours of life in seven of these infants. They all scored above the 25th percentile on AIMS at 4 months of age and exhibited no neurologic abnormalities at 12 months of age. BS or ictal activity persisted beyond 24 hours after birth in ten infants. Among infants with late normalisation of the dominating background pattern on aEEG, six had adverse outcome. Two of these six infants died prior to discharge from the NICU, and four had CP at 12 months of age. In the case of three infants with initial BS, the background pattern was found to normalise within 48 hours after birth. These three infants all scored above the 50th percentile on AIMS at four months of age. Abnormal motor outcome at 12 months of age was seen in all four infants who had scored below the 10th percentile on AIMS at 4 months of age. Although one infant was found to have slightly delayed gross-motor function, no infant who had scored above the 25th percentile on AIMS showed any signs of CP at 12 months of age. Figure 3 summarises aEEG findings in relation to outcome.

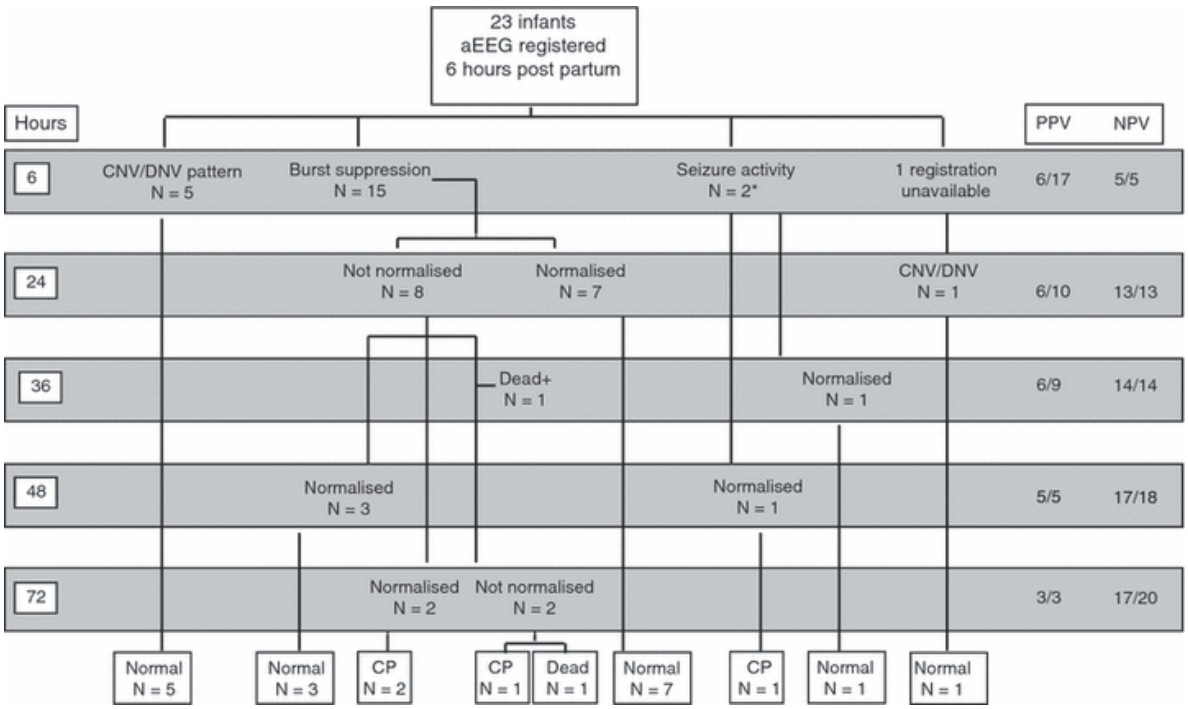

*Figure 3. Predominant background pattern on aEEG in infants treated with therapeutic hypothermia at 6-72 hours of age in relationship to predictive values and neurologic outcome at 12 months of age. * The infant with normal outcome at 12 months of age had BS background activity until the background pattern was normalised by 36 hours of age. The infant who later developed CP continued to have seizure activity until normalisation of the background pattern by 48 hours of age. + Intensive care withdrawn due to estimated poor prognosis. PPV, positive predictive value; NPV, negative predictive value. Reprint from Hallberg et al, The prognostic value of early aEEGS in asphyxiated infants undergoing systemic hypothermia treatment, ACTA Paediatrica, 2010, with permission [72].*

4.2 STUDY II

The aim of this study was to describe neurologic, neuromotor, cognitive and behavioural outcomes at early school-age and early adolescence following HIE treated with TH. A total of 66 infants treated with TH due HIE at Karolinska University Hospital between January 2007 to December 2009 were included.

4.2.1 Long-term outcome assessment

Mean age at the time of the first assessment was 7.4 years (SD 0.7 years), and at the time of the second assessment 11.2 years (SD 0.7 years). At early school age, outcome was known for 59 participants (91% of the cohort). One child had been excluded due to a genetic syndrome. Four children were lost to follow-up due to moving abroad. Two children residing in Sweden did not take part at this time, however one of them had been assessed in accordance with new national guidelines at 5.5 years of age. In early adolescence, outcome was known for 57 participants (88% of the cohort). One more child had moved abroad, and three families declined participation. Parents of a child with severe CP and multiple co-morbidities provided an update over telephone. Patient demographics at baseline are

summarised in Table 6. The flowchart in Figure 4 provides an overview of long-term outcomes at 6-8 and 10-12 years of age, respectively.

Patient characteristics	
Gestational age at birth in weeks + days, median (IQR; min - max)	40 + 2 (39 + 0 – 41 + 2; 34 + 0 – 42 + 1)
Birth weight in grams, median (IQR; min - max)	3500 (3183 – 4055; 2376 – 5828)
Apgar at 5 min, median (IQR, min - max)	3 (1 – 4; 0 – 10)
Apgar at 10 min, median (IQR, min -max)	4 (2 – 6; 0 – 10)
Boys/girls	31/34
Inborn/outborn	33/32
Singletons/part of a set of multiples	63/2
Mode of delivery o Vaginal delivery (unassisted) o Ventouse extraction o Forceps extraction o Caesarian section o Home birth (unassisted)	 15 (23.08%) 16 (24.62%) 1 (1.54%) 32 (49.23%) 1 (1.54%)
Clearly identifiable sentinel event around the time of birth o Shoulder dystocia o Placental abruption o Other massive haemorrhage o Uterine rupture o Maternal cardiac arrest o Other (compressed umbilical cord, failed attempt at assisted vaginal delivery)	 6 (9%) 8 (12%) 3 (5%) 3 (5%) 1 (2%) 7 (11%)
Intubation in the delivery room, no. (%)	48 (74%)
Continued need for resuscitation at 10 minutes, no. (%)	46 (71%)
Lowest pH within 60 min, median (IQR; min – max)	6.9 (6.8 – 7; 6.5 – 7.3)
Lowest BE (mmol/L) within 60 min, median (IQR, min – max)	-19 (-22.5 - -13.5; -36 – -4)
Time to initiation of TH in hours, median (IQR; min – max)	4 (2.3 – 5.4; 1 – 13)
Severity of HIE o Sarnat stage 1 o Sarnat stage 2 o Sarnat stage 3	 4 (6%) 50 (77%) 11 (17%)

Table 6. Patient characteristics at baseline. N=65. Abbreviations: IQR, inter-quartile range; min, minimum; max, maximum, TH, therapeutic hypothermia; HIE, hypoxic-ischaemic encephalopathy.

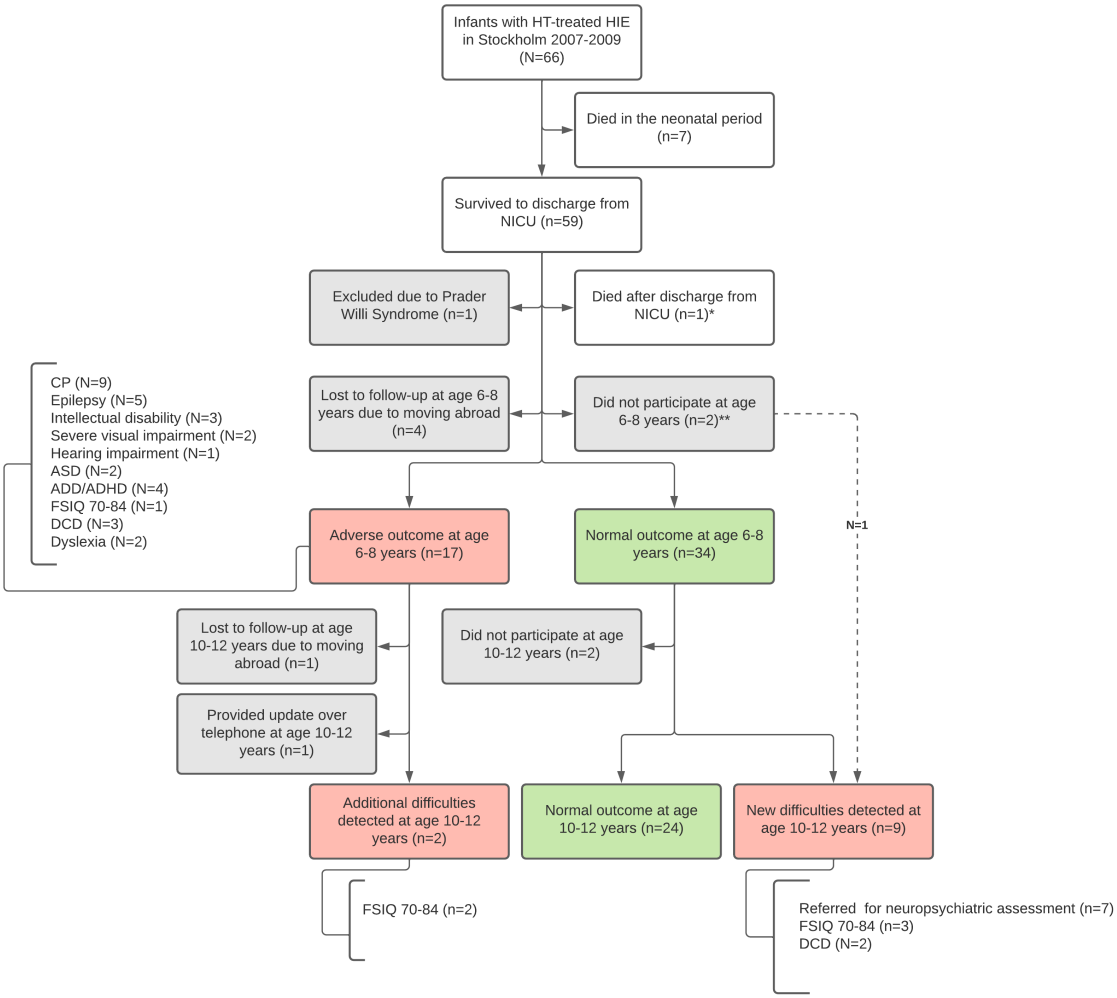

Figure 4. Flowchart illustrating outcomes at 6-8 years and 10-12 years of age.

Outcome in relation to Sarnat stage of HIE is summarised in Table 7.

	HIE Stage1 (n=4)	HIE Stage 2 (n=50)	HIE Stage 3 (n=11)
Normal outcome	1 (25%)	24 (48%)	1 (9%)
Adverse outcome (combined)	1 (25%)	23 (46%)	10 (91%)
Death	-	2 (4%)	6 (55%)
CP	-	8 (16%)	2 (18%)
Intellectual disability	-	3 (6%)	-
BIF	1 (25%)	6 (12%)	-
Profound hearing impairment	-	-	1 (9%)
CVI or other significant VI	-	2 (4%)	1 (9%)
ADD/ADHD			
o Confirmed	0	3 (6%)	1 (9%)
o Suspected	1 (25%)	6 (12%)	-
ASD	0	2 (4%)	-
DCD	0	4 (8%)	1 (9%)
Dyslexia	0	2 (4%)	1 (9%)
Lost to FU			
o Before age 6	2 (50%)	3 (6%)	0
o At age 6-8	0	0	0
o At age 10-12	0	3 (6%)	0

Table 7. Overview of long-term outcomes in relation to Sarnat grade of HIE. Total number of patients N=65. Overlap between diagnoses possible. Abbreviations: HIE, Hypoxic-Ischaemic Encephalopathy; CP, Cerebral palsy; BIF, Borderline Intellectual Functioning; ADD/ADHD, Attention Deficit Disorder/Attention Deficit and Hyperactivity Disorder; ASD, Autism Spectrum Disorder; DCD, Developmental Coordination Disorder; FU, Follow Up.

4.2.1.1 Neurologic outcome

Among study participants who survived to discharge from the NICU, ten (17%) later developed CP. One child with severe CP and epilepsy died at one year of age, bringing the total mortality in our cohort to 12%. Table 8 summarises Sarnat grade of HIE, subtype of CP, GMFCS level as well as neurologic co-morbidities. Most children with CP also had epilepsy (60%).

Sarnat stage of HIE	Subtype of cerebral palsy	Additional neurology co-morbidities
Severe	Bilateral spastic, GMFCS 1	
Severe	Dyskinetic, GMFCS 5	Epilepsy, CVI
Moderate	Unilateral spastic, GMFCS 1	Epilepsy, CVI, FSIQ < 85
Moderate	Dyskinetic, GMFCS 5	Epilepsy, severe intellectual disability
Moderate	Unilateral spastic, GMFCS 1	
Moderate	Unilateral spastic, GMFCS 1	ASD
Moderate	Unilateral spastic, GMFCS 1	Epilepsy, mild intellectual disability
Moderate	Dyskinetic, GMFCS 3	
Moderate	Bilateral spastic, GMFCS 5	Epilepsy, *ad mortem at one year of age*
Moderate	Dyskinetic, mixed form, GMFCS 5	Epilepsy, severe intellectual disability

Table 8. Subtype and severity in children with cerebral palsy. Any additional neurologic comorbidities are also listed here. Abbreviations: GMFCS, Gross Motor Function Classification System; CVI, Central/Cortical Visual Impairment; FSIQ, Full-Scale Intelligence Quotient; ASD, Autism Spectrum Disorder.

By early school age three children had been diagnosed with DCD. Upon repeated assessment in early adolescence, two more children were diagnosed with DCD, resulting in an incidence of 9% in our cohort.

A MABC-2 result ≤ 15th percentile was seen in 11/44 children (25%) at early school age. By early adolescence, this increased to 13/44 (30%), which is significantly higher compared to norms ($p < 0.006$). Findings are summarized in Table 9.

Motor Assessments	≤ 15th percentile	%	95% CI	p-value
MABC-2 at 6-8 years of age (n)	11/44	25	0.132 – 0.403	0.050
MABC-2 at 10-12 years of age (n)	13/44	30	0.168 – 0.452	**0.006**
McNemar test				0.791

Table 9. MABC-2 results at 6-8 and 10-12 years of age, children scoring at or below the 15th percentile, indicating a child at risk of motor disability (all children attended both assessments). Children with a history of mild/moderate/severe HIE, also including children with mild CP for whom the test could be administered. Abbreviations: MABC-2, Movement Assessment Battery for Children, 2nd edition; CI, Confidence Interval.

4.2.1.2 Developmental outcome and FTF questionnaire findings

By early school age, six children without CP experienced other difficulties affecting everyday life. Three children had a diagnosis of ADHD, one of them also had dyslexia and one also had DCD. One child had ASD. One child had profound bilateral hearing loss, requiring amplification. One child had unilateral blindness.

Upon repeated assessment in early adolescence, executive difficulties indicative of possible ADD/ADHD were evident in seven more children.

The FTF questionnaire results were unremarkable compared to norms at early school age. By early adolescence, however, the proportion of children with a score > the 90th percentile,

indicating obvious difficulties, was significantly increased compared to norms within several domains: Motor skills, Executive functions, Perception, Memory, and Language. Findings are summarised in Table 10.

	At age 6-8 years			**At age 10-12 years**		
Domain	>90th perc n (%)	95% CI for %	p-value	>90th perc n (%)	95% CI for %	p-value
Motor skills	6 (13.6%)	5.3 - 27.9	n.s.	13 (28.9%)	18.2 – 46.6	**<0.001**
Executive functions	4 (9.1%)	2.6 – 22.1	n.s.	15 (33.3%)	20.0 – 49.0	**<0.001**
Perception	2 (4.5%)	0.6 – 15.8	n.s.	12 (26.7%)	14.6 – 41.9	**<0.001**
Memory	5 (11.4%)	3.9 – 25.1	n.s.	13 (28.9%)	16.4 – 44.3	**<0.001**
Language	5 (11.4%)	3.9 – 25.1	n.s.	9 (20.0%)	9.6 – 34.6	**0.023**
Learning	6 (13.6%)	5.3 – 27.9	n.s.	8 (17.8%)	8.0 – 32.1	n.s.
Social	4 (9.1%)	2.6 – 22.1	n.s.	8 (17.8%)	8.0 – 32.1	n.s.
Emotional	3 (6.8%)	1.5 – 19.1	n.s.	8 (17.8%)	8.0 – 32.1	n.s.

Table 10. Proportion of children scoring > 90th percentile within the separate domains of the FTF questionnaire at age 6-8 and 10-12 years of age, respectively. Abbreviations: CI, Confidence Interval; n.s., not significant; perc, percentile.

4.2.1.3 Cognitive outcome

At early school age, 46 children completed WISC-IV. Mean FSIQ was 104 (SD 10.8, 95% CI 100.8 – 107.2). Mean Index scores were all within normal range. Borderline intellectual function was found in one child with ADHD and dyslexia. Children with either a diagnosis of or suspected ADD/ADHD had a mean FSIQ (89.3, SD 6.2, 95% CI 79.4 -99.1), which was significantly lower than children without such difficulties whose mean FSIQ was 105.4 (SD 10.1, 95% CI 102.2 – 108.5), ($p < 0.05$). They also had significantly lower Perceptual reasoning, Working memory, and Processing speed Index scores ($p < 0.05$).

In early adolescence, 45 children completed WISC-V. At the time of this assessment, mean FSIQ was 100.9 (SD 16.3, 95% CI 96 – 105.8). Mean Index scores were all within normal range. As was observed at the previous assessment, children with a diagnosis of or suspected ADD/ADHD had significantly lower mean FSIQ (91.6, SD 13.7, 95% CI 82.4 -100.9) compared to children without such difficulties whose mean FSIQ was 103.9 (SD 16.1, 95% CI 98.3 -109.5), ($p < 0.05$). They also had significantly lower mean Verbal reasoning and Processing Speed Index scores, ($p < 0.05$).

We found no significant difference when comparing boys to girls on either occasion. Mean Perceptual reasoning/Visual spatial Index score was significantly lower when the children were assessed at age 10-12 years (102.9, SD 15.2, 95% CI 98.3 – 107.5) compared to at age 6-8 years of age (106.7, SD 11.5, 95% CI 103.5 – 110.4). Figure 5 illustrates the distribution of FSIQ scores on both assessment occasions. Group level results from both assessment occasions are summarised in Table 11. At age 10-12 years, two children with previous diagnoses (ADHD and DCD in the case of one child, and CP, CVI and epilepsy in the case of

the second child) were found to have BIF. Furthermore, three children with favourable outcome at early school age now had borderline intellectual function. One child with CP (GMFCS 5) and CVI took the tests with the aid of an assistant. Testing revealed a cognitive ability equivalent to two-three years below the chronological age. Another child with CP (GMFCS 3) was not able to complete all the included tests, but those that could be completed indicated normal intelligence. Three children with CP (GMFCS 1) had results within normal range on both occasions.

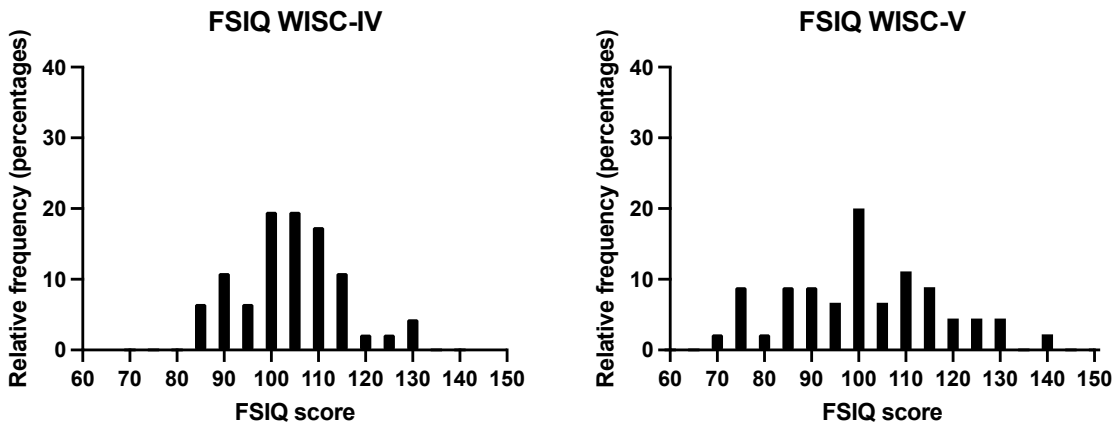

Figure 5. Histogram illustrating frequency distribution (depicted as relative frequency in percentages) of FSIQ scores among participants who completed WISC-IV (n=46) and WISC-V (n=45). Abbreviations: FSIQ, Full-Scale Intelligence Quotient; WISC, Wechsler Scales of Intelligence in Children.

Index	At age 6-8 years	At age 10-12 years	p-value
FSIQ			
○ **All**	103.8 (10.9, 100.4 – 107.1)	101.5 (16.6, 96.7 – 106.4)	n.s.
○ **Boys**	104.2 (12.3, 98.7 – 109.6)	100.7 (16.8, 93.3 – 108.2)	n.s.
○ **Girls**	103.3 (9.6, 99 – 107.6)	102.3 (15.3, 95.5 – 109.1)	n.s.
Verbal comprehension			
○ **All**	102.6 (9.4, 100.8 – 106.5)	104.1 (17.6, 98.7 – 109.4)	n.s.
○ **Boys**	104.8 (9.6, 100.6 – 109.1)	104.2 (17.6, 96.4 – 112)	n.s.
○ **Girls**	102.5 (9.2, 98.4 – 106.5)	103.9 (18.1, 95.9 – 111.9)	n.s.
Perceptual reasoning/Visual-spatial			
○ **All**	107 (11.5, 103.5 – 110.4)	102.9 (15.2, 98.3 – 107.5)	**< 0.05**
○ **Boys**	108.1 (12.8, 102.5 – 113.8)	102.3 (16.3, 95 – 109.5)	**< 0.05**
○ **Girls**	105.8 (10.1, 101.4 – 110)	103.5 (14.5, 97.1 – 109.9)	n.s.
Processing speed			
○ **All**	98.8 (16.4, 93.9 – 103.8)	100.8 (16.5, 95.8 – 105.9)	n.s.
○ **Boys**	98.5 (18, 90.5 – 106.5)	98.9 (13.6, 92.8 – 104.9)	n.s.
○ **Girls**	99.2 (14.9, 92.6 – 105.8)	102.8 (19.2, 94.3 – 111.3)	n.s.
Working memory			
○ **All**	98 (10.6, 94.7 – 101.2)	98.5 (12.4, 94.7 – 102.3)	n.s.
○ **Boys**	97 (12.8, 91.4 – 102.7)	97 (14.8, 90.4 – 103.6)	n.s.
○ **Girls**	98.9 (8.2, 95.3 – 102.6)	100 (9.6, 95.8 – 104.2)	n.s.
Fluid reasoning			
○ **All**	-	97.7 (14.7, 93.3 – 102.2)	-
○ **Boys**	-	98.3 (15.7, 91.5 – 105.1)	-
○ **Girls**	-	97.2 (14, 91 – 103.4	-

Table 11. Comparison of WISC-IV and WISC-V scores on a group level and by sex for all children who completed testing on both occasions (n=44), including children with a diagnosis of cerebral palsy. Results reported as mean value (with SD and 95% CI within brackets). A paired t-test was used to compare results between the two assessment occasions. Abbreviations: FSIQ, Full-Scale Intelligence Quotient.

STUDY III

The aim of this study was to investigate incidence and severity of AKI, using the neonatal modified KDIGO definition, in infants treated with TH due to HIE. We also investigated the association between AKI and neonatal outcomes. Furthermore, we assessed GFR as estimated from creatinine using the Schwartz-Lyon equation at 10-12 years of age. A total of 66 infants treated with TH due to HIE at Karolinska University Hospital between January 2007 and December 2009 were included. One infant was excluded as medical records of laboratory values were unavailable. Another infant was excluded due to a genetic syndrome. At the age of 10-12 years, five children were lost to follow-up due to moving abroad. Four

families declined participation in this assessment. Patient demographic characteristics at baseline are summarised in Table 12.

Patient characteristics	
Gestation age at birth in weeks + days, median (IQR; min-max)	40+2 (38+6 – 41+2; 34+0 – 42+1)
Birth weight in grams, median (IQR; min-max)	3498 (3145 – 4055; 2020 – 5828)
Apgar at 5 min, median (IQR, min-max)	3 (0 – 4; 0 – 10)
Apgar at 10 min, median (IQR, min-max)	4 (2 – 6; 0 – 10)
Sex (boys/girls)	31/34
Inborn/outborn	33/32
Lowest pH within 60 min, median (IQR; min-max)	6.9 (6.8 – 6.9; 6.5 – 7.3)
Lowest BE within 60 min, median (IQR; min-max)	-19 (-22.5 - -14; -36 - -4)
Age at start of TH in hours, median (IQR; min-max)	4 (2 – 5; 1 – 13)
Sarnat stage of HIE o Mild o Moderate o Severe	 4 (6%) 50 (77%) 11 (17%)

Table 12. Baseline patient demographic characteristics. N=65. Abbreviations: BE, base excess, TH, therapeutic hypothermia; IQR, interquartile range; min, minimum; max, maximum. Adapted from Robertsson Grossmann et al, Acute kidney injury in infants with hypothermia-treated hypoxic-ischaemic encephalopathy: An observational population-based study, ACTA Paediatrica 2022, with permission [265].

4.2.2 Creatinine trajectory in the neonatal period

Peak creatinine was observed within 48 hours after birth in 51 infants. The peak was then followed by a subsequent decline. Only three infants were found to have increasing creatinine after day three of life. In the case of two of these infants, creatinine peaked on day five at 260 and 248 µmol/L, respectively. In the third infant, a peak creatinine of 387 µmol/L was observed on day seven. On a group level, median creatinine reached a peak on day one after admission. A steady decline subsequently followed over the course of ten days. Findings are illustrated in Figure 6.

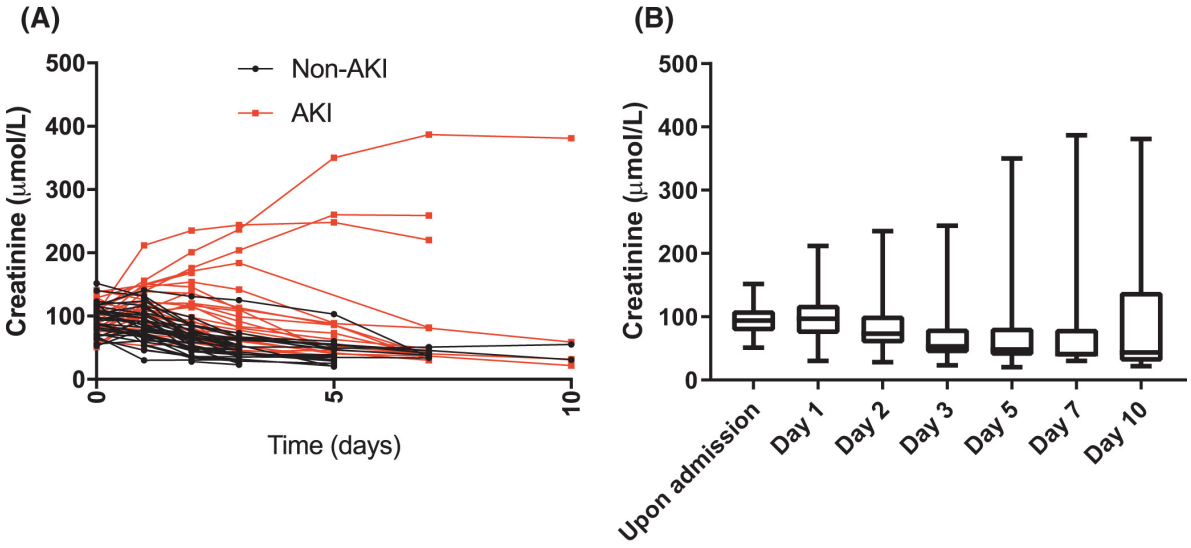

Figure 6. (A) Individual plasma creatinine values in µmol/L in infants with and without AKI from time of admission to the NICU until 10 days after birth. (B) Plasma creatinine levels in µmol/L (Median with range) from time of admission to the NICU until 10 days after birth. Abbreviations: AKI, Acute Kidney Injury. Reprint from Robertsson Grossmann et al, Acute kidney injury in infants with hypothermia-treated hypoxic-ischaemic encephalopathy: An observational population-based study, ACTA Paediatrica 2022, with permission [265].

4.2.3 Incidence of AKI

Twenty-nine infants (45%) developed AKI (any stage). Of them, nine infants (14%) fulfilled creatinine as well as UO criteria, whereas seven infants (11%) fulfilled only the creatinine criteria, and 13 infants (20%) fulfilled only the UO criteria. Findings are summarised in Table 13. Twenty-two infants (34%) were found to have suffered stage 1 AKI. Two infants (3%) had stage 2 AKI. Five infants (8%) had stage 3 AKI. One infant needed KST (continuous veno-venous haemodiafiltration) due to FO and electrolyte disturbances between days three to six of life. On days one and two of life, his creatinine peaked at 101 µmol/L. AKI in the remaining 28 infants was treated conservatively.

		Plasma creatinine AKI Status		
		No	Yes	Missing
UO AKI Status	No	36 (55%)	7 (11%)	
	Yes	13 (20%)	9 (14%)	
	Missing			1*

*Table 13. Absolute number of patients and percentage (within brackets). Patients fulfilling nKDIGO criteria for AKI highlighted in grey. *Excluded from all calculations due to unavailable data. Abbreviations: AKI, Acute Kidney Injury; UO, Urinary Output. Adapted from Robertsson Grossmann et al, Acute kidney injury in infants with hypothermia-treated hypoxic-ischaemic encephalopathy: An observational population-based study, ACTA Paediatrica 2022, with permission [265].*

4.2.4 AKI, other neonatal morbidity and mortality

Seven infants died before discharge from the NICU. Infants with AKI had significantly higher mortality (19%) compared to those without (8%) ($p < 0.05$). Infants with AKI were

also more likely to need transfusion of blood products than infants without AKI ($p < 0.05$). No difference between the two groups was observed with regards to need for intubation or need for full cardiopulmonary resuscitation after birth. Furthermore, we found no significant difference in duration of mechanical ventilation, incidence of persistent pulmonary hypertension of the new-born (PPHN), need for high-frequency oscillation ventilation (HFOV) or inhaled nitric oxide (NO), or need for inotropic support when comparing infants with AKI to those without. Findings are summarised in Table 14.

	No AKI (n=36)	**AKI (n=29)**	**p-value**
Neonatal mortality	1 (8%)	6 (19%)	**< 0.05**
Total mortality	2 (10%)	6 (19%)	n.s.
LOS, median (IQR)	11 (8-18)	13 (10-15)	n.s.
Need for KST	0 (0%)	1 (3%)	n.s.
Need for cardiac compressions during resuscitation	18 (50%)	19 (66%)	n.s.
Need for intubation during resuscitation	28 (78%)	21 (72%)	n.s.
Duration of MV in hours, median (IQR)	12 (1-70)	31 (1-105)	n.s.
Need for HFOV	5 (14%)	9 (31%)	n.s.
PPHN	6 (17%)	7 (24%)	n.s.
Need for inhaled NO	1 (3%)	4 (14%)	n.s.
Need for inotropic support	12 (33%)	15 (52%)	n.s.
Need for transfusion of blood products	25 (69%)	27 (93%)	**< 0.05**

Table 14. Incidences are reported in number of patients (with percentage within brackets) for dichotomous data. Median values (with IQR within brackets) are reported for non-categorical data. Abbreviations: AKI, Acute Kidney Injury; LOS, length of stay; KST, kidney support therapy; MV, mechanical ventilation; IQR, inter-quartile range; HFOV, high-frequency oscillation ventilation; PPHN, persistent pulmonary hypertension in the new-born; NO, nitric oxide; n.s., not significant. Adapted from Robertsson Grossmann et al, Acute kidney injury in infants with hypothermia-treated hypoxic-ischaemic encephalopathy: An observational population-based study, ACTA Paediatrica 2022, with permission [265].

4.2.5 AKI in relation to HIE severity

Two of the four infants with Sarnat stage 1 HIE suffered stage 1 AKI. Among the 50 infants with Sarnat stage 2 HIE, 15 (30%) suffered stage 1 AKI and three (6%) suffered stage 3 AKI. Among the 11 infants with Sarnat stage 3 HIE, six (55%) developed stage 1 AKI, two (18%) developed stage 2 and one (9%) developed stage 3 AKI. We found increased severity of HIE to be associated with more severe AKI ($p < 0.05$).

4.2.6 Renal function at age 10-12 years

Forty-eight children took part in a follow-up assessment at 10-12 years of age. One child had been excluded due to a genetic syndrome. Five families had emigrated. Four families actively declined participation. It was not possible to obtain a blood sample in the case of five children. Ultimately, information on neonatal AKI status as well as body height and creatinine at age 10-12 years was available for 42 patients (72% of all survivors in our cohort). Median plasma creatinine-based eGFR using the Schwartz-Lyon equation was 99.5 ml/min/1.73m^2 (IQR 90-109.8 ml/min/1.73m^2). Nine participants (21%) were found to have an eGFR below 90 ml/min/1.73m^2. Their eGFR ranged between 71 and 89 ml/min/1.73m^2.

The median eGFR and the incidence of decreased eGFR did not differ between children with and without a history of neonatal AKI. Boys and girls had similar median eGFR.

4.3 STUDY IV

The aim of this study was to describe renal outcomes following perinatal asphyxia and TH in greater detail. As reported in Study III, 48 children participated in a follow-up assessment at 10-12 years of age. Among them, 20/47 (43%) had a history of neonatal AKI as per the neonatal modified KDIGO definition. Blood samples could be obtained from 42 children and urine samples from 46 children. Forty-five children had their blood pressure measured and 32 children underwent MRI of the kidneys.

4.3.1 eGFR, albuminuria, and blood pressure

Decreased cyst C eGFR was found in two children (2/47). Subsequent examination with iohexol clearance confirmed decreased GFR (88 ml/min/1.73 m^2) in one child who had needed kidney support therapy in the neonatal period. KDIGO grade 2 albuminuria was observed in one child (1/46). This child had a urine albumin/creatinine ratio of 10.5 mg/mmol. An elevated office BP was observed in three children (3/45). Upon examination with ambulatory 24-hour BP-measurement, one of these children had high normal BP, whereas the other two had BP within normal range.

4.3.2 Renal volume on MRI

Fifteen of the 32 children who were examined with MRI (47%) had a history of neonatal AKI. In the case of one child, we found a unilateral hypoplastic kidney and compensatory enlargement of the contralateral kidney. The total kidney volume of this child was within normal range. Another participant was found to have bilateral minimal parenchymal scarring that did not affect the total kidney volume. Using nomograms based on ultrasonographical approximation of renal volume (in turn based on renal length using the ellipsoid formula), no child was found to have reduced renal volume. Mean Z-score was similar when comparing children with a history of neonatal AKI (-0.08, SD 1.18, 95% CI -0.74 – 0.57) to children without a history of neonatal AKI (0.09, SD 0.79, 95% CI -0.33 – 0.51). Figure 7 illustrates individual Z-scores according to neonatal AKI-status.

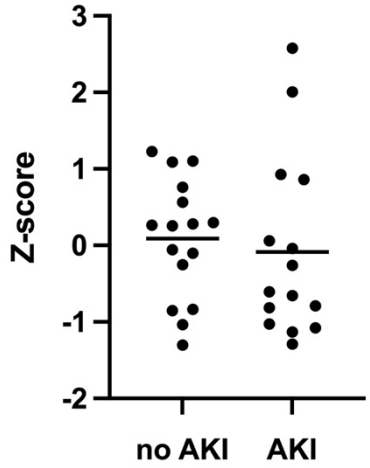

Figure 7. Individual total renal volume Z-score at 10-12 years of age according to neonatal AKI-status. Abbreviations: AKI, Acute Kidney Injury.

4.3.3 Fibroblast Growth Factor 23

Median FGF 23 value for all participants was 36.25 pg/ml (IQR 27.5 – 44.73 pg/ml). There was no significant difference in median FGF 23 values when comparing children with a history of neonatal AKI (median FGF 23 37.9 pg/ml, IQR 29.73 – 52.55 pg/ml) to those who had not suffered neonatal AKI (median FGF 23 34.85 pg/ml, IQR 26.38 – 42.88 pg/ml). No child was found to have a FGF 23 value above the 90th percentile for age [320, 321]. Figure 8 illustrates individual FGF 23 values according to neonatal AKI status.

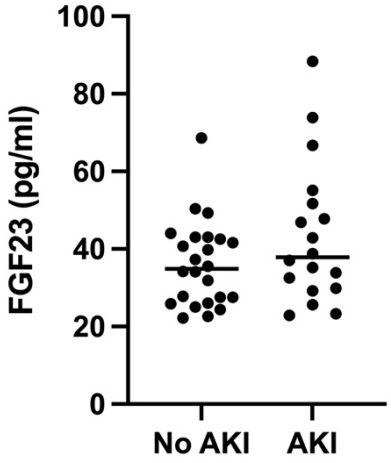

Figure 8. Individual FGF 23 levels in pg/ml at 10-12 years of age according to neonatal AKI-status. Abbreviations: AKI, Acute Kidney Injury, FGF, Fibroblast Growth Factor.

Table 15 summarises renal outcome findings.

	History of AKI (n=20)	No history of AKI (n=27)	p value
Cyst C eGFR <90 ml/min/1.73m^2 ○ GFR <90 ml/min/1.73m^2 confirmed by iohexol clearance	1/18 ○ 1	1/24 ○ 0	n.s.
KDIGO category A1 albuminuria	16/17	25/26	n.s.
KDIGO category A2 albuminuria	1/17	0/26	n.s.
KDIGO category A3 albuminuria	0/17	0/26	n.s.
Hypertension	0/20	0/25	-
High normal BP	0/20	1/25	-
Renal volume (total), mean Z-score (SD, 95% CI)	-0.08 (1.18, -0.74 – 0.57)	0.09 (0.79, -0.33 – 0.51)	n.s.
FGF 23 I pg/ml, median (IQR)	37.9 (29.73 – 52.55)	38.85 (26.38 – 42.88)	n.s.

Table 15. Summary of renal outcome characteristics at 10-12 years of age according to neonatal AKI-status. Abbreviations: Cyst C eGFR, cystatin C-estimated glomerular filtration rate; KDIGO, Kidney Disease: Improving Global Outcomes; BP, Blood Pressure; SD, standard deviation; CI, confidence interval; IQR, inter-quartile range, FGF, Fibroblast Growth Factor; n.s., not significant.

5 DISCUSSION

5.1 MAIN FINDINGS AND INTERPRETATIONS

5.1.1 The prognostic value of early aEEG in infants treated with TH due to HIE

This small population-based study was the first to demonstrate an altered predictive value of early aEEG in asphyxiated infants treated with TH. Normal outcome at 1 year of age was observed in 10/15 infants with a severely abnormal burst-suppression background pattern on aEEG at 6 hours post-partum. Severe aEEG abnormalities were significantly predictive for poor outcome (defined as overt signs of spasticity on neurologic examination or an AIMS score below the 5th percentile) after 36 hours. In the study by Thoresen et al, an abnormal aEEG background pattern at 3-6 of age had a positive predictive value of 86% in infants treated with normothermia compared to 59% in infants treated with TH. The time to normalisation of the aEEG background pattern was found to be the strongest predictor of outcome at 18 months of age. Furthermore, normalisation of the aEEG background pattern within 48 hours after birth was associated with favourable outcome among infants treated with TH. Prior to the advent of TH, an abnormal aEEG background pattern had a high predictive value for adverse neurodevelopmental outcome as early as before 6 hours post-partum in asphyxiated infants [70, 71] and had high impact on discussions regarding re-direction of care. The findings in our study and the study by Thoresen et al challenged this. More recently, a meta-analysis demonstrated that a persistently abnormal background pattern on aEEG at 48 hours of age or beyond was associated with adverse neurodevelopmental outcome (defined as death or moderate/severe disability at 1 year of age) [74]. The time to recovery of the background pattern on aEEG has been proposed as an alternative prognostic marker in hypothermia-treated infants with HIE [72, 73].

5.1.2 Long-term outcomes after HIE treated with TH

In this population-based study on long-term outcomes after hypothermia-treated HIE, survivors free of major neuromotor impairment were found to have a mean FSIQ within normal range. In early adolescence, four children with previously normal FSIQ had borderline intellectual function. At early school age, 67% of survivors had favourable outcome and 33% had adverse outcome. Upon repeated assessment in early adolescence, nine children (26%) with previously favourable outcome had developed seemingly new deficits. The number of children with a diagnosis of DCD increase from three to five between assessment occasions, bringing the incidence to 9%. Results of the MABC-2 and FTF questionnaire were unremarkable at early school age. In early adolescence, however, the proportion of children with a MABC-2 score < the 15th percentile was significantly increased compared to norms. Similarly, the proportion of children with an FTF questionnaire score indicating obvious difficulties was significantly increased compared to norms within several domains that may negatively impact educational outcomes. These findings align with the number of children with diagnosed or suspected ADD/ADHD increasing from four to eleven

between the two assessment occasions. By early adolescence, 19% of survivors in our cohort experiences difficulties indicative of ADD/ADHD. In comparison, the prevalence of ADD/ADHD in the general population is reported to be 5 to 7 per cent [333]. Children with diagnosed or suspected ADD/ADHD had significantly lower FSIQ on both assessment occasions compared to children without executive difficulties. In our cohort, the incidence of CP was 17%. This is somewhat lower than reported in the TOBY study (21%) [155] but similar to results in the NICHD study [87]. Among survivors with CP, 60% also suffered epilepsy. The mortality in our cohort (12%) was lower than reported in the TOBY and NICHD studies (29% and 26%, respectively), possibly due to the smaller proportion of infants with Sarnat stage 3 (severe) HIE. Except for one child, all children with a history of Sarnat stage 3 (severe) HIE had adverse outcomes. Outcomes after Sarnat stage 2 (moderate) HIE remain heterogenous in the era of TH, with 46% of children experiencing difficulties. In our cohort, four infants with Sarnat stage 1 (mild) HIE had been cooled. Two of them were later lost-to follow up but one child was found to have borderline intellectual function and executive difficulties. One of four children born at < 36 weeks' gestation who were nevertheless treated with TH died in the neonatal period, one was lost to follow-up, another child developed CP and epilepsy, and one child had borderline intellectual function in early adolescence.

Our findings indicate that more subtle impairments are common among survivors of HIE free of major neuromotor disability also in the era of TH. These findings align with recent reports describing that survivors of HIE treated with TH are less school ready than their healthy peers [177] and more frequently exhibit neuropsychological and cognitive sequelae [178]. Executive difficulties appear to be more common in this patient population than in the general population. Assessment around early school age may not allow for detection of more subtle impairments. Extended follow-up with repeated assessment in adolescence may therefore be warranted in this vulnerable patient population.

5.1.3 AKI in infants with hypothermia-treated HIE

In this population-based study, AKI remains a common complication among infants with HIE also in the era of TH. In our cohort, 45% of infants suffered AKI. Among infants with AKI, stage 1 was the most common. Severe AKI necessitating KST was rare. Only one child needed KST for a duration of three days. Peak creatinine was observed early in the first week of life. Interestingly, we observed a clearly elevated creatinine already at the time of NICU admission in the case of five infants. Despite creatinine values ranging between 116 and 152 µmol/L, these patients did not fulfil neonatal modified KDIGO creatinine criteria for AKI. A subsequent decline in creatinine was evident already on the next day in three of these infants. In two infants, creatinine continued to increase also on day two of life, remaining > 100 µmol/L also on day three and five of life, respectively. Three of these children were found to have AKI based on the UO criterion. Gupta et al have suggested an alternative approach for identifying AKI in this patient population, based on an expected rate of decline of creatinine during the first week of life rather than absolute or relative increases in creatinine [198].

Although there is international consensus for the neonatal modified KDIGO definition, there are infants with clearly impaired renal function who may still escape detection using this definition. In our cohort, 20% of infants with AKI were diagnosed based solely on the UO criterion. Historically, AKI in asphyxiated infants has been reported to be mainly non-oliguric [211]. Our findings indicate the importance of applying both criteria to avoid patients with AKI escaping detection. We found a similar incidence of AKI in our cohort compared to studies done before the advent of TH [208, 211, 334] and the largest multi-centre study of neonatal AKI in patients with NE to date [214]. The AWAKEN substudy, unfortunately, did not report severity of HIE or proportion of study participants who were treated with TH. Thus, comparisons are hampered. In comparison with studies by Selewski et al [212] and Bozkurt et al [213], the AKI incidence was somewhat higher in our cohort. AKI was associated with increased neonatal mortality, a finding that aligns with other reports. At age 10-12 years, mean creatinine-based eGFR was normal. We observed no difference in mean eGFR between children with and without a previous history of neonatal AKI. However, 21% of survivors were found to have decreased eGFR. There was no difference in the proportion of children with decreased eGFR when comparing children with and without a history of neonatal AKI.

We noted that although 29 infants had suffered AKI, only three infants had received a formal AKI diagnosis at the time of discharge from the NICU and none had been referred for follow-up with a paediatric nephrologist or elsewhere. Our findings align with several other reports [224, 225], indicating that AKI is an underrecognized and underreported entity in NICUs.

5.1.4 Renal outcomes in early adolescence following perinatal asphyxia and TH

In this prospective, population-based study we explored long-term renal outcomes in young adolescents with a history of perinatal asphyxia, HIE and TH. Decreased GFR, albuminuria, hypertension/elevated BP, and/or reduced renal volume assessed by MRI were rare at 10-12 years of age.

As reported in Study III, 21% of survivors in our cohort had decreased eGFR as estimated with the Schwartz-Lyon equation at this age. A more in-depth assessment of renal function could only confirm decreased cyst C eGFR in 5%, and decreased GFR on iohexol clearance in 2.5% of survivors in our cohort. Serum creatinine is widely used to estimate GFR, however, it can underestimate or overestimate GFR in certain populations. This has been discussed in a large cohort of apparently healthy adolescents [335]. The most commonly used Schwartz-Lyon equation was derived from paediatric CKD-patients. Equations developed in populations with decreased GFR have been suggested to underestimate GFR in adults, adolescents and children without renal disease [336]. This further supports the need for a more comprehensive assessment of renal function.

Very little is known about long-term renal outcomes after perinatal asphyxia and HIE, also from the pre-hypothermia era. Several studies on long-term renal outcomes have, however,

been done in other NICU-populations. In a cohort of children born before 28 weeks' gestational age, Sanderson et al reported that 50% had reduced renal volume, grade A2 albuminuria, and/or elevated BP at 15 years of age [337]. The proportion of children with renal sequelae was somewhat higher in this study compared to prior studies involving children with a history of preterm birth [338]. Recently, a systematic review on the effect of prematurity on long-term renal outcomes found that prematurity is associated with an increased risk for renal dysfunction and elevated/high BP in childhood and early adulthood [339]. A large study based on Swedish registry data reported that extremely preterm birth conferred a near-threefold increase in the risk of CKD, whereas preterm birth conferred a twofold increase in the risk of CKD [340]. New-born infants undergoing cardiac repair surgery constitute another NICU-population at high risk of AKI [341]. Huynh et al reported that 17% of patients had CKD, and 30% had hypertension at a median follow-up age of 6 years. An additional 15% of patients were found to have elevated BP [276].

No child in our cohort had reduced total renal volume. We measured renal volume using MRI and semi-automatic segmentation. Renal volume was compared to kidney size nomograms that are currently still based on calculation of renal volume by ellipsoid formula on ultrasound [300, 304, 308]. Compared to computed tomography (CT) or MRI segmentation, this method has been demonstrated to underestimate renal volume [309]. To date, there are no existing normal ranges of renal volume based on CT or MRI segmentation methods in paediatric populations, but Park et al determined the mean renal size on CT for each age and height group in 272 Korean children, suggesting that their results may serve as normative standards for assessment of renal growth [312].

Although renal abnormalities were rare in early adolescence among children in our cohort, long-term follow up is still warranted. In a study by Cherchi et al of 312 children with congenital anomalies of the kidney and urinary tract, renal deterioration could not be detected until late adolescence [342].

As CKD is usually asymptomatic in its early stages, there is frequently a delay in the diagnosis. Such a delay is associated with a more rapid disease progression and premature mortality in patients with ESRD [313]. Much research has been devoted to the search for early biomarkers of CKD. FGF 23 is a fairly novel marker of CKD-associated MBD, an important contributor to the increased risk of cardiovascular disease in this patient population [314, 315]. No child in our cohort was found to have FGF 23 above the 90th percentile for age [320]. Median FGF 23 levels did not differ between children with and without a history of neonatal AKI. As none of the children in our study had GFR < 70 ml/min/m^2, it is not surprising that FGF 23 levels were within normal range.

5.2 METHODOLOGICAL CONSIDERATIONS

For all studies included in this thesis, the ambition was to prospectively include all infants with moderate or severe HIE resulting from perinatal asphyxia at Karolinska University Hospital during the study period for the sample to be representative. TH was implemented

into regular clinical practice at Karolinska University Hospital on January 1st, 2007. In the two cohorts included in this thesis, four infants with Sarnat stage 1 (mild) HIE were treated with TH. Karolinska University Hospital did not have a policy of admitting infants with Sarnat stage 1 (mild) HIE for cooling; the fact that these four infants were cooled anyway illustrates one of the challenges associated with the implementation of a novel treatment into regular clinical practice. One can only speculate if these infants were subjected to TH as a result of fear among the treating physicians to miss a patient who might benefit from the therapy. Due to the population-based nature of the studies included in this thesis, we have reported outcomes also in these children with a history of Sarnat stage 1 HIE. In the analyses in Study II, adjusting for mild HIE did not significantly change results. The small sample size is the most obvious limitation with the studies in this thesis. This is related to the low incidence of infants fulfilling both A- and B-criteria for TH.

In Study III, serum creatinine was not measured according to a strict protocol. Furthermore, not all infants had a urinary catheter placed. Measuring UO by weighing diapers is known to be less precise. In Study III and IV, long-term outcome measures were determined at a single visit, which could be argued may not properly reflect a chronic trajectory in this sample. Using Dixon 3D sequences could have further improved measurement reliability.

5.2.1 Errors and biases

In epidemiologic research, errors are categorised as random or systematic. Random errors are unpredictable variations in data and sampling. The risk of random errors can be reduced by increasing the sample size. This is demonstrated statistically by narrower confidence intervals [343]. No sample size calculation was performed for the studies included in this thesis.

Systematic errors are independent of sample size. Often referred to as biases, systematic errors are repeatable and associated with flawed study design, or faulty equipment [343]. In any longitudinal study, loss to follow-up can introduce selection bias. Among non-participants, outcome is unknown. As the association between exposure and outcome remains unknown, selection bias can only be inferred under these circumstances [343]. In Study I, all survivors except for one were examined using AIMS at 4 months of age. Only one child was lost to follow-up at 12 months of age. In Study II, the participation rate among children still residing in Sweden was very high. Outcome was known for 91% of children at early school age, and for 88% of children in early adolescence. In Study III and IV, five children did not agree to a blood sample and one child did not prove a urine sample. In the case of two children with CP, it was not possible to obtain a reliable BP measurement due to involuntary movements. Among the 47 children (for whom neonatal AKI status was known) who took part in the assessment of renal functions in early adolescence, 32 children underwent MRI examination to measure renal volume. No missing data were imputed. Missing data can introduce bias. All studies included in this thesis are population-based, including all children who were treated with TH in the greater Stockholm region within a specific time frame (December 2006 to December 2007 for Study I, January 2007 to December 2009 for Study II, III and IV). This minimised the risk of introducing selection bias at the time of recruitment.

Bias resulting from the introduction of erroneous data from study subjects is called information bias [343]. For the studies in this thesis, prospectively collected perinatal data has subsequently been retrospectively validated. For Study I, interpretation of the dominating aEEG background pattern was done by two different assessors, both blinded to the other assessor's interpretation, with full congruency. For Study II, three psychologists performed the psychometric assessments. All assessors had been trained in the administration of the WISC-IV and WISC-V before performing any study assessments in order to minimise the risk of introducing information bias or confounding as a result of differences in performance. Neurologic examination and MABC-2 assessment were performed by the same assessors at early school age and in early adolescence. The paediatric neurologist and the paediatric physiotherapist performing the assessments were not blinded to the neonatal medical history of study participants. For Study IV, the radiologist calculating renal volume on MRI and the laboratory staff performing ELISA-analysis to measure FGF 23 levels were blinded to neonatal history and renal function at age 10-12 years.

Bias introduced by mixing the effect of the exposure with the effect of another variable is referred to as confounding [343]. In Study II, we report an incidence of executive difficulties suggestive of ADD/ADHD that appear to be substantially higher compared to the normal population [333]. We did not adjust for a parental diagnosis of ADD/ADHD or ASD. Genetics are known to play an a role in the aetiology of these conditions [344, 345]. Furthermore, we did not adjust for maternal level of education. Higher maternal educational level is known to be associated with better verbal skills and greater academic achievement in children [346, 347]. At the time of assessment around early school age, parental level of education was known. At the time of the repeated assessment, however, we did not inquire about this again. It is thus possible that parental level of education could have increased between assessments.

5.2.2 Strengths

Study I was the first study to demonstrate an altered prognostic value of the dominating background pattern on aEEG in infants treated with TH. Shortly after we had published our results, Thoresen et al reported similar findings. Among infants treated with TH, the positive predictive value of an abnormal aEEG background pattern at 3-6 hours of age was 59% compared to 84% among infants in the control group. Time to recovery of the aEEG background pattern was found to be the best predictor of adverse outcome at 18 months of age. Failure to develop SWC always predicted adverse outcome. A normalisation of the dominating background pattern on aEEG by 48 hours of age was associated with favourable outcome [73]. Recently, a meta-analysis of nine studies and a total of 520 study participants treated with TH concluded that a persistently abnormal background pattern on aEEG (defined as DNV, BS, flat trace or persistently low voltage) at 48 hours or more after birth is associated with adverse developmental outcome (defined as death or moderate/severe disability at 1 year of age). The positive predictive value of aEEG at 6 hours of age is poor in infants treated with TH. While a normal background pattern on aEEG at 6 hours of age has a

good negative predictive value, it does not exclude adverse outcomes [74]. These findings have influenced clinical practice, and a decision to redirect care is no longer based upon an abnormal aEEG background pattern at 6 hours of age.

Study II is, to the best of the author's knowledge, the first to evaluate outcomes in early adolescence after HIE treated with TH. Strengths of this study include its population-based design, standardised assessment approach and high participation rate. The cohort is well investigated and described and has been followed prospectively since birth.

Study III is one of still only few studies evaluating AKI in infants treated with TH due to HIE. Strengths of this study include its population-based design and application of both the creatinine and UO criteria of the neonatal modified KDIGO definition. Study III and IV are, to the author's knowledge, the first to report long-term renal outcomes in a population of adolescents with a history of perinatal asphyxia. An additional strength of Study IV is the multi-modal approach used to assess renal functions.

5.2.3 External validity

The efficacy of TH has been demonstrated in both several pre-clinical and clinical trials [84, 348]. While studies performed in LMICs have demonstrated the feasibility of cooling, they have also reported a significant increase of death among infants treated with TH [349, 350]. The phenotype among infants with encephalopathy described in the HELIX trial differs from what is generally reported in studies done in high-resource settings. In the HELIX trial, a relatively small proportion of infants had evidence of a sentinel event in close temporal proximity to delivery and clinical seizure debut was observed shortly after birth. Furthermore, the proportion of infants with white matter injuries was higher than expected. Although the inclusion criteria were less strict compared to most trials done in high-resource settings, the mortality rate was higher [350]. The findings and conclusions of the HELIX trial have stirred much debate regarding the use of TH in LMICs.

The findings reported in Study I have been shown to hold true also in other high resource settings as demonstrated by a meta-analysis [74]. To the best of the author's knowledge, there are currently no other reports on neurologic, motor, cognitive and behavioural outcomes in adolescence following HIE treated with TH. Thus, similar studies need to be done also in other settings. Studies on outcomes in early school-age have demonstrated that survivors of HIE are at increased risk of adverse clinical, cognitive and neuropsychological outcomes that can negatively affect education outcomes [95]. Over the last decade, AKI in the clinical setting of HIE and hypothermia has received increased attention. A recent meta-analysis concluded that the potential renoprotective effect of TH remains unclear, as comparisons between studies are hampered by the use of several different definition of AKI [217]. Long-term renal outcomes in this patient population remain poorly understood. Further studies extending into adolescence and preferably even adulthood are needed to elucidate long-term effects of perinatal asphyxia and TH on renal outcomes. The results of the studies in this thesis are likely not directly applicable to HIE patient populations in LMICs. All studies

included in this thesis are based on Swedish populations, using the Swedish versions of WISC-IV/V with Swedish/Scandinavian norms, and the FTF with Swedish norms. The MABC-2 has British norms. It is therefore difficult to assess the generalizability to other countries.

6 CONCLUSIONS

The overall aim of this thesis was to contribute to the improved treatment and care of infants with HIE by means of increased knowledge about the predictive value of early aEEG, neonatal AKI, and long-term outcomes in the era of TH.

- Treatment with systemic TH affects the predicitive value of early aEEG in infants with HIE. This needs to be considered in the context of early prognostication and discussions regarding possible re-direction of care.

- Survivors free of major neuromotor impairment after HIE treated with TH had cognitive abilities within normal range. More subtle deficits and difficulties may go undetected when children are assessed around early school-age. Repeated assessment in early adolescence revealed seemingly new deficits in 26% of children with previously favourable outcome. The incidence of executive difficulties appears to be increased in this patient population. Outcomes following moderate HIE remain heterogenous also in the era of TH, with adverse outcome occuring in 46% of children.

- AKI remains a common complication in infants with HIE also in the era of TH, occurring in 45% of infants in our cohort. Severe AKI necessatating KST, however, was rare. Peak plasma creatinine was observed within the first two days of life. A substantial proportion of infants with AKI only fulfilled the UO criterion. Applying only the creatinine criterion of the neonatal modified KDIGO definition may thus result in underrecognition of AKI. Infants with high initial plasma creatinine may also escape detection using this AKI definition. AKI was associated with increased mortality. By early adolescence, 21% of survivors had decreased eGFR as per the Schwartz-Lyon equation.

- A more in depth assessment of renal function demonstrated that renal sequelae were rare at age 10-12 years following perinatal asphyxia and TH. The Schwartz-Lyon equation appears to underestimate GFR in this patient population.

7 POINTS OF PERSPECTIVE

HIE and neuroprotection has received much attention over the last two decades. TH is standard of care for new-born infants with moderate or severe HIE in high-resource settings. Although TH has reduced mortality and improved neurodevelopmental outcomes in this patient population, brain injury and adverse outcomes remain common [84]. The HIE grading scale introduced by Sarnat and Sarnat in 1976 remains in continued use world-wide, albeit sometimes with modifications as in the major RCTs of TH [84]. In 2020, Harvey B. Sarnat himself proposed a revision of the grading scale, adding olfactory response and listing autonomic functions earlier in the table of clinical features to be assessed in the infant [351]. In 2019, Chalak et al performed a secondary analysis of the Prospective Research for Infants with Mild Encephalopathy (PRIME) study [78] with the aim to develop a new scoring system to stratify the risk of adverse outcome at 18-22 months of age [37]. In this secondary analysis, a numerical value was applied to each of the six domains of the NICHD system. The scores assigned for each of the six categories (Level of consciousness, Spontaneous activity, Posture, Tone, Primitive reflexes, Autonomic nervous system) were summed up to generate a total score. They reported that a total Sarnat score had high sensitivity and fair specificity to detect disability at age 18-22 months. Another alternative HIE grading system called the SIBEN Neurological Score has been proposed by Perez et al, intended to be used to assess infants already in the delivery room once the Apgar score is concluded [352]. The SIBEN Neurological Score assesses the same domains as the NICHD system. A study by Walsh et al comparing the two previously mentioned alternative scoring systems reported good agreement between the systems (K=0.86), although the SIBEN Neurological Score defined more infants as having moderate rather than mild HIE than did the NICHD score. Both numerical scores outperformed the standard grades in predicting later brain injury on MRI

Globally, NE is the most common cause of death and acquired brain injury among infants born at term gestational age [353]. Every year, the condition affects approximately 1 million new-born infants, the vast majority (over 90%) occurring low- and middle-income countries (LMICs) [353]. The recent HELIX trial stirred up debate as the authors concluded that, based on their findings, the use TH should immediately be suspended in LMICs [350]. Several concerns have been raised about factors that might have contributed to poorer outcomes reported in this trial [354-357]. The authors reported an earlier onset of seizures as well as a higher-than-expected incidence of white matter injuries, suggestive of antepartum injuries rather than peripartum complications, or possibly acute-on-chronic insults. In both the TH-group and the control group, the reported incidence of perinatal sentinel events was low. Cord pH-measurements were available only for a small proportion of included infants. Furthermore, many of the infants treated with TH were hypothermic already at the time of admission. Also, the abnormal total leukocyte count reported in most infants in both the TH-group and the control group raises the suspicion of possible early onset sepsis. Sepsis has been shown to limit the benefit of TH in both piglet and rat pup models of hypoxia-ischaemia [358, 359]. Further efforts are needed to improve antenatal and obstetric care in LMICs with the hope of preventing perinatal asphyxia.

Much research in the field during the past decade has been dedicated to the early recognition of infants for whom TH may be of benefit [360]. Thus far, no single biomarker sufficiently capable of predicting which new-born infants will develop significant HIE, foresee response to TH or outcome has been identified [360].

Important progress has been made in the clinical and research field of neonatal AKI over the last decade. There is now international expert consensus for a staged AKI definition, based on both absolute and relative increases in creatinine as well as UO, modified to better suit neonatal patient populations [194, 195]. Adopting a uniform definition for neonatal AKI will facilitate comparative research and audit. Moving forward, it is not unlikely that the definition will need to be adapted further for certain patient populations, such as extremely pre-term infants and infants with early onset AKI [173, 197]. The multi-centre AWAKEN-initiative has shed important light on the epidemiology of neonatal AKI [196]. It is also becoming increasingly evident that AKI is not a single organ disease; there are interfaces between the kidneys and several other organ systems [268, 361-363]. Whether or not TH has a reno-protective effect remains debatable. Several studies investigating the possible reno-protective effect and pharmacokinetics of non-selective adenosine receptor antagonists (theophylline, aminophylline, caffeine citrate) in infants with hypothermia-treated HIE are currently ongoing or planned. Already in 2011, KDIGO guidelines suggested that a single dose of theophylline be considered to prevent AKI in infants with HIE [195]. All clinical trials upon which this recommendation is based, however, were done prior to the widespread use of TH in this patient population. Studies have demonstrated association between high doses of theophylline or methylxanthines and increased risk of CNS excitation and seizures [364, 365].

Several studies have demonstrated that AKI is an underrecognized and underreported comorbidity among NICU-patients [220, 223, 224]. Collaborative efforts to integrate neonatologists and nephrologists in the care for our youngest and smallest patients are needed to improve AKI recognition, diagnosis and follow-up in this vulnerable population [225]. Neonates and children who suffer AKI are at increased risk of CKD [222, 269], but many are not monitored for these long-term renal complications [366]. Integration of follow-up programs for individuals with a history of AKI can allow for early detection of renal dysfunction, enabling early initiation of therapies to slow disease progression [367].

The author would like to acknowledge the following planned or active studies in infants with HIE and/or AKI:

- Neonatal Seizure Registry – Developmental Functional Evaluation (clinicaltrials.gov: NCT04337697), currently recruiting. A multi-centre, longitudinal study hoping to enroll 280 participants. Survivors of acute symptomatic seizures will be assessed for development, epilepsy, and parent well-being outcomes at school age. Developmental questionnaires and in-person testing at the age of 5.5 years. Parent well-being will also be assessed. Primary outcome is FSIQ as measured with the Wechsler Preschool and Primary Scales of Intelligence, 4th edition. Main secondary outcome is the change

in scores on the Vineland Adaptive Behavior Scale over time as assessed at multiple consecutive visits between the age of 3-8 years.

- TIME (Therapeutic hypothermia for Infants with Mild Encephalopathy) (clinicaltrials.gov: NCT04176471), not yet recruiting. A multi-centre RCT of TH (33.5°C $\pm$ 0.5°C for 72 hours) versus normothermia in term new-born infants with mild HIE. Primary outcome in this study is neurodevelopmental outcome at age 12-24 months.
- Caffeine for Hypoxic-Ischemic Encephalopathy (clinicaltrials.gov: NCT03913221), currently recruiting. A single-centre interventional trial where the first 9 infants with HIE will receive a loading dose of of caffeine citrate (20 mg/kg) followed by a lower maintenance dose of caffeine citrate (5 mg/mg, two daily doses) and the following 9 infants will receive the same initial loading dose followed by a higher maintenance dose (10 mg/kg, two daily doses). Primary outcome in this study the area under plasma concentration-time at Time t (AUC0-t) for caffeine, 7 samples will be collected. Secondary outcome measures are: the incidence of seizures and necrotizing enterocolitis from time of the first caffeine dose to 7 days following the last dose, number of participants with abnormal MRI brain findings (applying the NICHD Neonatal Research Network score) at 7-14 postnatal days, number of participants with a BSID-III composite score < 85 at 18-24 months of age.
- Effect of Allopurinol for Hypoxic-Icshemic Brain Injury on Neurocognitive Outcome (ALBINO) (clinicaltrials.gov: NCT03162653), currently recruiting. Multi-centre interventional trial hoping to enroll >800 near-term/term infants with HIE to be randomised to receive either two doses of Allopurinol (initial dose 20 mg/kg, the second dose 10 mg/kg) or placebo in addition to TH. The primary outcome measure for this study is death or severe neurodevelopmental impairment versus survival free of severe neurodevelopmental impairment at 24 months of age. Secondary outcome measures are death or neurodevelopmental impairment, incidence of death, incidence of CP, GMFCS score, and BSID-III composite score at 24 months of age.
- Carpediem Outcomes in Infants Through Collaboration (ICONIC) (clinicltrials.giv: NCT05161078), enrolling by invitation. A retrospective and prospective, multi-centre observational quality improvement study and research registry. Infants undergoing KST utilising CARPEDIEM for haemodialysis will be enrolled in the study. Outcome measures in the study are number of CARPEDIEM filters to meet prescribed treatment length, rate of patient survival through to hospital discharge, and rate of renal recovery (measured as per cent of patients treated with CARPEDIEM who recover baseline renal function, excluding patients with end stage renal disease).

With the introduction and implementation of novel treatments in infants, longitudinal studies extending beyond toddlerhood and early school-age are crucial to assess long-term outcomes. Given the nature of CKD, studies on long-term outcomes after perinatal asphyxia and neonatal AKI should ideally extend also into adulthood.

8 ACKNOWLEDGEMENTS

First and foremost, I want to thank the children and their parents who participated and contributed to the research. Without them, this thesis would not have been possible.

I would especially like to thank:

Mats Blennow, my principal supervisor. Thank you for welcoming me into your research group back when I was still just a medical student. You introduced me to clinical research and allowed what was initially meant to be an eight week-long student project to morph into something much bigger. Over the 14 years that we have worked together, you have allowed my research project to grow and change to better align with my specific clinical sub-interests, granting me plenty of freedom and allowing me to step up and take responsibility along the way. It has always been evident that you have truly cared about our patients and their families, about the quality of the research as well as the quality of my research education. Thank you for your patience and for genuinely looking out for me when I needed you to.

Milan Chromek, my co-supervisor, despite being one of the calmest and most collected persons I have ever met, you came into my life like a whirlwind and things haven't been the same since. Your enthusiasm for paediatric nephrology and your patients is contagious, and your endless curiosity and willingness to explore new paths in research is inspiring. I feel truly fortunate that I get to call you my colleague, research partner, and friend. Thank you for all the laughter, for many haiku moments, for sharing my interest in obscure Japanese films and for daring to join me on culinary adventures. I know that I've been a disappointment in the long-distance running department, but who knows – maybe we will run that marathon one day?

Lena Hellström-Westas, former co-supervisor, thank you for literally writing the book on neonatal aEEG and for being a wonderful role model in research. You prove that it is indeed possible to be kind-natured, down-to-earth and still become a world-leading authority in your research field. I look up to you!

Christian Speer, my mentor, whom I have known since I was a small child. Thank you for your encouragement and for being a great role model in the world of clinical research.

Björn Fischler, head of the Division of Paediatrics at CLINTEC. Whether in the clinic or at KI, it is always a pleasure to work with you. Thank you for standing up for me when I needed you to.

Agneta Wittlock and Anette Johansson, thank you both for your perpetual readiness to help me out with administrative questions/issues. I would have been lost without you.

Katarina Lindström, thank for all the work you have put into caring for the children in our cohort! It has been a privilege to collaborate with you, and I hope that we can join forces again in the future. You have truly set the standard for long-term follow up after perinatal asphyxia.

Mikael Finder, former fellow doctoral student now Ph.D., colleague, room-mate, and friend. We have been on this journey together for many years, sharing both joy and frustration. Thank you for making sure that we remained team-mates instead of letting things turn into a competition. Many thanks also for your perpetual readiness to provide technical support on everything from Word, EduRoam, Endnote, Mac OS and more, and for laughing *kindly* at my love for old school documentation. Traveling together to attend conferences has been great fun. Let's finish that fourth paper in your thesis and start making more plans!

Mimmi Eriksson Westblad, thank you for all your tremendous work of examining the children in our cohort, and for all the interesting discussions that have since followed. I love your great attention for detail and how you are always thorough and careful. Thank you for always speaking your mind and asking questions.

Gustaf Håkansson, Stockholm's best import from the island of Gotland. Thank you for sharing the ups and downs of doctoral studies, for your witty and kind humour. Thank you for being a good friend. Soon it is your turn to defend your thesis! I will be there to cheer you on.

Sari Kokkonen Nassef, thank you for focusing on the parental experience of hypothermia-treated HIE.

Michaela Melakari, working with you is a true delight! Thank you for keeping everything and everyone organised and for always approaching challenges with a smile.

Peter and **Suzanna Lagerroos,** what an amazing father-daughter psychologist duo! A warm thank you for joining forces with us, I very much appreciated working with both of you. Peter, I wish you a great retirement and many lovely days on the golf course.

Fellow PhD-students and PhDs **Susanna Klevebro**, **Caroline-Aleksi Mägi**, **Jenny Svedenkrans**, **Viveka Nordberg**, **Agnes Linnér**, **Anne Elwin**, **Essi Whaites-Heinonen**, **Kolbrún Gunnarsdottir**, **Sonja Baldursdottir**, **Nikica Tomasic**, **Elena Palleri, Josef Brandström, Ewa Henckel –** it has been a privilege to share the journey of doctoral studies with you.

I am fortunate to get to work both at the Neonatal Department as well as the Paediatric Nephrology Department. Thank you to my bosses **Peter Bárány** and **Béatrice Skiöld** and director of studies **Emma Elsmén Steen** for generously granting me time off from clinical work so that I could focus on my research and write this thesis. Thank you also for paving new ground by making it possible for me to combine my two major clinical interests. Many thanks also to my former boss **Wouk Stannervik**, I nearly didn't want my residency to end because you are such a great boss.

Thank you to my paediatric nephrology colleagues **Mia Herthelius**, **Kajsa Åsling Monemi**, **Bogna Faryna Niwinska**, **Valya Georgieva**, **Åsa Laestadius**, **Nilüfer Kuru**. You patiently answer all my questions and provide mentoring and encouragement, even at times when we are all overwhelmed with things to do. Thank you for making work a true joy! **Svante**

Swerkersson, we miss you dearly. Paediatric nephrology matriarch **Ulla Berg**, thank you for being such an inspiration and for introducing the tradition of "onsdagslunch med delad chokladboll". All the paediatric nephrology nursing staff – thank you for taking such great care of all our patients and for creating a warm and friendly work environment.

The neonatology department is large and full of enthusiastic and dedicated co-workers– it would take a book to thank each and every one of you. I would particularly like to thank **Lars Navér**, **Kajsa Bohlin**, **Alexander Rakow**, **Veronica Frimmel**, **Paraskevi Kosma**, **Leif Evaggelidis**, and **Giulia Aquilano** for sharing your knowledge and expertise during days and nights in the NICU. **Jessica Westman**, thank you for patiently guiding me on my first trip to the Swedish snow-covered mountains and for trusting me to take care of **Hera**, who took on the role of emotional support dog during my final days of writing this thesis. **Sofia Levén**, I am so glad to have you on the neo-nephrology team, and I very much look forward to joining forces both "on the floor" as well as in research. **Dirk "Dirkules" Wackernagel**, Du bist ein Unikat. You are in a league of your own when it comes to level of enthusiasm, devotion to your patients, and being teaching-minded at all times. You have made the NICU and Karolinska University Hospital a better place. Ich werde Dich sehr vermissen. May the ultrasound machine always be with you.

Hanna Björck – what on earth would we do without you? Our patients are so lucky to have you. **Lina Norberg Larsson**, you are truly a rock to lean on! **Rachelle Lidholm**, thank you for great team-work in the NICU and many hours of nightly chats while on call, for your friendship outside of work and for being a formidable Wordfeud-opponent. I can't thank you enough for reading my mind that one time.

Emelie Sundkvist, it is hard to wrap my head around the fact that you will soon finish your residency in paediatrics. It has been an honour to watch you grow from a clever teenager and gymnast into a full-fledged paediatrician. Thanks for letting me be a part of your journey.

Diana Treis and **Silvia Malenicka**, thank you for great friendship both at and outside of work. I can't wait to cheer you on when it's your turn to write and defend your thesis!

My friends from the first semester at KI – **Elisabeth Harrison**, **Niklas Poijes**, **Najla Ahmed** and **Mehrnoush Khoshnevis**. Thank you for reminding me of everything that is important outside of work and research! **Simon Wiener**, I am so glad you decided to sit next to me in Fysik B. Twenty years of friendship – and still going strong. **Sofie Näslund**, thank you for being YOU. Words will never be enough. **Paul Castillo**, our paths have crossed numerous times since that first day of my rotation in the anaesthesiology department when you were a (very!) coffee-deprived resident. Thank you for trying to teach me everything you know since then and for all the chats when we have run into each other. **Gustav Nilsonne,** many thanks for numerous great dinners/parties, interesting discussions and for your gracious help when I felt lost during my first course in medical statistics. My dance sisters **Maria Granström** and **Ülkü Holago** – thank you for many, many great hours of dance! My instructor/idol **Sabriye Tekbilek** – you ARE the music, watching you dance is one of my favourite things to do.

Thank you for everything you do for this wonderful art form. **Shiho Tanaka** and **Fille Angele**, my favourite people to run into in Vasastan! Thank you for your friendship and for letting us join you in Nagano. My dear childhood friend **Claudia Fessler**, you and your lovely family have enriched my life for so long! Thank you for all the fun memories from Stockholm, Mátra Vidéki Erömü, Budapest, Vienna and Dénia. We all hope to be able to visit you again soon. **Davide Medici**, thank you for being a gentle giant during karate practice and for all the home-cooked Sunday dinners, movies, hours of talking about everything between heaven and earth. Thank you for flying to Barcelona to meet up, just thinking about how you did that still overwhelms me! I hope that we can soon resume our yearly reunions. **Soo-Kyun Kim**, you are one of the strongest, bravest people I have ever met. Thank you for all your support! I miss you dearly and hope to one day see you and your family return to Stockholm. **Ingrid Ekström**, thank you for reaching out to me to reconnect several years after we first met during one of many doctoral courses at KI. I love exchanging thoughts on research, life, family, and all things Germany/Austria-related with you. Your brilliant mind is impressive, and your generosity is second to none. **Elham Berglund Rostami**, Persian princess - it was love at first sight! Looking back, we have shared some incredible adventures and met fascinating people. Thank you for being *you*, and for always being there for me and my family, no matter what.

My Japanese family – **Yukiko** and **Tsutomu Kobayashi**, **Masatomo Kobayashi** and **Kyoko Komai**. Thank you for opening your home and your hearts to me. Kanazawa will forever be my other home. I can't wait to see you again.

My parents **Gertie Grossmann** and **Bengt Robertson** – thank you for raising me, for bringing me along as you explored the world, for introducing me to new countries, languages and cultures. Thank you for always keeping our home open for friends as well as guest researchers (Curosurf family FTW). Thank you for the simple life and many delicious berry pies on Hasselö, for playing Maple Leaf Rag on the piano when we were being too slow, and for dancing two-step to every type of beat. Your encouragement and support have meant the world to me. Pappa Bengt, there are not enough words to describe how much I miss you. One day, we shall meet again by the pond in Kenroku-en. My sisters **Petra**, **Camilla** and **Andrea** – strong, spirited, fascinating ladies! I love you all and I am very proud of you. Thank you for being such great aunts to my children. **Craig**, **Anders** and **Marcus**, thank you for being part of this big and boisterous family. Världens vackraste moster, **Bibbi Grossmann**, thank you for showcasing that concert pianists can simultaneously be public servants and drive huge buses – your open-mindedness is inspiring. My late grandparents **Doris** and **Max**, thank you for taking such great care of us grandchildren.

Andreas Hurme Lundin, my other half and partner in life. Thank you for singing "kudd-sången", and learning how to make melon pan – is there even a greater way to show love? Thank you for being so devoted to our family, for sharing both ups and downs in equal measure, for providing words of encouragement as well as important points of perspective when stress has at times rendered me near-sighted. Thank you for providing ad hoc technical

support and salty liquorice when I needed it, and for bringing Robert Parker into our lives. Let's make more music! My children **Matilda**, **Ferdinand** and **Jonathan**, I love you more than anything and I am so very proud of you. Thank you for being you and for letting me join you as you explore life and the world. You have been very patient with me these last couple of months, I can't wait to have more time to be together again.

This thesis would not have been possible without the generous support and grants from Stiftelsen Frimurare Barnhuset, Jerringfonden, Martin Rinds Stiftelse, H.K.H. Kronprinsessan Lovisas Förening för Barnasjukvård/Stiftelsen Axel Tielmans Minnesfond, Njurfonden, Region Stockholm (ALF project and Centre for Innovative Medicine), and the Neonatal Department at Karolinska University Hospital.

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