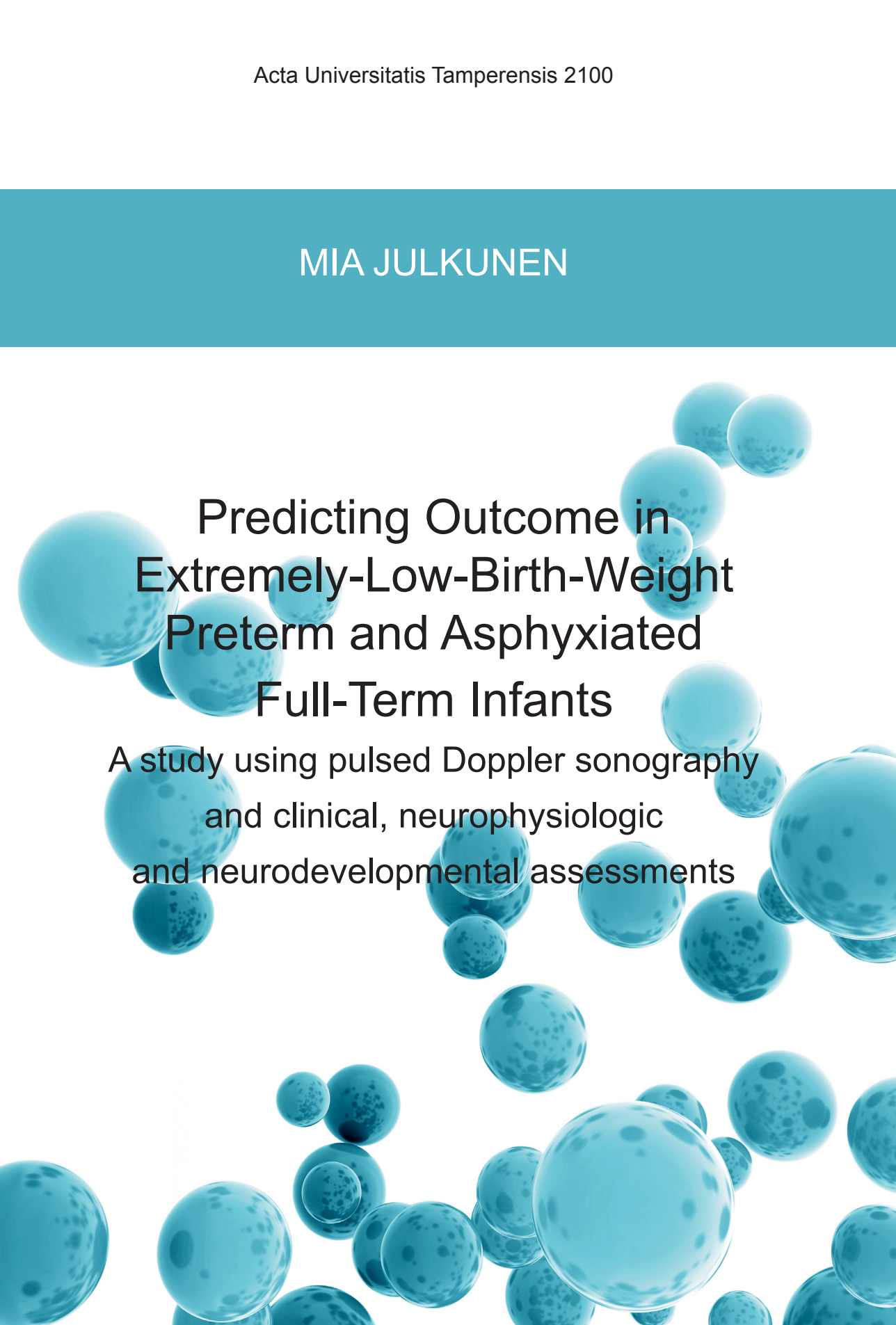# MIA JULKUNEN

Predicting Outcome in Extremely-Low-Birth-Weight Preterm and Asphyxiated Full-Term Infants

A study using pulsed Doppler sonography
and clinical, neurophysiologic
and neurodevelopmental assessments

MIA JULKUNEN

Predicting Outcome in Extremely-Low-Birth-Weight Preterm and Asphyxiated Full-Term Infants

A study using pulsed Doppler sonography
and clinical, neurophysiologic
and neurodevelopmental assessments

■

UNIVERSITY OF TAMPERE

MIA JULKUNEN

Predicting Outcome in Extremely-Low-Birth-Weight Preterm and Asphyxiated Full-Term Infants

A study using pulsed Doppler sonography
and clinical, neurophysiologic
and neurodevelopmental assessments

TAMPEREEN
YLIOPISTO

The originality of this thesis has been checked using the Turnitin OriginalityCheck service
in accordance with the quality management system of the University of Tampere.

Cover design by
Mikko Reinikka

Layout by
Sirpa Randell

441 729
Painotuote

CONTENTS

ABSTRACT

Background: The risk of brain injury is increased among extremely-low-birth-weight and asphyxiated full-term infants. It is therefore important to evaluate the methods to identify high-risk infants as early as possible.

Aims: The aims of the study were (1) to evaluate by pulsed Doppler ultrasound the incidence of end-diastolic block in the cerebral circulation and its connection to the development of intraventricular hemorrhage (IVH) in preterm infants of birth weight less than 1000 g, and

(2) to evaluate whether postnatal pulsed Doppler findings in cerebral arteries in the first day of life, hypoxic ischemic encephalopathy (HIE), Apgar score, obstetric parameters (cardiotocography (CTG), cord blood gas analysis), neurophysiologic examinations (electroencephalogram (EEG), evoked potentials) can predict the one-year neurological outcome of asphyxiated full-term infants. We also sought to establish the usefulness of three validated neurodevelopmental assessments in predicting the outcome of asphyxiated full-term infants.

Subjects and methods: Seventy-five preterm infants to birth weight <1000 g were born in Tampere University Hospital between the years 1999 and 2002. The gestational age ranged between 24 to 31 weeks. Of these infants 55, who underwent pulsed Doppler ultrasound, cranial structural ultrasound and echocardiography examinations one to four times during the first four days of life, constituted the study group. The study population was divided into a block group, with an end-diastolic block diagnosed in the intracranial arteries at least once during the study period, and a control group with normal findings in each of the examinations.

Thirty full-term (gestational age 37 weeks or more) infants with asphyxia and 30 healthy gestational age- and sex-matched infants born in Tampere University Hospital between the years 2000 and 2003 were enrolled. Each infant underwent pulsed Doppler examinations, cranial structural ultrasound and echocardiography at about 0–8 hours, at 10–12 hours and at 24 hours of age and thereafter daily if needed. Cerebral blood flow velocities and resistive indices in the anterior cerebral and basilar arteries were measured by Doppler examination. EEGs were recorded on average 1.5 days after birth and evoked potentials (BAEP, SEP, VEP) during the first week of life. The concentrations of the biochemical markers serum creatine kinase (CK), serum neuron-specific enolase (NSE) and plasma lactate were measured in the asphyxia group. General movements (GMs) were assessed by a physiotherapist before discharge and at 3 months of age. The Alberta Infants Motor Scale (AIMS) was applied by a physiotherapist as a part of the follow-up at the age of 3, 6 and 12 months. The overall neurological outcome was assessed clinically at 3, 6 and

12 months of age by a pediatric neurologist using modified Griffiths Developmental Scales. In addition, the neuromotor scoring (NMS) was applied at 3 months of age by a pediatric neurologist. Brain magnetic resonance imaging (MRI) was undertaken at one year of age in the asphyxia group.

Results: An end-diastolic block was found by pulsed Doppler in 22 preterm infants at least once during the first four days of life, whereas no block was detected in 33 preterm infants. End-diastolic block in the intracranial arteries and a mean arterial pressure (MAP) of 30 mmHg or less was associated with an increased risk of grade I–IV IVH, whereas all block cases with MAP > 30 mmHg remained free of IVH. Patent ductus arteriosus (PDA) was a significant risk factor for IVH.

Increased (> mean + 3SD) systolic cerebral blood flow velocities (CBFV) in the anterior cerebral or basilar arteries at the age of 24 hours were detected in 20% of the asphyxiated full-term infants. The sensitivity of a combination of increased systolic CBFV and moderate or severe HIE in predicting an abnormal 1-year outcome in the asphyxiated infants was 100% and the specificity 95%. Pathological CTG, low cord blood pH or base excess results, low Apgar scores and biochemical biomarkers showed a low predictive value.

Abnormal EEG background activity predicted a poor outcome in the asphyxia group with 67% sensitivity and 81% specificity, and abnormal somatosensory evoked potential (SEP) with 75% and 79%, respectively. The predictive values of brainstem auditory evoked potential (BAEP) and visual evoked potential (VEP) were poor. Seizures after asphyxia seemed not to be an independent predictor of abnormal outcome. The combination of normal EEG, normal SEP and normal systolic CBFV at 24 hours of age predicted a good outcome with a sensitivity of 100% and a positive predictive value (PPV) of 100%.

Normal GMs at 3 months of age predicted a good outcome among asphyxiated full-term infants with a sensitivity of 95% and a specificity of 75%. Abnormal GMs at 3 months of age predicted a poor outcome with a sensitivity of 75% and a specificity of 95%. AIMS at 6 months of age predicted a poor 1-year outcome with a sensitivity of 100% and a specificity of 89%. The predictive value of NMS was low.

Conclusions: An end-diastolic block in the cerebral circulation as detected by pulsed Doppler ultrasonography, together with a mean arterial pressure 30 mmHg or less is associated with a high risk of IVH in extremely-low-birth-weight preterm infants. The presence of a PDA during the first four days of life is a significant risk factor for IVH.

An increased systolic cerebral blood flow velocity at 24 hours of age in infants with moderate or severe HIE predicts an abnormal one-year outcome in asphyxiated infants better than CTG, cord blood gas analyses, Apgar scores or biochemical biomarkers. Normal EEG and normal SEP combined with normal systolic CBFV at about 24 hours of age may be valuable in the prediction of a normal 1-year outcome among asphyxiated infants. Seizures after asphyxia were not independent predictors of a poor outcome. Abnormal GMs at 3 months predicted a poor one-year outcome and normal GMs a good 1-year outcome among asphyxiated infants.

TIIVISTELMÄ

Taustaa: Hyvin pienipainoisina ja ennenaikaisina syntyneet ja asfyktiset täysiaikaiset lapset ovat alttiita aivovauriolle. Tärkeätä on tunnistaa mahdollisimman varhain vastasyntynyt, jolla on suuri riski saada neurologisia komplikaatioita.

Tutkimuksen tarkoitus: (1) Selvittää hyvin pienipainoisena (syntymäpaino alle 1000 g) syntyneiden aivoverenkierron loppudiastolisen virtauskatkoksen (end-diastolic block) esiintyvyyttä ja merkitystä aivoverenvuodon kehittymisen kannalta käyttäen Doppler-ultraäänitutkimusta.

(2) Tutkia täysiaikaisilla asfyktisillä vastasyntyneillä aivoverenkierron muutoksia ensimmäisen elinpäivän aikana Doppler-ultraäänitutkimuksella ja selvittää lisäksi hypoksis-iskeemisen enkefalopatian (HIE) vaikeusasteen, synnytyksen aikaisen sikiön sykekäyrän (KTG), verikaasuanalyysin, Apgarin pisteiden, biokemiallisten merkkiaineiden ja neurofysiologisten tutkimusten (EEG ja herätevasteet) kykyä ennustaa lapsen neurologista tilannetta 12 kuukauden iässä. Tutkimuksessa arvioitiin myös kolmen kliinisen neurologisen mittausmenetelmän soveltuvuutta asfyktisena täysiaikaisena syntyneen lapsen neurologisen kehityksen seurannassa.

Aineisto ja menetelmät: Tampereen yliopistollisessa sairaalassa vuosina 1999–2002 syntyi 75 ennenaikaista lasta (raskausviikot 24–31), joiden syntymäpaino oli alle 1000 g. Tutkimukseen rekrytoitiin 55 lasta, joille oli tehty aivoverenkierron Doppler-ultraäänitutkimus, aivojen rakenteellinen ultraäänitutkimus ja sydämen ultraäänitutkimus ainakin kerran ensimmäisen neljän päivän aikana normaalina potilastyönä. Tutkimusryhmä jaettiin kahteen ryhmään Doppler-löydöksen mukaan: lapset, joilla oli todettu loppudiastolinen virtauskatkos aivoverenkierrossa ainakin kerran ja kontrolliryhmä, joilla Doppler-mittaukset olivat normaalit.

Asfyksiatutkimukseen osallistui 30 asfyktista lasta ja 30 tervettä kontrollilasta, jotka syntyivät täysiaikaisina (raskausviikot vähintään 37) Tampereen yliopistollisessa sairaalassa vuosina 2000–2003. Vastasyntyneille tehtiin aivojen ultraäänitutkimus, aivoverenkierron Doppler-tutkimus ja sydämen ultraäänitutkimus 0–8, 10–12 ja 24 tunnin iässä ja sen jälkeen tarvittaessa päivittäin. Aivoverenkierron Doppler-ultraäänitutkimuksella määritettiin virtausnopeudet ja resistenssi-indeksit kallonpohjavaltimosta ja etummaisesta aivovaltimosta. Vastasyntyneille tehtiin EEG-rekisteröinti keskimäärin 1,5 vuorokauden iässä ja herätevastetutkimukset (BAEP, SEP, VEP) ensimmäisen elinviikon aikana. Asfyktisilta vastasyntyneiltä määritettiin aivovaurion merkkiaineina plasman laktaatti, seerumin neuronispesifinen enolaasi (NSE) ja seerumin kreatiinikinaasi (CK).

Fysioterapeutti arvioi tutkimukseen osallistuvien vastasyntyneiden liikunnallista kehitystä General Movements (GMs) -menetelmällä kotiinlähtövaiheessa ja 3 kuukauden iässä sekä 3, 6 ja 12 kk:n iässä Alberta Infant Motor Scale (AIMS) -testillä. Lastenneurologi arvioi lasten kehitystä 3, 6 ja 12 kk:n iässä käyttäen modifioitua Griffiths Scale -menetelmää neurologisen arvioinnin tukena ja lisäksi 3 kk:n iässä Neuromotor scoring (NMS) -menetelmää. Asfyktisina syntyneille lapsille tehtiin aivojen MRI-tutkimus vuoden iässä.

Tulokset: Aivoverenkierron loppudiastolinen virtauskatkos todettiin 22 hyvin pienipainoisella ennenaikaisella lapsella. 33 lapsella ei virtauskatkosta todettu. Lapsilla, joilla oli aivoverenkierron loppudiastolinen virtauskatkos ja joilla keskiverenpaine oli 30 mmHg tai alhaisempi, oli suurempi riski saada aivoverenvuoto (gradus I–IV) kuin lapsilla, joilla oli virtauskatkos, mutta keskiverenpaine yli 30 mmHg. Avoin valtimotiehyt todettiin merkittäväksi aivoverenvuodon riskitekijäksi.

Asfyktisista täysiaikaisista vastasyntyneistä 20 %:lla todettiin kiihtynyt aivoverenkierron virtausnopeus (suurempi kuin keskiarvo + 3 SD) joko kallonpohjavaltimosta tai etumaisesta aivovaltimoista 24 tunnin iässä. Kiihtynyt aivoverenkierron virtausnopeus 24 tunnin iässä ja kohtalainen tai vaikea hypoksis-iskeeminen enkefalopatia ennustivat kehitysviivettä 12 kuukauden iässä 100 % sensitiivisyydellä ja 95 % spesifisyydellä. KTG:n, verikaasuanalyysin ja Apgarin pisteiden ennusteellinen arvo oli huono.

Poikkeava EEG ennusti kehitysviivettä asfyktisena syntyneelle lapselle 67 % sensitiivisyydellä ja 81 % spesifisyydellä ja poikkeava somatosensorinen herätevaste (SEP) 75 % sensitiivisyydellä ja 79 % spesifisyydellä. Muilla herätevastetutkimuksilla (BAEP, VEP) ei ollut ennusteellista merkitystä. Asfyksian jälkeiset epileptiset kohtaukset eivät yksinään ennustaneet poikkeavaa kehitystä vuoden iässä. Normaali EEG, normaali SEP ja normaali aivoverenkierron virtausnopeus Doppler-tutkimuksessa 24 tunnin iässä ennustivat normaalia 12 kk:n iän kehitystä asfyktisena syntyneelle lapselle 100 % spesifisyydellä ja 100 % positiivisella ennustearvolla.

Normaalit GMs-liikkeet 3 kuukauden iässä ennustivat normaalia kehitystä 95 % sensitiivisyydellä ja 75 % spesifisyydellä. Poikkeava GMs ennusti huonoa kehitystä 75 % sensitiivisyydellä ja 95 % spesifisyydellä. AIMS-menetelmän sensitiivisyys oli 100 % 6 kk iässä asfyktisina syntyneiden lasten kehitysseurannassa ja menetelmän spesifisyys oli 89 %. NMS-menetelmän ennusteellinen arvo oli huono tässä tutkimuksessa.

Johtopäätökset: Jos hyvin pienipainoisella ennenaikaisella lapsella todetaan sekä aivoverenkierron loppudiastolinen virtauskatkos (end-diastolic block) ja matala keskiverenpaine (30 mmHg tai alempi), aivoverenvuodon vaara on huomattava. Avoin valtimotiehyt ensimmäisen neljän vuorokauden aikana on merkittävä aivoverenvuodon riskitekijä.

Asfyktisella täysiaikaisella vastasyntyneellä kiihtynyt systolinen aivoverenkierron virtausnopeus Doppler-tutkimuksessa 24 tunnin iässä ja kohtalainen tai vaikea hypoksis-iskeeminen enkefalopatia ennustavat kehityksen viivästymistä 12 kuukauden iässä paremmin kuin KTG, verikaasuanalyysi, Apgarin pisteet tai biokemialliset merkkiaineet.

Normaali EEG, normaali SEP ja normaali aivoverenkierron Doppler-mittaus asfyktisella täysiaikaisella vastasyntyneellä saattavat olla hyödyksi hyvän ennusteen arvioimisessa. Asfyksian jälkeiset epileptiset kohtaukset eivät yksinään ennustaneet poikkeavaa kehitystä vuoden iässä.

Tutkimuksessa käytetyistä kolmesta kliinisestä neurologisesta mittausmenetelmästä kolmen kuukauden iässä tehty GMs osoittautui käyttökelpoiseksi asfyktisen lapsen kehityksen seurannassa. Poikkeavat GMs-liikkeet 3 kuukauden iässä ennustivat kehitysviivettä asfyktisena syntyneillä lapsilla 12 kuukauden iässä, mutta normaalit GM-liikkeet taas viittasivat siihen, että lapsen kehitys jatkuu normaalina myös 12 kuukauden ikään.

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original articles, which are referred to in the text by their Roman numerals I–IV. Some additional unpublished data are also presented.

I   Julkunen M, Parviainen T, Janas M, Tammela O. 2008. End-diastolic block in cerebral circulation may predict intraventricular hemorrhage in hypotensive extremely-low-birth-weight infants. Ultrasound Med Biol 34:538-45.

II   Julkunen MK, Uotila J, Eriksson K, Janas M, Luukkaala T, Tammela O. 2012. Obstetric parameters and Doppler findings in cerebral circulation as predictors of 1 year neurodevelopmental outcome in asphyxiated infants. J Perinatol 32:631-8.

III   Julkunen MK, Himanen SL, Eriksson K, Janas M, Luukkaala T, Tammela O. 2014. EEG, evoked potentials and Doppler in asphyxiated term infants. Clin Neurophysiol 125:1757-63.

IV   Julkunen MK, Eriksson K, Lehto U, Kähärä V, Luukkaala T, Tammela O. Hypoxic ischemic encephalopathy and neurodevelopmental assessments in the prediction of 1 year outcome in asphyxiated infants. Submitted

These articles have printed here with the permission of their copyright holders.

ABBREVIATIONS

AAP	American Academy of Pediatrics
ACA	Arteria cerebri anterior
ACOG	American College of Obstetricians and Gynecologists
aEEG	Amplitude-integrated electroencephalogram
AIMS	Alberta Infant Motor Scale
BA	Basilar artery
BAEP	Brainstem auditory evoked potential
BDNF	Brain-derived neurotrophic factor
BGT	Basal ganglia and thalami
CBF	Cerebral blood flow
CBFV	Cerebral blood flow velocity
CK	Creatine kinase
CP	Cerebral palsy
CS	Cesarean section
CSF	Cerebral spinal fluid
CTG	Cardiotocography
CUS	Cranial ultrasonograhy
DWI	Diffusion-weighted imaging
EEG	Electroencephalogram
ELBW	Extremely-low-birth-weight
EPs	Evoked potentials
ERG	Electroretinogram
FIGO	The International Federation of Gynecology and Obstetrics
GFAP	Glial fibrillary acidic protein
GMs	General movements
HI	Hypoxic ischemic
HIE	Hypoxic ischemic encephalopathy
IVH	Intraventricular hemorrhage
ICA	Internal carotid artery
IL	Interleukin

MAP	Mean arterial pressure
MCA	Middle cerebral artery
MRI	Magnetic resonance imaging
NE	Neonatal encephalopathy
NIRS	Near-infrared spectroscopy
NMS	Neuromotor scoring
NPV	Negative predictive value
NSE	Neuron-specific enolase
$PaCO_2$	Arterial partial pressure of CO_2
PaO_2	Arterial partial pressure of O_2
PDA	Patent ductus arteriosus
PHVD	Post-hemorrhagic ventricular dilation
PLIC	Posterior limb of the internal capsule
PPV	Positive predictive value
PVHI	Periventricular hemorrhagic infarction
PVL	Periventricular leukomalacia
RI	Resistive index
RR	Relative risk
SCBFV	Systolic cerebral blood flow velocity
SEP	Somatosensory evoked potential
SWC	Sleep-wake cycling
UCHL-1	Ubiquitin carboxyl-terminal hydrolase L-1
VEP	Visual evoked potential
VLBW	Very-low-birth-weight
WM	White matter

1   INTRODUCTION

Birth asphyxia is defined as a condition of impaired gas exchange between mother and infant during labor, leading to hypoxemia, hypercapnia and metabolic acidosis (Low 1997). Intrapartum hypoxia followed by hypoxic ischemic encephalopathy (HIE) is a major cause of death and disability. Twenty-three % of 4 million neonatal deaths worldwide are caused by asphyxia (Lawn et al. 2005).

The incidence of birth asphyxia is from 1 to 6 cases in 1000 live births (de Haan et al. 2006). In developing countries the figure may be 10-fold higher (Golubnitschaja et al. 2011). The incidence of HIE is estimated to be 1.5 per 1000 live births (Kurinczuk et al. 2010). HIE in the perinatal period is a prominent cause of cerebral palsy (CP) in 29% and associated with cognitive, general developmental delay or learning difficulties in as many as of 45% of term infants with HIE (Mwaniki et al. 2012). The degree of subsequent neurological problems has been found to be related to the severity of HIE (Hagberg et al. 2001). Despite advances in neonatal intensive care and the introduction of therapeutic hypothermia, moderate or severe HIE still causes death or disability among term infants (Edwards et al. 2010).

Disturbances in cerebral blood flow (CBF) are significant in the pathogenesis of brain injury (Ballabh 2014). Impaired cerebral autoregulation is considered to be a risk factor for ischemic brain injury in preterm infants (Khwaja and Volpe 2008) and in full-term infants after a hypoxic ischemic (HI) event (Archer et al. 1986, Pryds et al. 1990a, Boylan et al. 2000). Fluctuation in CBF has been shown to be a risk factor for developing intraventricular hemorrhage (IVH) in preterm infants (Ballabh 2014), likewise periventricular leukomalacia (PVL) due to a pressure-passive cerebral circulation (Volpe 2008).

Early identification of infants who are vulnerable to neurodevelopmental problems, and prediction of the long-term outcome shortly after birth are a challenge for clinicians. Neurological monitoring in high-risk infants might thus provide useful prognostic information, important for counselling parents and making management decisions.

The main purpose of this study was to evaluate the clinical value of bed-side Doppler ultrasonography as a non-invasive assessment tool in predicting short-term outcome in extremely-low-birth-weight (ELBW) preterm and asphyxiated full-term infants. Further, Doppler findings, obstetric parameters, biochemical markers, severity of HIE, neurophysiologic examinations (electroencephalogram (EEG), evoked potentials) and neurodevelopmental assessments were compared and evaluated in predicting the neurological outcome at 1 year in asphyxiated full-term infants.

2 REVIEW OF THE LITERATURE

2.1 Regulation of cerebral blood flow (CBF)

The brain is supplied by four large arteries: two internal carotid (ICA) and two vertebral arteries. Each internal carotid artery arises from the common carotid artery in the neck and divides into the anterior cerebral (ACA) and the middle cerebral (MCA) arteries. The first branches of the left and right subclavian arteries are the vertebral arteries, which ascend through the neck to the brainstem and form the basilar artery (BA) at the level of the pons. The BA branches into two posterior cerebral arteries. The internal carotid arteries and BA divide into several branches and form an anastomotic structure called the Circle of Willis.

The ACA and the MCA provide blood to the frontal and parietal lobes, the major area of the temporal lobe and deep grey matter. Blood to the cerebellum and brain stem is supplied by the branches of the BA, except for parts of the cerebellum and medulla oblongata which are supplied by the branches of the vertebral arteries. The BA supplies blood to a part of the temporal lobe, the occipital lobe and the thalamus. The posterior cerebral artery supplies the inferior surface of the brain and the occipital lobe.

At term neonatal CBF varies between 10 and 20 mL/100g/min and represents approximately 40% of adults values. CBF increases with postnatal age in parallel with the increasing cerebral metabolic rate and energy demand of the growing brain (Chalak et al. 2014a). The cerebral circulation is regulated by a number of homeostatic mechanisms, including autoregulation, the arterial partial pressure of CO_2 ($PaCO_2$) and O_2 (PaO_2), blood glucose, neuronal activity (Volpe 2008), blood viscosity and flow-metabolism coupling on the CBF (Udomphorn et al. 2008).

2.1.1 Autoregulation

Cerebral autoregulation is a homeostatic mechanism where arteries dilate and constrict to maintain CBF near constant over a broad range of perfusion pressures, known as the autoregulation plateau (Greisen 2005, Liem and Greisen 2010, Tasker 2013).

Autoregulation maintains a constant CBF if the mean arterial pressure (MAP) is within certain limits (approximately 24 to 39 mmHg) in preterm infants of gestational age between 24 to 30 weeks (Tyszczuk et al. 1998). Although the mean arterial pressure limits of the autoregulation plateau have not been established, the approximate autoregulatory

range ranges lies between 25 mmHg and 50 mmHg. The upper and lower limits of MAP vary according to gestational age (Volpe 2008).

It has been recommended that the MAP in mmHg should not fall below the gestational age of the infant in weeks to maintain autoregulation (Joint Working Party of the British Association of Perinatal Medicine and the Research Unit of the Royal College of Physicians 1992). Beyond these limits of the autoregulation plateau, CBF depends on MAP in a pressure-passive manner, which means that hypotension results in cerebral ischemia and hypertension causes cerebral hyperemia (Udomphorn et al. 2008). A pressure-passive cerebral circulation is frequent in premature infants in the first days after birth. It is not, however, an "all-or-none" phenomenon. In very-low-birth-weight (VLBW) preterm infants, CBF monitored by NIRS (near-infrared spectroscopy) is reported to be pressure-passive for an average of 20% of the recording time. Impaired cerebral autoregulation is associated with low gestational age and birth weight, systemic hypotension and maternal hemodynamic factors in preterm infants of birth weight less than 1500 g (Soul et al. 2007).

The deficit is considered to be a risk factor for ischemic brain injury in preterm infants (Khwaja and Volpe 2008), but the role of impaired autoregulation in the development of IVH has not been established (Ballabh 2014). The autoregulation of CBF has been found using Xenon clearance or Doppler ultrasonography to be impaired in full-term infants with asphyxia. Cerebral hyperemia after asphyxia is common, and is associated with a poor outcome (Archer et al. 1986, Pryds et al. 1990a, Boylan et al. 2000).

2.1.2 Other regulatory factors

Carbon dioxide (CO_2) is the most potent cerebral vasodilator. CBF is sensitive to changes in $PaCO_2$ (Udomphorn et al. 2008, Liem and Greisen 2010, Tasker 2013, Vutskits 2014). The hypercapnia induced increase in CBF is approximately 6% per mmHg change in $PaCO_2$, and hypocapnia reduces CBF by approximately 3% per mmHg change in $PaCO_2$ (Tasker 2013). Hypercapnia > 55 mmHg (> 8 kPa) is associated with impaired cerebral autoregulation in VBLW infants (Kaiser et al 2005). The association between PVL and severe hypocapnia is well documented (Greisen et al. 1987, Okumura et al. 2001, Shankaran et al. 2006). Even mild hypocapnia (< 4.5 kPa) is associated with leukomalacia and CP in preterm (Collins et al. 2001) and with poor outcome after HIE in term infants (Pappas et al. 2011).

The influence of PaO2 on the cerebral circulation is of lesser clinical significance. There are minimal changes in CBF with changes in PaO2 above 50 mmHg (6.7 kPa). Below a PaO2 threshold of 50 mmHg (7 kPa), CBF increases to maintain adequate cerebral oxygen delivery (Udomphorn et al. 2008, Tasker 2013).The arterial oxygen concentration is related not only to PaO2 but also to the hemoglobin concentration and the oxygen affinity to hemoglobin. Oxygen delivery to the brain may be affected not only by hemoglobin concentration, but also by the blood viscosity (Volpe 2008).

In a recent Doppler study term infants with polycythemia had significantly lower blood flow velocity in MCA, whereas infants with anemia had significantly higher cerebral blood flow velocity (CBFV) compared to normocythemic infants (Weissman et al. 2012). Preterm infants with anemia have increased CBF, while in polycythemia CBF decreases as measured by NIRS (Liem et al. 1997).

Hypoglycemia (< 1.7 mmol/L) has been shown to increase CBF in preterm infants to maintain glucose supply to the brain (Pryds et al. 1990b). Immature vascular anatomy is also one of the important determinants of CBF in newborns (Brew et al. 2014).

2.1.3 Neuronal activation

Further research is needed on the impact of neuronal activation, seizures or drug-induced central nervous system depression on infants' cerebral hemodynamics or metabolism (Brew et al. 2014). However, coupling of neuronal activity to CBF has been reported in sleep states and seizures. A decrease in CBF during sleep in preterm (Greisen et al. 1985) and a marked increase in CBF with the neuronal activity of a seizure in asphyxiated term infants (Perlman et al. 1985) have been reported.

2.2 Doppler ultrasonography in the evaluation of CBF

2.2.1 Technical principles

According to the Doppler principle, ultrasound waves emitted from the Doppler transducer are transmitted and reflected by moving red blood cells within the cerebral vessels. The measured Doppler frequency shift is the difference between emitted and reflected waves. (Figure 1.)
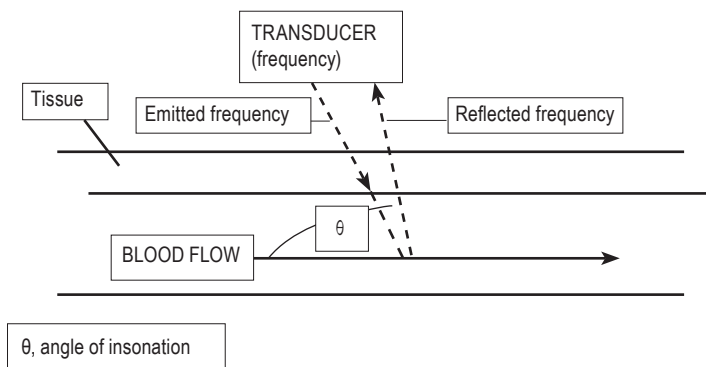
Figure 1. The difference in the frequency between the emitted and reflected waves, referred to as the "Doppler frequency shift", is directly proportional to the speed of the moving red blood cells (blood flow velocity).

When the angle of insonation is known and the Doppler frequency shift is measured, the blood flow velocity can be calculated using the formula: V = Fd x c/2 x Fo x cos θ, where

V = velocity of blood flow (cm/s)

Fo = emitted frequency (transducer frequency)

Fd = Doppler frequency shift (the difference between emitted and reflected frequency)

c = constant: velocity of sound in brain (1540 m/s)

θ = angle of insonation (angle between emitted ultrasound beam and direction of flow)

(Govaert and de Vries, 2001).

The angle of insonation has an impact on the velocity measured. An angle of zero or the emitted wave parallel to the direction of blood flow (cosine 0 = 1) gives an optimal measurement. The larger the angle, the larger will be the cosine of the angle; the greater the error in blood flow velocity measurement. It is important to minimize the angle of insonation to less than 30 degrees to keep the error below 15% (Purkayastha and Sorond 2012).

The anterior fontanelle is the standard acoustic window in neonatal Doppler and cranial studies. Thereby the ACA, BA and ICA arteries can be identified. The CBFV in the MCA can be measured through the temporal acoustic window (Govaert and de Vries 2001).

Three Doppler indices can be calculated from the cerebral blood flow curve (Figure 2). These indices are angle-independent:

S/D ratio = Peak systolic velocity / End-diastolic velocity (Stuart et al. 1980)

Pulsatility index PI = (Peak systolic velocity – End-diastolic velocity) / Mean velocity (Gosling and King 1971)

Resistive index RI = (Peak systolic velocity – End-diastolic velocity) / Peak systolic velocity (Pourcelot 1975).
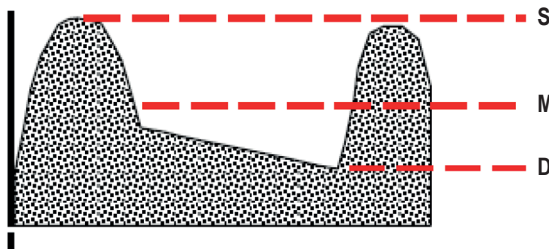
Figure 2. Cerebral blood flow curve by Doppler. Peak systolic velocity (S), mean velocity (M) and end-diastolic velocity (D).

CBF velocity can be used to obtain beat-to-beat changes in systolic, diastolic and mean CBF velocity. The peak systolic CBFV, end-diastolic CBFV and RI are the most common measurements used in monitoring cerebral circulation in infants. An increase or decrease in the CBFVs by Doppler corresponds to an increase or decrease in brain perfusion measured by Xenon-133 clearance (Greisen et al. 1984). A high RI correlates with increased cerebral vascular resistance and a decreased diastolic blood velocity and a low RI with decreased resistance and increased blood velocity in the cerebral diastolic circulation (Lowe and Bailey 2011). Various factors can influence Doppler measurements. Factors which have been shown to affect the cerebral circulation flow velocity in Doppler ultrasonography examinations are presented in Table 1.

Table 1. Factors and changes in cerebral blood flow velocity and resistive index by Doppler in infants*.

	Cerebral blood flow velocity (CBFV)	Resistive Index (RI)
Intracranial abnormalities		
Intracranial bleeding		Increased
PVL		Increased
Cerebral edema		Increased
Hydrocephalus	Decreased	Increased
Brain death	Decreased	Increased (then no flow)
Asphyxia	Increased	Decreased
Extracranial abnormalities		
PCO2 (hypercapnia)	Increased	Decreased
Heart rate (increased)		Decreased
PDA	Decreased	Increased
Pneumothorax		Increased
Polycythemia	Decreased	Increased
Anemia	Increased	Decreased
Apnea	Decreased	
Seizures	Increased	Decreased
Medications		
Caffeine	Decreased	
Dexamethasone	Increased	
Indomethacin	Decreased	Increased
Surfactant	Increased	

*Modified from Pezzati et al. 2002, Volpe 2008, Bulas 2009, Deeg et al. 2015.

2.3 Cerebral Doppler ultrasonography in infants

The first neonatal Doppler ultrasonography study was reported in 1979 (Bada et al. 1979). Normal neonatal reference values and postnatal changes in peak systolic and end-diastolic CBFV have been determined in several studies (Gray et al. 1983, Archer et al. 1985, Deeg and Ruprecht 1989, Cheung et al. 1994, Meek et al. 1998, d'Orey et al. 1999, Pezzati et al. 2002, Romagnoli et al. 2006). CBFV increases linearly during the first hours and days of

life in both preterm and term infants (Cheung et al. 1994, Meek et al. 1998, d'Orey et al. 1999) and the velocities increase with gestational age and birth weight (d'Orey et al. 1999). Peak systolic velocities are 10-20% higher in the ICA than in the BA or ACA. In contrast to the CBFV, the RI is independent of age and weight (Deeg et al. 1989). There should be no significant differences in RIs among the different cerebral arteries (Bulas 2009). RI ranging between 0.6–0.8 is regarded as normal (Deeg et al. 1989, Allison et al. 2000).

2.3.1 Preterm infants

Two different patterns of CBFV by Doppler ultrasonography in premature infants on the first day of life have been observed: (1) a stable CBFV and (2) a fluctuating CBFV (continuous alteration in systolic and diastolic CBFV) pattern. Preterm infants with fluctuating CBFV have had a higher incidence of IVH than infants with stable CBFV patterns (Perlman et al. 1983).

Very low velocities by Doppler also constitute a risk for severe IVH in preterm infants (Deeg et al. 1990). Low CBFV (Fukuda et al. 2006) and fluctuating CBFV (Volpe 2008) in the cerebral circulation are likewise risk factors for developing PVL due to a pressure-passive cerebral circulation. In preterm infants with a symptomatic patent ductus arteriosus (PDA), the diastolic cerebral blood flow has been seen to decrease because of a ductal stealing effect and cerebral blood flow normalized after closure of PDA (Perlman et al. 1981). Absence of diastolic cerebral blood flow (end-diastolic block) or retrograde diastolic flow in cerebral arteries are associated with hemodynamically significant PDA in preterm infants (Kupterschmid et al. 1988) and hydrocephalus with increased intracranial pressure (Bulas 2009). Loss of diastolic flow, retrograde diastolic flow or no detectable flow in cerebral arteries in a small group of pediatric patients of ages ranging from newborn to 4 years has been related to a high risk of death, although survival without sequela is also possible (Chiu et al. 2003).

2.3.2 Term infants after a hypoxic ischemic event

Hypoxic ischemic brain injury is a process initiated by an HI insult leading to a decreased flow to the brain (primary lesions), followed by the restoration of blood flow in the brain and the initiation of a biochemical cascade (reperfusion injury) (Wintermark et al. 2011).

A decrease in CBFV detected by Doppler 12 hours after an HI event has been reported in asphyxiated infants (van Bel et al. 1998, Ilves et al. 2004). Asphyxiated term infants with moderate to severe HIE have evinced very low CBFV (van Bel et al. 1998, Ilves et al. 2004, 2009a, 2009b). Low cerebral blood flow volumes among infants with mild to moderate HIE during the first 28 days is associated with a risk of developing CP (Fukura et al. 2008).

Increased CBFV and RI below 0.55 have been associated with poor outcome among asphyxiated term infants at ages from 24 to 72 hours in a number of studies (Levene et al. 1989, Deeg et al. 1990, Pryds et al. 1990a, Stark and Seibert 1994, Bennhagen et al. 1998, Meek et al. 1999, Ilves et al. 2004). The reason for the high CBFV and the low RI during days 2 to 3 after asphyxia may be a severe vasoparalysis, a form of irreversible cerebral vascular injury (Levene et al. 1989, Pryds et al. 1990a, Meek et al. 1999).

An RI less than 0.55 in the cerebral arteries has had a positive predictive value for death or disability of 75% and a negative predictive value of 100% among term infants from 24 hours of age with moderate or severe HIE at normal temperature (Archer et al. 1986). The prognostic value of RI is reduced when hypothermia is used. In one recent study, the low cerebral RI (< 0.55) was not a good predictor of poor outcome in infants with HIE during hypothermia. Low RI predicted death or severe disability at 18 months of age in 60% of term HIE infants (Elstad et al. 2011). On the other hand, after rewarming, RI 0.55 or below seemed to be of value, in predicting a poor outcome in 100% among hypothermia-treated infants (Skranes et al. 2014).

2.4   Intraventricular hemorrhage (IVH)

The pathogenesis of IVH is primarily attributed to the fragility of the germinal matrix vasculature, disturbance and fluctuation in the CBF, and platelet and coagulation disorders. The subependymal germinal matrix is highly vascular and vulnerable to hemorrhage. Fragility of the germinal matrix vasculature can be attributed to a lack of pericytes and immaturity of the basal lamina, with low fibronectin in the angiogenic vessel and reduced glial fibrillary acidic protein expression in the astrocyte end-feet in the germinal matrix vasculature. After 24 gestational weeks, the thickness of the germinal matrix is decreased, and it almost disappears by 36 to 37 gestational weeks. Vaginal delivery, low Apgar score, severe respiratory distress syndrome, pneumothorax, hypercapnia, hypoxia, seizures, PDA and infection increase fluctuations in CBF and thus represent risk factors for the development of IVH. (Ballahb 2014)

The incidence of IVH is 20%–30% among very preterm infants (Larroque et al. 2003, Bolisetty et al. 2014). However, the incidence of severe IVH grade III and IV has remained at 6% (Groenendaal et al. 2010, Bolisetty et al. 2014). Prenatal glucocorticoids (Ballabh 2014) and delayed cord clamping (Chiruvolu et al. 2015, Jelin et al. 2015) have been shown to be effective interventions to prevent IVH. IVH is characteristically seen by cranial ultrasonography (CUS) in the germinal matrix within 72 hours of life (Linder et al. 2003, Ballabh 2010). Grading of IVH according to Papile and associates (1978) is presented in Table 2.

Table 2. Grading of intraventricular hemorrhage (IVH).

Grade I	Hemorrhage limited to subependymal matrix
Grade II	Hemorrhage extending into ventricular system without ventricular dilatation
Grade III	Hemorrhage extending into ventricular system with acute ventricular dilatation
Grade IV	Intraventricular hemorrhage with parenchymal hemorrhage

One prominent complication of IVH is periventricular hemorrhagic infarction (PVHI). This results from venous infarction due to the obstruction of the veins by the hemorrhage (van Wezel-Meijler et al. 2010). The incidence of PVHI would appear to be 6% in very preterm infants with IVH (Leijer et al. 2009). The most serious complication of IVH is post-hemorrhagic ventricular dilatation (PHVD). About 25–30% of preterm infants with IVH develop PVHD (Murphy et al. 2002, Lee et al. 2009). The presence of hemorrhagic parenchymal infarction and PHVD increase the risk of cerebral palsy from 40% to 80–90% (Whitelaw and Aquilina 2012).

2.5   Birth asphyxia in term infants

2.5.1   Definitions

Birth asphyxia is defined as a condition of impaired gas exchange between mother and infant during labor, leading to progressive hypoxemia, hypercapnia and metabolic acidosis (Low 1997).

There is a lack of common, international criteria for birth asphyxia. The guidelines of the American College of Obstetricians and Gynecologists (ACOG) and the American Academy of Pediatrics (AAP) in 2003 considered four criteria essential in diagnosing intrapartum asphyxia as a cause of brain injury: (1) metabolic acidosis (pH < 7.00 and base deficit > 12 mmol/l) in a cord arterial sample, (2) moderate or severe encephalopathy, (3) cerebral palsy or dyskinetic type and (4) exclusion of other etiologies. The five additional criteria were the following: (1) sentinel event, (2) abrupt fetal heart rate change, (3) multisystem failure within 72 hours of birth, (4) Apgar score 0–3 longer than 5 minutes, (5) early imaging evidence (American College of Obstetricians and Gynecologists and American Academy of Pediatrics 2003).

The ACOG recently updated the guidelines for neonatal encephalopathy and neurologic outcome. In the revised report, the neonatal signs consistent with an acute peripartum or intrapartum hypoxic ischemic event are: (1) Apgar scores of less than 5 at 5 minutes and 10 minutes, (2) fetal umbilical artery pH less than 7.00 or base deficit greater than or equal to 12 mmol/l or both, (3) neuroimaging evidence of acute brain injury seen in brain magnetic resonance imaging or magnetic resonance spectroscopy consistent with HI, (4) presence of multiorgan failure with HIE (American College of Obstetricians and Gynecologists 2014).

Neonatal encephalopathy (NE) is the clinical presentation of disordered brain function, manifested in respiratory difficulty, depression of tone and reflexes, subnormal level of consciousness and often seizures (Nelson and Leviton 1991). Over 70% of NE cases give no evidence of intrapartum asphyxia. Several different etiologies, for example genetic, metabolic and infective diseases or trauma are possible causes of NE (Badawi et al. 1998).

The disorder is defined as HIE if there is evidence that intrapartum asphyxia is the cause of the encephalopathy (Robertson and Perlman 2006). A clinical staging of HIE has been given by Sarnat and Sarnat (1976) to grade the severity of neurological abnormalities (consciousness, muscle tone, posture, reflexes, autonomic function, seizures). According to the classic Sarnat staging, the severity of HIE is classified into three stages (mild, moderate or severe) combined with EEG findings and outcome (Sarnat and Sarnat 1976). The modified Sarnat grading by Levene and associates (1985) is based on clinical observation and is that usually used for grading HIE (Table 3).

Table 3. Modified Sarnat grading for hypoxic ischemic encephalopathy (HIE)*

		Mild HIE (Grade 1)	Moderate HIE (Grade 2)	Severe HIE (Grade 3)
Level of consciousness		Irritability	Lethargic	Stupor
Muscle tone		Normal	Mild hypotonia	Flaccid
Primitive reflexes				
	Suck	Normal/Weak	Weak/Absent	Absent
	Moro	Strong	Weak/Incomplete	Absent
Seizures		Absent/Hyperalert	Common	Frequent

* Levene et al. 1985.

Thompson scoring is a numeric system for the assessment of HIE during the neonatal period for prognostication of neurodevelopmental outcome. The system is based on nine aspects of neurological examination of infants with HIE: the total score ranges from 0–22. A maximum score > 10 during the first 7 days of life predicts an abnormal outcome with 100% sensitivity and 61% specificity (Thompson et al. 1997).

Apgar score was presented by the American anaesthesiologist Virginia Apgar in 1953 (Apgar 1953). The Apgar score has a limitation in identifying birth asphyxia and predicting neurological outcome (AGOG 1996). However, low Apgar scores from 0 to 3 at 5 minute have more prognostic value when combined with fetal acidemia (Freeman and Nelson 1988) and symptoms of neonatal encephalopathy (Moster et al. 2002). Prolonged depression of the Apgar score is related to severe neurodevelopmental outcome or death (Nelson and Ellenberg 1981, Harrington et al. 2007). The likelihood of death or moderate to severe disability is approximately 80% with Apgar scores < 3 at 10 minutes of life (Laptook et al. 2009).

The incidence of birth asphyxia is from 1 to 6 cases in 1000 live births (de Haan et al. 2006, Kurinczuk et al. 2010). In developing countries the figure is much higher

(Golubnitschaja et al. 2011). Birth asphyxia accounts for 23% of 4 million neonatal deaths worldwide (Lawn et al. 2005).

The incidence of HIE has remained at 3–5 infants per 1000 term births (Gonzales and Ferriero 2008). Moderate-to-severe HIE occurs in 1–2 infants per 1000 term live births (Low et al. 1997). In a recent study, the incidence of HIE was estimated to be lower, 1.5 per 1000 live births (Kurinczuk et al. 2010).

2.5.2 Pathogenesis of hypoxic ischemic encephalopathy (HIE)

The fetal circulation responds to hypoxemia and asphyxia by a centralization and autoregulation of blood flow to the vital organs (brain, heart, adrenals), at the expense of flow to less vital organs (e.g. kidney, intestine, muscle). The timing, severity, pattern and duration of the fetal insult determine the outcome after the asphyxia event (Chakak et al. 2014a). Hypoxic ischemic brain injury is a result of both initial damage (primary phase of injury) caused by a decrease in blood flow and a subsequent delayed process in which the damage continues after the restoration of blood flow (reperfusion injury) (Wintermark et al. 2011).

Acute severe hypoxia impairs the oxidative metabolism, leading to neuronal depolarization and ischemia and initial damage within minutes, called the primary phase of injury. Some neurons die during the acute ischemic insult, this being thought to be followed by a "latent phase" (from 6 to 15 hours) of improved tissue oxygenation. The third phase is a delayed process of cell damage (from 6 hours to days or even weeks) (Drury et al. 2010). Therapeutic interventions such as hypothermia utilize the window provided by the latent phase and delay in cell damage.

The pathogenetic mechanism underlying brain injury in HIE resulting from hypoxia and ischemia is deprivation of glucose and oxygen supply, which causes a primary energy failure and initiates at cellular level a series of biochemical events (Lai and Yang 2011) which may seen as an excito-oxidative cascade (Johnston et al. 2011). Ischemia reduces delivery of the glucose necessary for the anaerobic mechanism, which powers neurotransmitter reuptake pumps on perisynaptic astrocytes. This leads to an excessive concentration of excitatory amino acids in the synaptic cleft and neuronal depolarization. Glutamate is the most important excitatory amino acid in the brain. Opening of glutaminergic NMDA receptor channels and other calcium channels leads to an excess calcium influx into the neurons. The calcium influx through NMDA channels activates the enzyme nitric oxide synthetase, leading to high levels of the toxic free radical neurotransmitter nitric oxide. Other free radicals and several enzymes also attack the mitochondria and other cellular machinery. (Johnston et al. 2011, Rennie 2012)

Hypoxia-ischemia and reperfusion activates many inflammatory pathways, especially microglia and macrophages, production of cytokines, and infiltration of neutrophils into the brain (Hudome et al. 1997). Activated microglias begin to accumulate in the first 4

hours after reperfusion and continue to increase over the next 48 hours (Bona et al. 1999). Microglias release cytokines, glutamate and free radicals, which are neurotoxic. Lactic acid is also accumulated when oxidative phosphorylation within the mitochondria is impaired. An increased lactate concentration and acidosis are neurotoxic (Volpe 2008).

Cerebral edema occurs when the pumps required for water homeostasis are impaired by reduced energy supplies owing to damaged mitochondria (Johnston et al. 2011). There are two types of brain edema: vasogenic and cytotoxic. Cytotoxic edema, which follows cerebral ischemia, due to failure of cellular membrane pumps, results of intracellular accumulation of water, Na^+ and Ca^{++}. In vasogenic edema, there is an increase in leakiness of the blood-brain barrier, allowing entry of serum protein into the brain parenchymal. The resulting increase in intracerebral osmotic pressure leads to the accumulation of fluid within the extracellular compartment of the brain. (Levene and Chervenak 2009)

With moderate to severe HI injury, the third phase (from approximately 6–15h) is associated with stereotypic seizures, cytotoxic edema and accumulation of excitotoxins, failure of cerebral mitochondrial activity and spreading cell death (Wassink et al. 2014). Acute ischemia and severe exhaustion of energy supplies leads to necrosis. However, apoptosis is the major type of cell death after asphyxia. Apoptosis or programmed cell death is an active, energy-dependent process. It has multiple pathways in neonatal asphyxia. Proapoptotic factors released from damaged mitochondria play an important role in apoptosis (Johnston et al. 2011, Rennie 2012).

The incidence of seizures in term newborns is reported to be approximately 1 to 5 per 1000 live births (Glass et al. 2014). The etiology for neonatal seizures is broad. Global cerebral hypoxic ischemic insult is the cause in 40% of cases (Tekgul et al. 2006). Neonatal seizures in birth asphyxia are associated with poorer developmental outcome (Tekgul et al. 2006, Glass et al. 2009). The occurrence of seizures in infants with asphyxia is a risk factor independent of the severity of hypoxic-ischemic brain injury as evaluated using MRI and neurodevelopmental assessment (Miller et al. 2002, Glass et al. 2009).

2.5.3 Outcome after birth asphyxia

The degree of subsequent neurological disorders has been found to be related to the severity of HIE (Hagberg et al. 2001). The risk of CP is about 14% among infants with HIE (Graham et al. 2008). Mild HIE is usually associated with a good outcome (de Haan et al. 2006), although a risk of CP (Rosenbloom 1994) and learning and memory difficulties (Marlow et al. 2005) have been reported. The rates of motor and cognitive impairment or death are 32% for moderate HIE and almost 100% for severe HIE (Pin et al. 2009). In a trial of hypothermia, 48% of infants with moderate HIE and 85% of infants with severe HIE died or survived with moderate or severe disability (Shankaran et al. 2005). A recent systematic review has reported that cognitive, general developmental delay or learning difficulties were noted in 45%, CP in 29%, deafness or hearing loss in 9%, impaired vision

or blindness in 26%, gross motor and coordination problems in 17%, epilepsy in 12% and behavioral problems in 1% among infants with HIE. Multiple impairments were reported in 20.5% of these infants (Mwaniki et al 2012).

2.5.4 Predicting the outcome of birth asphyxia

2.5.4.1 Sensitivity, specificity and predictive values

The capacity of diagnostic tests, that is their ability to detect an infant with disease or exclude an infant without disease, is usually described by terms such as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

The sensitivity of a test is defined as the proportion of subjects with disease who will yield a positive result. The specificity of a test is the proportion of subjects without disease who will yield negative results. In other words, sensitivity is the proportion of true positives and specificity the proportion of true negatives correctly identified by the test. Positive and negative predictive values describe a patient's probability of having a given disease once the results of the test are known. The PPV of a test is defined as the proportion of subjects with a positive result who actually have the disease, and the NPV of a test is the proportion of subjects with negative test results who do not have disease. (Akobeng 2006)

Calculation of sensitivity, specificity, PPV and NPV is presented in Table 4.

Table 4. Calculation of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

	Infant with disease	Infant without disease
Test positive	a	b
Test negative	c	d

Sensitivity = a/(a+c)
Specificity = d/(b+d)
PPV = a/(a+b)
NPV = d/(c+d)

2.5.4.2 Cardiotocography

Cardiotocography (CTG) is a screening tool used to assess fetal condition during labor and to identify the possibility of asphyxia. However, the high false-positive rate of CTG makes it a poor tool in predicting the outcome of asphyxiated infants (Finer et al. 1981, Nelson et al. 1996, Toh 2000).

The value of different CTG patterns in identifying encephalopathy is low, although associations exist with late decelerations and decreased variability. For the identification

of HIE, sensitivity and specificity were 15.4% and 98.9%, respectively, for bradycardia in the CTG and 53.8% and 79.8%, respectively, for decreased variability during the last hour before delivery (Larma et al. 2007).

Infants with HIE who developed seizures are reported to have a significantly longer duration of abnormal fetal heart rate patterns (72 +/-12 versus 49 +/-12 minutes) (Williams and Gaulerneua 2004). In one recent study, both severe encephalopathy and neonatal death were more frequent with an abnormal admission CTG pattern (Jonsson et al. 2014).

2.5.4.3 Cord pH and base deficit

Normal term infants have mean (SD) cord arterial pH 7.27 +/-0.07 and base deficit (BE) -2.7 +/-2.8 mmol/L (Riley and Johnson 1993). An increased risk of neurological morbidity is found when the umbilical arterial pH is < 7.00 or BE is > 12 mmol/l (Low et al. 1997). In a systematic review, 23% of infants with cord pH < 7.00 are reported to have neurological morbidity and mortality (Graham et al. 2008).

2.5.4.4 Biomarkers of brain injury

The value of biochemical markers in outcome prediction and early interventions and use in routine practice is limited and calls for more research (Ramaswamy et al. 2009, Bennet et al. 2010, Douglas-Escobar and Weiss 2012).

S100B is released predominantly after astrocyte death (Douglas-Escobar and Weiss 2012). The S100 (BB and A1B dimers) concentration in the first urine (cut-off > 1 µg/L) has suggested an increased risk of neonatal death among asphyxiated infants with a sensitivity and specificity of 100% (Gazzolo et al. 2009). A cord blood concentration of S100B greater than 2.02 µg/L predicts the development of moderate or severe HIE (Qian et al. 2009). An association of serum S100B levels with outcome after asphyxia has been shown at the age of 6 hours after asphyxia (Roka et al. 2012). Elevated serum S100B in infants with neonatal encephalopathy is associated with brain injury (Massaro et al. 2012) and poor neurodevelopmental outcome at 15 months of age (Massaro et al. 2014).

Neuron-specific enolase (NSE) is specific for neurons and neuroectodermal cells. Elevated serum NSE levels are reported after perinatal asphyxia (Celtik et al. 2004, Roka et al. 2012), being associated with an increasing outcome severity at 15 months (Massaro et al. 2014).

Glial fibrillary acidic protein (GFAP) is only released into the blood after astrocyte death. A high GFAP in cerebral spinal fluid (CSF) has correlated with death in neonatal encephalopathy (Blennow et al. 2001). Serum GFAP levels have been elevated in infants with HIE and predicted abnormal brain magnetic resonance imaging (MRI) findings (Ennen et al. 2011) as well as an abnormal neurological outcome (Chalak et al. 2014b).

Serum interleukin 1-beta (IL-1beta) and CSF IL-1beta measured before 96 hours of age have been significantly predictive of long-term outcome in asphyxiated term infants (Ramaswamy et al. 2009). The umbilical cord concentration of interleukin 6 (IL-6) has been 5.5-fold higher in infants with HIE than in those without. IL-6 concentrations have also related significantly to the severity of HIE and neurological outcome at the age of 2 years (Chiesa et al. 2003). Serum IL-6 and IL-8 were greater in infants with moderate or severe HIE than with mild HIE. Elevated levels were associated with abnormal neurological outcomes (Chalak et al. 2014b).

Creatine kinase (CK) and its isoenzymes are used as a biomarker after an asphyxia event, but the predictive value for outcome has been reported to be poor (Sweet et al. 1999). Activin A, adrenomedullin (Bennet al. 2010), brain-derived neurotrophic factor (BDNF), pNF-H and ubiquitin carboxyl-terminal hydrolase L-1 (UCHL-1) (Douglas-Escobar and Weiss 2012) have been suggested to be useful for HIE.

2.5.4.5   Structural cranial ultrasound (CUS)

Acute findings on CUS after a severe HI insult may include diffuse brain swelling with echogenic subcortical white matter on days 1–2 after the insult. This is followed by a loss of grey-white matter differentiation and increasing echogenicity of basal ganglia and thalami (BGT) and white matter (WM) over the next 3–4 days. BGT echogenicity is usually bilateral and increases daily by serial CUS examinations (Leijser et al. 2006).

Several patterns of hypoxic-ischemic brain injury in full-term infants can be detected by CUS as follows: (1) predominant injury to the deep grey matter, (2) predominant injury to the cortex and subcortical white matter ("watershed pattern"), (3) global brain injury and (4) arterial infarction (van Wezel-Meijler et al. 2010). A good correlation between CUS and MRI findings in the brain among infants with NE has been found. The diagnostic accuracy of CUS was 95.7% compared to MRI (Epelman et al. 2010). High-quality CUS is a good screening test for use in infants too critically ill to be transported to the MRI (Epelman et al. 2010). However, MRI is indicated to confirm CUS findings (van Wezel-Meijler et al. 2010, Epelman et al. 2010).

Intraventricular hemorrhage is rare in term infants. The incidence of IVH detected by CUS in term healthy asymptomatic infants is approximately 3.5% (Heibel et al. 1993). There are several risk factors for IVH, including severe asphyxia, traumatic delivery, instrumental delivery and venous thrombosis (Wu et al. 2003). In one recent study, hemodynamic instability, hemostasis disturbances and hypoglycemia were risk factors for IVH in severe HIE during hypothermia and rewarming (Al Yazidi et al. 2014). The incidence of hemorrhagic brain lesions has been from 30% to 40% among noncooled or cooled infants with HIE (Rutherford et al. 2005).

2.5.4.6 Brain magnetic resonance imaging (MRI)

Neonatal brain MRI provides useful information regarding the site and severity of brain injury and the outcome of infants with HIE (Martinez-Biarge et al. 2010, Martinez-Biarge et al. 2011). The optimal timing of serial conventional MRI imaging to visualize perinatal brain lesions after HI insult seems to be between 1 and 2 weeks from delivery. Earlier MRI scanning during the first week should include diffusion-weighted imaging (DWI), as DWI is ideal for early identification of ischemic tissue and lasts for 7–14 days (Rutherford et al. 2010a). The accuracy of MRI for the prediction of outcome among HIE infants has not been altered by therapeutic hypothermia (Rutherford et al. 2010b).

Neurodevelopmental problems are correlated with the severity of brain lesions (Cowan et al. 2003, Martinez-Biarge et al. 2012). Central grey matter damage is a sign of acute perinatal hypoxia-ischemia in term infants (Okereafor et al. 2008). Lesions of BGT and the posterior limb of the internal capsule (PLIC) are notable causes of death and CP (Okereafor et al. 2008, Rutherford et al. 1998). CP as a consequence of BGT injury affects 70–75% of survivors (Martinez-Biarge et al. 2011). Brainstem injury is often connected with HIE and BGT injury (Martinez-Biarge et al. 2010).

2.5.4.7 Neurophysiologic examinations

2.5.4.7.1 Electroencephalogram (EEG)

Severely abnormal background activity on the EEG is generally associated with an adverse outcome after an HIE event (Sinclair et al. 1999, El-Ayouty et al. 2007, Leijser et al. 2007). The sensitivity of an abnormal background in predicting poor outcome was 93% and specificity with 75%, and PPV 88% (Leijser et al. 2007). Background amplitude < 30 uV, inter-burst interval of > 30 s, electrographic seizures and absence of sleep-wake cycling (SWC) at 48 hours of age have been associated with abnormal outcome in HIE (Murray et al. 2009). The return of SWC as detected by EEG has been associated with a good outcome, especially if it occurs within 36 hours after birth (Osredkar et al. 2005). Never-developing SWC always predicts adverse outcome (Thoresen et al. 2010). The presence of an epileptiform discharge on the EEG is not reported to be predictive of an adverse outcome in term infants with HIE (Biagioni et al. 2001, Leijser et al. 2007). A persistent discontinuous pattern on the EEG after the first week after an asphyxia event is markedly related to a poor outcome (Biagioni et al. 1999, Menache et al. 2002). A normal outcome is seen in infants with normal or mildly abnormal EEG patterns (Wertheim et al. 1994, Zeinstra et al. 2001, El-Ayouty et al. 2007, Leijser et al. 2007, Murray et al. 2009). Normal or mildly abnormal EEG within 24 hours after birth had 100% PPV for normal outcome and an NPV value of 76% among infants with HIE (Murray et al. 2009). In a recent systematic review, the

accuracy of EEG for the prediction of outcome in infants with HIE after birth asphyxia was good (sensitivity 92%, specificity 83%) (van Laerhoven et al. 2013).

2.5.4.7.2   Evoked potentials (EPs)

Brainstem auditory evoked potential (BAEP)

BAEP reflects the electrophysiological activity of neurons in the brainstem auditory pathway following acoustic stimulation. The utility of neonatal BAEP assessments in prognostication is limited. A normal BAEP does not ensure a normal outcome, while severely abnormal BAEP would appear to predict an abnormal outcome at 12-24 months of age in high-risk infants (Majnermer et al. 1988, Scalais et al. 1998). Abnormal BAEP has predicted an adverse outcome with a sensitivity of 41% and a specificity of 88% (Jiang et al. 2008).

Somatosensory evoked potential (SEP)

SEP is a tool for assessing the functional integrity of the somatosensory pathways of the peripheral (median nerve) and central nervous system. Early SEPs have a relatively high sensitivity and specificity in predicting outcome in infants with neonatal encephalopathy (Gibson et al. 1992, Taylor et al. 1992, Scalais et al. 1998, Mandel et al. 2002). A normal SEP finding at 1 week of age is generally a sign of normal outcome in asphyxiated infants and NE, whereas an abnormal SEP predicts an adverse outcome in 90% of cases (Taylor et al. 1992, Gibson et al. 1992, Kontio et al. 2013). The sensitivity of abnormal SEP in asphyxiated term infants has varied from 90 to 95%, and specificity 86–92% (Eken et al. 1995, Swarte et al. 2012). Bilateral loss of cortical SEP seems to be a strong indicator of poor outcome in acute encephalopathy in adults and in older children (Goodwin et al. 1991, Wolrab et al. 2001, Robinson et al. 2003) and in infants with HIE, with a positive predictive value of 100% (Mandel et al. 2002). Bilateral loss of cortical SEP at birth is reported to predict CP at two years in survivors of neonatal encephalopathy (Suppiej et al. 2010). A prolonged SEP latency on day 3 has shown a correlation with neurocognitive outcome at school age (Kontio et al. 2013).

Visual evoked potential (VEP)

VEP is a cortical response elicited by flashing light, light-emitting diode or by pattern visual stimuli. VEP has a good correlation with neurodevelopmental outcome in term asphyxiated infants assessed within the first week of life (Kato and Watanabe 2006, van Laerhoven et al. 2013). The sensitivity of an abnormal VEP in asphyxiated term infants in predicting abnormal outcome has varied from 78% to 91% and specificity from 67% to 100% (Muttitt et al. 1991, Taylor et al. 1992, Eken et al. 1995, Kato and Watanabe 2006, van Laerhoven et al. 2013).

2.5.4.8   Neurodevelopmental assessments

General movements (GMs)

General movements (GMs) is a non-invasive mode of assessment, based on evaluation of the quality of the spontaneous movements of the infants. Two patterns of normal GMs are observed: writhing and fidgety movements. Writhing movements, seen at term age to the first two months post-term, are small- to moderate-amplitude movements. Normal fidgety movements are defined as circular movements of small amplitude, moderate speed and acceleration of neck, trunk and limbs in all directions from 6 weeks to 20 post-term weeks (Prechtl et al. 1997).

Abnormal movements are defined as having (1) poor-repertoire general movements (the sequence of successive movement components is monotonous and movements of different body parts do not occur in complex), (2) cramped-synchronized general movements (all limb and trunk muscles contract and relax almost simultaneously or are chaotic) or (3) absent fidgety movements (Prechtl et al. 1997, Einspieler and Prechtl 2005). Normal fidgety GMs at 3 months predict a good outcome in high-risk infants (Prechtl et al. 1997, Ferrari et al. 2011). Definitely abnormal GMs at 3 months post-term have a relationship with CP (Adde et al. 2007, Hadders-Algra 2014). The median sensitivity of GMs at 3 months post-term is 98% (range 50–100%) and median specificity 94% (range 35–100%) (Hadders-Algra 2014).

Alberta Infant Motor Scale (AIMS)

The Alberta Infants Motor Scale (AIMS) is an observational measure of gross motor development from birth to 18 months of age (Piper and Darrah 1994). The 58-item scale is designed to assess gross motor skills in four positions: prone, supine, sitting and standing. The score for each position is summed to obtain total raw scores, and converted into an age-related percentile ranking. The risk of abnormal motor developmental performance is defined as a score on AIMS below the 10th percentile at 4 months or below the 5th percentile at 8 months of age. AIMS at 4 months has predicted the outcome with a sensitivity of 77% and specificity 82%, and at 8 months with a sensitivity of 87% and a specificity 93%. The AIMS is a practical, reliable and valid assessment tool used to measure developing term infants (Darrah et al. 1998).

Neuromotor scoring (NMS)

The neuromotor scoring system (NMS) is applied at 3 and 12 months of age. The system includes an assessment of mental status, cranial nerve II-XII function, active and passive tone and power in the trunk and extremities, deep tendon reflexes and primitive reflexes. The summary findings are presented in Table 5. NMS scores have correlated with the NMS at 3 months and the results of neurological examination at 12 months of age. (Hajnal et al. 1999)

Table 5. Neuromotor score (NMS).

Score	Description
0	Normal
1	Mild abnormality of tone or reflexes
2	Mild abnormality of tone and reflexes
3	Abnormality of tone or reflexes, or both, with decreased power in the trunk or extremities
4	Any motor abnormality and cranial nerve involvement
5	Spastic quadriparesis

3   AIMS OF THE STUDY

The purpose of the present study was to evaluate the accuracy of pulsed Doppler ultrasonography of the cerebral circulation in extremely-low-birth-weight (ELBW) preterm infants and compared with clinical, neurophysiologic and neurodevelopmental assessments in the prediction of the outcome in asphyxiated full-term infants.

The specific aims were as follows:

1. To establish whether end-diastolic block in the cerebral circulation as detected by pulsed Doppler ultrasonography predicts intraventricular hemorrhage in ELBW infants (Study **I**).

2. To assess whether postnatal pulsed Doppler findings in the cerebral arteries, symptoms of hypoxic ischemic encephalopathy and obstetric parameters are useful predictors of the 1-year neurological outcome of asphyxiated full-term infants (Study **II**).

3. To assess whether Doppler findings in the cerebral arteries, EEG and evoked potentials are of value as predictors of the 1-year outcome in asphyxiated full-term infants (Study **III**).

4. To evaluate the accuracy of neurodevelopmental assessments in the prediction of the 1-year outcome in asphyxiated full-term infants (Study **IV**).

4 PATIENTS AND METHODS

4.1 Patients and study design (Studies I–IV)

Study **I** was a prospective cohort study from birth to four days of age. Seventy-five consecutive ELBW infants with birth weights < 1000 g were born in Tampere University Hospital between June 1999 and August 2002. The gestational age ranged between 24 to 31 weeks. Twenty infants were excluded from the analysis: 13 died during the first or second day of life, three had not undergone Doppler ultrasonography examination and in one case, pulsed Doppler ultrasound examination was first performed on the fifth day of life. Relevant data were missing in three cases. The remaining 55 infants, who underwent pulsed Doppler ultrasound examinations one to four times during the first four days of life, constituted the study group. The study population was divided into a block group, with an end-diastolic block diagnosed in the intracranial arteries at least once during the study period, and a control group with continuous flow found in each of the examinations.

The asphyxia study (Studies **II–IV**) was a prospective longitudinal follow-up study carried out in the neonatal intensive care unit in Tampere University Hospital. Altogether 30 full-term (gestational age 37 weeks or more) infants with asphyxia and 30 healthy gestational age- and sex-matched infants, born between July 2000 and September 2003, were enrolled. Infants were included in the asphyxia group when five-minute Apgar scores were 5 or less and if they fulfilled at least one of following criteria: 1) signs of fetal distress such as abnormal CTG or meconium-stained amniotic fluid, 2) cord arterial pH < 7.10 or 3) symptoms of HIE within 48 hours of life.

For each asphyxiated infant a non-asphyxiated control infant was enrolled in the postnatal ward. Infants with major congenital malformations likely to affect Apgar scores were excluded. One asphyxiated infant with grade 2 HIE, in whom Kabuki syndrome was diagnosed according to the characteristic features at 2 years and 9 months of age, was included in the analysis.

4.2 Methods

The timing of ultrasound, electrophysiologic and neurodevelopmental assessments in the full- term asphyxiated and control infants is summarized in Table 6 (Studies **II–IV**).

Table 6. Timing of ultrasound, electrophysiologic and neurodevelopmental assessments in the asphyxiated and control infants.

	Asphyxiated infants		Controls	
Ultrasonography examinations				
Cerebral Doppler	0–8 hrs	10–12 hrs	24 hrs*	0–8 hrs	10–12 hrs	24 hrs*
Cranial ultrasound	0–8 hrs	10–12 hrs	24 hrs*	0–8 hrs	10–12 hrs	24 hrs*
Echocardiography	0–8 hrs	10-12 hrs	24 hrs	0–8 hrs	10–12 hrs	24 hrs
Electrophysiologic examinations				
Electroencephalogram (EEG)	1–4 d		1–2 d	
Brainstem auditory evoked potential (BAEP)	1–8 d		1–3 d	
Somatosensory evoked potential (SEP)	1–8 d		1–3 d	
Visual evoked potential (VEP)	1–8 d		1–3 d	
Neurodevelopmental assessments				
General movements (GMs)	Before discharge	3 mos	Before discharge	3 mos
Alberta Infant Motor Scale (AIMS)	3 mos	6 mos	3 mos	6 mos
Neuromotor scoring (NMS)	3 mos	12 mos	3 mos	12 mos

* Cerebral Doppler and cranial ultrasound were undertaken three times during the first day of life and then daily until cerebral blood velocity was normalized.

4.2.1 Ultrasonography examinations (Studies I–IV)

Each infant underwent pulsed Doppler examinations and structural ultrasonography performed by an attending neonatologist (**I**) or by the investigators (**II–IV**), using a 7.5 MHz transducer (PowerVision 6000, Toshiba, Nasu, Japan). Examinations were made of ELBW infants one to four times during the first four days of life (**I**). The asphyxiated term infants study, following the study protocol, included Doppler examinations at about 8 hours (median 6; range 1–9 hours), at 10–12 hours (median 10; range 9–14 hours) and at 24 hours of age (median 29; range 19–49 hours) and thereafter daily until CBFV appeared to be normalized (**II–IV**).

CBFV (cm/s) in the ACA and BA arteries was measured from the midsagittal section through the anterior fontanelle. The flow of blood in the ACA was measured in the front of the third ventricle and the flow in the BA in the front of the pons cerebri in the sagittal section. A resistive index (RI = (peak-systolic velocity – end-diastolic velocity)/peak systolic velocity) was calculated from these data. An RI ranging between 0.6 and 0.8 was regarded as normal (Deeg and Ruprecht 1989, Allison et al. 2000). End-diastolic block was diagnosed when end-diastolic flow was found to be absent from the Doppler waveforms of at least 20 consecutive cardiac cycles in either or both cerebral arteries. (**I–IV**.) The mean values of peak systolic and diastolic CBFV measurements were considered abnormal if they were three standard deviations (3 SD) above the means in healthy control infants (**II–IV**).

Cerebral ultrasonography was performed to detect structural abnormality, intraventricular hemorrhage (IVH) or changes in parenchymal echodensity or edema. Hemorrhages were graded according to Papile and colleges (1978). Cranial ultrasound was considered normal when no IVH, anomalies or increased echodensities were detected (I–IV).

Standard echocardiography was performed with the same equipment either immediately before or after brain ultrasonography to define the direction of the ductal flow and the time of ductal closure from the parasternal and suprasternal view. Diagnosis of hemodynamically significant PDA was made on clinical findings (**I–IV**). PDA was considered hemodynamically significant if there were three of the following findings: (1) a characteristic systolic murmur, (2) bounding peripheral pulses, (3) hyperactive precordium, (4) cardiac enlargement in chest X-ray and (5) respiratory deterioration. In such cases the PDA was closed either by means of indomethacin treatment or by surgical ligation, at the discretion of the attending neonatologist (I).

4.2.2 Hemodynamic monitoring (Study I)

Blood pressure was monitored via an indwelling peripheral arterial catheter using a multichannel neonatal monitor (HP Model 64S, Hewlett-Packard GmbH, Boeblingen Germany). MAP was recorded simultaneously with the Doppler ultrasonography. Blood

gas analyses, blood glucose and hematocrit were determined within 2 to 4 hours of cerebral ultrasound examination. Hypocapnia was defined as a partial pressure of carbon dioxide (pCO_2) < 4.5 kPa in arterial blood gas analyses and hypoglycemia as a blood glucose concentration < 2.6 mmol/L.

Hypotension was defined as a MAP in mmHg less than gestational age (Joint Working Party of the British Association of Perinatal Medicine and the Research Unit of the Royal College of Physicians 1992). Infants with low MAP levels for their current gestational age received a 10 mL/kg intravenous infusion of Ringer's solution or fresh frozen plasma in a period of 30 to 60 minutes. In cases of poor response or recurrent MAP drop, treatment was initiated with inotropics (dopamine 5–10 microg/kg/min, combined if necessary with dobutamine 5–10 µg/kg/min). If hypotension persisted despite maximum dosage of dopamine and dobutamine, hydrocortisone 5 to 10 mg/kg/d divided into three intravenous doses was administered.

4.2.3 Grading of HIE (Studies II–IV)

The severity of HIE was graded from 1 to 3 according to modified Sarnat criteria (Sarnat and Sarnat 1976). Grading was based on clinical observation (level of consciousness, muscle tone, posture, reflexes, autonomic functions and seizures) during the first 48 hours of life. Infants with grade 1 HIE had normal muscle tone and usually recovered well within 12–24 hours without seizure activity. Infants who developed grade 2–3 HIE had altered levels of consciousness and seizure activity 12–24 hours after the hypoxic-ischemic event. Loss of reflex activity, flaccid muscle tone, respiratory failure and coma were criteria for grade 3 HIE.

4.2.4 Obstetric parameters (Study II)

Obstetric data were collected and analysed by an obstetrician who was blinded to the Doppler findings and the outcomes of the infants. Data from CTG were available in 29 (97%) of the asphyxia cases and in 28 (93%) of the controls, either during the whole labor or at admission before the decision to undertake cesarean section (CS). CTG was classified according to the FIGO criteria (1987) as normal, non-reassuring, pathological or preterminal, taking into account the given time criteria for the occurrence of decreased variability (more than 40 or 60 minutes for non-reassuring or pathological, respectively). Prolonged bradycardia (< 110 beats per minute) of more than 10 minutes' duration was included in the criteria for pathological CTG. According to the available CTG changes and other obstetric data the obstetrician further determined whether the fetus suffered an asphyxia episode lasting longer than one hour. Apgar scores were evaluated at the age

of one and five minutes (Apgar 1953) and cord artery pH and base excess were measured according to the routine practice of the hospital.

4.2.5 Biochemical asphyxia markers (Study II)

The concentrations of serum CK, serum NSE and plasma lactate were measured as biochemical asphyxia markers in the asphyxia group on day 1 of life using the standard laboratory methods of the hospital. The following cut-off values were used for asphyxia: s-NSE above 40 ug/L (Nagdyman et al. 2003, Celtik et al. 2004), plasma lactate above 7.5 mmol/L (Shah et al. 2004) and s-CK above 1000 U/L (Omokhodion et al. 1991).

4.2.6 Electrophysiology (Study III)

EEGs were taken on average at 1.5 (range 1–4) days of age in the asphyxia group and 1.0 (range 1–2) days of age in the control group. SEPs were recorded on average at 5.0 (range 1–8) days of age in the asphyxia group and 2.0 (range 1–3) days of age in the control group. BAEPs and VEPs were measured on average at 4.0 (range 1–8) days of age in the asphyxia group and 1.0 (range 1–3) days of age in the control group, respectively. In one asphyxiated infant BAEP and VEP were not taken until 30 day's and SEP 37 day's age.

EEG and EP recordings were carried out according to routine clinical procedures at the Department of Clinical Neurophysiology, Tampere University Hospital. The laboratory rooms are sound-proof and equipped for clinical EEG and EP measurements. The room temperature was kept at 20–22°C. Data were analyzed by a clinical neurophysiologist who was blinded to the outcome of the infants. If evoked potential latency was more than 3 SD from the normative values of the laboratory, the response was considered abnormal.

EEG
EEG was carried out using a Nervus M40 device (Taugagreining, Iceland). The EEG contained only 9 electrodes according to the International 10-20 system, due to the small size of the infant's head. In addition electrocardiogram, respiratory belt and video were recorded. For visual analysis 16 bipolar EEG-derivations were used (Fp1-T3, T3-O1, Fp1-C3, C3-O1, Fp1-Cz, Cz-O1, Fp2-T4, T4-O2, Fp2-C4, C4-O2, Fp2-Cz, Cz-O1, T4-C4, C4-Cz, Cz-C3, C3-T3). A clinical neurophysiologist divided the EEG outcomes into four groups according to the methods introduced by Leijser and co-workers (Leijser et al. 2007). The first level represents normal EEG and EEG levels 2–4 are considered abnormal (Table 7).

BAEP

In BAEP recordings a Nicolet Viking IV device (Nicolet Biomedical Instruments) was used. The BAEP responses were recorded with surface active electrodes on both mastoid processes, the reference being placed at the vertex (Liveson and Ma 1992a). The electrode impedances were kept under 5.0 kΩ. The BAEPs were elicited with a rarefaction click stimulus of 80 dB sound pressure level and 100 µs duration, given at a rate of 9.9 Hz, a masking noise of 40 dB being delivered to the contralateral ear. Responses were amplified with the high- and low-pass filters set at 100 Hz and 3 kHz, respectively. The BAEP was recorded at least twice to ascertain reproducibility. The peak latencies of waves I, III and V, as well as the inter-wave intervals (I–III, I–V, III–V) were measured from ipsilateral responses on both sides. Waves I, III and V were identified and marked manually on the computer screen by the nurse and the results confirmed and visually analyzed by a clinical neurophysiologist who divided the BAEPs into 5 groups (Table 7).

SEP

SEP responses were elicited by electrical stimulation applied on both median nerves on the wrist using a constant current square wave pulse (0.1 ms width, cathode proximal) at a repetition rate of 1 Hz. The stimulus intensity was regulated to produce a small thumb twitch. Responses were recorded from ERB, cervical level (C2-Fpz) and cortically from CP3-Fz, CP3-M2, CP4-Fz and CP3-M1-derivations (Liveson and Ma 1992b). In addition, the derivations CP3-M2 and CP4-M1 are in routine use in our laboratory. Two trials of at least 100 artifact-free samples (automatic artifact rejection) were recorded with an analysis time of 100 ms. The SEP outcomes were divided into three groups, the first representing normal responses (Table 7).

VEP

Non-corneal electroretinogram (ERG) and VEP were recorded in a partially darkened room and were elicited by binocular stimulation with a stroboscopic un-patterned flash (frequency 0.9 Hz) placed about 25 cm from the eyes. ERG responses were recorded from EOGleft – Fz and EOGright – Fz derivations in order to ensure normal retinal function. All ERG responses were normal and VEPs were further analyzed. VEPs were recorded from Fz-O1, Fz-Oz and Fz-O2 - derivations. The ground electrode was at midline (Cz) (Odom et al. 2010). Two trials of at least 50 artifact-free responses (automatic artifact rejection) were recorded within 512 ms after stimulus. For VEPs the P2 latencies of both eyes were measured. Results were divided into two groups, normal and abnormal responses (Table 7).

Table 7. Classification of the neurophysiologic measures.

EEG	
level 1 (Normal)	Normal background, no epileptiform discharges
level 2 (EEG-2)	Normal background with epileptiform discharges
level 3 (EEG-3)	Abnormal background, no epileptiform discharges
level 4 (EEG-4)	Abnormal background with epileptiform discharges
BAEP	
Normal	Normal latencies bilaterally
BAEP-2	Unilaterally normal, absent responses on the other side
BAEP-3	Lengthened peripheral latencies unilaterally or bilaterally (wave I)
BAEP-4	Bilaterally absent responses
BAEP-5	Lengthened central latencies unilaterally or bilaterally (wave III, V, interval III–V)
SEP	
Normal	Bilaterally normal cortical responses
SEP-2	Unilaterally lengthened cortical latencies with normal cervical responses
SEP-3	Bilaterally lengthened cortical latencies with normal cervical responses
VEP	
Normal	Bilaterally normal responses
Abnormal	Absence of responses (bilaterally or unilaterally)

Abbreviations: EEG, electroencephalogram; BAEP, Brainstem auditory evoked potential; VEP: Visual evoked potential; SEP, Somatosensory evoked potential. EEG classification is made according to the method introduced by Leijser and co-workers (Leijser et al 2007). EEG-3: abnormal background = discontinuous and/or asymmetrical and/or low voltage EEG (amplitude ≤30 µV)

4.2.7 Neurodevelopmental assessments (Study IV)

Alberta Infant Motor Scale (AIMS)

The AIMS is an observational measure of motor development from birth to 18 months of age (Piper and Darrah 1994). The 58-item scale is designed to assess gross motor skills in four positions: prone, supine, sitting and standing. Total raw scores were here calculated and converted into an age-related percentile ranking. The risk of abnormal motor developmental performance is defined as a score on AIMS below the 10th percentile at 4 months or below the 5th percentile at 8 months of age (Darrah et al. 1998). The assessments were made by physiotherapists with 2–15 years' clinical experience. The schedule of the AIMS assessments is seen in Table 6.

General movements (GMs)

The GMs of infants were videotaped before discharge and at 3 months of age. All recordings were made during active wakefulness, the infants lying supine and partially dressed. The recordings were assessed by a physiotherapist, who was certified by the General Movement Trust in performing the Prechtl methodology and was not aware of the medical history of

the infants. Two patterns of normal GMs are observed: writhing and fidgety movements (Prechtl et al. 1997). Accordingly, movements here classified as abnormal if the normal GMs were absent at 3 months of age.

Neuromotor scoring system (NMS)

The neuromotor scoring system was applied at 3 months of age by a pediatric neurologist who was blinded to the perinatal history of the infants. The NMS at 3 months (NMS-3) included an assessment of mental status (adaptive capacity to sound, light and the examiner, alertness, vocalization, curiosity and consolability), cranial nerve II–XII function, active and passive tone and power in the trunk and extremities, deep tendon reflexes, primitive reflexes (asymmetric tonic neck, Moro's reflex, palmar and plantar grasp, automatic walking, placing, rooting, sucking, cortical thumbing and Babinski's reflex). The summary findings were scored from 0 to 5 as follows: 0 = normal; 1 = mild abnormality of tone or reflexes; 2 = mild abnormality of tone and reflexes; 3 = abnormality of tone or reflexes or both and decreased power in the trunk and extremities; 4 = any motor abnormality and cranial nerve involvement; 5 = spastic quadriparesis (Table 5). (Hajnal et al. 1999)

4.2.8 One-year neurological outcome (Studies II–IV)

The overall neurological outcome among the asphyxiated term infants was assessed clinically at 1 year of age by a pediatric neurologist who was blinded to the history of the infants. Griffiths Developmental Scales (Alin-Åkerman and Nordberg 1991) were used to structure the assessment, but only raw scores were recorded. According to these raw scores and neuropediatric clinical evaluation the outcome at the age of 1 year was divided into three groups (1) normal, (2) motor disability, (3) both motor and mental disability. Poor outcome was defined as any neurological impairment or death.

4.2.9 MRI (Study IV)

Brain MRI was performed using 1.5T Siemens Avanto (Erlangen, Germany) at 1 year of age in asphyxiated term infants. MR images were evaluated by two neuro-radiologists blinded to the clinical data of the asphyxiated infants.

4.2.10 Statistical analyses (Studies I-IV)

Statistical analyses were made using SPSS 11.0 and 17.0 for Windows (SPSS, Chicago, IL, USA). The statistical analyses between the block group and the control group were performed by independent *t*-test, chi-square, Fisher's exact test or Mann-Whitney *U*-test,

as appropriate. A p-value below 0.05 was considered significant. Stepwise logistic regression analysis was used to identify risk factors for end-diastolic block (birth weight, IVH, MAP 30 mmHg or less, pCO_2 4.5 kPa or less, hematocrit 0.40% or less, blood glucose 2.6 mmol/l or less, PDA), and to identify risk factors for IVH (birth weight, block, MAP 30 mmHg or less, pCO_2 4.5 kPa or less, hematocrit 0.40% or less, blood glucose 2.6 mmol/l or less, PDA). Relative risk (RR) for IVH with presence of block and MAP 30 mmHg or less was calculated (**I**).

Differences between infants with asphyxia and healthy controls, and between normal and abnormal outcome within the asphyxia group were tested for statistical significance by independent t-test or Brown-Forsythe robust test of equality due to the non-homogeneity of variances if continuous variables were normally distributed. Mann-Whitney U-test or Kruskal-Wallis test were used for skew-distributed continuous variables, and categorical variables were tested by Pearson's chi-square or Fisher's exact test if expected values were too small. A p-value below 0.05 (with Bonferroni correction if appropriate) was considered significant (**II–IV**).

Sensitivity and specificity for CTG classification, Apgar scores, cord blood pH, biomarkers, HIE grading and Doppler findings or their combinations were calculated to assess their accuracy in the prediction of abnormal neurological outcome at 1 year of age or death in asphyxiated infants. Sensitivity and specificity for CTG classification, Apgar scores, HIE grading and Doppler findings or their combinations were calculated in asphyxiated infants with normal 1-year outcome (**II**).

Sensitivity, specificity, PPV and NPV of EEG grading, grading of BAEP, SEP, VEP, HIE grading and Doppler findings, and various combinations of these covariates in predicting neurological outcome at 1 year of age were calculated in asphyxiated infants (**III**). Sensitivity, specificity, NPV and PPV for a neurological outcome at 1 year of age or death were calculated for HIE grading, GMs at discharge and at age 3 months, NMS at 3 months and AIMS assessments at 3 months and 6 months of age in asphyxiated infants (**IV**). Sensitivity, specificity, NPV and PPV with 95% confidence intervals were calculated by the Confidence Interval Analysis program version 2.1.2. (University of Southampton, UK) (**II–IV**).

4.2.11 Ethics (Studies I–IV)

The study protocols were approved by the Research Ethics Committee of Pirkanmaa Hospital District, Tampere, Finland (decision numbers R00045, R01541). In Study **I** the ultrasound examinations were performed according to the routine follow-up protocol used in the unit. The parents were well informed on the timing and results of the examinations. In Studies **II–IV** at least one parent of the infant gave written informed consent.

5   RESULTS

5.1   Clinical characteristics of the extremely-low-birth-weight (ELBW) preterm infants (Study I)

A total of 147 pulsed Doppler ultrasound examinations were performed among the ELBW preterm infants during the first four days of life. Of these infants, 22 developed an end-diastolic block at least once, whereas no block was detected in 33 infants. The block group and the control group were similar in birth weight, gestational age and male/female ratio (Table 8).

Dopamine or dobutamine treatment did not differ between the groups (data not shown). In infants with hypotension, glucocorticoid was administered almost statistically significantly more often in the block group than in the control group, (p = 0.057). The median dosage of morphine did not differ between the two groups on day 1, but the infants with block detected on days 2–4 received statistically a significantly higher median dosage of morphine (14.3 (range 2.6–32) μg/kg/h) than the controls (8.7 (range 2.1–20) μg/kg/h), (p = 0.021). Blood glucose, hematocrit, pCO_2 and pO_2 values did not differ between the groups. Seven (32%) infants in the block group and 6 (18%) in the control group died, (p = 0.244).

Table 8. Clinical characteristics of the ELBW study groups.

	Block group (n = 22)	Control group (n = 33)	p-value
Birth weight (g) mean (SD)	757 (137)	809 (151)	0.204
Gestational age (wk) mean (SD)	27 (2.0)	27 (1.7)	0.926
SGA (n) (%)	10 (73)	10 (30)	0.250
Delivery route			0.808
Vaginal (n) (%)	6 (27)	10 (30)	
Cesarean section (n) (%)	16 (73)	23 (70)	
Apgar scores at 1 min 5 or less (n) (%)	15 (68)	16 (49)	0.149
Apgar scores at 5 min 5 or less (n) (%)	7 (32)	17 (52)	0.564
Male/female ratio	12/10	19/14	0.824
Antenatal steroids (n) (%)	21 (95)	31 (94)	0.933
Dopamine treatment (n) (%)	19 (86)	27 (82)	0.727
Dose of dopamine (ug/kg/min) Median (range)	12.0 (2.2–13.5)	9.6 (2.7–17.2)	0.697

5.2   End-diastolic block and systolic cerebral blood flow velocity in cerebral arteries in ELBW preterm infants (Study I)

An end-diastolic block in the ACA or in the BA was detected in 17 (35%) ELBW preterm infants on day 1. Eight infants had a block only in the ACA, three only in the BA and six simultaneously in both arteries; 32 infants evinced continuous flow. During days 2–4 an end-diastolic block was detected at least once in 13 (24%) infants. Two infants had a block only in the ACA, six only in the BA and five simultaneously in both arteries, and one infant had a block in the BA on day 3 and in the ACA on day 4; 42 infants evinced continuous flow. Retrograde diastolic flow was detected once in two infants in the block group during the first four days of life.

During the first 24 hours, the peak systolic cerebral blood flow velocity (SCBFV) was measured in 53% of the infants in the block group and in 81% in the control group. The SCBFVs did not differ between these study groups. The peak SCBFV in the BA was marginally lower in the block group (mean (SD) 20.9 (8.4) cm/s) than in the control group (25.5 (7.6) cm/s), (p = 0.059), but in the ACA was fairly similar in both groups (24.1 (7.2) cm/s) vs. 21.4 (6.4) cm/s, respectively, (p = 0.363). During days 2 to 4, peak SCBFVs in the ACA or in the BA did not differ between the two groups.

5.3   Hemodynamic values in ELBW preterm infants (Study I)

The mean (SD) of the MAP recordings measured during Doppler ultrasound examinations in the block group infants was significantly lower (30 mmHg; SD 5; range 16–40 mmHg) than in those without (34 mmHg; SD 5; range 25–52 mmHg), (p = 0.01). On day 1, the concomitant mean of MAP in the infants with end-diastolic block was marginally lower (33 mmHg; SD 7.4; range 24–59) than in those without (35 mmHg; SD 5.3; range 25–52) at the time of the Doppler examination, (p = 0.060) (Figure 3).

The mean MAP at the time of the Doppler examination on days 2 and 3 did not differ between the study groups as is seen in Figure 3, but on day 4 MAP in the block group infants was again significantly lower (34 mmHg; SD 5; range 24–40) than in the controls (41 mmHg; SD 8.2; range 31–67), (p = 0.019).
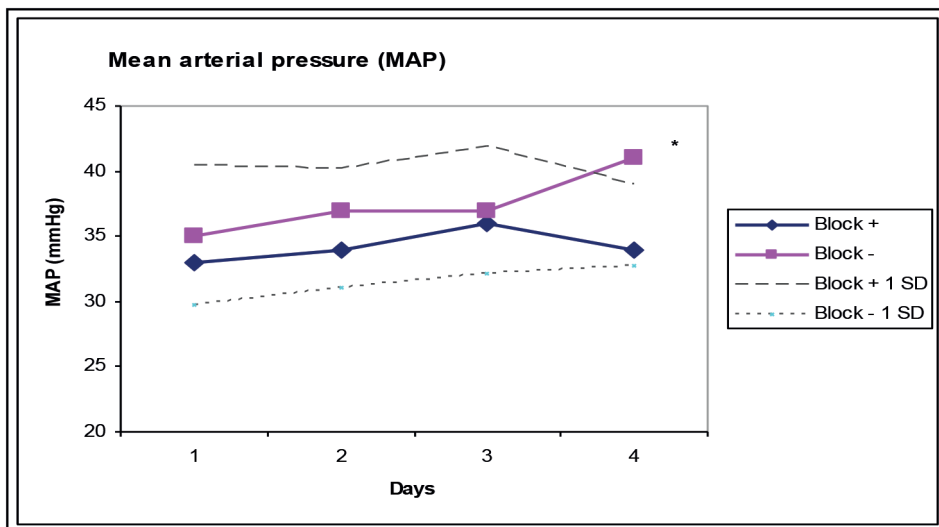
Figure 3. The mean (SD) of mean arterial pressure (MAP) in the ELBW infants with an end- diastolic block (block +) in the cerebral circulation at least once during the first four days and in the control group without block (block -). *p < 0.05.

5.4   Patent ductus arteriosus (PDA) and end-diastolic block in ELBW preterm infants (Study I)

The ductus arteriosus was open in 93% of cases in the block group and in 81% in the controls in the day 1 echocardiographic examination. On the following days, spontaneous closure of PDA occurred earlier in the infants without block. Right-to-left ductal shunting was not seen in any case, but bidirectional shunting was still present in three of the block

cases and in two controls on day four. PDA was surgically ligated in five (23%) infants in the block group and in two (6%) in the control group. Three (14%) infants in the block group received indomethacin to close PDA. PDA closed spontaneously in 11 (50%) infants in the block group and in 27 (82%) in the control group, (p = 0.010).

5.5 IVH and end-diastolic block in ELBW preterm infants (Study I)

Six infants (27%) in the block group and 10 (30%) among the controls developed IVHs, (p = 0.808). Grade I–II IVHs occurred in 2 (9%) cases in the block group and in 6 (18%) controls, p = (0.692) and grade III–IV IVHs in 4 (18%) vs. 4 (12%) of the block and control cases, respectively, (p = 0.707).

5.6 Predictors of IVH in ELBW preterm infants (Study I)

End-diastolic block in the intracranial arteries at least once during the first four days of life connected to a MAP of 30 mmHg or less was associated with an increased risk of IVH. IVHs occurred in 46% of such cases, whereas all block cases with mean arterial pressures higher than 30 mmHg remained without IVHs (Figure 4), (p = 0.046). The calculated relative risk (RR) for developing IVH with presence of end-diastolic block and MAP 30 mmHg or less was 1.9 (95% confidence interval (Cl) 1.1–3.1) (unpublished data).

Eighteen per cent of the 33 infants without block had mean arterial pressures 30 mmHg or less and 33% of them developed IVH. The remaining 82% of such infants had mean arterial pressures over 30 mmHg, and 30% of these developed IVHs (Figure 4); (p = 1.000).

Stepwise logistic regression analysis was used to identify risk factors for IVH. In this analysis, PDA was the only significant risk factor for intraventricular hemorrhage (odds ratio (OR) 20.0; 95% CI 2.0–196.0, p = 0.01), whereas of the potential risk factors for the occurrence of end-diastolic block, none reached statistical significance in a corresponding analysis. The method of PDA closure was not associated with the occurrence of IVH.
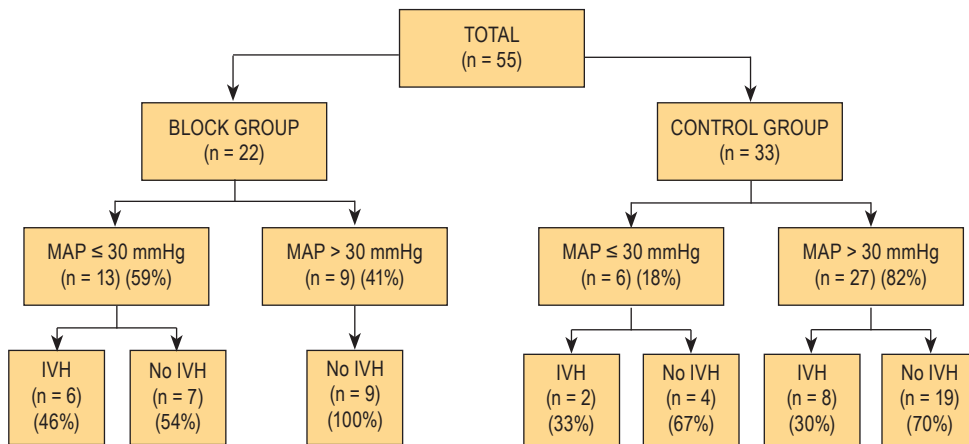
Figure 4. Mean arterial pressure (MAP) as a threshold value in the assessment of risk of IVH in the ELBW infants with and without an end-diastolic block in the cerebral circulation. *$p < 0.05$, MAP > 30 mmHg and IVH *vs.* MAP < 30 mmHg and IVH in the block group; $p = 1.000$ in the control group.

5.7 Full-term asphyxia study groups (Studies II–IV)

Clinical characteristics of the asphyxia study groups are presented in Table 9. Characteristics of asphyxiated infants with normal or abnormal outcome are shown in Table 10.

Table 9. Clinical characteristics of the asphyxiated and control infants.

	Asphyxia group (n = 30)	Control group (n = 30)	p value[a]
Birth weight (g), Median (range)	3325 (2295–4240)	3676 (2920–4440)	0.004
SGA[b], (n) (%)	1 (3.3)	0 (0)	0.317
Gestational age (wk), Median (range)	39 (37–42)	40 (37–42)	0.311
Male/female ratio	26/4	27/3	0.690
Intrapartum complication			
Shoulder dystocia, (n) (%)	1 (3.3)	0 (0)	0.317
Meconium staining of amniotic fluid, (n) (%)	16 (53.3)	5 (17.2)	0.004
Cord complications, (n) (%)	6 (20)	0 (0)	0.010
Delivery route			< 0.001
Vaginal, (n) (%)	13 (43.3)	29 (96.7)	
Cesarean section, (n) (%)	17 (56.7)	1 (3.3)	
CTG criteria[c]			0.001
Normal, (n) (%)	3 (10.3)	14 (50)	
Non-reassuring, (n) (%)	10 (34.5)	14 (50)	
Pathological, (n) (%)	16 (55.2)	0 (0)	
Bradycardia > 10 min in the CTG, (n) (%)[d]	15 (53.6)	0 (0)	< 0.001
Asphyxia episodes lasting			
Less than 1 hour, (n) (%)	19 (65.5)		
More than 1 hour, (n) (%)	10 (34.5)		
HIE	9 (30.0)		
No HIE, (n) (%)	11 (36.7)		
Grade 1, (n) (%)	10 (33.3)		
Grade 2–3, (n) (%)			
Apgar scores at 5 min, Median (range)	4 (1–5)	9 (8–10)	< 0.001
Cord arterial pH 7.05 or less, (n) (%)[e]	8 (27.6)	0 (0)	0.012
Cord arterial base deficit 10 or more, (n) (%)[f]	8 (32.0)	1 (3.8)	0.021
Closure of PDA at 48 hrs (n) (%)	27 (90)	30 (100)	0.334

[a] Asphyxia group vs control group
[b] SGA: small for gestational age; < 2500 g in term infant.
[c] One CTG in the asphyxia group and two in the control group were missing.
[d] Three missing data in the asphyxia group.
[e] One pH was not available in the asphyxia group and two in the control group.
[f] Five cord blood base deficits were not available in both study groups.

Table 10. Clinical characteristics of the asphyxiated infants with normal or abnormal outcome.

	Asphyxia group outcome[a] (n = 27)		p value[b]
	Normal (n = 21)	Abnormal[c] (n = 6)	
Birth weight (g), Median (range)	3350 (2530–4240)	3420 (2295–3500)	0.579
SGA[d], (n) (%)	0 (0)	1 (16.7)	0.222
Gestational age (wk), Median (range)	40 (37–42)	39 (37–40)	0.050
Male/female ratio	18/3	6/0	0.335
Intrapartum complications			
Shoulder dystocia, (n) (%)	1 (4.8)	0 (0)	0.593
Meconium staining of amniotic fluid, (n) (%)	11 (52.4)	5 (83.3)	0.182
Cord complications, (n) (%)	3 (14.3)	1 (16.7)	0.887
Delivery route			1.000
Vaginal, (n) (%)	9 (42.9)	3 (50.0)	
Cesarean section, (n) (%)	12 (57.1)	3 (50.0)	
CTG criteria[e]			1.000
Normal, (n) (%)	2 (10.0)	0 (0)	
Non-reassuring, (n) (%)	8 (40.0)	2 (33.3)	
Pathological, (n) (%)	10 (50.0)	4 (66.7)	
Bradycardia > 10 min in the CTG, (n) (%)[f]	10 (47.6)	4 (66.7)	0.648
Asphyxia episodes lasting			0.644
Less than 1 hour, (n) (%)	13 (65.0)	3 (50.0)	
More than 1 hour, (n) (%)	7 (35.0)	3 (50.0)	
HIE			0.025
No HIE, (n) (%)	6 (28.6)	1 (16.7)	
Grade 1, (n) (%)	10 (47.6)	0 (0))	
Grade 2–3, (n) (%)	5 (23.8)	5 (83.3)	
Apgar scores at 5 min, Median (range)	4 (2–5)	4 (1–5)	0.432
Cord arterial pH 7.05 or less, (n) (%)[g]	4 (20.0)	3 (50.0)	0.293
Cord arterial base deficit 10 or more, (n) (%)[h]	5 (27.8)	2 (50.0)	0.565

[a] Three infants (two HIE 0 and one HIE 1) in the asphyxia group were lost to the follow-up.
[b] Normal vs abnormal outcome in the asphyxia group.
[c] Abnormal outcome was defined by pediatric neurologist using a modified Griffith`s scale at 1 year of age or death.
[d] SGA: small for gestational age; < 2500 g in term infant.
[e] One CTG in the asphyxia group was missing.
[f] Three missing data in the asphyxia group.
[g] One pH was not available in the asphyxia group.
[h] Five cord blood base deficits were not available in the asphyxia group.

5.8   Outcome of the asphyxiated and control infants (Studies II–IV)

In all, 9 (30%) out of 30 infants with asphyxia recovered well without HIE symptoms. A total of 21/27 (78%) follow-up infants had a normal outcome at 1 year of age, 10 (23.8%) with grade 1 and 6 (47.6%) with HIE grade from 2 to 3 and 6 (28.6%) with no HIE symptoms. One infant with grade 3 HIE died at the age of 9 days. In all, two asphyxiated infants were lost to follow-up and 27 (90%) were followed up to 1 year of age. Six (22%) had an abnormal outcome. One infant with grade I HIE and two with grade 2 HIE had a delay in motor development. One infant with grade 2 HIE had spastic diplegia and one with grade 3 HIE had a severe cognitive and motor developmental delay.

None of the control infants evinced neurological symptoms on the postnatal ward. A total of 22 (78%) of the 30 controls were followed up to 1 year of age and one of them showed delayed motor development.

5.9   MRI in the asphyxiated infants (Study IV)

Twenty-three (77%) out of thirty asphyxiated term infants underwent brain MRI examinations at 1 year of age. Three (13%) yielded abnormal MRI findings. One infant with grade 2 HIE had minor white matter lesions and one white lesions with a thin corpus callosum on MRI. One infant with grade 3 HIE had white matter reduction and lesions, ventricular dilatation and a thin corpus callosum. Five infants with grade 2 HIE, eight with grade I HIE and seven without any HIE symptoms had a normal MRI.

5.10   Doppler findings in cerebral circulation (Studies II–IV)

All asphyxiated and control infants underwent a pulsed Doppler examination three times during the first day of life. The CBFVs measured within the first 12 hours of life did not differ significantly between the groups. The trends in Doppler findings in the cerebral arteries in the asphyxia group with normal and abnormal 1-year outcome and in the control group during the first day of life are seen in Figure 5 (unpublished data).

ACA and BA (Peak Diastolic CBFV)
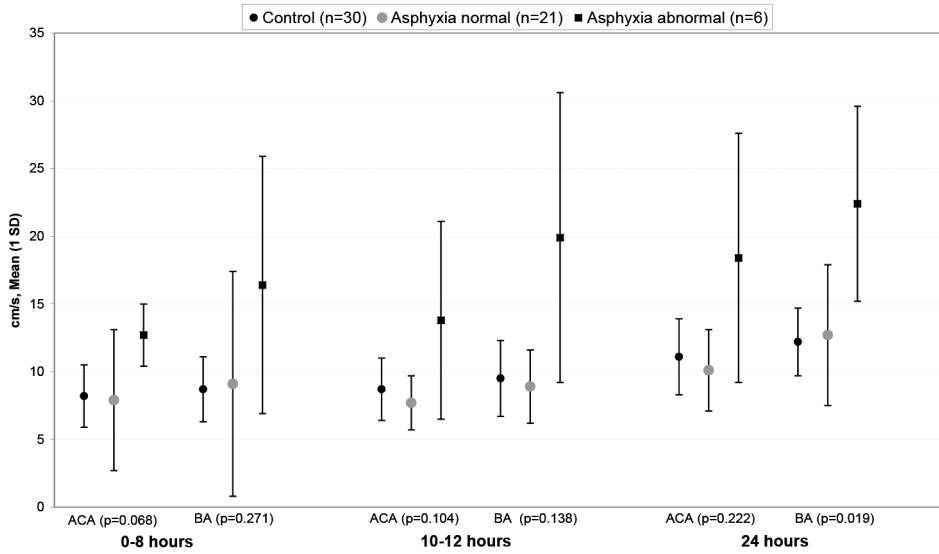
ACA and BA (Peak Systolic CBFV)
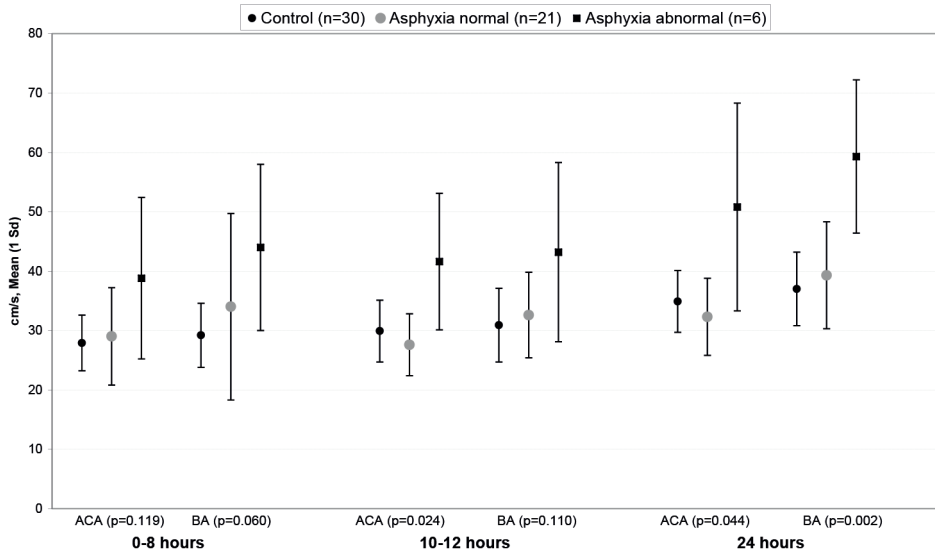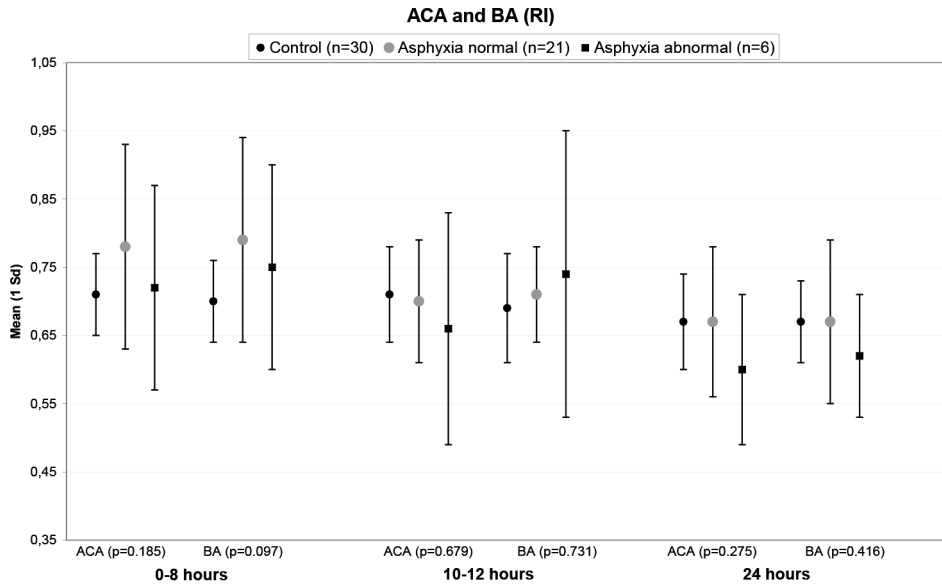
Figure 5. The peak diastolic and systolic CBFV and RIs in the ACA and BA during the first day of life in the asphyxia group with normal or abnormal outcome at 1 year of age and in the control group. Differences (p) between abnormal and normal outcome in the asphyxia group were assessed by Brown-Forsythe statistic test.

5.11 Doppler findings at 24 hours of age and neurological outcome (Studies II–IV)

Peak systolic CBFV over 50.0 cm/s (mean + 3SD) in the ACA or over 55 cm/s (mean + 3SD) in the BA around 24 hours of age (at 27–37 hours of age) was found in six (20%) out of thirty asphyxiated infants, none in the control cases. Five of the six asphyxiated infants with increased peak systolic CBFV had an abnormal outcome at 1 year of age or died in the neonatal period. The pathologically increased CBFVs normalized at 3–11 days of age, except in the infant with grade 3 HIE who died at 9 days of age.

The associations of the peak systolic and diastolic CBFVs and RIs in the ACA and BA around 24 hours of age with one-year outcome in the asphyxia group and in the control group are presented in Table 11 (unpublished data).

Table 11. The values of peak systolic and diastolic CBFVs and RIs in the ACA and BA around 24 hours of age in the asphyxia group with outcome at 1 year of age and in the control group[a].

| | Asphyxia group outcome[b] (n = 27) | | | | | |
	Normal (n = 21)	Abnormal[c] (n = 6)	p value[d]	Control group (n = 30)	p value[e]	p value[f]
ACA						
Systolic CBFV (cm/s) mean (SD)	32.3 (6.5)	50.8 (17.5)	0.048	34.9 (5.2)	0.115	0.076
Diastolic CBFV (cm/s) mean (SD)	10.1 (3.0)	18.4 (9.2)	0.195	11.1 (2.8)	0.260	0.222
RI, mean (SD)	0.67 (0.11)	0.60 (0.11)	0.168	0.68 (0.07)	0.932	0.035
BA						
Systolic CBFV (cm/s) mean (SD)	39.3 (9.0)	59.3 (12.9)	< 0.001	37.0 (6.2)	0.294	0.007
Diastolic CBFV (cm/s) mean (SD)	12.7 (5.2)	22.4 (7.2)	0.002	12.2 (2.5)	0.702	0.033
RI, mean (SD)	0.67 (0.12)	0.62 (0.09)	0.298	0.67 (0.06)	0.883	0.061

[a] Differences between the groups were tested by t test.
[b] Three infants in the asphyxia group were lost to the follow-up.
[c] Abnormal outcome at 1 year of age or death.
[d] p value abnormal outcome versus normal outcome in the asphyxia group.
[e] p value control group versus asphyxia group with normal outcome
[f] p value control group versus asphyxia group with abnormal outcome.

5.12 HIE grading and Doppler findings (Studies II–IV)

Systolic CBFV and RI at 24 hours of age in asphyxiated infants according to HIE grading are shown in Table 12 (Julkunen et al. 2006).

Table 12. Systolic CBFV and RI in the ACA and BA at 24 hours of age according to HIE grading.

	No HIE (n = 9)	HIE I (n = 11)	HIE II–III (n = 10)
ACA (cm/s)			
Mean (SD)	32.1 (6.2)	33.2 (7.1)	42.7 (17.1)
RI	0.68 (0.06)	0.67 (0.08)	0.64 (0.17)
BA (cm/s)			
Mean (SD)	35.5 (6.3)	39.1 (7.2)	52.6 (16.5)*
RI	0.68 (0.08)	0.67 (0.11)	0.67 (0.06)

* p < 0.05 HIE II–III vs. No HIE, HIE I

5.13 The accuracy of HIE and Doppler findings in predicting outcome in the full-term asphyxia study groups (Studies II–IV)

In all, 20% of the asphyxiated infants but none in the control group had increased peak systolic CBFVs (mean + 3 SD) in the ACA or BA around 24 hours of life. The sensitivity of increased systolic CBFV to predict abnormal outcome in the asphyxia group was 83%, specificity 95%, PPV 83% and NPV 95%, and the sensitivity of the combination of HIE grade from 2 to 3 and increased systolic CBFV in the ACA or BA was 100%, specificity 95%, PPV 83% and NPV 100%, respectively.

5.14 Cranial ultrasound imaging in the full-term study groups (Studies II–IV)

No congenital brain abnormalities were detected in cranial ultrasound imaging in the study groups during the first day of life. One infant with grade 3 HIE developed ventricular dilatation after 14 days of severe asphyxia.

5.15 Obstetric parameters in the full-term asphyxia study groups (Study II)

Pathological CTG recordings were concentrated in the asphyxia group. Less severe, non-reassuring CTG tracings were equally distributed in the asphyxia and control groups. The criteria for a normal CTG were fulfilled more frequently in the control than the asphyxia group. However, the classification of CTG had no association with the neurological outcome within the asphyxia group. The distributions of CTG findings, the severity of HIE and the occurrence of increased systolic CBFVs around 24 hours of age and abnormal outcome at 1 year of age are presented in Table 13.

Table 13. CTG patterns, HIE, Doppler findings and outcome in the asphyxia group.

| | CTG[a] | | | | Asphyxia episodes lasting | |
	Normal	Non-reassuring	Pathological	Bradycardia > 10 min	Less than 1 h	More than 1 hour
No HIE (n = 9)	1 (12.5%)	4 (50.0%)	3 (37.5%)	2 (22.2%)	5 (62.5%)	3 (37.5%)
HIE grade 1 (n = 11)	1 (9.1%)	4 (36.4%)	6 (54.6%)	6 (54.5%)	10 (90.9%)	1 (9.1%)
HIE grade 2–3 (n = 10)	1 (10.0%)	2 (20.0%)	7 (70.0%)	7 (70.0%)	4 (40.0%)	6 (60.0%)
sCBFV (mean +3SD) (n = 6)	0 (0%)	2 (33.3%)	4 (66.7%)	4 (66.7%)	3 (50.0%)	3 (50.0%)
Abnormal outcome at 1 year of age[b] (n = 6)	0 (0%)	2 (33.3%)	4 (66.7%)	4 (66.7%)	3 (50.0%)	3 (50.0%)

Abbreviations: CTG, cardiotocograhy; HIE, hypoxic ischemic encephalopathy.
[a] One CTG was missing.
[b] Abnormal outcome was defined by a pediatric neurologist using a modified Griffith`s scale at 1 year of age or death.

5.16 Biochemical biomarkers in asphyxiated full-term infants (Study II)

Plasma lactate was higher than 7.5 mmol/L in four (19%) asphyxiated infants with normal outcome and in one (17%) with abnormal outcome, p = 1.000. Serum NSE was more than 40 µg/L in eight cases (38%) with normal outcome and four (37%) with abnormal outcome, p = 0.357. Serum CK was > 1000 U/L in 11 (55%) cases with normal outcome and 3 cases (50%) with abnormal outcome, p = 1.000.

The accuracy of biochemical asphyxia markers in predicting abnormal 1-year outcome was low. The sensitivity of plasma lactate (cut-off value > 7.5 mmol/L) at around 24 hours was 20% and specificity 71%. The sensitivity of serum NSE (cut-off value > 40 µg/L) was 67% and specificity 70% and the sensitivity of serum CK (cut-off value > 1000 U/L) 50% and specificity 42%.

5.17 EEG and evoked potentials (Study III)

EEG and evoked potentials (BAEP, SEP, VEP) assessments as predictors of a 1-year outcome were evaluated in the asphyxia study groups.

Seizures and electrophysiologic findings are presented in Table 14. Seven of the asphyxiated infants received anticonvulsive drugs because of clinical seizures, of all which occurred one day after the asphyxia event. Two infants with seizures had an abnormal EEG background without and two infants with epileptiform discharges. Only one infant had

prolonged inter-burst intervals of almost 10 s. Three had normal EEGs. Five of the seven asphyxiated infants with seizures had a normal 1-year outcome. SEPs were normal in four, and two asphyxiated infants with seizures had unilaterally lengthened cortical latencies with normal cervical responses. VEPs were normal among infants with seizures. BAEPs were performed in six of the seven asphyxiated infants with seizures. Two asphyxiated infants suffering seizures had lengthened latencies and one had missing responses bilaterally.

Table 14. HIE, seizures and clinical brain monitor findings in the study groups.

	Asphyxia group (n =30)	Control group (n = 30)	p value	Asphyxia outcome[d] (n = 27)		p value
				Normal (n = 21)	Abnormal (n = 6)	
HIE						0.025
No HIE, (n) (%)	9 (30.0)			6 (28.6)	1 (16.7)	
Grade 1, (n) (%)	11 (36.7)			10 (47.6)	0 (0)	
Grade 2–3, (n) (%)	10 (33.3)			5 (23.8)	5 (83.3)	
Seizures, (n) (%)	7 (23.3)	0 (0)	<0.001	5 (23.8)	2 (33.3)	0.645
EEG, (n) (%)			0.002			0.002
Normal	21 (70)	30 (100)		17 (81)	1 (16.6)	
EEG-2	1 (3.3)	0 (0)		0 (0)	1 (16.6)	
EEG-3	6 (20)	0 (0)		2 (9.5)	4 (66.7)	
EEG-4	2 (6.7)	0 (0)		2 (9.5)	0 (0)	
BAEP, (n) (%)[a]			0.023			0.155
Normal	22 (78.6)	28 (100)		18 (85.7)	3 (60)	
BAEP-2	0 (0)	0 (0)		0 (0)	0 (0)	
BAEP-3	2 (7.1)	0 (0)		2 (9.5)	0 (0)	
BAEP-4	1 (3.6)	0 (0)		0 (0)	1 (20)	
BAEP-5	3 (10.7)	0 (0)		1 (4.8)	1 (20)	
SEP, (n) (%)[b]			0.046			0.031
Normal	17 (68)	24 (92)		15 (79)	1 (25)	
SEP-2	7 (28)	2 (8)		4 (21)	2 (50)	
SEP-3	1 (4)	0 (0)		0 (0)	1 (25)	
VEP, (n) (%)[c]	28 (100)	27 (100)	–	21 (100)	5 (100)	–
Normal	0 (0)	0 (0)		0 (0)	0 (0)	
Abnormal						
Systolic CBFV > 3 SD in the ACA or BA at 24 hrs, (n) (%)	6 (20)	0 (0)	0.010	1 (4.8)	5 (83)	0.002

Abbreviations:
BAEP, brainstem auditory evoked potential; EEG, electroencephalogram; HIE, hypoxic ischemic encephalopathy; SEP, somatosensory evoked potential; Systolic CBFV, systolic cerebral blood flow velocity; VEP, visual evoked potential.
[a] BAEP: Two in the asphyxia and two in the control group were missing.
[b] SEP: Five in the asphyxia group and four in the control group were missing.
[c] VEP: Two in the asphyxia group and three in the control group were missing.
[d] In all, 3 of the 30 asphyxiated infants were lost to follow-up and 4 out of 27 follow-up infants had no SEP; one had no VEP or BAEP.
Determinations of EEG, BAEP and SEP grading, see text.

An abnormal EEG background predicted poor 1-year outcome in the asphyxia group with a sensitivity of 67% and specificity of 81%, and an abnormal SEP with 75% and 79%, respectively. The accuracy of abnormal BAEP and VEP were low in predicting abnormal 1-year outcome. Normal EEG and SEP predicted a good outcome in the asphyxia group, with sensitivities from 79% to 81%, and specificities of 67% and 75%, respectively. The combination of normal EEG, normal SEP and systolic CBFV < 3 SD predicted a good outcome with a sensitivity of 74% and 100% specificity and PPV was 100% (Table 15).

Table 15. Predictive value of EEG, EPs, HIE and Doppler findings in predicting poor and good outcome of asphyxiated infants. [a]

Ability to predict poor outcome	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV% (95% CI)
EEG-3/EEG-4, (n = 27)	67 (30–90)	81 (60–92)	50 (22–79)	89 (69–97)
BAEP-4/BAEP-5, (n = 26)	40 (12–77)	95 (77–99)	67 (21–94)	87 (68–96)
SEP-2/SEP-3, (n = 23)	75 (30–95)	79 (57–91)	43 (12–80)	94 (68–100)
SEP-3, (n = 23)	25 (5–70)	100 (83–100)	100 (21–100)	86 (67–95)
Abnormal VEP, (n = 26)	0 %	100 %		
EEG-3/EEG-4 and SEP-2/SEP-3, (n = 23)	50 (15–83)	89 (69–97)	50 (15–85)	89 (69–97)
HIE grade 2–3, (n = 27)	83 (44–97)	76 (55–89)	50 (24–76)	94 (73–99)
sCBFV >mean 3 SD in the ACA or BA at 24 hrs, (n = 27)	83 (44–97)	95 (77–99)	83 (44–97)	95 (77–99)
EEG-3/EEG-4 and sCBFV > mean 3 SD in the ACA or BA at 24 hrs, (n = 27)	50 (19–81)	100 (85–100)	100 (44–100)	88 (69–96)
SEP-2/SEP-3 and sCBFV >mean 3 SD in the ACA or BA at 24 hrs, (n = 23)	75 (30–95)	100 (83–100)	100 (31–100)	95 (73–100)
EEG-3/EEG-4 and HIE grade 2–3, (n = 27)	67 (30–90)	90 (71–97)	67 (30–90)	90 (72–97)
SEP-2/SEP-3 and HIE grade 2–3, (n = 23)	75 (30–95)	95 (75–99)	75 (22–99)	95 (72–100)
sCBFV > mean 3 SD in the ACA or BA at 24 hrs and HIE grade 2–3, (n = 27)	100 (57–100)	95 (78–99)	83 (44–97)	100 (85–100)

Ability to predict good outcome	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV% (95% CI)	NPV% (95% CI)
Normal EEG, (n = 27)	81 (60–92)	67 (30–90)	89 (69–97)	50 (21–79)
Normal BAEP, (n = 26)	86 (65–95)	40 (12–77)	86 (63–96)	67 (12–98)
Normal SEP, (n = 23)	79 (57–91)	75 (35–95)	94 (72–99)	43 (16–75)
sCBFV < mean 3 SD in the ACA or BA at 24 hrs, (n = 27)	95 (77–99)	83 (44–97)	95 (77–99)	83 (44–97)
Normal EEG and sCBFV < mean 3 SD, (n = 27)	71 (50–86)	100 (61–100)	100 (80–100)	50 (25–75)
Normal SEP and sCBFV < mean 3 SD, (n = 23)	74 (51–88)	75 (30–95)	93 (66–100)	38 (10–74)
Normal EEG, normal SEP and sCBFV < mean 3 SD in the ACA or BA at 24 hrs, (n = 23)	74 (51–88)	100 (51–100)	100 (73–100)	44 (15–77)
Normal EEG, HIE 0-1 and sCBFV < mean 3 SD in the ACA or BA at 24 hrs, (n = 27)	67 (45–83)	100 (61–100)	100 (78–100)	46 (23–71)

[a] 3 of the 30 asphyxiated infants were lost to follow-up.
BAEP-4, missing responses bilaterally; BAEP-5, lengthened cervical latencies unilaterally;
EEG -3, abnormal EEG background and no epileptiform discharges; EEG-4, abnormal EEG background with epileptiform discharges;
HIE, hypoxic ischemic encephalopathy; SEP-2, unilaterally lengthened cortical latencies with normal cervical responses;
SEP-3, bilaterally lengthened cortical latencies with normal cervical responses; systolic cerebral blood flow velocity (sCBFV) in the artery cerebral anterior (ACA) or basilaris (BA).

5.18 Neurodevelopmental assessments in predicting neurological outcome at 1 year (Study IV)

GMs, AIMS and NMS findings in the asphyxia study groups are shown in Table 16.

Table 16. Neurodevelopmental assessment findings in the study groups.

	Asphyxia group (n = 30)	Control group (n = 30)
GMs before discharge[a]		
Normal, (n) (%)	4 (16)	14 (58)
Abnormal, (n) (%)	21 (84)	10 (42)
GMs at 3 months[b]		
Normal, (n) (%)	24 (88.9)	19 (95)
Abnormal, (n) (%)	3 (11.1)	1 (5)
AIMS at 3 months[c]		
Normal, (n) (%)	24 (85.7)	20 (100)
≤ 10th centile, (n) (%)	4 (14.3)	0 (0)
AIMS at 6 months[d]		
Normal, (n) (%)	19 (76)	19 (95)
≤ 10th centile, (n) (%)	6 (24)	1 (5)
NMS at 3 months[e]		
Normal, (n) (%)	18 (75)	15 (93.7)
≥ 3 scores, (n) (%)	6 (25)	1 (6.3)

[a] Five missing data in the asphyxia group and six in the control group.
[b] Three missing data in the asphyxia group and ten in the control group.
[c] Two missing data in the asphyxia group and ten in the control group.
[d] Five missing data in the asphyxia group and ten in the control group.
[e] Six missing data in the asphyxia group and fourteen in the control group.

Of the five asphyxiated infants with abnormal outcome at 1 year of age, three had abnormal 3-month GMs. One infant in the control group with normal GMs at 3 months had an abnormal outcome and one control infant with abnormal GMs at 3 months of age had a normal outcome.

The AIMS test was applied to 28 (93%) asphyxiated infants and 20 (67%) controls at 3 months of age, and to 25 (83%) asphyxia cases and 20 (67%) controls at six months. Four (14%) asphyxiated infants had total scores below the 10th percentile at three months of age, and three (75%) of these had an abnormal one-year outcome. At age six months total scores below the 10th percentile were found in six (64%) asphyxia infants and at 12 months below the 5th percentile in 5 (21%) asphyxia infants. Of these four had an abnormal one-year outcome at 1 year of age. One control infant with an abnormal outcome had total scores on AIMS below the 10th percentile at six months of age and below the 5th percentile at 12 months of age. The NMS test was performed in 24 (80%) cases with asphyxia and in 16 (53%) control infants at 3 months of age. Six (25%) asphyxia cases had scores of 3 or above

and two of these (33%) had an abnormal outcome. One control infant with score 3 had a normal outcome.

Neurodevelopmental assessments and their accuracy in predicting abnormal and good outcome at 1 year of age in the asphyxiated infants in comparison to HIE classification are presented in Table 17.

Table 17. Predictive values of neurodevelopmental assessments (GMs, AIMS, NMS) and HIE in predicting poor and good 1-year outcome in asphyxiated infants[a].

	Sensitivity [95 %CI]	Specificity [95 %CI]	Positive predictive value [95 %CI]	Negative predictive value [95 %CI]
Ability to predict poor outcome				
GMs at 0 month (abnormal/normal), (n =23)	100% [51–100]	16% [6–38]	20% [8–42]	100% [44-100]
GMs at 3 months (abnormal/normal), (n=25)	75% [30–95]	95% [77–99]	75% [30–95]	95% [77–99]
AIMS at 3 months ≤ 10th percentile, (n=26)	60% [23–88]	100% [84–100]	100% [44–100]	91% [73–98]
AIMS at 6 months ≤ 10th percentile, (n=23)	100% [51–100]	89% [69–97]	67% [30–90]	100% [82–100]
NMS at 3 months ≥ 3 scores, (n=23)	40% [12–77]	78% [55–91]	33% [10–70]	82% [59–94]
HIE (grade 2–3), (n=27)	83% [44–97]	76% [55–89]	50% [24–76]	94% [73–99]
Ability to predict good outcome				
GMs at 3 months (normal/abnormal)	95% [77–99]	75% [30–95]	95% [77–99]	75% [30–99]
AIMS at 3 months > 10th percentile, (n=26)	100% [84–100]	60% [23–88]	91% [73–98]	100% [44–100]
AIMS at 6 months > 10th percentile, (n=23)	89% [69–97]	100% [51–100]	100% [82–100]	67% [30–90]
HIE grade 0–1, (n=27)	76 % [55–89]	83% [44–97]	94% [73–99]	50% [38–96]

[a] Three of the 30 asphyxiated infants were lost to follow-up.

6 DISCUSSION

6.1 End-diastolic block in cerebral circulation as a predictor of outcome in ELBW preterm infants (Study I)

The prominent finding in this study was that during the first four days of life, the occurrence of an end-diastolic block in either the ACA or BA, mean arterial pressure (MAP) less than 30 mmHg and the presence of PDA during the first four days of life might be associated with IVH in ELBW infants. The presence of an end-diastolic block in the cerebral arteries connected with MAP below 30 mmHg would appear to signify impaired cerebral autoregulation and inadequate perfusion in vulnerable brain areas. Preterm infants with impaired autoregulation are prone to develop IVH (Pryds et al. 1989, Tsuij et al. 2000) and periventricular leukomalacia (Volpe 2001). Impaired autoregulation with a pressure-passive cerebral circulation is associated with low gestational age and birth weight, systemic hypotension and maternal hemodynamic factors in preterm infants with birth weights < 1500 g (Soul et al. 2007). A MAP range of 23.7 to 39.3 mmHg seems to be adequate to maintain cerebral perfusion as measured by NIRS in infants of gestational ages between 24 to 30 weeks (Tyszczuk et al. 1998), and it has been recommended that the MAP in mmHg should not fall below the gestational age of the infants in weeks (Joint Working Party of the British Association of Perinatal Medicine and the Research Unit of the Royal College of Physicians 1992). Our results confirm that besides MAP, other factors affect the cerebral circulation. One should thus look not only at the hypotensive mean arterial blood pressure levels in the treatment of patients in whom impaired autoregulation in the cerebral circulation is suspected and/or detected.

6.2 Doppler findings in cerebral circulation as a predictor of outcome in asphyxiated full-term infants (Studies II–IV)

Hyperperfusion in the cerebral circulation is regarded as a sign of permanent brain damage in asphyxiated infants (Levene et al. 1989, Ilves et al. 2004, Ilves et al. 2009a). Increased CBFV by the age of 12 hours in infants with severe HIE or poor outcome has been reported (Ilves et al. 1998, Ilves et al. 2004). Increased CBFV from 24 to 72 hours has been a sign of

permanent brain damage with poor prognosis in asphyxiated infants (Levene et al. 1989, Deeg et al. 1990, Stark and Seibert 1994).

Increased systolic CBFV in the ACA or BA at around 24 hours of life seemed to be associated with a poor 1-year outcome in the present asphyxia group, confirming previous findings. In contrast, if no pathological increase in the systolic CBFV was observed within the first 27 to 37 hours of life, the outcome seemed to be good.

6.3   Predictive value of obstetric parameters in asphyxiated full-term infants (Study II)

The values of CTG, Apgar score and acid base status in predicting outcome seemed in the present study to be poor. All of the asphyxiated term infants with a poor neurological outcome had abnormal CTG, but only half of them had a clear pathological CTG recording, while in the other half the CTG was classified as only moderate, "non-reassuring". Half of the asphyxiated infants with normal outcome had pathological CTGs, and half of the healthy control infants had abnormal CTGs. This is consistent with earlier studies suggesting a high false-positive rate of CTG (Finer et al. 1981, Nelson et al. 1996, Toh 2000).

Apgar score is a useful basis for assessing the general condition of infants after birth, but it has limitations in identifying birth asphyxia and predicting neurological outcome. The specificity of a combination of 5 min Apgar scores below 5 and the presence of HIE grade from 2 to 3 to predict abnormal outcome was high, but the sensitivity was low in this study. Infants with Apgar scores from 0 to 3 at 5 minutes and symptoms of NE had an increased risk of subsequent neurodevelopmental impairments and learning difficulties whereas a similar Apgar score without neurological symptoms had no association with a high risk of later impairments compared with the healthy control infants (Moster et al. 2002).

6.4   Electrophysiologic assessments as predictors of outcome in asphyxia (Study III)

Abnormal neurophysiologic assessments such as EEG and EPs performed during the first week of life seemed to be of limited value in predicting 1-year outcome in the present study. Combining abnormal EEG or EPs findings with high systolic CBFV did not alter the prediction. In contrast, normal EEG and normal SEP with normal CBFV by Doppler at age 24 hours improved specificity in predicting a normal 1-year outcome compared to a normal EEG or normal SEP only. Seizures after asphyxia seemed not to be independent predictors of poor outcome.

The sensitivity of abnormal SEPs in asphyxiated term infants has varied from 90% to 95% (Eken et al. 1995, Swarte et al. 2012). An abnormal SEP at 1 week of age predicts an

adverse outcome in 90% of asphyxiated infants, whereas a normal SEP is generally a sign of normal outcome (Gibson et al. 1992, Taylor et al. 1992). In the present study unilaterally or bilaterally absent SEPs predicted a poor outcome and a normal SEP a good outcome. The predictive values of BAEP and VEP were poor.

6.5   Neurodevelopmental assessments in asphyxia (Study IV)

GMs at 3 months of age seemed here to be a valuable tool in predicting a good or a poor 1-year outcome. Abnormal GMs predicted a poor outcome among asphyxiated infants with moderate sensitivity and high specificity. Normal fidgety GMs at 3 months predicted a good outcome in high-risk infants with a very good sensitivity, confirming the results of previous studies (Prechtl et al. 1997, Ferrari et al. 2011).

In one recent work, normal MRI or normal GMs predicted a normal outcome with a negative predictive value of 100%, but in contrast the pattern of central gray matter damages observed in early MRI correlated with cramped-synchronized GMs and poor motor outcome among term infants with HIE (Ferrari et al. 2011). In this study the majority of asphyxiated cases who underwent brain MRI at the age of 1 year yielded normal imaging, and almost all had normal GMs at 3 months of age. MRI was not performed during the neonatal period in our cases. AIMS was a good predictor at age 6 months, but NMS seems to be of low value in the follow-up of asphyxiated infants.

6.6   Strengths and limitations of the study

Pulsed Doppler ultrasonography is a non-invasive means of examining the cerebral circulation at the bedside, even in sick infants. The findings in pulsed Doppler ultrasonography reflected the blood flow in only two intracranial arteries (ACA, BA). However, the CBFV of ACA represented the carotid and the CBFV of BA the vertebrobasilar circulation and thus yielded reliable information on the cerebral circulation. The limitation of this approach was that the period of each measurement was only a few minutes and continuous monitoring was not possible (**I–IV**).

A limitation of study **I** was that a rather high percentage (27%) of eligible ELBW infants were not included in the evaluation, this possibly leading to a patient selection bias. Blood pressures were recorded nearest to the time of pulsed Doppler ultrasonography, not continuously. Moreover, the possibility cannot be excluded that a diastolic block in the cerebral circulation might also have been present in the control cases outside the time-point of examination (**I**).

The strength of the asphyxia study was the healthy sex- and age-matched control group, which allowed longitudinal comparison of CBFV during the first 24 hours. By using healthy controls the effects of examiner and the ultrasound equipment on the results can

be standardized (**II–IV**). Asphyxiated and control infants also underwent recordings of EEG and evoked potentials (**III**).

A limitation was that the numbers of infants were relatively small in the study groups and the follow-up at 1 year of age was not complete. Power analysis could not performed by the reason of the lack of comparable patient populations in earlier studies. However, statistically significant differences were found even in this small population. Neurodevelopmental assessment at one year of age is not as reliable as assessment at pre-school or school age (**II–IV**).

6.7   Future considerations

Early identification of infants who are at high risk of neurodevelopmental problems, and the prediction of the long-term outcome after birth are a challenge to clinicians. Predicting outcome in asphyxiated infants is difficult during the first days of life. A combination of diagnostic assessments is necessary in making decisions regarding clinical care.

According to the present findings, a combination of normal Doppler findings in the cerebral circulation at 24 hours, normal EEG and normal SEP predicted a good 1-year outcome among asphyxiated term infants with a specificity of 100% and 100% PPV. This information is important in counselling parents and planning follow-up.

Hypothermia is a standard made of care for moderate-to-severe HIE among term infants (Perlman et al. 2010). The Doppler findings and prediction outcome were obtained with asphyxiated infants at normal temperature in this study. It would be important to investigate whether prediction of outcome from high systolic CFBV at 24 hours has changed in the era of hypothermia. In recent studies, low RI detected in the cerebral arteries was not as good a predictor of poor outcome in infants with HIE during hypothermia as previously reported in normothermic infants (Elstad et al. 2011, Skranes et al. 2014). Bedside neurological monitoring with Doppler, NIRS and amplitude-integrated electroencephalogram (aEEG) in high-risk infants might provide useful prognostic information. The accuracy of a combination of Doppler, NIRS and aEEG during hypothermia in predicting the outcome would be valuable to determine.

7 CONCLUSIONS

An end-diastolic block in the cerebral circulation detected by pulsed Doppler ultrasonography, together with a mean arterial pressure of 30 mmHg or less and the presence of patent ductus arteriosus during the first four days of life might predict an intraventricular hemorrhage in extremely-low-birth-weight infants. This may indicate a need for treatment of patent ductus arteriosus (**I**).

An increased systolic cerebral blood flow velocity by Doppler ultrasonography combined with hypoxic ischemic encephalopathy (HIE) grade 2 to 3 seemed to predict a poor one-year outcome and the absence of an increased systolic flow and HIE grade 1 a good one-year outcome in asphyxiated infants better than CTG, acid basement status, Apgar scores or biochemical markers (**II**).

Combining increased systolic cerebral blood flow velocity with abnormal EEG or evoked potential findings did not improve prediction of a poor 1-year outcome in asphyxiated infants. On the other hand, normal EEG and normal somatosensory evoked potentials combined with systolic cerebral blood flow velocity less than 3SD at about 24 hours can be valuable in the prediction of a normal 1-year outcome among such infants. Seizures after asphyxia seemed not be independent predictors of poor outcome (**III**).

General movements at 3 months of age appeared to offer a suitable basis for assessment in predicting the 1-year outcome of asphyxiated term infants. Abnormal general movements at 3 months predicted a poor outcome among asphyxiated infants with average sensitivity and good specificity. Normal fidgety general movements at 3 months seemed to predict a good outcome with a good sensitivity. The Alberta infant motor scale is a good predictor later, at 6 months of age, but neuromotor score seems to be of low value in the follow-up of asphyxiated infants (**IV**).

8 ACKNOWLEDGEMENTS

Tampere, September 2015

Mia Julkunen

9 REFERENCES

ACOG Committee on Obstetric Practise, American Academy of Pediatrics Committee on Fetus and Newborn. 1996. Use and abuse of the Apgar score. Pediatrics 98:141-142.

Adde L, Rygg M, Lossius K, Oberg GK, Stoen R. 2007. General movements assessment: Predicting cerebral palsy in clinical practise. Early Hum Dev 83:13-18.

Akobeng AK. 2006. Understanding diagnostic tests 1: sensitivity, specificity and predictive values. Acta Paediatr 96: 338-341.

Alin-Åkerman B, Nordberg L.1991. Griffith's utvecklingsskalor I och II. Manual för administering och utvärdering, PsykologiFörlaget AB, Göteborg, Sverige.

Allison JW, Faddis LA, Kinder DL, Robertson PK, Glasier CM, Seibert JJ. 2000. Intracranial resistive index (RI) values in normal term infants during the first day of life. Pediatr Radiol 30:618-620.

Al Yazidi G, Srour M, Whitemark P. 2014. Risk factors for intraventricular hemorrhage in term asphyxiated newborns treated with hypothermia. Pediatr Neurol 50:630-635.

American College of Obstetricians and Gynecologists (ACOG) and American Academy of Pediatrics (AAP). 2003. Neonatal encephalopathy and cerebral palsy: Defining the pathogenesis and pathophysiology. Am Coll of Obstetricians Gynecologists, Washington, DC, USA.

American College of Obstetricians and Gynecologists. 2014. Neonatal Encephalopathy and Neurologic Outcome Second Edition. Obstet Gynecol 123:896-901.

Apgar V. 1953. A proposal for a new method of evaluation of the newborn infant. Curr Res Aneth Analg 32:260-7.

Archer LNJ, Evans DH, Levene MI. 1985. Doppler ultrasound examinations of the anterior cerebral arteries of normal newborn infants. the effect of postnatal age. Early Hum Dev 10:255-60.

Archer L, Levene M, Evans D. 1986. Cerebral artery Doppler ultrasonography for prediction of outcome after perinatal asphyxia. Lancet 15:1116-1118.

Bada HS, Haijar W, Chua C, Sumner DS. 1979. Noninvasive diagnosis of neonatal asphyxia and intraventricular hemorrhage by Doppler ultrasound. J Pediatr 95:775-9.

Badawi N, Kurinczuk JJ, Keogh JM, Alessandri LM, O'Sullivan F, Burton PR, Pemberton PJ, Stanley FJ. 1998. Intrapartum risk factors for newborn encephalopathy: the Western Australian case-control study. BMJ. 317:1554-1558.

Ballabh P. 2010. Intraventricular hemorrhage in premature infants: mechanism of disease. Pediatr Res 67:1-8.

Ballabh P. 2014. Pathogenesis and prevention of intraventricular hemorrhage. Clin Perinatol 41:47-67.

Bennet L, Booth L, Gunn AJ. 2010. Potential biomarkers for hypoxic-ischemic encephalopathy. Semin Fetal Neonatal Med 15:253-260.

Bennhagen RG, Weintraub RG, Lundström RG, Svenningsen NW. 1998. Hypoxic-ischemic encephalopathy is associated with regional changes in cerebral blood flow velocity and alterations in cardiovascular function. Biol Neonate 73:275-86.

Biagioni E, Bartalena L, Boldrini A, Pieri R, Cioni G. 1999. Constantly discontinuous EEG patterns in full-term neonates with hypoxic-ischemic encephalopathy. Clin Neurophysiol 110:1510-1515.

Biagioni E, Mercuri E, Rutherford M, Cowan F, Azzopradi D, Frisone MF, Cioni G, Dubowitz L. 2001. Combined use of electroencephalogram and magnetic resonance imaging in full-term neonates with acute encephalopathy. Pediatrics 107: 461-468.

Blennow M, Savman K, Ilves P, Thoresen M, Rosengren L. 2001. Brain-specific proteins in the cerebrospinal fluid of the severely asphyxiated newborn infants. Acta Paediatr Scand 90:1171-5.

Bolisetty S, Dhawan A, Abdel-Latif M, Bajuk B, Stack J, Lui Kei. 2014. Intraventricular hemorrhage and neurodevelopmental outcomes in extreme preterm. Pediatrics 133:55-62.

Bona E, Andersson A, Blomgren K , Giland E, Puka-Sundvall M, Gustafson K, Hagberg H. 1999. Chemokine and inflammation cell response to hypoxia-ischemia in immature rats. Pediatr Res 45:500-509.

Boylan G, Young K, Panerai R, Rennie JM, Evans DH. 2000. Dynamic cerebral autoregulation in sick newborn infants. Pediatr Res 48:12-17.

Brew N, Walker D, Wong FY. 2014. Cerebral vascular regulation and brain injury in preterm infants. Am J Physiol Regul Integr Comp Physiol 306:R773-R786.

Bulas DI. 2009. Transcranial Doppler: Applications in Neonates and Children. Ultrasound Clin 4:533-551.

Celtik C, Acunas B, Öner N, Pala Ö. 2004. Neuron-specific enolase as a marker of the severity and outcome of hypoxic ischemic encephalopathy. Brain Dev 26:398-402.

Chalak LF, Tarumi T, Zhang R. 2014a. The "neurovascular unit approach" to evaluate mechanisms of dysfunctional autoregulation in asphyxiated newborns in the era of hypothermia. Early Hum Dev 90:687-694.

Chalak LF, Sanchez PJ, Adams-Huet B, Laptook AR, Heyne RJ, Rosenfeld CR. 2014b. Biomarkers for severity of neonatal hypoxic-ischemic encephalopathy and outcomes in newborns receiving hypothermia therapy. J Pediatr 164:468-74.

Cheung YF, Lam PKL, Yeung CY. 1994. Early postnatal cerebral Doppler changes in relation to birth weight. Early Hum Dev 37:57-66.

Chiesa C, Pellegrini G, De Luca T, Assuma M, Signore F, Pacifico L. 2003. Umbilical cord interleukin-6 levels are elevated in term neonates with perinatal asphyxia. Eur J Clin Invest 33:352-358.

Chiruvolu A, Toila VN, Quin H, Stone GL, Rich GL, Conant RJ, Inez RW. 2015. Effect on delayed cord clamping on very preterm infants. Am J Obstet Gynecol 18. pii: S0002-9378(15)00752-8. doi: 10.1016/j.ajog.2015.07.016.

Chiu NC, Shen YE, Ho CS. 2003. Outcome in children with significantly abnormal cerebral blood flow detected by Doppler ultrasonography: Focus on the survivors. J Neuroimaging 13:53-56.

Collins MP, Lorenz JM, Jetton JR, Paneth N. 2001. Hypocapnia and other ventilation-related risk factors for cerebral palsy in low birth weight infants. Pediatr Res 50:712-719.

Cowan F, Rutherford M, Groenendaal F, Eker P, Mercuri E, Bydder GM, Meiners LC, Dubowitz LM, de Vries LS. 2003. Origin and timing of brain lesions in term infants with neonatal encephalopathy. Lancet 361:736-742.

Darrah J, Piper M, Watt M. 1998. Assessment of gross motor skills of at risk infants: predictive validity of the Alberta Infant Motor Scale. Dev Med Child Neurol 40:485-491.

Deeg KH, Rupprecht TH. 1989. Pulsed Doppler sonographic measurement of normal values for the flow velocities in the intracranial arteries for healthy newborns. Pediatr Radiol 19:71-8.

Deeg KH, Rupprecht TH, Zeilinger G. 1990. Doppler sonography classification of brain edema in infants. Pediatr Radiol 20:509-514.

Deeg KH, Rupprecht T, Hofbeck M (ed). 2015. Doppler Sonography in Infancy and Childhood, Springer, Switzerland.

de Haan M, Wyatt JS, Roth S, Vargha-Khadem F, Gadian D, Mishkin M. 2006. Brain and cognitive-behavioural development after asphyxia at term birth. Dev Sci 9:350-8.

d'Orey C, Mateus M, Guimaraes H, Ramos I, Melo MJ, Silva J, Ramos E, Montenegro N, Barros H, Santos N. 1999. Neonatal cerebral Doppler: Arterial and venous flow velocity measurements using colour and pulsed Doppler system. J Perinatol Med 27:352-361.

Douglas-Escobar M, Weiss MD. 2012. Biomarkers of hypoxic-ischemic encephalopathy in newborns. Front Neurol 3:1-5.

Drury PP, Bennet L, Gunn AJ. 2010. Mechanisms of hypothermic neuroprotection. Sem Fetal Neonatal Med 15:287-93.

Edwards AD, Brocklehurst P, Gunn AJ, Halliday H, Juszczak E, Levene M, Strohm B, Thoresen M, Whitelaw A, Azzopardi D. 2010. Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. BMJ 340:c363 doi:10.1136/bmj.c363.

Einspieler C, Prechtl HFR. 2005. Prechtl's assessment of general movements: a diagnostic tool for the functional assessment of young nervous system. Ment Retard Dev Disabil Res Rev 11:61-7.

Eken P, Toet MC, Groenendaal F, de Vries LS. 1995. Predictive value of early neuroimaging, pulsed Doppler and neurophysiology in full term infants with hypoxic-ischemic encephalopathy. Arch Dis Child 75:F75-80.

El-Ayouty M, Abdel-Hady H, El-Mogy S, Zaghlol H, El-Beltagy M, Aly H. 2007. Relationship between electroencephalography and magnetic resonance imaging findings after hypoxic-ischemic encephalopathy at term. Am J Perinatol 24:467-73.

Elstad M, Whitelaw A, Thoresen M. 2011. Cerebral resistance index is less predictive in hypothermic encephalopathic newborns. Acta Paediatr 100:1344-1349.

Ennen CS, Huisman TA, Savage WJ, Northington FJ, Jennings JM, Everett AD, Graham EM. 2011. Glial fibrillary acidic protein as a biomarker for neonatal hypoxic-ischemic enecphalopathy treated with whole-body cooling. Am J Obstet Gynecol 205:251.e1-7. doi: 10.1016/j.ajog.2011.06.025.

Epelman M, Daneman A, Kellenberger CJ, Aziz A, Konen O, Moineddin R, Whyte H, Blaser S. 2010. Neonatal encephalopathy: a prospective comparison of head US and MRI. Pediatr Radiol 40:1640-1650.

Ferrari F, Todeschini A, Guidotti I, Marinez-Biarge M, Roversi MF, Berardi A, Ranzi A, Cowan FM, Rutherford MA. 2011. General movements in full-term infants with perinatal asphyxia are related to basal ganglia and thalamic lesions. J Pediatr 158:904-911.

FIGO. 1987. Guidelines for the use of fetal monitoring. Int J Gynaecol Obstet 12:159-167.

Finer NN, Robertson CM, Richards RT, Pinnell LE, Peters KL. 1981. Hypoxic-ischemic encephalopathy in term infants: perinatal factors and outcome. J Pediatr 98:112-117.

Freeman JM, Nelson KB. 1988. Intrapartum asphyxia and cerebral palsy. Pediatrics 82:240-249.

Fukuda S, Kato T, Kakita H, Yamada Y, Hussein MH, Kato I, Suzuki S, Togari H. 2006. Hemodynamics of the cerebral arteries of infants with periventricular leukomalacia. Pediatrics 117:1-8.

Fukuda S, Mizuno K, Kawai S, Kakita H, Goto T, Hussein MH, Daoud GA, Ito T, Kato I, Suzuki S, et al. 2008. Reduction of cerebral blood flow volume in infants complicated with hypoxic ischemic encephalopathy resulting in cerebral palsy. Brain Dev 30:246-253.

Gazzolo D, Frigiola A, Bashir M, Iskander I, Mufeed H, Aboulgar H, Venturini P, Marras M, Serra G, Frulio R et al. 2009. Diagnostic accuracy of S100B urinary testing at birth in full-term asphyxiated newborns to predict neonatal death. PLos ONE 4: e4298.

Gibson NA, Graham M, Levene M. 1992. Somatosensory evoked potentials and outcome in perinatal asphyxia. Arch Dis Child 67:393-8.

Glass HC. 2014. Neonatal seizures. Advances in mechanisms and management. Clin Perinatol 41:177-190.

Glass HC, Glidden D, Jeremy RJ, Barkovich AJ, Ferriero DM, Miller SP. 2009. Clinical neonatal seizures are independently associated with outcome in infants at risk for hypoxic-ischemic brain injury. J Pediatr 155:318-23.

Golubnitschaja O, Yeghiazaryan K, Cebioglu M, Morelli M, Herrera-Marschitz M. 2011. Birth asphyxia as the major complication in newborns: moving toward improved individual outcomes by prediction, targeted prevention and tailored medical care. EPMA Journal 2:197-210.

Gonzalez FF, Ferriero DM. 2008. Therapeutics for neonatal brain injury. Pharmacol Ther 120:43-53.

Goodwin SR, Freidman WA, Bellefleur M.1991. Is it time to use evoked potentials to predict outcome in comatose children and adults? Crit Care Med 19:518-24.

Gosling RG, Dunbar G, King DH, Newman DL, Side CD, Woodcock JP, Fitzgerald DE, Keates JS, MacMillan D. 1971. The quantitative analysis of occlusive peripheral arterial disease by a non-instrusive technique. Angiology 22:52-55.

Govaert P, de Vries LS (ed). 2001. An Atlas of Neonatal Brain Sonography, Mac Keith Press, London, UK.

Graham EM, Ruis KA, Hartman AL, Northington FJ, Fox HE. 2008. A systematic review of the role of intrapartum hypoxia-ischemia in causation of neonatal encephalopathy. Am J Obstet Gynecol 199:587-95.

Gray PH, Griffin EA, Drumm JE, Fitzgerald DE, Duigan NM. 1983. Continuous wave Doppler ultrasound in evaluation of cerebral blood flow in neonates. Arch Dis Child 58:677-81.

Greisen G, Johansen K, Ellison PH, Fredrikson PS, Mali J, Friis-Hansen B. 1984. Cerebral blood flow in the newborn infant: comparison of ultrasound and Xenon-133 clearance. J Pediatr 104:411-418.

Greisen G, Hellström-Westas L, Lou H, Rosen I, Svenningsen N. 1985. Sleep-waking shifts and cerebral blood flow in stable preterm infants. Pediatr Res 19:1156-1159.

Greisen G, Munck H, Lou H. 1987. Severe hypocarbia in preterm infants and neurodevelopmental deficit. Acta Paediatr Scand 76:401-4.

Greisen G. 2005. Autoregulation of cerebral blood flow in newborn babies. Early Hum Dev 81:423-428.

Groenendaal F, Termote JU, Heide-Jalving M, van Haastert, de Vries LS. 2010. Complications affecting preterm neonates from 1991 to 2006: what have we gained? Acta Paediatr 99:354-358.

Hadders-Algra M. 2014. Early diagnosis and early intervention in cerebral palsy. Front Neurol 5:185.

Hagberg B, Hagberg G, Beckung E, Uvebrant P. 2001. Changing panorama of cerebral palsy in Sweden: VIII. Prevalence and origin in the birth year period 1991–1994. Acta Paediatr 90:271-277.

Hajnal BL, Sahebkar-Moghaddam F, Barnwell AJ, Barkovich AJ, Ferriero DM. 1999. Early prediction of neurological outcome after perinatal depression. Pediatr Neurol 21:788-93.

Harrington DJ, Redman CW, Moulden M, Greenwood CE. 2007. The long-term outcome in surviving infants with Apgar zero at 10 minutes: a systematic review of the literature and hospital-based cohort. Am J Obstetr Gynecol 196:463.e1-463.e5.

Heibel M, Heber R, Bechinger D, Kornhuber HH. 1993. Early diagnosis of perinatal cerebral lesions in apparently normal full-term newborns by ultrasound of the brain. Neuroradiology 35:85-91.

Hudome S, Palmer C, Roberts R, Mauger D, Housman C, Towfighi J. 1997. The role of neutrophils in the production of hypoxic-ischemic brain injury in the neonatal rat. Pediatr Res 41:607-616.

Ilves P, Talvik R, Talvik T. 1998. Changes in Doppler ultrasonography in asphyxiated term infants with hypoxic-ischemic encephalopathy. Acta Paediatr 87:680-684.

Ilves P, Lintrop M, Metsvaht T, Vaher U, Talvik T. 2004. Cerebral blood-flow velocities in predicting outcome of asphyxiated newborn infants. Acta Paediatr 93:523-528.

Ilves P, Lintrop M, Talvik I, Muug K, Maipuu L, Metsvaht T. 2009a. Low cerebral blood flow velocity and head circumference in infants with severe hypoxic ischemic encephalopathy and poor outcome. Acta Paediatr 98:459-465.

Ilves P, Lintrop M, Talvik I, Muug K, Maipuu L. 2009b. Changes in cerebral and visceral blood flow velocities in asphyxiated term infants with hypoxic ischemic encephalopathy. J Ultrasound Med 28:1471-1480.

Jelin AC, Zlatnik MG, Kuppermann M, Gregorich SE, Nakagawa S, Clyman R. 2015. Clamp late and maintain perfusion (CLAMP) policy: delayed cord clamping in preterm infants. J Matern Fetal Neonatal Med 17:1-5.

Jiang ZD, Liu XY, Shi BP, Lin L, Bu CF, Wilkinson AR. 2008. Brainstem auditory outcomes and correlation with neurodevelopment after perinatal asphyxia. Pediatr Neurol 39:189-95.

Johnston MV, Faterni A, Wilson MA, Northington F. 2011. Treatment advances in neonatal neuroprotection and neurointensive care. Lancet Neurol 10:372-382.

Joint Working Party of the British Association of Perinatal Medicine and the Research Unit of the Royal College of Physicians. 1992. Development of audit measures and guidelines for good practice in the management of neonatal respiratory distress syndrome. Arch Dis Child 67:1221-1227.

Jonsson M, Ågren J, Norden-Lindeberg S, Ohlin A, Hanson U. 2014. Neonatal encephalopathy and the association to asphyxia in labor. Am J Obstet Gynecol 211:667.e1-667.e8.

Julkunen M, Janas M, Eriksson K, Tammela O. 2006. Cerebral blood flow velocity by pulsed Doppler in full-term infants with asphyxia. Poster presented in the 2006 Pediatric Societies' Annual Meeting, Barcelona, Spain.

Kaiser JR, Gauss CH, Williams DK. 2005. The effects of hypercapnia on cerebral autoregulation in ventilated very low birth weight infants. Pediatr Res 58:931-935.

Kato T, Watanabe K. 2006. Visual evoked potentials in the newborn. Does it have predictive value? Seminars in Fetal Neonatal Med 11:459-463.

Khwaja O, Volpe JJ. 2008. Pathogenesis of cerebral white matter injury of prematurity. Arch Dis Child Fetal Neonatal Ed 93:F153-F161.

Kontio T, Toet MC, Hellström-Westas L, van Handel M, Groenendaal F, Stjerna S, Vanhatalo S, de Vries LS. 2013. Early neurophysiology and MRI in predicting neurological oucome at 9–10 years after birth asphyxia. Clin Neurophysiol 124:1089-1094.

Kupferschmid C, Lang D, Pohlandt F. 1988. Sensitivity, specificity and predictive value of clinical findings, m-mode echocardiography and continuous-wave Doppler sonography in the diagnosis of symptomatic patent ductus arteriosus in preterm infants. Eur J Pediatr 147:279-82.

Kurinczuk JJ, White-Koning M, Badawi N. 2010. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. Early Hum Dev 86:329-338.

Lai M-C, Yang S-N. 2011. Perinatal hypoxic-ischemic encephalopathy. J Biomed Biottechnol 609813. doi: 1155/2011/609813.

Laptook AR, Shankaran S, Ambalavanan N, Carlo WA, McDonald SA, Higgins RD, Das A. 2009. Outcome of term infants using Apgar scores at 10 minutes following hypoxic-ischemic encephalopathy. Pediatrics 124:1619-1626.

Larma JD. Silva AM, Holcroft CJ, Thompson RE, Donohue PK, Graham CA. 2007. Intrapartum electronic fetal heart rate and the identification of metabolic acidosis and hypoxic-ischemic encephalopathy. Am J Obstet Gynecol 197:301.e1-301.e8.

Larroque B, Marret S, Ancel PY, Arnaud C, Marpeau L, Supernant K, Pierrat V, Roze JC, Matis J, Cambonie G, et al. 2003. White matter damage and intraventricular hemorrhage in very preterm infants. the EPIPAGE study. J Pediatr 143:477-83.

Lawn J, Cousens S, Zupan J, Team for the Lancet Neonatal Survival Steering Team. 2005. 4 million neonatal deaths: When? Where? Why? Lancet 365:891-900.

Lee IC, Lee HS, Su PH, Liao WJ, Hu JM, Chen JY. 2009. Posthemorrhagic hydrocephalus in newborns: clinical characteristics and role of ventriculoperitoneal shunts. Pediatr Neonatol 50:26-32.

Leijser LM, de Vries LS, Cowan FM. 2006. Using cerebral ultrasound effectively in the newborn infant. Early Hum Dev 82:827-35.

Leijser LM, Vein AA, Liauw L, Strauss T, Veen S, van Wezel-Meijler G. 2007. Prediction of short-term neurological outcome in full-term neonates with hypoxic-ischemic encephalopathy based on combined use of electroencephalogram and neuroimaging. Neuroped 38: 219-27.

Leijser LM, de Bruine FT, Steggerda SJ, van der Grond J, Walther FJ, van Wezel-Meijker G. 2009. Brain imaging findings in very preterm infants through the neonatal period: Part I. Incidences and evolution of lesions, comparison ultrasound and MRI. Early Hum Dev 85:101-109.

Levene ML, Kornberg J, Williams THC. 1985. The incidence and severity of post-asphyxial encephalopathy in full-term infants. Early Hum Dev 11:21-6.

Levene MI, Fenton AC, Evans DH, Archer LNJ, Shortland DB, Gibson NA. 1989. Severe birth asphyxia and abnormal cerebral blood-flow velocity. Dev Med Child Neurol 31:427-434.

Levene MI, Chervenak FA (ed). 2009. Fetal and Neonatal Neurology and Neurosurgery (4th Ed). Churchill Livingstone Elsevier, UK.

Liem KD, Hopman JC, Osesburg B, de Haan AF, Kollee LA. 1997. The effect of blood transfusion and haemodilution on cerebral oxygenation and haemodynamics in newborn infants investigated by near infrared spectroscopy. Eur J Pediatr 156:305-310.

Liem KD, Greisen G. 2010. Monitoring of cerebral haemodynamics in newborn infants. Early Hum Dev 86:155-158.

Linder N, Haskin O, Levin O, Klinger G, Prince T, Naor N, Turner P, Karmazyn B, Sirota L. 2003. Risk factors for intraventricular hemorrhage in very low birth weight premature infants: a retrospective case-control study. Pediatrics 111:e590-e595.

Liveson JA, Ma DM. 1992a. Auditory evoked responses. In: Laboratory Reference for Clinical Neurophysiology, Oxford University Press, Oxford, UK.

Liveson JA, Ma DM. 1992b. Somatosensory evoked responses. In: Laboratory References for Clinical Neurophysiology, Oxford University Press, Oxford, UK.

Low JA. 1997. Intrapartum fetal asphyxia: definition, diagnosis, and classification. AM J Obstet Gynecol 100:1004-14.

Low JA, Lindsay BG, Derrick EJ. 1997. Threshold of metabolic acidosis associated with newborn complications. Am J Obstet Gynecol 177:1391-4.

Lowe LH, Bailey Z. 2011. State-of-the-Art Cranial Sonography: Part I, Modern Techniques and Image Interpretation. AJR 196:1028-1033.

Majnermer A, Rosenblatt B, Riley P. 1988. Prognostic significance of the auditory brainstem evoked response in high-risk neonates. Dev Med Child Neurol 40:43-52.

Mandel R, Martinot A, Delepuolle F, Lamblin MD, Laureau E, Valee L, Leclerc F. 2002. Prediction of outcome after hypoxic-ischemic encephalopathy: A prospective clinical and electrophysiologic study. J Pediatr 141:45-50.

Marlow N, Rose AS, Rands CE, Draper ES. 2005. Neurophysiological and educational problems at school age associated with neonatal encephalopathy. Arch Dis Child Fetal Neonatal Ed 90:F380-7.

Martinez-Biarge M, Diez-Sebastain J, Rutherford MA, Cowan FM. 2010. Outcomes after central grey matter injury in term perinatal hypoxic-ischaemic encephalopathy. Early Hum Dev 86:675-682.

Martinez-Biarge M, Diez-Sebastian J, Kapellou O, Gindner D, Allison JM, Rutherford MA, Cowan FM. 2011. Predicting motor outcome and death in term hypoxic-ischemic encephalopathy. Neurology 76:2055-2061.

Martinez-Biarge M, Bregant T, Wusthoff CJ, Chew ATM, Diez-Sebastian J, Rutherford MA, Cowan MA. 2012. White matter and cortical injury in hypoxic-ischemic encephalopathy: Antecedent factors and 2-year outcome. J Pediatr 161:799-807.

Massaro AN, Chang T, Kadom N, Tsuchia T, Scafidi J, Glassa P, McCarter R, Baumgart S, Vezina G, Nelson KB. 2012. Biomarkers of brain injury in neonatal encephalopathy treated with hypothermia. J Pediatr 161:434-440.

Massaro AN, Chang T, Baumgart S, McCarter R, Nelson KB, Glass P. 2014. Biomarkers S100B and neuron-specific enolase predict outcome in hypothermia-treated encephalopathic newborns. Pediatr Crit Care Med 15:615-22.

Meek JH, Tyszcuzuk L, Elwell CE, Wyatt JS. 1998. Cerebral blood flow increases over the first three days of life in extremely preterm neonates. Arch Dis Child Fetal Neonatal 78:F33-F37.

Meek JH, Elwell CE, Mc Cormick DC, Edwards AD, Townsend JP, Stewart AL, Wyatt JS. 1999. Abnormal cerebral haemodynamics in perinatally asphyxiated infants. Arch Dis Child Fetal Neonatal 81:F110-F115.

Menache CC, Bourgeois BF, Volpe JJ. 2002. Prognostic value of neonatal discontinuous EEG. Pediatr Neurol 27:93-101.

Miller SP, Weiss J, Barnwell A, Ferriero DM, Latal-Hajnal B, Ferrer-Rogers A, Newton N, Partridge JC, Glidden DV, Vigneron DB et al. 2002. Seizure-associated brain injury in term newborns with perinatal asphyxia. Neurology 58:542-548.

Moster D, Lie RT, Markestad T. 2002. Joint association of Apgar scores and early neonatal symptoms with minor disabilities at school age. Arch Dis Child Fetal Neonatal Ed 86:F16-F21.

Murphy BP, Inder TE, Rooks V, Taylor GA, Andersson NJ, Mogridge N, Horwood LJ, Volpe JJ. 2002. Posthaemorrhage ventricular dilatation in the premature infant: natural history and predictors of outcome. Arch Dis Child Fetal Neonatal Ed 87:F37-F41.

Murray DM, Boylan GB, Ryan CA, Connolly S. 2009. Early EEG findings in hypoxic-ischemic encephalopathy predict outcomes at 2 years. Pediatrics 124:e459-67.

Muttit SC, Taylor MJ, Kobayashi JS, MacMillan L, Whyte HE. 1991. Serial visual evoked potentials and outcome in term birth asphyxia. Pediatr Neurol 7:86-90.

Mwaniki MK, Atieno M, Lawn JE, Newton CRJC. 2012. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet 379: 445-52.

Nagdyman N, Grimmer I, Scholz T, Muller C, Obladen M. 2003. Predictive value of brain specific proteins in serum for neurodevelopmental outcome after birth asphyxia. Pediatr Res 54:270-275.

Nelson K, Ellenberg J. 1981. Apgar scores as predictors of chronic neurologic disability. Pediatrics 68:36-44.

Nelson KB, Leviton A. 1991. How much of neonatal encephalopathy is due to birth asphyxia? Am J Dis Child 145:1325-31.

Nelson KB, Dambrosia JM, Ting TY, Grether JK. 1996. Uncertain value of electronic fetal monitoring in predicting cerebral palsy. N Engl J Med 334:613-618.

Odom JV, Bach M, Brigell M, Holder GE, McCulloch DL, Tormene AP, Vaegan. 2010. ISCEV standard for clinical visual poentials (update 2009). Doc Ophthalmol 120:111-9.

Okereafor A, Allsop J, Counsell SJ, Fitzpatrick J, Azzopardi D, Rutherford MA, Cowan FM. 2008. Patterns of brain injury in neonates exposed to perinatal sentinel events. Pediatrics 121:906-914.

Okumura A, Hayakawa F, Kato T, Itomi K, Maruyama K, Ishihara N, Kubota T, Suzuki M, Sato Y, Kuno K, et al. 2001. Hypocarbia in preterm infants with periventricular leukomalacia: The relation between hypocarbia and mechanical ventilation. Pediatrics 107:469-75.

Omokhodion SI, Losekoot TG, Jaiyesimi F. 1990. Serum creatine kinase and creatine kinase-MB isoenzyme activities in perinatally asphyxiated newborns. Eur Heart J 12:980-984.

Osredkar D, Toet MC, van Rooij LGM, van Huffeken AC, Groenendaal F, de Vries LS. 2005. Sleep-wake cycling on amplitude-integrated electroencephalography in term newborns with hypoxic-ischemic encephalopathy. Pediatrics 115:327-332.

Papile LA, Burstein J, Burstein R, Koffler H. 1978. Incidence and evolution of subependymal and intraventricular hemorrhage: A study of infants with birth weight less than 1,500 gms. J Pediatr 92:529-534.

Pappas A, Shankaran S, Laptook AR, Langer JC, Bara R, Ehrenkranz RA, Goldberg RN, Das A, Higgins RD, Tyson JE, et al. 2011. Hypocarbia and adverse outcome in neonatal hypoxic-ischemic encephalopathy. J Pediatr 158:752-758.

Perlman JM, Hill A, Volpe JJ. 1981. The effect of patent ductus arteriosus on flow velocity in the anterior cerebral arteries: Ductal steal in the preterm newborn infant. J Pediatr 99:767-771.

Perlman JM, MacMenamin JB, Volpe JJ. 1983. Fluctuating cerebral blood flow velocity in respiratory distress syndrome. N Engl J Med. 309:204-209.

Perlman JM, Hersovitch P, Kreusser KL, Raichle ME, Volpe JJ. 1985. Positron emission tomography in the newborn: Effect of seizure on regional cerebral blood flow in an asphyxiated infant. Neurology 35:244-247.

Perlman JM, Wyllie J, Kattwinkel J, Atkins DL, Chameides L, Goldsmith JP, Guinsburg R, Hazinski MF, Morley C, Richmond S et al. 2010. Neonatal resuscitation: 2010 international consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations. Pediatrics 126:1319-1344.

Pezzati M, Dani C, Biadaioli R, Filippi L, Biagiotti R, Giani T, Rubaltelli FF. 2002. Early postnatal Doppler assessment of cerebral blood flow velocity in healthy preterm and term infants. Dev Med Child Neurol 44:745-752.

Pin TW, Eldridge B. Galea MP. 2009. A review of developmental outcomes of term infants with post-asphyxia neonatal encephalopathy. Eur J Paeditr Neurol 13:224-34.

Piper MC, Darrah J. 1994. Motor assessment of the developing infant, Saunders, Philadelphia, USA.

Pourcelot L. 1975. Application clinique de l'examen Doppler trancutane: In: Peronneau P, ed. Velocimetre Ultrasonore Doppler. Paris: Inserm. 213-240.

Prechtl HFR, Einspieler C, Cioni G, Bos AF, Ferrari F, Sontheimer D. 1997. An early marker for neurological deficits after perinatal brain lesions. Lancet 349:1361-63.

Pryds O, Greisen G, Lou H, Friis-Hansen. 1989. Heterogeneity of cerebral vasoreactivity in preterm infants supported by mechanical ventilation. J Pediatr 115:638-45.

Pryds O, Greisen G, Lou H, Friis-Hansen B. 1990a. Vasoparalysis associated with brain damage in asphyxiated term infants. J Pediatr 117:119-125.

Pryds O, Christensen NJ, Friis-Hansen HB. 1990b. Increased cerebral blood flow and plasma epinephrine in hypoglycemic, preterm neonates. Pediatrics 85:172-176.

Purkayastha S, Sorond F. 2012. Transcranial Doppler ultrasound: Technique and Application. Semin Neurol 32:411-420.

Qian J, Zhou D, Wang Y-W. 2009. Umbilical artery blood S100B protein: a tool for the early identification of neonatal hypoxic-ischemic encephalopathy. Eur J Pediatr 168:71-77.

Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. 2009. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. Pediatr Neurol 40:215-226.

Rennie JM (ed). 2012. Rennie & Roberton's Textbook of Neonatolgy (5th Ed). Churchill Livingstone Elsevier, UK.

Riley RJ, Johnson JW. 1993. Collecting and analyzing cord blood gases. Clin Obstet Gynecol 36:13-23.

Robertson CMT, Perlman M. 2006. Follow-up of the term infant after hypoxic-ischemic encephalopathy. Paediatr Child Health 11:278-82.

Robinson LR, Micklesen PJ, Tirschwell DL, Lew HL. 2003. Predictive value of somatosensory evoked potentials for awakening from coma. Crit Care Med 31:960-7.

Roka A, Kelen D, Halasz J, Beko G, Azzopardi D, Szabo M. 2012. Serum S100B and neuron-specific enolase levels in normothermic and hypothermic infants after perinatal asphyxia. Acta Paediatr 101:319-323.

Romagnoli C, Giannantonio C, De Carolis MP, Gallini F, Zecca E, Papacci P. 2006. Neonatal color Doppler US study: Normal values of cerebral blood flow velocities in preterm infants in the first month of life. Ultrasound in Med & Biol 32:321-331.

Rosenbloom L. 1994. Dyskinetic cerebral palsy and birth asphyxia. Dev Med Child Neurol 36:285-9.

Rutherford MA, Pennock JM, Counsell SJ, Mercuri E, Cowan FM, Dubowitz LM, Edwards AD. 1998. Abnormal magnetic resonance signal in the internal capsule predicts poor neurodevelopmental outcome in infants with hypoxic-ischemic encephalopathy. Pediatrics 102:323-328.

Rutherford MA, Azzopardi D, Whitelaw A, Cowan F, Renowden S, Edwards AD, Thoresen M. 2005. Mild hypothermia and the distribution of cerebral lesions in neonates with hypoxic-ischemic encephalopathy. Pediatrics 116:1001- 1006.

Rutherford M, Malamateniou C, McGuinness A, Allsop J, Matinez Biarge M, Counsell S. 2010a. Magnetic resonance imaging in hypoxic-ischaemic encephalopathy. Early Hum Dev 86:351-360.

Rutherford M, Ramenghi LA, Edwards AD, Brocklehurst P, Levene M, Strohm B, Thoresen M, Whitelaw A, Azzopradi D. 2010b. Assessment of brain tissue injury after moderate hypothermia in neonates with hypoxic-ischaemic encephalopathy: a nested substudy of a randomised controlled study. Lancet Neurol 9: 39-45.

Sarnat HB, Sarnat MS. 1976. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol 33:696-705.

Scalais E, Francois-Adant A, Nuttin C, Bachy A, Guerit JM. 1998. Multimodality evoked potentials as a prognostic tool in term asphyxiated infants newborns. Electroencephalogr Clin Neurophysiol 108:199-207.

Shah S, Tracy M, Smyth J. 2004. Postnatal lactate as an early predictor of short-term outcome after intrapartum asphyxia. J Perinatol 24:16-20.

Shankaran S, Laptook AR, Ehrenkrantz RA, Tyson JE, McDonald SA, Donovan EF, Fanaroff AA, Poole WK, Wright LL, Higgings RD, Finer NN, Carlo WA et al. 2005. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. N Engl J Med 353:1574-84.

Shankaran S, Langer JC, Kazzi SN, Laptook AR, Walsh M. 2006. Cumulative index of exposure to hypocarbia and hyperoxia as risk factors for periventricular leukomalacia in low birth weight infants. Pediatrics 118:1654-9.

Sinclair DB, Campbell M, Byrne P, Prasertsom W, Robertson CMT. 1999. EEG and long-term outcome of term infant with neonatal hypoxic-ischemic encephalopathy. Clin Neurophysiol 110:655-9.

Skranes JH, Elstad M, Thoresen M, Cowan FM, Stiris T, Fugelseth D. 2014. Hypothermia makes cerebral resistance index a poor prognostic tool in encephalopathy newborns. Neonatology 106:17-23.

Soul JS, Hammer PE, Tsuji M, Saul PP, Bassan H, Limperopoulus C, Disalvo DN, Moore M, Akins P, Ringer S, et al. 2007. Fluctuating pressure-passitivity is common in the cerebral circulation of sick premature infants. Pediatr Res 61:467-473.

Stark JE, Seibert JJ. 1994. Cerebral artery Doppler ultrasonography for prediction of outcome after perinatal asphyxia. J Ultrasound Med 13:595-600.

Stuart B, Drumm J, Fitzgerald DE, Duignan NM. 1980. Fetal blood velocity waveforms in neonatal pregnancy. Br J Obstet Gynaecol 87:780-785.

Suppiej A, Cappellari A, Franzoi M, Traverso A, Ermani M, Zanardo V. 2010. Bilateral loss of cortical somatosensory evoked potential at birth predicts cerebral palsy in term and near-term newborns. Early Hum Dev 86:93-8.

Swarte RMC, Cherian PJ, Lequn M, Visser GH, Govaert P. 2012. Somatosensory evoked potentials are of additional prognostic value in certain patterns of brain injury in term birth asphyxia. Clin Neurophysiol 123:1631-8.

Sweet DG, Bell AH, McClure G, Wallace IJ, Shields MD. 1999. Comparison between creatine kinase isoenzyme (CKBB) activity and Sarnat score for adverse outcome following perinatal asphyxia. J Perinat Med 27:478-83.

Tasker RC. 2013. Brain vascular and hydrodynamic physiology. Sem in Pediatr Surg 22:168-173.

Taylor MJ, Murphy WJ, Whyte HE. 1992. Prognostic reliability of somatosensory and visual evoked potentials of asphyxiated term infants. Dev Med Child Neurol 34:507-15.

Tekgul H, Gsauvreau K, Soul J, Murphy L, Robertson R, Stewart J, Volpe J, Bourgeois B, du Plessis AJ. 2006. The current etiologic profile and neurodevelopmental outcome of seizures in term newborn infants. Pediatrics 117:1270-1280.

Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, Malan AF. 1997. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. Acta Paediatr 86:757-761.

Thoresen M, Hellström-Westas L, Liu X, de Vries LS. 2010. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. Pediatrics 126:e131-9.

Toh V. 2000. Early predictors of adverse outcome in term infants with post-asphyxial hypoxic ischaemic encephalopathy. Acta Paediatr 89:343-347.

Tsuij M, Saul P, du Plessis A, Eichenwald E, Sobh J, Crocker R, Volpe JJ. 2000. Cerebral intravascular oxygenation correlates with mean aterial pressure in critically ill premature infants. Pediatrics 106:625-632.

Tyszczuk L, Meek J, Elwell C, Wytt JS. 1998. Cerebral blood flow is independent of mean arterial pressure in preterm infants undergoing intensive care. Pediatrics 102:337-341.

Udomphorn Y, Armstead WM, Vavilala MS. 2008. Cerebral blood flow and autoregulation after pediatric traumatic brain injury. Pediatr Neurol 38:225-234.

van Bel F, Shadid M, Moison RM, Dorrepaal CA, Fontijn J, Monteiro L, van De Bor M, Berger HM. 1998. Effect of allopurinol on postasphyxial free radical formation, cerebral hemodynamics, and electrical brain activity. Pediatrics 101:28-38.

van Laerhoven H, de Haan TR, Offringa M, Post B, van der Lee JH. 2013. Prognostic tests in term neonates with hypoxic-ischemic encephalopathy: A systematic review. Pediatrics 131:88-98.

van Wezel-Meijler G, Steggerda SJ, Leijser LM. 2010. Cranial ultrasonography in neonates: Role and limitations. Semin Perinatol 34:28-38.

Volpe JJ. 2001. Neurobiology of periventricular leukomalacia in the preterm infant. Pediatr Res 50:553-562.

Volpe JJ. (ed). 2008. Neurology of the Newborn (5th Ed), Saunders, Philadelphia, USA.

Vutskits L. 2014. Cerebral blood flow in the neonate. Pediatr Anesthesia 24:22-29.

Wassink G, Gunn ER, Drury PP, Bennet L, Gunn AL. 2014. The mechanisms and treatment of asphyxial encephalopathy. Front NeuroSci 8:1-11.

Weissman A, Olanovski I, Weiner Z, Blazer S. 2012. Doppler middle cerebral artery peak systolic velocity for diagnosis of neonatal anemia. J Ultrasound Med 31:1381-1385.

Wertheim D, Mercuri E, Faundez JC, Rutherford M, Acolet D, Dubowitz L. 1994. Prognostic value of continuous electroencephalographic recording in full term infants with hypoxic ischaemic encephalopathy. Arch Dis Child 71:F97-F102.

Whitelaw A, Aquilina K. 2012. Management of posthaemorrhagic ventricular dilatation. Arch Dis Child Fetal Neonatal Ed 97:F229-F233.

Williams KP, Galerneau F. 2004. Comparison of intrapartum fetal heart rate tracings in patients with neonatal seizures vs. no seizure: what are the differences? J Perinatal Med 32:422-425.

Wintermark P, Hansen A, Gregas MC, Soul J, Labrecque M, Robertson RL, Warfield SK. 2011. Brain perfusion in asphyxiated newborns treated with therapeutic hypothermia. AJNR 32:2023-29.

Wolrab G, Botshauser E, Schmitt B. 2001. Neurological outcome in comatose children with bilateral loss of cortical somatosensory evoked potentials. Neuropediatrics 32:271-4.

Wu YW, Hamrick SEG, Miller SP, Haward MF, Lai MC, Callen PW, Barkovich AJ, Ferriero DM. 2003. Intraventricular hemorrhages in term neonates caused by sinovenous thrombosis. Ann Neurol 54:123-126.

Zeinstra E, Fock JM, Begeer JH, van Weereden TW, Maurits NM, Zweens MJ. 2001.The prognostic value of serial EEG recordings following acute neonatal asphyxia in full-term infants. Eur J Paediatr Neurol 5:155-60.

ORIGINAL PUBLICATIONS

ELSEVIER

● *Original Contribution*

END-DIASTOLIC BLOCK IN CEREBRAL CIRCULATION MAY PREDICT INTRAVENTRICULAR HEMORRHAGE IN HYPOTENSIVE EXTREMELY-LOW-BIRTH-WEIGHT INFANTS

Mia Julkunen,*[†] Tiina Parviainen,[‡] Martti Janas,[†] and Outi Tammela*[†]

*Pediatric Research Centre, University of Tampere; [†]Department of Paediatrics, Tampere University Hospital; and [‡]School of Public Health, University of Tampere and Research Unit, Tampere University Hospital, Tampere, Finland

Abstract—We investigated the association of intracranial arterial end-diastolic block with mean arterial pressure (MAP), patent ductus arteriosus (PDA) and intraventricular hemorrhage (IVH) in infants with birth weights <1000*g*. End-diastolic block was diagnosed when end-diastolic flow was found to be absent from the Doppler waveforms in cerebral arteries by pulsed Doppler ultrasound examinations. Cranial ultrasonography and pulsed Doppler examination of anterior cerebral and basilar arteries were performed in 55 preterm infants (gestational age range 24–31 wk) one to four times during the first four days of life. Of these, 22 (40%) developed an end-diastolic block at least once (block group); in 33 no block was detected (control group). Echocardiography was performed and MAP recorded concomitantly. The block group infants had significantly lower mean MAPs than the controls and 59% of those had MAP of 30 mm Hg or lower. In the block group, IVH developed more often in infants with MAP <30 mm Hg (46%) than in infants with MAPs >30 mm Hg (0%). However, in the control group IVH developed equally frequently in infants with MAP ≤30 mm Hg (33%) and in infants with MAP >30 mm Hg (30%). PDA was a significant risk factor for IVH. An end-diastolic block in the cerebral circulation, together with a MAP of ≤30 mm Hg or less and the presence of PDA during the first four days of life, might be associated with IVH in extremely-low-birth-weight infants. (E-mail: outi.tammela@uta.fi)   © 2008 World Federation for Ultrasound in Medicine & Biology.

Key Words: Blood pressure, Cerebral circulation, Intraventricular hemorrhage, Patent ductus arteriosus, Pulsed Doppler ultrasound.

INTRODUCTION

Intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL) are the most severe forms of brain injury in preterm infants. Disturbances in cerebral blood flow (CBF) are significant in the pathogenesis of IVH (Lou et al. 1979; Perlman et al. 1983). Episodes of hypotension (Milligan 1980; Miall-Allen et al. 1987), hypertension (Grönlund et al. 1994) and fluctuating blood pressure (Perlman et al. 1983) have been associated with failure of cerebral autoregulation in preterm infants. Also a large patent ductus arteriosus (PDA) has been associated with IVH (Evans and Kluckow 1996) and low blood pressure (Evans and Iyer 1993).

Pulsed Doppler ultrasound is a noninvasive means of examining cerebral circulation at the bedside, even in sick preterm infants under intensive care. Loss of diastolic flow, retrograde diastolic flow or no detectable flow in cerebral arteries in a small group of pediatric patients of ages ranging from newborn to 4 y has been related to a high risk of death, although survival without sequelae is also possible (Chiu et al. 2003). Absence of diastolic flow in intracranial arteries can therefore be regarded as a significant disturbance in cerebral blood flow, possibly constituting a risk factor for adverse outcome and development of cerebral injury.

The aim of this study was to establish by pulsed Doppler ultrasound the incidence of end-diastolic block in the cerebral circulation and its connection with the development of IVH in extremely-low-birth-weight (ELBW) infants. End-diastolic block is diagnosed when end-diastolic flow is found to be absent from the Doppler waveforms in cerebral arteries. We also sought to determine risk factors associated with the blockage and its relationship to the concomitant systemic mean arterial

Address correspondence to: Dr Outi Tammela, Department of Paediatrics, Tampere University, Hospital, P.O. Box 2000, FIN- 33521, Tampere, Finland. E-mail: outi.tammela@uta.fi

pressure (MAP), as well as the presence of PDA in such infants during the first days of life. To our knowledge, the relation between end-diastolic block in the cerebral circulation, MAP and IVH has not so far been examined.

PATIENTS AND METHODS

This prospective longitudinal follow-up study was carried out in the neonatal intensive care unit in Tampere University Hospital. The Ethics Committee of Tampere University Hospital had approved the research study protocol. The ultrasound examinations were performed according to the routine follow-up protocol used in the unit. The parents were well informed on the timing and results of the examinations.

Seventy-five consecutive infants with birth weights <1000*g* were born in Tampere University Hospital between June 1999 and August 2002. The gestational ages ranged from 24 to 31 weeks. Twenty were excluded from the analysis: 13 died during the first or second day of life, three had not undergone Doppler ultrasound examination and in one case, pulsed Doppler examination was first performed on the fifth day of life. Relevant data were missing in three cases. On the first day, pulsed Doppler ultrasonography was performed in 49 infants. Six had not undergone pulsed Doppler examination during the first 24 h. Altogether 55 infants thus underwent Doppler ultrasound examinations one to four times during the first four days of life. The study population was divided into a block group, with an end-diastolic block diagnosed in intracranial arteries at least once during the study period, and a control group with continuous flow found in each of the examinations. The study focused on the pulsed Doppler examinations on days 1 to 4 because IVH has been shown to develop during the first days in preterm infants (Bracci et al. 2006).

Each infant underwent pulsed Doppler examinations and cranial ultrasonography conducted by an attending neonatologist, using a 7.5-MHz transducer (PowerVision 6000, Toshiba, Nasu, Japan). The infants were lying supine, either sleeping or awake but quiet. Cerebral blood flow velocity (CBFV) (cm/s) in the anterior cerebral (ACA) and basilar (BA) arteries was measured from the midsagittal section through the anterior fontanelle. The CBFV of ACA represented the carotid and the CBFV of BA the vertebrobasilar circulation. The flow in the ACA was measured in the front of the third ventricle and the flow in the BA in the front of the *pons cerebri* in the sagittal section. There was no need to use angle of inosonation. A resistive index (RI: peak-systolic velocity − end-diastolic velocity/peak-systolic velocity) was calculated from these data. An RI ranging between 0.6 and 0.8 was regarded as normal (Deeg and Rupprecht 1989; Mires et al. 1994). End-diastolic block was diag-

nosed when end-diastolic flow was found to be absent from the Doppler waveforms of at least 20 consecutive cardiac cycles in either or both cerebral arteries.

Cerebral ultrasonography was performed to detect structural abnormalities, IVH or changes in parenchymal echodensity. Hemorrhages were graded according to Papile et al. (1978) and PVL according to de Vries et al. (1992).

Standard echocardiography was performed with the same equipment either immediately before or after cranial ultrasonography to define the direction of the ductal flow and the time of ductal closure from the parasternal and suprasternal view. The PDA was considered hemodynamically significant in cases yielding at least three of the following findings: a characteristic systolic murmur, bounding peripheral pulses, hyperactive precordium, cardiac enlargement in chest X-rays and respiratory deterioration. Diagnosis of PDA was made by clinical findings. Echocardiography was used to determine the size of the PDA and direction of the ductal shunt and to ensure that there was no contraindication for closure. In such cases the PDA was closed either by means of indomethacin treatment or by surgical ligation, at the discretion of the attending neonatologist.

Blood pressure was monitored *via* an indwelling peripheral arterial catheter using a multichannel neonatal monitor (HP Model 64S, Hewlett-Packard GmbH, Boeblingen, Germany). At the time of Doppler ultrasonography, mean arterial pressure (MAP) was recorded and blood gas analyses, blood glucose and hematocrit were determined within 2 to 4 h of ultrasound examination. Hypocapnia was defined as a partial pressure of carbon dioxide (pCO_2) value <4.5 kPa in arterial blood gas analyses and hypoglycemia as a blood glucose value <2.6 mmol/L. Infants with low MAP levels for their current gestational age received a 10 mL/kg IV infusion of Ringer's solution or fresh frozen plasma in a period of 30 to 60 min. In cases of poor response or recurrent MAP drop, treatment was initiated with inotropics (dopamine infusion 5–10 μg/kg/min, combined if necessary with dobutamine 5–10 μg/kg/min). Besides inotropics, hydrocortisone was used to elevate blood pressure in hypotensive preterm infants (Seri 2006). If hypotension persisted when the infant was receiving a maximum dosage of both dopamine and dobutamine, hydrocortisone 5 to 10 mg/kg/d divided into three IV doses was administered.

Statistical analyses were performed using SPSS 11.0 for Windows (SPSS, Chicago, IL, USA). The statistical analyses between the block group and the control group were performed by independent *t*-test, chi-square, Fisher's exact test or Mann-Whitney *U* test, as appropriate. Stepwise logistic regression analysis was used to identify risk factors for end-diastolic block (birth weight, IVH, MAP ≤30 mm Hg, $pCO_2 \leq 4.5$ kPa, hematocrit

Table 1. Clinical characteristics of study groups

	Block group (n = 22)	Control group (n = 33)	p value
Birth weight (g) mean (SD)	757 (137)	809 (151)	.204
Gestational age (wk) mean (SD)	27 (2.0)	27 (1.7)	.926
SGA (n) (%)	10 (73)	10 (30)	.250
Delivery route			.808
Vaginal (n) (%)	6 (27)	10 (30)	
Cesarean section (n) (%)	16 (73)	23 (70)	
Apgar scores at 1 min 5 or less (n) (%)	15 (68)	16 (49)	.149
Apgar scores at 5 min 5 or less (n) (%)	7 (32)	17 (52)	.564
Male/female ratio	12/10	19/14	.824
Antenatal steroids (n) (%)	21 (95)	31 (94)	.933
Dopamine treatment (n) (%)	19 (86)	27 (82)	.727
Dose of dopamine (μg/kg/min) Median (range)	12.0 (2.2–13.5)	9.6 (2.7–17.2)	.697
Dobutamine treatment (n) (%)	10 (46)	14 (42)	.824
Dose of dobutamine (μg/kg/min) Median (range)	11.3 (10.1–12.3)	11.3 (4.2–15.2)	.591
Glucocorticoid treatment (n) (%)	10 (45)	7 (21)	.057
Morphine treatment (n) (%)	22 (100)	27 (82)	.071
Dose of morphine (μg/kg/h) Median (range)	12.1 (2.3–32.0)	10.3 (2.1–20.0)	.732

$\leq 0.40\%$, blood glucose ≤ 2.6 mmol/L, PDA) and to identify risk factors for IVH (birth weight, block, MAP ≤ 30 mm Hg, pCO_2 ≤ 4.5 kPa, hematocrit $\leq 0.40\%$, blood glucose ≤ 2.6 mmol/L, PDA). A p-value < 0.05 was considered significant.

RESULTS

Clinical characteristics and outcome

A total of 147 pulsed Doppler ultrasound examinations were performed in 55 ELBW infants one to four times per infant during the first four days of life. Of these infants, 22 developed an end-diastolic block at least once, whereas no block was detected in 33 infants.

The block group and the control group were similar in birth weight, gestational age and male-to-female ratio. Data on clinical characteristics are shown in Table 1. The median duration of administration of dopamine was 2 d (range 1–6 in the block group) and 4 d (range 1–18 in the control group) (p = 0.164) and duration of administration of dobutamine 2.5 d (range 1–4 in the block group) and 1 d (range 1–4 in the control group) (p = 0.72). The median duration of administration of morphine was 6 d (range 1–31 in the block group) and 8 d (range 0–24 in the control group) (p = 0.586). The median dosage of morphine did not differ between the two groups on day 1, but the infants with block detected on days 2 to 4 received statistically a significantly higher median dosage of morphine (14.3 μg/kg/h; range 2.6–32.0) than the controls (8.7 μg/kg/h; range 2.1–20.0), p = 0.021.

Blood glucose, hematocrit, pCO_2 and partial pressure of oxygen (pO_2) values did not differ between the study groups. The durations of assisted ventilation, ox-

ygen supplementation, primary hospitalization and occurrence of adverse outcome were similar in the groups (Table 2). Seven (32%) infants in the block group and six (18%) in the control group died, p = 0.244.

Occurrence of end-diastolic block in cerebral circulation

The numbers of pulsed Doppler ultrasound examinations in the study groups on days 1 to 4 are seen in Table 3. An end-diastolic block in the ACA or the BA was detected in 17 (35%) infants on day 1. Eight infants had a block only in the ACA, three infants only in the BA and six simultaneously in both arteries; 32 infants

Table 2. Outcome

	Block group (n = 22)	Control group (n = 33)	p value
Duration of Ventilator treatment (days) mean (SD)	10 (9)	16 (18)	
Median (range)	7 (1–33)	8 (0–63)	.764
Duration of Oxygen supplementation (days) mean (SD)	42 (29)	48 (31)	
Median (range)	48 (1–96)	45 (1–120)	.453
Duration of Hospitalisation (days) mean (SD)	63 (50)	73 (35)	
Median (range)	73 (1–199)	80 (3–137)	.525
Oxygen supplementation at gestational week 36 (n) (%)	4 (18)	7 (21)	1.000
Adverse outcome (n) (%)*	18 (82)	19 (58)	.081

* Infants with at least one of the following: bronchopulmonary dysplasia, death, intraventricular hemorrhage, necrotising enterocolitis, periventricular leucomalacia.

Table 3. Numbers of pulsed Doppler examinations in the study groups on days 1 to 4

	Day 1	Day 2	Day 3	Day 4
Block group, n (%)	20 (91)	16 (73)	15 (68)	10 (45)
Control group, n (%)	29 (88)	24 (73)	16 (48)	17 (52)

evinced continuous flow. During days 2 to 4 an end-diastolic block was detected at least once in 13 (24%) infants. Two infants had a block only in the ACA, six only in the BA and five simultaneously in both arteries, and one infant had a block in the BA on day 3 and in the ACA on day 4; 42 infants evinced continuous flow. During the first four days, an end-diastolic block was thus detected at least once in 22 (40%) of 55 infants; in 33 infants, no block was detected during the examinations.

Retrograde diastolic flow was detected once in two infants in the block group during the first four days of life. There were no cases with nondetectable flow in cerebral arteries by pulsed Doppler ultrasound examination.

Hemodynamic values

During the first 24 h, the peak systolic cerebral blood flow velocity (SCBFV) was measured in 53% of the infants in the block group and in 81% in the control group. The velocities in both cerebral arteries did not differ between the study groups. The peak SCBFV in the BA was marginally lower in the block group (mean [SD] 20.9 [8.4] cm/s) than in the control group (25.5 [7.6] cm/s), $p = 0.059$, but in the ACA fairly similar in both groups (24.1 [7.2] cm/s *vs.* 21.4 [6.4] cm/s, respectively, $p = 0.363$). During days 2 to 4, peak SCBFVs in the ACA or in the BA did not differ between the two groups (Fig. 1).

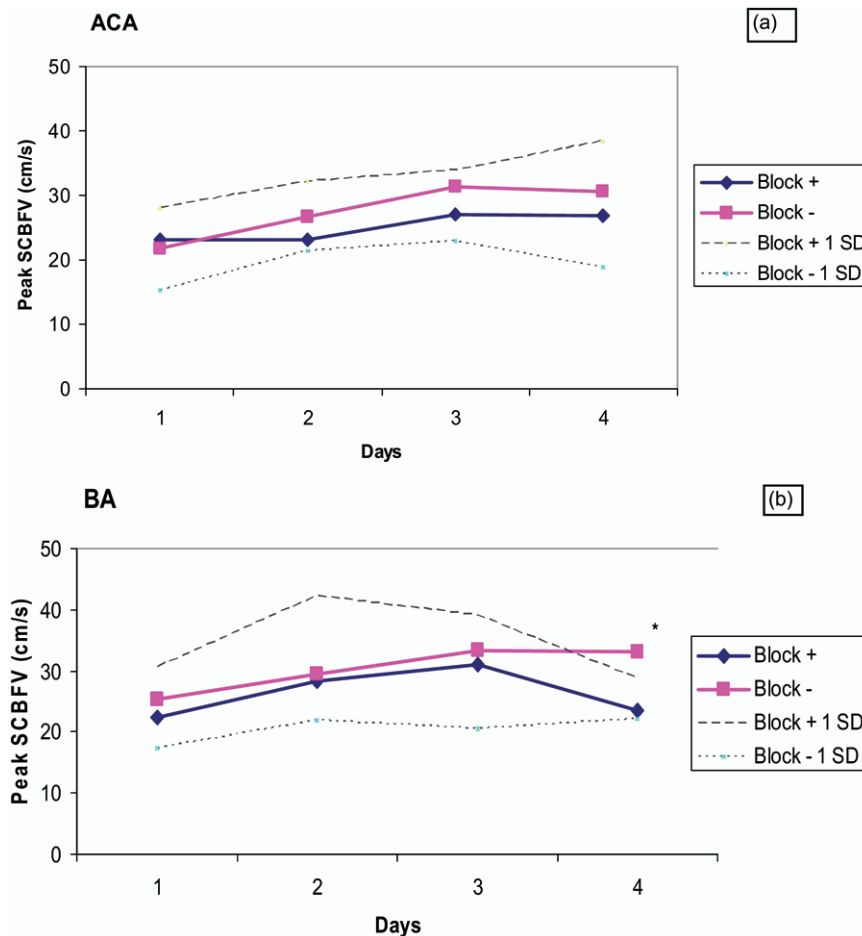The mean (SD) of the lowest MAP recordings mea-

Fig. 1. The SCBFV in the ACA and the BA in infants with an end-diastolic block in the cerebral circulation (block +) and in the control group (block −) (mean [SD]). *$p < 0.05$.
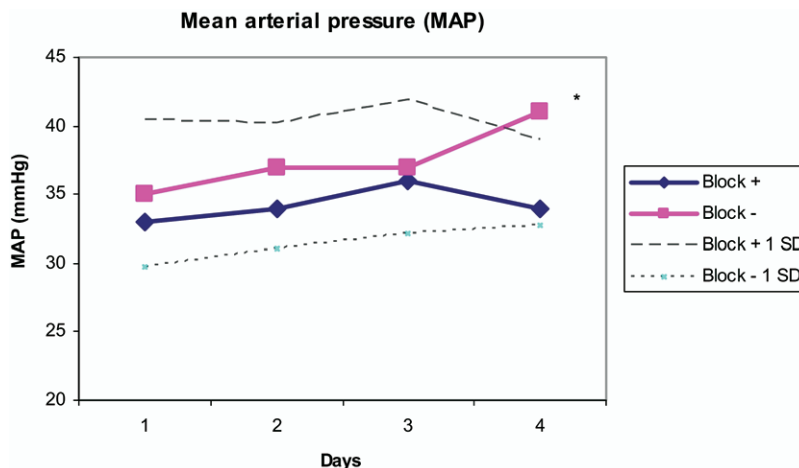
Mean arterial pressure (MAP)

Fig. 2. The mean (SD) of MAP in infants with an end-diastolic block (block +) in the cerebral circulation at least once during the first four days and in the control group (block −). *$p < 0.05$.

sured during Doppler ultrasound examinations in infants with end-diastolic block was significantly lower (30 [5] mm Hg; range 16–40 mm Hg) than in those without (34 [5] mm Hg; range 25–52 mm Hg), $p = 0.01$. On day 1, the concomitant mean (SD) of MAP in the infants with end-diastolic block was marginally lower (33 [7.4] mm Hg; range 24–59) than in those without (35 [5.3] mm Hg; range 25–52) at the time of Doppler examination, $p = 0.060$ (Fig. 2). The mean MAP at the time of Doppler examination on days 2 and 3 did not differ between the study groups as is seen in Fig. 2, but on day 4 the mean arterial blood pressure in the block group infants was again significantly lower (34 [5.0] mm Hg; range 24–40) than in the controls (41 [8.2] mm Hg; range 31–67), $p = 0.019$.

PDA and end-diastolic block

PDA was open in 93% of cases in the block group and in 81% in the controls at the day 1 echocardiographic examination. On the following days, spontaneous closure of PDA occurred earlier in the infants without block. Right-to-left ductal shunting was not seen in any case, but bidirectional shunting was still present in three of the block cases and two controls on day four. PDA was surgically ligated in five (23%) infants in the block group and in two (6%) in the control group. Three (14%) infants in the block group and one (3%) in the control group received indomethacin to close PDA. PDA closed spontaneously in 11 (50%) infants in the group with block and in 27 (82%) in the control group, $p = 0.010$.

Predictors of intraventricular hemorrhages

Six infants (27%) in the block group and 10 (30 %) among the controls developed IVH, $p = 0.808$. Grade

I-II IVH occurred in 2 (9%) cases in the block group and in 6 (18%) controls, $p = 0.692$ and grade III-IV IVH in 4 (18%) *vs.* 4 (12 %) of the block and control cases, respectively; $p = 0.707$. One infant in the control group developed cystic PVL, whereas no such cases were found in the block group.

End-diastolic block in the intracranial arteries at least once during the first four days of life connected to a MAP ≤30 mm Hg was associated with an increased risk of IVH. Intraventricular hemorrhages developed in 46% of such cases, whereas all block cases with MAPs >30 mm Hg remained without IVH (Fig. 3), $p = 0.046$. Eighteen per cent of the 33 infants without block had MAPs ≤30 mm Hg, and 33% of them developed IVH. The remaining 82% of such infants had MAPs >30 mm Hg, and 30% of these infants developed IVH (Fig. 3); $p = 1.000$.

In logistic regression analysis, a significant PDA emerged as the only significant risk factor for IVH (odds ratio [OR] 20.0; 95% confidence interval [CI] 2.0–196.0, $p = 0.01$), whereas of the potential risk factors for the occurrence of end-diastolic block, none reached statistical significance in corresponding analysis. There was no difference in IVH relative to the methods of PDA closure in the study groups.

DISCUSSION

This study involved several methodological problems. A rather high percentage of eligible infants were not included in the evaluation, which might make for a patient selection bias. The findings in Doppler ultrasound examination assessed the blood flow in only two intracranial arteries, and the period of each observation was
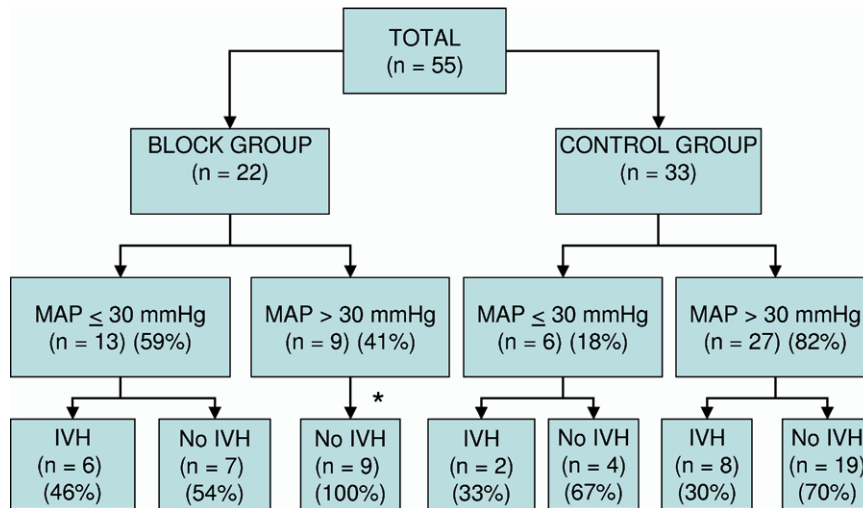
Fig. 3. MAP as a threshold value in the assessment of risk of IVH in the infants with and without an end-diastolic block in cerebral circulation. *$p < 0.05$, MAP >30 mm Hg and IVH *vs.* MAP <30 mm Hg and IVH in the block group; $p = 1.000$, in the control group.

only a few minutes. Moreover, the possibility cannot be excluded that a diastolic block might also have been present in the intracranial arteries in the control cases outside the moment of examinations. Thirdly, blood pressures were recorded nearest to the time of pulsed Doppler ultrasound, not continuously. Our findings should therefore be regarded as cross-sectional observations within a very narrow window of time.

The most important finding of the study was that during the first four days of life, the occurrence of an end-diastolic block in the flow of either the BA or ACA and MAP <30 mm Hg would appear to be correlated with the development of IVH in infants with birth weights <1000*g*. The cerebral circulation in preterm infants is a low-resistive system in which the diastolic blood flow is an important component (Lee et al. 1999). The infants with end-diastolic block had significantly lower mean MAPs than those without, which suggests that, with a decreasing MAP the RI in intracranial arteries increases until the diastolic flow becomes insufficient and a diastolic block in the cerebral circulation ensues.

The blood pressure level sufficient to avoid cerebral injuries in ELBW infants has yet to be determined. There are in practice two definitions of early hypotension. Earlier work has suggested that a MAP <30 mm Hg might increase the risk of IVH in infants of 26 to 30 weeks' (Miall-Allen et al. 1987) and below 31 weeks' (Hambleton and Wigglesworth 1976) gestation. According to more recent data, during the first 24 h after birth the lower limits of MAP for infants between 26 to 32 weeks of gestation were numerically similar to the gestational ages (Miall-Allen et al. 1989). A MAP in the

range of 23.7 to 39.3 mm Hg was adequate to maintain cerebral perfusion as measured by near infrared spectroscopy (NIRS) in infants of gestational ages between 24 to 30 weeks (Tyszczuk et al. 1998), and it has been recommended that the MAP in mm Hg should not fall below the gestational age of the infants in weeks (Joint Working Party of British Association of Perinatal Medicine and the Reseach Unit of the Royal College of Physicians 1992).

Autoregulation of the cerebral circulation is a protective mechanism against disturbances in arterial blood pressure. A recent study using NIRS demonstrated that cerebral autoregulation seemed to be functional in normotensive but not in hypotensive ELBW infants. The breakpoint lay at about 30 mm Hg in the cerebral blood flow–mean arterial pressure (CBF-MAP) auto-regulation curve (Munro et al. 2004). Our results likewise suggest that the presence of an end-diastolic block in BA or ACA connected with a MAP <30 mm Hg during the first days of life might be a sign of such failure, and of inadequate perfusion in vulnerable brain areas. CBFV did not differ between the groups, and duration and dosage of inotropics were similar in both. Further research is needed to establish whether an intervention to elevate MAP above 30 mm Hg by means of volume expanders or inotropics might be useful in an ELBW infant in such situations.

A trial study by Hall et al. (2005) showed an association between morphine therapy and hypotension among preterm infants, but further analysis found no increased risk of IVH or death. In our study, the median dosage of morphine was significantly higher in the infants with block on days 2 to 4 than in the controls. These

infants might have been more fragile and therefore needed higher dosages of morphine. Nonetheless we could speculate that a higher dosage of morphine might increase a tendency to hypotension, which might have a negative effect on the cerebral circulation and lead to the development of an end-diastolic block.

Studies of cerebral blood flow velocity in preterm infants with clinically significant PDA have suggested that there is in such situations an alteration in cerebral circulation and blood pressure. In a recent study, the incidence of IVH in preterm infants with a significant PDA was increased and all cases of severe IVH occurred in infants with RI increased above 0.8 in the cerebral circulation (Jim et al. 2005). In preterm infants with a symptomatic PDA, the diastolic cerebral blood flow has been seen to decrease because of a ductal stealing effect and cerebral blood flow normalized after closure of PDA (Perlman et al. 1981). A symptomatic PDA also had a significant effect on average blood pressure, and systemic blood pressure was enhanced by successful medical PDA closure in infants of 24 to 30 weeks of gestational age (Evans and Iyer 1993). Larger, early PDA shunts as well as lack of antenatal steroids have been significantly associated with IVH in preterm infants (Evans and Kluckow 1996). In our analysis, a significant PDA emerged as a risk factor for IVH, and PDA closed spontaneously significantly less often in infants with end-diastolic block than in those without, supporting these previous findings. In contrast, the presence of PDA and a retrograde diastolic flow in the intracranial arteries detected by Doppler ultrasound on at least one occasion during the first week of life has been found to be associated with the development of PVL in infants of gestational ages <31 weeks, whereas no association emerged between PDA and IVH (Shortland et al. 1990). The cases with symptomatic and asymptomatic PDA were not analyzed separately, which might explain the controversial result in this study. It can thus be suggested that a symptomatic PDA has significant effects on the cerebral and systemic circulation, and there seems to be a significant association between such a PDA and the development of IVH.

In a recent review, Evans (2006) hypothesized that the early preterm transitional period from the low-resistance intrauterine state to the high-resistance ex-utero state compounded by large PDA shunts could cause low systemic blood flow. We assume that in our study an end-diastolic block in the cerebral circulation on day 1 might be a sign of an insufficient adaptation to a higher vascular resistance state. The block group also had lower blood pressure than the controls, which might be explained by a lower blood flow in the systemic circulation. PDA was closed earlier and more often in the control group than in the block group, this being related to a delayed transitional period seen in the block group on days 2 to 4.

The present study suggests possible benefits of maintaining MAP over 30 mm Hg in ELBW infants with end-diastolic block in cerebral circulation and PDA during the first four days of life. Further research is needed to confirm whether this might prevent the block and be beneficial in the prevention of IVH in such infants. Also great caution must be stressed in the administration of vasoactive medication and medication for sedation and pain relief for ELBW infants, because these medications might have a significant influence on the cerebral circulation in critically ill ELBW infants.

REFERENCES

Bracci R, Perrone S, Buonocore G. The timing of neonatal brain damage. Biol Neonate 2006;90:145–155.

Chiu NC, Shen YE, Ho CS. Outcome in children with significantly abnormal cerebral blood flow detected by Doppler ultrasonography: Focus on the survivors. J Neuroimaging 2003;13:53–56.

Deeg KH, Rupprecht T. Pulsed Doppler sonographic measurement of normal values for the flow velocities in the intracranial arteries of healthy newborns. Pediatr Radiol 1989;19:71–78.

de Vries LS, Eken P, Dubowitz LMS. The spectrum of leucomalacia using cranial ultrasound. Behav Brain Res 1992;49:1–6.

Evans N, Iyer P. Change in blood pressure after treatment of patent ductus arteriosus with indomethacin. Arch Dis Child 1993;68:584–587.

Evans N, Kluckow M. Early ductal shunting and intraventricular haemorrhage in ventilated preterm infants. Arch Dis Child 1996;75:F183–F186.

Evans N. Which inotrope for which babies? Arch Dis Child Fetal Neonatal Ed 2006;91:F213–F220.

Grönlund JU, Korvenranta H, Kero P, Jalonen J, Välimäki IAT. Elevated arterial blood pressure is associated with periventricular haemorrhage. Eur J Pediatr 1994;153:836–841.

Hall RW, Kronsberg SS, Barton MS, Kaiser JR, Anand KJS. Morphine, hypotension and adverse outcome among preterm infants: Who's to blame? Secondary results from the NEOPAIN trial. Pediatrics 2005;115:1351–1359.

Hambleton G, Wigglesworth JS. Origin of intraventricular haemorrhage in the preterm infant. Arch Dis Child 1976;51:651–659.

Jim W-T, Chiu N-C, Chen M-R, Hung H-Y, Kao H-A, Hsu C-H, Chang J-H. Cerebral hemodynamic change and intraventricular hemorrhage in very low birth weight infants with patent ductus arteriosus. Ultrasound in Med Biol 2005;31:197–202.

Joint Working Party of British Association of Perinatal Medicine and the Research Unit of the Royal College of Physicians. Development of audit measures and guidelines for good practice in the management of neonatal respiratory distress syndrome. Arch Dis Child 1992;67:1221–1227.

Lee J, Rajadurai VS, Tan KW. Blood pressure standards for very-low-birthweight infants during the first day of life. Arch Dis Child Fetal Neonatal Ed 1999;81:F168–F170.

Lou H, Lassen NA, Friis-Hansen B. Impaired autoregulation of cerebral blood flow in the distressed newborn infant. J Pediatr 1979;94:118–121.

Miall-Allen VM, De Vries LS, Whitelaw AG. Mean arterial blood pressure and neonatal cerebral lesions. Arch Dis Child 1987;62:1068–1069.

Miall-Allen VM, De Vries LS, Dubowitz LMS, Whitelaw AGL. Blood pressure fluctuation and intraventricular hemorrhage in the preterm infant of less than 31 weeks gestation. Pediatrics 1989;83:657–661.

Milligan DWA. Failure of autoregulation and intraventricular haemorrhage in preterm infants. Lancet 1980;1:896–898.

Mires GJ, Patel NB, Forsyth JS, Howie PW. Neonatal cerebral Doppler flow velocity waveforms in the uncomplicated pre-term infants: Reference values. Early Hum Dev 1994;36:205–212.

Munro MJ, Walker AM, Barfield CP. Hypotensive extremely low birth weight infants have reduced cerebral blood flow. Pediatrics 2004; 114:1591–1596.

Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: A study of infants with birth weights less than 1,500 gm. J Pediatr 1978; 92:529–534.

Perlman JM, Hill A, Volpe JJ. The effect of patent ductus arteriosus on flow velocity in the anterior cerebral arteries: Ductal steal in the preterm newborn infant. J Pediatr 1981;99:767–771.

Perlman JM, McMenamin JB, Volpe JJ. Fluctuating cerebral blood-flow velocity in respiratory-distress syndrome. Relation to the development of intraventricular hemorrhage. N Engl J Med 1983;309: 204–209.

Seri I. Hydrocortisone is effective in treatment of vasopressor-resistant hypotension in very low birth weight neonates. J Pediatr 2006;149: 422–423.

Shortland DB, Gibson NA, Levene MI, Archer LNJ, Evans DH, Shaw DE. Patent ductus arteriosus and cerebral circulation in preterm infants. Dev Med Child Neurol 1990;132:386–393.

Tyszczuk L, Meek J, Elwell C, Wyatt JS. Cerebral blood flow is independent of mean arterial blood pressure in preterm infants undergoing intensive care. Pediatrics 1998;102:337–341.

ORIGINAL ARTICLE

Obstetric parameters and Doppler findings in cerebral circulation as predictors of 1 year neurodevelopmental outcome in asphyxiated infants

MK Julkunen[1,2], J Uotila[3], K Eriksson[4], M Janas[2], T Luukkaala[5] and O Tammela[1,2]

[1]*Pediatric Research Centre, University of Tampere, Tampere, Finland;* [2]*Department of Pediatrics, Tampere University Hospital, Tampere, Finland;* [3]*Department of Obstetrics and Gynecology, Tampere University Hospital, Tampere, Finland;* [4]*Department of Child Neurology, Tampere University Hospital, Tampere, Finland and* [5]*Science Center, Pirkanmaa Hospital District and Tampere School of Public Health, University of Tampere, Tampere, Finland*

Objective: To establish the association of cardiotocography (CTG) and other obstetric parameters with pulsed Doppler findings in cerebral arteries during the first day of life, and to compare the cerebral artery Doppler with other determinants of asphyxia in predicting 1-year neurological outcome in asphyxiated full-term infants.

Study Design: Cerebral blood flow velocity (CBFV) were measured from the anterior cerebral (ACA) and basilar (BA) arteries in 30 asphyxiated and 30 healthy-term infants using pulsed Doppler ultrasonography at approximately 24 h of age. CTG, cord artery pH, Apgar scores, biochemical asphyxia markers and symptoms of hypoxic-ischemic encephalopathy (HIE) were compared with the Doppler findings in respect of the ability to predict the outcome, defined by death or impaired neurological performance at 1 year of age.

Result: In all, 20% of the asphyxiated infants but none in the control group had increased peak systolic CBFVs (mean + 3 s.d.) in the ACA or BA. The sensitivity of increased systolic CBFV to predict abnormal outcome in the asphyxia group was 83%, specificity 95% and the sensitivity of the combination of HIE grade from 2 to 3 and increased systolic CBFV in the ACA or BA was 100% and specificity was 95%, respectively. Pathological CTG and low cord artery pH or low Apgar scores showed low predictive power.

Conclusion: Grade from 2 to 3 HIE and the systolic CBFV (mean + 3 s.d.) in the ACA or BA by Doppler ultrasound seemed to predict the outcome in asphyxiated infants at 1 year of age better than CTG, acid basement status, Apgar scores or asphyxia markers. If an increase of more than + 3 s.d. in the systolic CBFV does not occur within the first 24 h of life, a good 1-year neurological outcome may be anticipated.

Journal of Perinatology (2012) **32**, 631–638; doi:10.1038/jp.2011.151; published online 20 October 2011

Correspondence: Dr MK Julkunen, Department of Pediatrics, Tampere University Hospital, P.O. Box 2000, FIN-33521, Tampere, Finland.
E-mail: mia.julkunen@fimnet.fi

Introduction

The incidence of birth asphyxia has been estimated to be from 3 to 5/1000 live births in the Western world.[1,2] Half of these infants develop moderate or severe hypoxic-ischemic encephalopathy (HIE) symptoms[1–3] and 25% will develop neurological impairments.[4,5]

The degree of subsequent neurological problems has been found to be related to the severity of HIE.[5] This varies from grade 1, associated with normal outcome, to grade 3, associated with high mortality and severe neurodevelopmental abnormalities. The HIE grade 2 outcome varies widely from normal development to severe impairment.[3,6,7]

The prediction of long-term neurodevelopmental outcome has been difficult. The relationship between conventional clinical parameters (Apgar scores, cord blood pH and HIE symptoms) and outcome has been assessed in a number of studies.[6,8–10] The presence and severity of HIE by Sarnat criteria has proved a good determinant of neurological outcome,[6,8] whereas Apgar scores and cord blood pH have been poor predictors of the outcome.[8–10] Cardiotocography (CTG) is a screening tool used to assess the fetal condition during the labor and to identify the possibility of asphyxia. CTG has a high sensitivity but low specificity for fetal hypoxia or asphyxia.[11]

Increased cerebral blood flow velocity (CBFV) and resistive index reduced below 0.55 by pulsed Doppler ultrasonography, have been associated with poor outcome in birth asphyxia assessed at the age from 24 to 72 h.[12–14]

We have previously shown a strong association of increased systolic CBFV in the anterior cerebral (ACA) or in the basilar (BA) arteries at around 24 h of age with adverse neurological outcome at the age of 1 year, whereas reduced resistive index failed to show

association with poor outcome (submitted/unpublished data). In this study, we sought to analyse the obstetric parameters, biochemical asphyxia markers, HIE symptoms or their combinations, in predicting the neurological outcome at 1 year in full-term asphyxiated infants.

Patients and methods

Patients

This longitudinal prospective follow-up study was carried out in the neonatal intensive care unit in Tampere University Hospital. The Ethics Committee of Tampere University Hospital had approved the study protocol. Informed consent was obtained from the parents.

In all, 30 full-term (gestational age 37 weeks or more) infants with asphyxia and 30 healthy gestational age- and sex-matched infants, born between July 2000 and September 2003, were enrolled.

Infants were included in the asphyxia group when Apgar scores were 5 or less at the 5th minute of life and if they fulfilled at least one of following criteria for asphyxia: (1) signs of fetal distress such as abnormal CTG or meconium-stained amniotic fluid, (2) cord arterial pH < 7.10 or (3) symptoms of HIE within 48 h of life.

For each asphyxiated infant, a non-asphyxiated control infant was enrolled in the postnatal ward. Infants with major congenital malformations likely to affect Apgar scores were excluded. One asphyxiated infant with grade 2 HIE, in whom Kabuki syndrome was diagnosed according to the characteristic features at 2 years and 9 months of age, was included in the analysis.

HIE grading

The severity of HIE was graded (grade from 1 to 3) according to the modified Sarnat criteria.[6] Grading was based on clinical observations (level of consciousness, muscle tone, posture, reflexes, autonomic functions and seizures) during the first 48 h of life. Infants with grade 1 HIE had normal muscle tone and usually recovered well within 12 to 24 h without seizure activity. Infants with altered levels of consciousness and seizure activity from 12 to 24 h after the hypoxic-ischemic event developed grade from 2 to 3 HIE. Further deterioration to grade 3 HIE led to loss of reflex activity, flaccid muscle tone, respiratory failure and coma.

Pulsed Doppler ultrasound examination

The diastolic and systolic CBFV in the ACA and BA arteries was measured through the anterior fontanel, using a 7.5-MHz. transducer (PowerVision 6000, Toshiba, Nasu, Japan) at about 24 h of age (median 29.0; range from 19 to 40 h). The CBFV in the ACA represented the carotid and that in the BA the vertebrobasilar circulation. The CBFV in the ACA was measured in front of the third ventricle and that in the BA in front of the pons cerebri in sagittal section. Peak CBFVs (cm per s) in systole and diastole were measured from 1 to 3 cardiac cycles once a stable velocity

recording was obtained. There was no need to use the angle of insonation.

Obstetric parameters

Obstetric data were collected and analyzed by one obstetrician (JU) who was blinded to the Doppler findings in the newborn and the outcomes of the infants. Data from CTG were available in 29 (97%) asphyxia cases and in 28 (93%) controls, either during the whole labor or at admission before the decision to undertake the cesarean section. CTG was classified according to the FIGO criteria (1987)[15] as normal, non-reassuring, pathological or preterminal, taking into account the given time criteria for the occurrence of decreased variability (more than 40 or 60 min for non-reassuring or pathological, respectively). Prolonged bradycardia (< 110 beats per min) of more than 10 min duration was included in the criteria for pathological CTG. According to the available CTG changes and other obstetric data, the obstetrician further determined if the fetus had suffered from asphyxia episode lasting longer or less than 1 h. The Apgar scores were evaluated at the age of 1 and 5 min. The cord artery pH and base excess were measured according to the routine practice of the hospital.

Biochemical asphyxia markers

The concentrations of serum creatine kinase, serum neuron-specific enolase and plasma lactate were measured as biochemical asphyxia markers in the asphyxia group on day 1.

The following cut-off values were used for asphyxia: serum neuron-specific enolase used above 40 μg per l,[16,17] plasma lactate above 7.5 mmol per l[18] and serum creatine kinase above 1000 U per l.[19]

Neurological outcome

The overall neurological outcome of the infants was assessed clinically at 1 year of age by a pediatric neurologist (KE), who was blinded to the history of the infants. A modified Griffith's scale was used to structure the assessment and only raw scores were noted. Neurological outcome was classified as normal, motor disability or motor and mental disability. Abnormal outcome was defined as any neurological impairment or death.

Statistical analysis

Statistical analyses were performed using SPSS 11.0. and 17.0 for Windows (SPSS, Chicago, IL, USA). Differences between the infants with asphyxia and the healthy control infants, and between the normal and abnormal neurological outcome in the asphyxia group were tested. Mann-Whitney U was used for skew-distributed continuous variables and categorical variables were tested by Pearson's χ^2-square or Fisher's exact test if expected values were too small. A *P*-value below 0.05 was considered significant.

Sensitivity and specificity of predictive abnormal neurological outcome at 1 year of age or death were calculated for CTG

classification, Apgar scores, cord blood pH, biomarkers, HIE grading and Doppler findings, or their combinations in asphyxiated infants. Predictive values with 95% confidence intervals were calculated by confidence interval analysis program version 2.1.2 (University of Southampton, UK).

Results

Clinical characteristics in the study groups and in the asphyxia group with normal and abnormal outcome are seen in Table 1. In all, 3 asphyxiated infants were lost to the follow-up and 27 (90%) asphyxiated infants were followed up to 1 year of age. Altogether six (22%) had an abnormal outcome. One infant with grade 1 HIE and two with grade 2 HIE evinced delay in motor development at the age of 1 year, one infant with grade 2 HIE had spastic diplegia and 1 with grade 3 HIE had severe cognitive and motor developmental delay at 1 year of age. One infant with grade 3 HIE died at the age of 9 days.

In all, 9 (30%) out of 30 infants with asphyxia recovered well without the HIE symptoms. A total of 21/27 (78%)

Table 1 Clinical characteristics of the study groups

	Asphyxia group (n = 30)	Control group (n = 30)	P-value[a]	Asphyxia group outcome (n = 27)[b]		P-value[c]
				Normal (n = 21)	Abnormal (n = 6)[d]	
Birth weight (g), median (range)	3325 (2295–4240)	3676 (2920–4440)	0.004	3350 (2530–4240)	3420 (2295–3500)	0.579
SGA, (n) (%)[e]	1 (3.3)	0 (0)	0.317	0 (0)	1 (16.7)	0.222
Gestational age (week), median (range)	39 (37–42)	40 (37–42)	0.311	40 (37–42)	39 (37–40)	0.050
Male/female ratio	26/4	27/3	0.690	18/3	6/0	0.335
Intrapartum complications						
Shoulder dystocia, (n) (%)	1 (3.3)	0 (0)	0.317	1 (4.8)	0 (0)	0.593
Meconium staining of amniotic fluid, (n) (%)	16 (53.3)	5 (17.2)	0.004	11 (52.4)	5 (83.3)	0.182
Cord complications, (n) (%)	6 (20)	0 (0)	0.010	3 (14.3)	1 (16.7)	0.887
Delivery route			< 0.001			1.000
Vaginal, (n) (%)	13 (43.3)	29 (96.7)		9 (42.9)	3 (50.0)	
Cesarean section, (n) (%)	17 (56.7)	1 (3.3)		12 (57.1)	3 (50.0)	
CTG criteria[f]			0.001			1.000
Normal, (n) (%)	3 (10.3)	14 (50)		2 (10.0)	0 (0)	
Non-reassuring, (n) (%)	10 (34.5)	14 (50)		8 (40.0)	2 (33.3)	
Pathological, (n) (%)	16 (55.2)	0 (0)		10 (50.0)	4 (66.7)	
Bradycardia >10 min in the CTG, (n) (%)[g]	15 (53.6)	0 (0)	< 0.001	10 (47.6)	4 (66.7)	0.648
Asphyxia episodes lasting						0.644
Less than 1 h, (n) (%)	19 (65.5)			13 (65.0)	3 (50.0)	
More than 1 h, (n) (%)	10 (34.5)			7 (35.0)	3 (50.0)	
HIE						0.025
No HIE, (n) (%)	9 (30.0)			6 (28.6)	1(16.7)	
Grade 1, (n) (%)	11 (36.7)			10 (47.6)	0 (0))	
Grade 2–3, (n) (%)	10 (33.3)			5 (23.8)	5 (83.3)	
Apgar scores at 5 min, median (range)	4 (1–5)	9 (8–10)	< 0.001	4 (2–5)	4 (1–5)	0.432
Cord arterial pH 7.05 or less, (n) (%)[h]	8 (27.6)	0 (0)	0.012	4 (20.0)	3 (50.0)	0.293
Cord arterial base deficit 10 or more, (n) (%)[i]	8 (32.0)	1 (3.8)	0.021	5 (27.8)	2 (50.0)	0.565

Abbreviations: CTG, cardiotocography; HIE, hypoxic-ischemic encephalopathy; SGA, small for gestational age.
[a]Asphyxia group vs control group.
[b]Three infants (two HIE 0 and one HIE 1) in the asphyxia group were lost to the follow-up.
[c]Normal vs abnormal outcome in the asphyxia group.
[d]Abnormal outcome was defined by pediatric neurologist using a modified Griffth's scale at 1 year of age or death.
[e]SGA: small for gestational age; < 2500 g in term infant.
[f]One CTG in the asphyxia group and two in the control group were missing.
[g]Three missing data in the asphyxia group.
[h]One pH was not available in the asphyxia group and two in the control group.
[i]Five cord blood base deficits were not available in both the study groups.

follow-up-asphyxiated infants had normal outcome at 1 year of age, 10 (23.8%) with HIE grade 1 and 6 (47.6%) with HIE grade from 2 to 3 and 6 (28.6%) with no HIE symptoms. None of the control infants evinced neurological symptoms at the postnatal ward. A total of 22 (73%) of the 30 controls were followed up to 1 year of age and one of them had delayed motor development at the age of 1 year.

The mean raw scores for Griffith's scale at 1 year of age were 124.4 in those with abnormal neurological outcome and 146.7 for those with normal neurological outcome. However, the Griffith's scale was used merely as a supportive tool for the neurological assessment, and therefore the clinical significance of the difference in these raw scores is minimal.

Pathological CTG recordings were concentrated in the asphyxia group, while less severe, non-reassuring CTG tracings were equally distributed in both the groups. The criteria for totally normal CTG were fulfilled more frequently in the controls than among the asphyxia group. However, the classification of CTG had no association with the neurological outcome within the asphyxia group. Likewise, low Apgar scores, cord blood pH and base excess were more frequent in the asphyxia group, but their distribution did not differ according to the outcome. Neither was the duration of asphyxia episodes related to the outcome (Table 1).

Plasma lactate was more than 7.5 mmol per l in four (19%) asphyxiated cases with normal outcome and in one case (17%) with abnormal outcome measured at around 24 h, $P = 1000$; serum NSE more than 40 µg per l in eight cases (38%) with normal outcome and four (37%) with abnormal outcome,

respectively, $P = 0.357$; serum CK>1000 U per l in 11 (55%) asphyxiated cases with normal outcome and in 3 cases (50%) with abnormal outcome, $P = 1.000$.

Peak systolic CBFV over 50.0 cm per s (mean + 3 s.d.) in the ACA or over 55 cm per s (mean + 3 s.d.) in the BA around 24 h of age (from 27 to 37 h of age) was found in 6 (20%) of 30 asphyxiated infants, but none in the control group. Five of these six asphyxiated infants with increased peak systolic CBFV had abnormal outcome at 1 year of age or died in the neonatal period. Peak diastolic CBFV over 20.0 cm per s (mean + 3 s.d.) in the ACA or in the BA around 24 h of age was measured in five (18%) of the asphyxiated infants, four (67%) of them having abnormal outcome. The CBFVs measured within the first 12 h of life did not differ significantly between the groups. The pathologically increased CBFVs normalized from 3 to 11 days of age, except in the infant with grade 3 HIE who died at 9 days of age. An imagine of high systolic CBFV in the BA by Doppler ultrasound in an aspyhxiated infant with severe HIE is seen in Figure 1.

Clinical parameters among the asphyxiated cases with increased systolic CBFVs in the ACA or BA at around 24 h of age and adverse neurological outcome are shown in Table 2.

The distributions of CTG findings in the asphyxiated infants as assessed by the severity of HIE, increased systolic CBFVs around 24 h of age and abnormal outcome at 1 year of age are seen in Table 3.

For prediction of abnormal outcome at 1 year of age or death in the asphyxia group, increased systolic CBFV (mean + 3 s.d.) in the ACA or BA at around 24 h of age was the best independent predictor
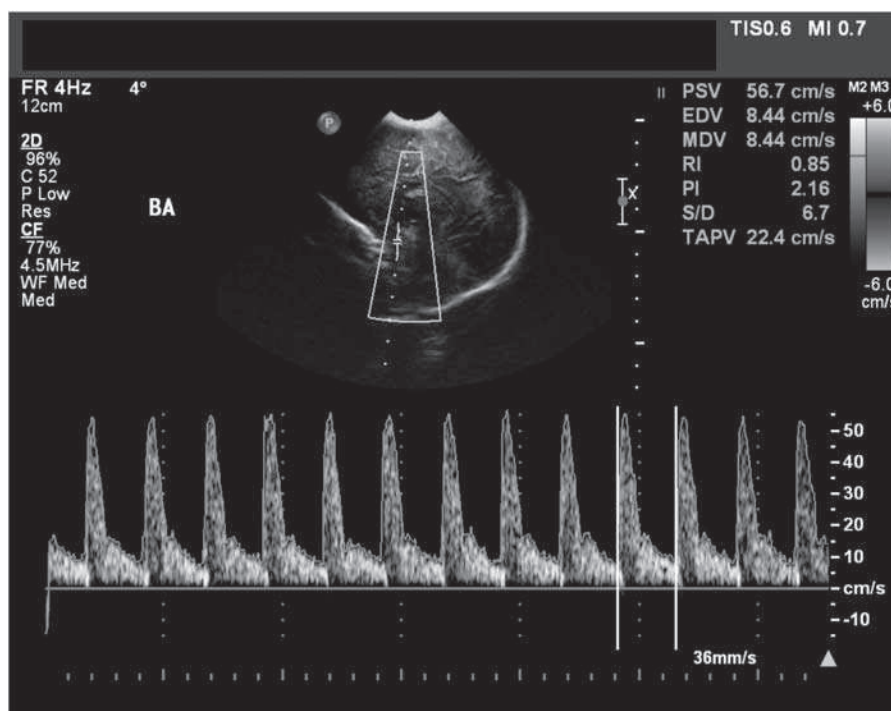

Figure 1 The high systolic CBFV in the BA by Doppler ultrasound in an asphyxiated infant with severe HIE.

Table 2 Clinical parameters among asphyxiated cases with increased sCBFV in the ACA or BA around 24 h or adverse outcome at 1 year of age

Cases	CTG	Asphyxia episodes lasting	Cord artery pH	Cord arterial base deficit	Apgar at 5 min	HIE grade	sCBFV in the ACA at 24 h (cm per s)	sCBFV in the BA at 24 h (cm per s)	s-CK U per l at 24 h	P-lactate mmol per l at 24 h	s-NSE μg per l at 24 h	Outcome at 1 year of age
1	Non-reassuring	Less than 1 h	7.21	8.00	5	2	34.2	64.4	2040	10.7	63	Normal
2	Non-reassuring	Less than 1 h	7.18		5	2	74.2	47.6	410	5.5	45	Motor disability
3	Pathological	Less than 1 h	7.21	10.00	2	2	63.6	51.0	645	8.0	38	Motor disability
4	Pathological	More than 1 h	7.04	6.00	5	2	26.0	65.8	557	2.6	55	Motor disability
5	Pathological	More than 1 h	6.96	13.00	3	3	45.1	78.7	1727	7.0	135	Motor disability
6	Pathological	More than 1 h	6.61		1	3	66.2	56.6	1130	3.6	40	Death
7	Non-reassuring	Less than 1 h	7.13	9.3	5		39.4	46.5	8725	2.6	83	Motor disability

Abbreviations: ACA, anterior cerebral artery; BA, basilar artery; CTG, cardiotocography; HIE, hypoxic-ischemic encephalopathy; S-CK, serum creatine kinase; S-NSE, serum neuron-specific enolase.

Table 3 CTG patterns, HIE, Doppler findings and outcome in the asphyxia group

	CTG[a]				Asphyxia episodes lasting	
	Normal	Non-reassuring	Pathological	Bradycardia >10 min	Less than 1 h	More than 1 h
No HIE (n=9)	1 (12.5%)	4 (50.0%)	3 (37.5%)	2 (22.2%)	5 (62.5%)	3 (37.5%)
HIE grade 1 (n=11)	1 (9.1%)	4 (36.4%)	6 (54.6%)	6 (54.5%)	10 (90.9%)	1 (9.1%)
HIE grade 2–3 (n=10)	1 (10.0%)	2 (20.0%)	7 (70.0%)	7 (70%)	4 (40.0%)	6 (60.0%)
sCBFV (mean+3 s.d.) At 24 h (n=6)	0 (0%)	2 (33.3%)	4 (66.7%)	4 (66.7%)	3 (50.0%)	3 (50.0%)
Abnormal outcome At 1 year of age[b] (n=6)	0 (0%)	2 (33.3%)	4 (66.7%)	4 (66.7%)	3 (50%)	3 (50%)

Abbreviations: CTG, cardiotocography; HIE, hypoxic-ischemic encephalopathy.
[a]One CTG was missing.
[b]Abnormal outcome was defined by pediatric neurologist using a modified Griffith's scale at 1 year of age or death.

(Table 4). It found that 5/6 cases with adverse neurological outcome, as did also the presence of HIE grade from 2 to 3, but increased systolic CBFV showed much higher specificity than the HIE grade from 2 to 3. The combination of HIE stage from 2 to 3 and increased systolic CBFV found all cases with adverse outcome. The predictive values of pathological CTG, pH 7.05 or less, the Apgar scores below 4 to 5 min and biochemical asphyxia markers were poor. The absence of pathological increase in systolic CBFVs within the first 24 h of life was the best independent predictor of the normal neurological outcome, even in presence of HIE symptoms (Table 5).

Discussion

The main finding in this study was that increased systolic CBFVs in the ACA or in the BA around 24 h of life seemed to be associated with poor 1 year outcome in the asphyxia group more clearly than the CTG, acid basement status, Apgar scores or biochemical asphyxia markers, or HIE. The combination of moderate or severe HIE (grade from 2 to 3) and increased systolic CBFV around 24 h of age predicted abnormal outcome even better. On the other hand, if no pathological increase in systolic CBFVs is observed within the first 27 to 37 h of life, the 1-year outcome seems to be

Table 4 Predictive value of CTG patterns, cord arterial pH, Apgar scores, HIE, Doppler findings and asphyxia markers to predict poor outcome of asphyxiated infants[a]

	Sensitivity% (95% CI)	Specificity% (95% CI)
Severely pathological CTG, ($n = 26$)	67 (30–90)	50 (30–70)
Bradycardia >10 min in the CTG, ($n = 25$)	67 (30–90)	53 (30–70)
Cord arterial pH < 7.05, ($n = 26$)	50 (19–81)	75 (53–89)
Apgar < 4 at 5 min, ($n = 27$)	50 (19–81)	86 (65–95)
HIE grade 2–3, ($n = 27$)	83 (44–97)	76 (55–89)
Apgar <4 at 5 min and HIE 2–3, ($n = 27$)	33 (10–70)	95 (77–95)
Systolic CBFV >mean 3 s.d. in the ACA or BA around 24 h, ($n = 27$)	83 (44–97)	95 (77–99)
HIE grade 2–3 and systolic CBFV >mean 3 s.d. in the ACA or BA around 24 h, ($n = 27$)	100 (57–100)	95 (78–99)
s-NSE at 24 h (cut-off value >40 μg per l), ($n = 26$)	67 (30–90)	70 (48–86)
P-lactate at 24 h (cut-off value >7.5 mmol per l), ($n = 21$)	20 (4–62)	71 (50–90)
s-CK at 24 h (cut-off value >1000 U per l), ($n = 26$)	50 (19–81)	45 (26–66)
Pathological CTG and cord pH <7.05 and Apgar <4 at 5 min and systolic CBFV>mean+3 s.d. in the ACA or BA at 24 h and HIE 2–3, ($n = 25$)	33 (10–70)	95 (75–99)
Golden standard (abnormal outcome by modified of Griffith's scale	100	100

Abbreviations: ACA, anterior cerebral artery; BA, basilar artery; CTG, cardiotocography; CI, confidence interval; HIE, hypoxic-ischemic encephalopathy; S-CK, serum creatine kinase; S-NSE, serum neuron-specific enolase.
[a]In all, 3 of the 30 asphyxiated infants were lost to follow-up.

Table 5 Apgar score, cord arterial pH, CTG patterns, HIE and Doppler findings predicting normal outcome in the asphyxia group[a]

	Sensitivity% (95% CI)	Specificity% (95% CI)
Apgar 4 or more at 5 min, ($n = 27$)	52 (32–72)	50 (19–81)
Cord arterial pH >7.10, ($n = 26$)	70 (48–86)	50 (19–81)
CTG normal or non-reasurring, ($n = 26$)	50 (30–70)	67 (30–90)
No HIE symptoms, ($n = 27$)	29 (14–50)	83 (44–97)
HIE grade 0–1, ($n = 27$)	76 (55–89)	83 (44–97)
Systolic CBFV < mean+3 s.d. in the ACA or BA around 24 h, ($n = 27$)	95 (77–99)	83 (44–97)
Systolic CBFV < mean+3 s.d. in the ACA or BA around 24 h and HIE 0–1, ($n = 27$)	76 (55–89)	83 (44–97)

Abbreviations: ACA, anterior cerebral artery; BA, basilar artery; CTG, cardiotocography; CI, confidence interval; HIE, hypoxic-ischemic encephalopathy.
[a]Three of the 30 asphyxiated infants were lost to follow-up.

good. Only one out of six (17%) asphyxiated infants with increased CBFV had normal outcome at 1 year of age, while 5 out of 10 newborns with HIE grade from 2 to 3 (50%) appeared normal at age of 1 year after the asphyxia event. Assessment of the infants' CBFVs between 27 and 37 h of age after birth asphyxia thus seems to provide useful information for discussion with the parents.

CBFVs were measured in two cerebral arteries (ACA and BA) by pulsed Doppler ultrasonography. The brain is supplied by four large arteries, two internal carotid arteries and two vertebral arteries. The internal carotid artery divides into two large branches, the ACA and the middle cerebral artery. The two vertebral arteries combine into the unpaired BA artery. The Doppler findings in the ACA and in the BA thus represented both the carotid and vertebrobasilar circulation and yielded reliable information on the cerebral circulation.

Hyperperfusion in the cerebral circulation has been held to be a sign of permanent brain damage in the asphyxiated infants in several Doppler studies.[12,20,21] Increased CBFV in the cerebral circulation by the age of 12 h in infants with severe HIE or poor outcome has been reported.[20,22] Increased CBFV from 24 to 72 h has been taken as a sign of permanent brain damage with poor prognosis in asphyxiated infants,[12–14] and the present results confirm the findings concerning increased CBFVs.

The criteria for asphyxia are variable in different studies. Even moderate degree of acidemia, pH from 7.10 to 7.15, is associated with an increased risk of low Apgar scores.[23] In a recent General Movements study the cord arterial pH<7.20 was one inclusion criteria for the asphyxia group.[24] The International Cerebral Palsy Task Force (1999)[25] have suggested that intrauterine hypoxia, severe enough to be the cause of HIE and/or cerebral palsy should meet following criteria: Apgar scores from 0 to 6 longer than 5 min, cord arterial pH below 7.00 and base deficit above 12 mmol per l, sentinel event or abrupt fetal heart rate change, moderate or severe neonatal encephalopathy of early onset, affection of other

organ systems and early imaging evidence of acute cerebral abnormality. The ACOG committee (2002)[26] criteria for acute perinatal asphyxia were quite similar, but Apgar scores were lower, 3 or below at 5 min. The ACOG committee criteria for asphyxia are fullfiled quite rarely, only in severely asphyxiated infants. Criteria for birth asphyxia similar to ours have been previously used in the literature. Thus, we chose less strict inclusion criteria, compared with the ACOG criteria.

The Apgar score is a useful basis for assessing the general condition of infants at birth, but it has a limitation in identifying birth asphyxia and predicting neurological outcome.[27] However, infants with low Apgar score from 0 to 3 at 5 min and symptoms of neonatal encephalopathy had an increased risk of subsequent neurodevelopmental impairments and learning difficulties; whereas a similar low Apgar score without neonatal symptoms had no association with a high risk of later impairments compared with the healthy control infants.[28] In another study, the specificity of the combination of the presence of HIE, a base deficit over 20 mmol per l, and an Apgar score below 4 at the age of 5 min for death or adverse outcome was 100% and the sensitivity was 67%.[29] In our study, the specificity of combination of low 5 min Apgar scores and the presence of HIE grade from 2 to 3 to predict adverse outcome was high, but the sensitivity was poor, only 33%.

According to one study, the infants with neonatal encephalopathy had significantly more abnormal (89%) CTG traces (absent accelerations and low variability), but 52% of control CTG traces were also abnormal.[30] In our study, all of the asphyxiated cases with a poor neurological outcome had abnormal CTG, but only half of them had clear pathological recording, while the other half had a CTG of only moderate, 'non-reassuring' classification. At the same time, half of the asphyxiated cases with normal outcome had pathological CTG, and also half of the non-asphyxiated control cases had abnormal CTG. Thus, our findings are consistent with the earlier studies suggesting high false-positive rate of CTG, making it a poor tool in predicting the outcome of asphyxiated children.[7,11,29]

Several serum, urine or cerebrospinal fluid biomarkers have been examined as markers of outcome in HIE, but none has been studied extensively enough to warrant routine clinical use.[31] In this study, there was likewise no correlation between the blood biomarkers according to the outcome in the asphyxiated.

Conclusion

The values of pathological CTG, Apgar scores, acid basement status and biochemical asphyxia markers in predicting the 1-year outcome of the asphyxiated infants were poor, showing either low sensitivity or low specificity. Severe HIE grade from 2 to 3 was sensitive in predicting adverse neurological outcome, but as the false positive rate was high, the specificity was only 76%. Increased systolic CBFV was superior in predicting adverse outcome, with a

high sensitivity and high specificity. Thus, absence of a pathological increase in systolic CBFV after birth asphyxia might be a useful predictor of favorable 1-year neurological outcome.

Conflict of interest

The authors declare no conflict of interest.

References

1 Thornberg E, Thiringer K, Odeback A, Milsom I. Birth asphyxia: incidence, clinical course and outcome in a Swedish population. *Acta Paediatr* 1995; **84**: 927–932.

2 Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, Davis P. Cooling for newborn with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev* 2007; (4): CD003311.

3 Levene MI, Sands C, Grindulis H, Moore JR. Comparison of two methods of predicting outcome in perinatal asphyxia. *Lancet* 1986; **1**: 67–69.

4 Freeman JM, Nelson KB. Intrapartum asphyxia and cerebral palsy. *Pediatrics* 1988; **12**: 240–249.

5 Hagberg B, Hagberg G, Beckung E, Uvebrant P. Changing panorama of cerebral palsy in Sweden: VIII. Prevalence and origin in the birth year period 1991–1994. *Acta Paediatr* 2001; **90**: 271–277.

6 Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. *Arch Neurol* 1976; **36**: 308–313.

7 Finer NN, Robertson CM, Richards RT, Pinnell LE, Peters KL. Hypoxic-ischemic encephalopathy in term infants: perinatal factors and outcome. *J Pediatr* 1981; **98**: 112–117.

8 Finer NN, Robertson CM, Peters KL, Coward JH. Factors affecting in hypoxic-ischemic encephalopathy in term infants. *Am J Dis Child* 1983; **137**: 21–25.

9 Yudkin PL, Johnson A, Clover LM, Murphy KW. Clustering of perinatal markers of birth asphyxia and outcome at age five years. *Br J Obstet Gynaecol* 1994; **101**: 774–781.

10 Nelson KB, Grether JK. Potentially asphyxiating conditions and spastic cerebral palsy in infants of normal birth weight. *Am J Obstet Gynecol* 1998; **179**: 507–513.

11 Nelson KB, Dambrosia JM, Ting TY, Grether JK. Uncertain value of electronic fetal monitoring in predicting cerebral palsy. *N Engl J Med* 1996; **334**: 613–618.

12 Levene MI, Fenton AC, Evans DH, Archer LNJ, Shortland DB, Gibson NA. Severe birth asphyxia and abnormal cerebral blood-flow velocity. *Dev Med Child Neurol* 1989; **31**: 427–434.

13 Deeg KH, Rupprecht Th, Zeilinger G. Doppler sonographic classification of brain edema in infants. *Pediatr Radiol* 1990; **20**: 509–514.

14 Stark JE, Seibert JJ. Cerebral artery Doppler ultrasonography for prediction of outcome after perinatal asphyxia. *J Ultrasound Med* 1994; **13**: 595–600.

15 FIGO. Guidelines for the use of fetal monitoring. *Int J Gynaecol Obstet* 1987; **12**: 159–167.

16 Nagdyman N, Grimmer I, Scholz T, Muller C, Obladen M. Predictive value of brain-specific proteins in serum for neurodevelopmental outcome after birth asphyxia. *Pediatr Res* 2003; **54**: 270–275.

17 Celtik C, Acunas B, Öner N, Pala Ö. Neuron-specific enolase as a marker of the severity and outcome of hypoxic ishemic encephalopathy. *Brain Dev* 2004; **26**: 398–402.

18 Shah S, Tracy M, Smyth J. Postnatal lactate as an early predictor of short-term outcome after intrapartum asphyxia. *J Perinatol* 2004; **24**: 16–20.

19 Omokhodion SI, Losekoot TG, Jaiyesimi F. Serum creatine kinase and creatine kinase-MB isoenzyme activities in perinatally asphyxiated newborns. *Eur Heart J* 1991; **12**: 980–984.

20 Ilves P, Lintrop M, Metsvaht T, Vaher U, Talvik T. Cerebral blood-flow velocities in predicting outcome of asphyxiated newborn infants. *Acta Paediatr* 2004; **36**: 308–313.

21 Ilves P, Lintrop M, Talvik T, Muug K, Maipuu L, Metsvaht T. Low cerebral blood flow velocity and head circumference in infants with severe hypoxic ischaemic encephalopathy and poor outcome of asphyxiated infants. *Acta Paediatr* 2009; **98**: 459–465.

22 Ilves P, Talvik R, Talvik T. Changes in Doppler ultrasonography in asphyxiated term infants with hypoxic-ischaemic encephalopathy. *Acta Paediatr* 1998; **87**: 680–684.

23 Goldaber KG, Gilstrap 3rd LC, Leveno KJ, Dax JS, McIntire DD. Pathological fetal acidemia. *Obstet Gynecol* 1991; **78**: 1103–1107.

24 van Iersel PA, Bakker SC, Jonker AJ, Hadders-Algra M. Quality of general movements in term infants with asphyxia. *Early Hum Dev* 2009; **85**: 7–12.

25 MacLennan A. A template for defining a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. *BMJ* 1999; **319**: 1054–1059.

26 American College of Obstetricians and Gynegologists, American Academy of Pediatrics. *Neonatal encephalopathy and cerebral palsy. Defining of pathogenesis and pathophysiology*. Washington (DC): American College of Obstetrics and Gynecology, 2003.

27 ACOG Committee on Obstetric Practise, American Academy of Pediatrics Committee on Fetus and Newborn. Use and abuse of the Apgar score. *Pediatrics* 1996; **98**: 141–142.

28 Moster D, Lie RT, Markestad T. Joint association of Apgar scores and early neonatal symptoms with minor disabilities at school age. *Arch Dis Child Fetal Neonatal Ed* 2002; **86**: F16–F21.

29 Toh VC. Early predictors of adverse outcome in term infants with post-asphyxial hypoxic ischaemic encephalopathy. *Acta Paediatr* 2000; **89**: 343–347.

30 Spencer JA, Badawi N, Burton P, Keogh J, Pemberton P, Stanley F. The intrapartum CTG prior to neonatal encephalopathy at term: a case-control study. *Br J Obstet Gynaecol* 1997; **104**: 25–28.

31 Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. *Pediatr Neurol* 2009; **40**: 215–226.
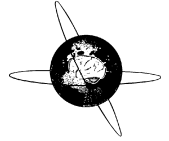
EEG, evoked potentials and pulsed Doppler in asphyxiated term infants

Mia K. Julkunen [a,b,*], Sari-Leena Himanen [c,d], Kai Eriksson [a,e], Martti Janas [b],
Tiina Luukkaala [f], Outi Tammela [a,b]

[a] Pediatric Research Centre, University of Tampere, Tampere, Finland
[b] Department of Pediatrics, Tampere University Hospital, Tampere, Finland
[c] Department of Clinical Neurophysiology, Tampere University Hospital, Tampere, Finland
[d] Faculty of Medicine, University of Tampere, Tampere, Finland
[e] Pediatric Neurology Unit, Tampere University Hospital, Tampere, Finland
[f] Science Center, Pirkanmaa Hospital District, Tampere School of Public Health, University of Tampere, Tampere, Finland

A R T I C L E   I N F O

H I G H L I G H T S

- Normal EEG combined with systolic cerebral blood flow velocity (<mean 3 SD) measured by Doppler ultrasonography at about 24 h can be valuable in the prediction of normal 1-year outcome among asphyxiated term infants.
- The SEP might be a useful examination in identification of asphyxiated infants with good outcome.
- The predictive values of BAEPs and VEPs were poor in predicting 1-year outcome in asphyxiated term infants.

A B S T R A C T

Objective: To evaluate electroencephalograms (EEG), evoked potentials (EPs) and Doppler findings in the cerebral arteries as predictors of a 1-year outcome in asphyxiated newborn infants.
Methods: EEG and EPs (brain stem auditory (BAEP), somatosensory (SEP), visual (VEP) evoked potentials) were assessed in 30 asphyxiated and 30 healthy term infants during the first days (range 1–8). Cerebral blood flow velocities (CBFV) were measured from the cerebral arteries using pulsed Doppler at 24 h of age. EEG, EPs, Doppler findings, symptoms of hypoxic ischemic encephalopathy (HIE) and their combination were evaluated in predicting a 1-year outcome.
Results: An abnormal EEG background predicted poor outcome in the asphyxia group with a sensitivity of 67% and 81% specificity, and an abnormal SEP with 75% and 79%, respectively. Combining increased systolic CBFV (mean + 3SD) with abnormal EEG or SEP improved the specificity, but not the sensitivity. The predictive values of abnormal BAEP and VEP were poor. Normal EEG and SEP predicted good outcome in the asphyxia group with sensitivities from 79% to 81%. The combination of normal EEG, normal SEP and systolic CBFV < 3SD predicted good outcome with a sensitivity of 74% and 100% specificity.
Conclusions: Combining abnormal EEG or EPs findings with increased systolic CBFV did not improve prediction of a poor 1-year outcome of asphyxiated infants. Normal EEG and normal SEP combined with systolic CBFV < 3SD at about 24 h can be valuable in the prediction of normal 1-year outcome.
Significance: Combining systolic CBFV at 24 h with EEG and SEP examinations can be of use in the prediction of normal 1-year outcome among asphyxiated infants.

1. Introduction

Perinatal asphyxia is an important cause of brain injury. It may lead to hypoxic-ischemic encephalopathy (HIE), which is the most common cause of encephalopathy in full-term infants

* Corresponding author at: Department of Pediatrics, Tampere University Hospital, P.O. Box 2000, FIN-33521 Tampere, Finland. Tel.: +358 3 311 64057; fax: +358 3 311 64394.
E-mail address: mia.julkunen@fimnet.fi (M.K. Julkunen).

(Pierrat et al., 2005). The incidence of HIE has been estimated to be from 1 to 6 /1000 live term births (Volpe, 2008).

The presence and severity of HIE by Sarnat criteria has been proven to be a good determinant of neurological outcome (Sarnat and Sarnat, 1976; Finer et al., 1983). The degree of subsequent neurological problems has been found to be related to the severity of HIE (Hagberg et al., 2001), which varies from mild, associated with normal outcome, to severe, associated with high mortality and severe neurodevelopmental problems. The outcome of moderate HIE varies widely from normal development to severe impairment (Sarnat and Sarnat, 1976; Finer et al., 1981; Levene et al., 1986).

Prediction of the long-term outcome of asphyxiated term infants shortly after birth is challenging. The prognostic value of electroencephalography (EEG) in full-term infants with HIE has been well documented (Selton and Andre, 1997; El-Ayouty et al., 2007; Murray et al., 2009). Severely abnormal background EEG is generally associated with adverse outcome after an HIE event (Sinclair et al., 1999; El-Ayouty et al., 2007; Leijser et al., 2007). On the other hand, normal outcome is seen in infants with normal or mildly abnormal EEG patterns (Wertheim et al., 1994; Zeinstra et al., 2001; El-Ayouty et al., 2007; Leijser et al., 2007).

Evoked potentials (EPs) have been assessed in identifying the risk of adverse outcome in asphyxiated infants. Bilateral loss of cortical somatosensory evoked potentials (SEP) seems to be a good predictor of poor outcome in acute encephalopathy in adults and older children (Goodwin et al., 1991; Robinson et al., 2003; Wolrab et al., 2001). A normal SEP response at 1 week of age is generally a sign of normal outcome, whereas an abnormal SEP predicts an adverse outcome in ~90% of asphyxiated infants (Gibson et al., 1992; Taylor et al., 1992). Brain stem auditory evoked potential (BAEP) or visual evoked potential (VEP) have been poorer predictors among asphyxiated infants (Jiang et al., 2008; Suppiej et al., 2010).

Pulsed Doppler ultrasound is a non-invasive means of evaluating changes in cerebral blood circulation. Increased cerebral blood flow velocity (CBFV) and a resistive index reduced below 0.55 upon pulsed Doppler examination have been associated with poor outcome in birth asphyxia assessed at ages from 24 to 72 h (Deeg et al., 1990; Levene et al., 1989; Stark and Seibert, 1994).

The aim of the present study was to assess EEG, EPs, symptoms of HIE and compared and combined with Doppler findings in cerebral circulation in predicting the 1-year neurodevelopmental outcome of the term asphyxiated infants. To our knowledge, the predictive values of EEG, BAEP, SEP, VEP with and without Doppler assessments in term asphyxiated infants and healthy controls have not previously been compared. Our hypothesis was that the predictive value of neurophysiologic findings is improved by combining them with Doppler findings.

2. Patients and methods

2.1. Patients

This longitudinal prospective follow-up study was carried out in the neonatal intensive care unit in Tampere University Hospital. The Ethics Committee of Tampere University Hospital had approved the study protocol. Informed consent was obtained from the parents in each case.

Data on the study subjects have been explained in detail in our previous publication (Julkunen et al., 2012). In brief: 30 full-term (gestational age 37 weeks or more) infants with asphyxia (Apgar scores 5 or less at the 5th minute of life and at least one of the following criteria for asphyxia: (1) signs of fetal distress such as abnormal cardiotocography (CTG) or meconium-stained amniotic fluid, (2) cord arterial pH < 7.10 or (3) symptoms of HIE within 48 h of life), and 30 healthy gestational age- and sex-matched in-

fants were enrolled. Systolic CBFV > mean 3SD in the cerebral arteries at around 24 h of age was found in 6 (20%) of the 30 asphyxiated infants, but none in the control group. 5 (83%) of these six asphyxiated infants with increased peak systolic CBFV had abnormal outcome or died in the neonatal period. The overall neurological outcome was assessed clinically at 1 year of age by pediatric neurologist who was blinded to the history of the infants. Griffith's Developmental Scales (Alin-Åkerman and Nordberg, 1991) were used to structure the assessment but only raw scores were recorded. According to these raw scores and neuropediatric clinical evaluation the neurological outcome at the age of 1 year was classified as normal, motor disability or motor and mental disability. Abnormal outcome was defined as any neurological impairment or death. Altogether 6 (22%) asphyxiated infants had an abnormal outcome, defined as any neurological impairment or death. One infant died at the age of 9 days. A total of 21/27 (78%) follow-up asphyxiated infants had normal outcome. One control infant had delayed motor development. In asphyxiated infants the sensitivity of increased systolic CBFV (> mean 3SD) at around 24 h of age was 83% and specificity 95% in predicting adverse outcome. The combination of increased systolic CBFV and HIE grade from 2 to 3 predicted abnormal outcome among asphyxiated infants even better, sensitivity being 100% and specificity 95% (Julkunen et al., 2012).

2.2. HIE grading

The severity of HIE was graded (grade 1–3) according to modified Sarnat criteria (Sarnat and Sarnat, 1976). Grading was based on clinical observations (level of consciousness, muscle tone, posture, reflexes, autonomic functions and seizures) during the first 48 h of life. Infants with grade 1 HIE had normal muscle tone and usually recovered well within 12–24 h without seizure activity. Infants with altered levels of consciousness and seizure activity from 12 to 24 h after the hypoxic-ischemic event developed grade from 2 to 3 HIE. Further deterioration to grade 3 HIE led to loss of reflex activity, flaccid muscle tone, respiratory failure and coma.

2.3. Pulsed Doppler ultrasound examination

The diastolic and systolic CBFV in the ACA and BA arteries was measured through the anterior fontanel using a 7.5 MHz transducer (PowerVision 6000, Toshiba, Nasu, Japan) at around 24 h of age (median 29.0; range 19–40 h). The pulsed Doppler ultrasound technique has been described in detail elsewhere (Julkunen et al., 2012).

2.4. Electrophysiology

EEGs were taken on average at day 1.5 (range 1–4) in the asphyxia group and day 1.0 (range 1–2) in the control group. SEPs were recorded on average at day 5.0 (range 1–8) in the asphyxia group and day 2.0 (range 1–3) in the control group. BAEPs and VEPs were measured on average at day 4.0 (range 1–8) in the asphyxia group and day 1.0 (range 1–3) in the control group, respectively, except that in one asphyxiated infant BAEP and VEP were taken on day 30 and SEP on day 37.

EEG and EP-recordings were carried out according to routine clinical procedures at the Department of Clinical Neurophysiology, Tampere University Hospital. The laboratory rooms are sound-proof and equipped for clinical EEG and EP measurements. The room temperature was kept at 20–22 °C. Data were analyzed by a clinical neurophysiologist who was blinded to the outcome of the infants. If evoked potential latency was more than 3 SD from the normative values of the laboratory, the response was considered abnormal.

2.5. EEG

EEG was carried out using a Nervus M40 device (Taugagreining, Iceland). The EEG contained only 9 electrodes according to the International 10–20 system, due to the small infant's head. In addition we recorded electrocardiogram, respiratory belt and video. For visual analysis 16 bipolar EEG-derivations were used (Fp1-T3, T3-O1, Fp1-C3, C3-O1, Fp1-Cz, Cz-O1, Fp2-T4, T4-O2, Fp2-C4, C4-O2, Fp2-Cz, Cz-O1, T4-C4, C4-Cz, Cz-C3, C3-T3). A clinical neurophysiologist divided the EEG outcome into four groups according to the methods introduced by Leijser et al. (2007). The first group represents normal EEG and the EEG-levels 2–4 are considered abnormal (Table 1). Examples of abnormal EEG are seen in Figs. 1a and 1b.

2.6. BAEP

In BAEP recordings headphones (Telephonics TDH-39P) delivered auditory stimuli and a Nicolet Viking IV device (Nicolet Biomedical Instruments) was used. The BAEP responses were recorded with surface active electrodes on both mastoid processes, the reference being placed at the vertex (Liveson and Ma, 1992a,b). The electrode impedances were kept under 5.0 kΩ. The BAEPs were elicited with a rarefaction click stimulus of 80 dB pSPL intensity and 100 μs duration given at a rate of 9.9 Hz, a masking noise of 40 dB pSPL being delivered to the contralateral ear. Responses were amplified with the high- and low-pass filters set at 100 Hz and 3 kHz, respectively. For both sides, the BAEP was recorded at least twice to ascertain reproducibility. The peak latencies of waves I, III and V, as well as the inter-wave intervals (I–III, I–V, III–V) were measured from ipsilateral responses on both sides. Waves I, III and V were identified and marked manually by a nurse on the computer screen and the results confirmed and visually analyzed by a clinical neurophysiologist who divided the BAEPs into five groups (Table 1).

Table 1
Classification of the neurophysiologic measures.

EEG	
Level 1 (Normal)	Normal background, no epileptiform discharges
Level 2 (EEG-2)	Normal background with epileptiform discharges
Level 3 (EEG-3)	Abnormal background, no epileptiform discharges
Level 4 (EEG-4)	Abnormal background with epileptiform discharges
BAEP	
Normal	Normal latencies bilaterally
BAEP-2	Unilaterally normal, absent responses on the other side
BAEP-3	Lengthened peripheral latencies unilaterally or bilaterally (wave I)
BAEP-4	Bilaterally absent responses
BAEP-5	Lengthened central latencies unilaterally or bilaterally (wave III, V, interval III–V)
SEP	
Normal	Bilaterally normal cortical responses
SEP-2	Unilaterally lengthened cortical latencies with normal cervical responses
SEP-3	Bilaterally lengthened cortical latencies with normal cervical responses
VEP	
Normal	Bilaterally normal responses
Abnormal	Absence of responses (bilaterally or unilaterally)

Abbreviations: EEG, electroencephalogram; BAEP, Brainstem auditory evoked potential; VEP: Visual evoked potential; SEP, Somatosensory evoked potential.
EEG classification is made according to the method introduced by Leijser et al. (2007).
EEG-3: abnormal background = discontinuous and/or asymmetrical and/or low voltage EEG (amplitude ⩽ 30 μV).

2.7. SEP

SEP responses were elicited by electrical stimulation applied on both median nerves at the wrist using a constant current square wave pulse (0.1 ms width, cathode proximal) at a repetition rate of 1 Hz. The stimulus intensity was regulated to produce a small thumb twitch. Responses were recorded from ERB, cervical level (C2-Fpz) and cortically from CP3-Fz, CP3-M2, CP4-Fz and CP3-M1-derivations (Liveson and Ma, 1992a,b). In addition the derivations CP3-M2 and CP4-M1 are in routine use in our laboratory. Two trials of at least 100 artifact-free samples (automatic artifact rejection) were recorded with an analysis time of 100 ms. The SEPs outcomes were divided into three groups, the first group representing normal responses (Table 1).

2.8. VEP

Noncorneal electroretinogram (ERG) and VEP were recorded in a partially darkened room and were elicited by binocular stimulation with a stroboscopic un-patterned flash (frequency 0.9 Hz) placed about 25 cm from the eyes. ERG responses were recorded from EOGleft – Fz and EOGright – Fz derivations in order to ensure normal retinal function. All ERG responses were normal and VEPs were further analyzed. VEPs were recorded from Fz-O1, Fz-Oz and Fz-O$_2$ – derivations. The ground electrode was at midline (Cz) (Odom et al., 2010). Two trials of at least 50 artifact-free responses (automatic artifact rejection) were recorded within 512 ms after stimulus. For VEPs the P2 latencies of both eyes were measured. Results were divided into two groups, normal and abnormal responses (Table 1).

2.9. Statistical analysis

Statistical analyses were performed using SPSS 11.0 and 17.0 for Windows (SPSS, Chicago, IL, USA). Differences between infants with asphyxia and healthy controls, and between normal and abnormal neurological outcome in the asphyxia group were tested. Mann–Whitney U was used for skew-distributed continuous variables and categorical variables were tested by Pearson's chi-square or Fisher's exact test if expected values were too small. A P-value below 0.05 (with Bonferroni correction if appropriate) was considered significant.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in predicting neurological outcome at 1 year of age or death were calculated for EEG grading, grading of BAEP, SEP, VEP, HIE grading and Doppler findings, or their combination in asphyxiated infants. Predictive values with 95% confidence intervals were calculated by the Confidence Interval Analysis Program (CIA) version 2.1.2.50 by the Wilson method (University of Southampton).

3. Results

3.1. Electrophysiology

The results of EEGs and EPs are presented in Table 2. An example of a low-amplitude EEG is seen in Fig. 1a. An example of a unilaterally abnormal SEP response is presented in Fig. 2.

3.2. Cases with seizures

Seven of the asphyxiated infants received antiepileptic drugs because of clinical seizures, which all occurred on day 1 after the asphyxia event. Status epilepticus was not detected in any case (Table 2). Two infants with seizures had an abnormal EEG
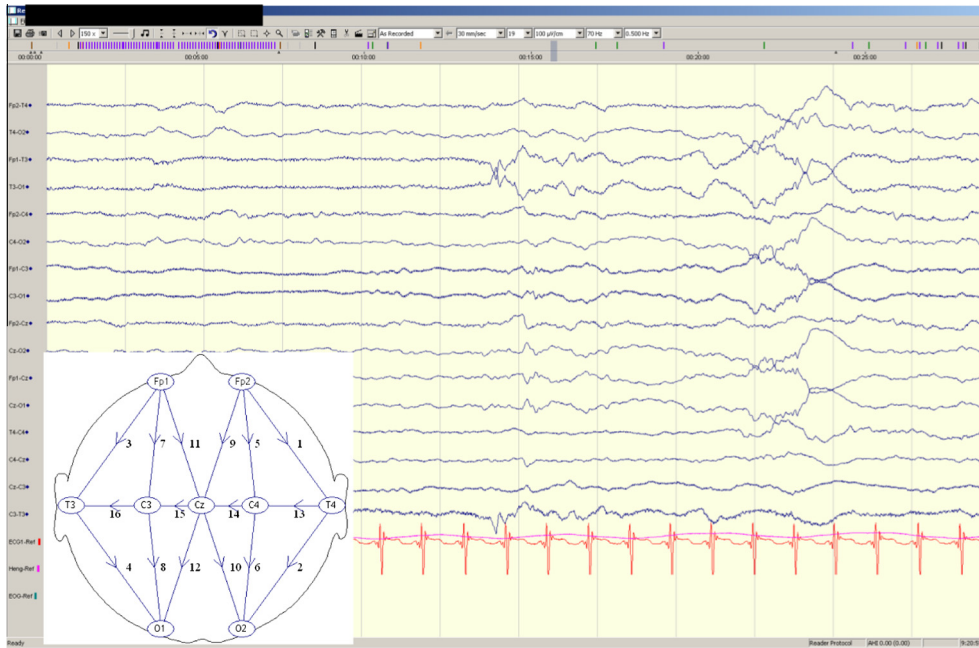
Fig. 1a. An example of an abnormal EEG (duration 12 s, 30 mm/s, sensitivity 100 uV/cm) with low-amplitude activity and asynchronous patterns in asphyxiated term infants. The EEG-montage is presented in the lower left corner of the figure. The additional channels are ECG and respiration with belt sensor.
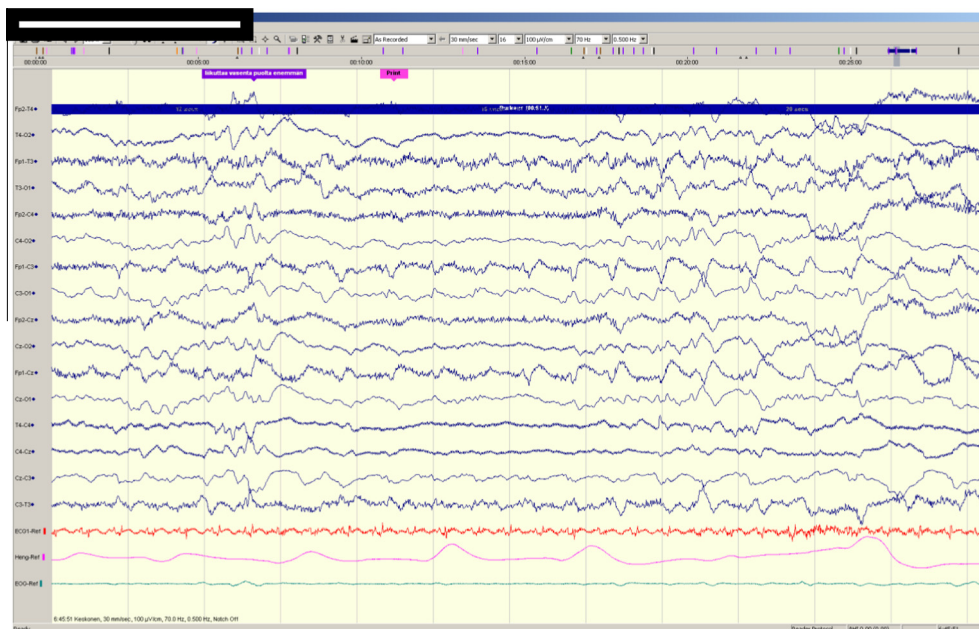
Fig. 1b. An example of an abnormal EEG with an epileptic discharge in the left hemisphere derivations. The settings and channels are as in Fig. 1a.

background without epileptiform discharges and two infants with epileptiform discharges. Only one infant had prolonged interburst intervals of almost 10 s. Three had normal EEGs.

SEPs were normal in four and two asphyxiated infants had unilaterally lengthened cortical latencies with normal cervical responses. VEPs were normal. BAEPs were performed in six of the seven asphyxiated infants with seizures. Two asphyxiated infants with seizures had lengthened latencies and one infant had missing BAEP responses bilaterally. Five of these seven asphyxiated infants with seizures had normal 1-year outcome.

Antiepileptic drugs are known to modify EP results. However, most often they induce diffuse alteration and one could assume

that they affect the EP outcomes bilaterally. In our data only one asphyxiated infant showed bilaterally abnormal SEP or BAEP responses. Therefore it seems unlikely that the medication is responsible for the abnormal EP findings in this study.

3.3. Prediction

The predictive values of EEG evoked potentials, Doppler findings and HIE grading in predicting poor and good outcome at 1 year of age in the asphyxia group are presented in Table 3. Combining Doppler findings with EEG or SEP results improved the predictive value of a good outcome, but not a poor outcome.

Table 2
HIE, seizures and clinical brain monitor findings in the study groups.

	Asphyxia group ($n = 30$)	Control group ($n = 30$)	p Value	Asphyxia outcome [d] ($n = 27$)		p Value
				Normal ($n = 21$)	Abnormal ($n = 6$)	
HIE						
No HIE, (n) (%)	9 (30.0)			6 (28.6)	1 (16.7)	0.025
Grade 1, (n) (%)	11 (36.7)			10 (47.6)	0 (0)	
Grade 2–3, (n) (%)	10 (33.3)			5 (23.8)	5 (83.3)	
Seizures, (n) (%)	7 (23.3)	0 (0)	<0.001	5 (23.8)	2 (33.3)	0.645
EEG, (n) (%)			0.002			0.002
Normal	21 (70)	30 (100)		17 (81)	1 (16.6)	
EEG-2	1 (3.3)	0 (0)		0(0)	1 (16.6)	
EEG-3	6 (20)	0 (0)		2 (9.5)	4 (66.7)	
EEG-4	2 (6.7)	0 (0)		2 (9.5)	0 (0)	
BAEP, (n) (%) [a]			0.023			0.155
Normal	22 (78.6)	28 (100)		18 (85.7)	3 (60)	
BAEP-2	0 (0)	0 (0)		0 (0)	0 (0)	
BAEP-3	2 (7.1)	0 (0)		2 (9.5)	0 (0)	
BAEP-4	1 (3.6)	0 (0)		0 0)	1 (20)	
BAEP-5	3 (10.7)	0 (0)		1 (4.8)	1 (20)	
SEP, (n) (%) [b]			0.046			0.031
Normal	17 (68)	24 (92)		15 (79)	1 (25)	
SEP-2	7 (28)	2 (8)		4 (21)	2 (50)	
SEP-3	1 (4)	0 (0)		0 (0)	1 (25)	
VEP, (n) (%) [c]				–		
Normal	28 (100)	27 (100)		21 (100)	5 (100)	
Abnormal	0 (0)	0 (0)		0 (0)	0 (0)	
Systolic CBFV > 3SD in the ACA or BA at 24 h, (n) (%)	6 (20)	0 (0)	0.010	1 (4.8)	5 (83)	0.002

Abbreviations: BAEP, brain stem auditory evoked potential; EEG, electroencephalogram; HIE, hypoxic ischemic encephalopathy; SEP, somatosensory evoked potential; Systolic CBFV, systolic cerebral blood flow velocity; VEP, visual evoked potential.
Determinations of EEG, BAEP and SEP grading, see Table 1.

[a] BAEP: two in the asphyxia and two in the control group were not performed.
[b] SEP: four in the asphyxia group were not performed and one was unobtainable. Three in the control group were not performed and one was missing.
[c] VEP: two in the asphyxia group and three in the control group were not performed.
[d] In all, 3 of the 30 asphyxiated infants were lost to follow-up; and 4 out of 27 follow-up infants had no SEP; one had no VEP or BAEP.

4. Discussion

We found abnormal neurophysiologic assessments such as EEG and EPs performed during the first week of life to be of apparently limited value in predicting the 1-year outcome in asphyxiated term infants. Combining abnormal EEG and EPs findings with increased systolic CBFV did not alter the prediction of poor outcome. Instead, normal EEG and normal SEP with systolic CBFV < 3SD at 24 h of age improved specificity in predicting good neurological 1-year outcome compared to a normal EEG or normal SEP only. Combining normal EEG or SEP with increased CBFV < 3SD at 24 h of age improved the specificity in predicting good outcome, compared to a normal EEG or SEP only. Seizures after asphyxia seemed not to be independent predictors of poor outcome.

The predictive value of EEG in term infants with HIE has been well documented (Selton and Andre, 1997; El-Ayouty et al., 2007; Murray et al., 2009). Infants with severe HIE had a highly abnormal EEG background in 90%, while infants with mild HIE produced normal or mildly abnormal EEG recordings (Selton and Andre, 1997). EEG patterns which have been associated with abnormal outcomes in HIE are background amplitude < 30 uV, inter-burst interval of > 30 s, electrographic seizures and absence of sleep-wake cycling at 48 h of age (Murray et al., 2009). A persistent discontinuous pattern in the EEG after the first week after an asphyxia event is strongly related to poor outcome (Biagioni et al., 1999; Menache et al., 2002).

Abnormalities in the background activity upon EEG performed in the first week of life have been shown to be strong predictors of adverse outcome (Sinclair et al., 1999; El-Ayouty et al., 2007; Leijser et al., 2007), while a normal or mildly abnormal EEG recording has predicted a favorable outcome in 78–100% of cases (Wertheim et al., 1994; Zeinstra et al., 2001; El-Ayouty et al., 2007; Leijser et al., 2007). The sensitivity of a normal EEG in predicting favourable outcome in our study was good, confirming the results of previous studies (Wertheim et al., 1994; Zeinstra et al., 2001; El-Ayouty et al., 2007; Leijser et al., 2007).

Seven infants (28%) in the asphyxia group had abnormal SEPs unilaterally and only one asphyxiated infant had bilaterally abnormal SEP responses in this study. In recent studies, unilaterally abnormal SEP was found in only 5% of infants with neonatal encephalopathy (Suppiej et al., 2010) and the incidence of bilaterally absent SEP varied from 24% to 55% (Suppiej et al., 2010; Swarte et al., 2012). In our cases unilaterally abnormal SEPs were much more frequent. It might be that our asphyxia cases were less severe than in most previous publications. The timing of assessments did not explain the differences.

Bilateral loss of cortical SEP seems likewise to be a strong indicator of poor outcome in acute encephalopathy in adults and in older children (Goodwin et al., 1991; Robinson et al., 2003; Wolrab et al., 2001). The sensitivity of abnormal SEPs in asphyxiated term infants has been reported to vary from 90% to 95% and the specificity from 73% to 85% (Eken et al., 1995; Swarte et al., 2012). An abnormal SEP assessment at 1 week of age predicts an adverse outcome in approximately 90% of asphyxiated infants, whereas a normal SEP is generally a sign of normal outcome (Gibson et al., 1992; Taylor et al., 1992). In the present study the sensitivity and specificity of unilaterally or bilaterally absent SEPs in predicting a poor outcome was similar to previous studies. Normal SEP predicted well a good outcome also in our cases, and combination of normal Doppler findings with normal SEP even improved the specificity.

The sensitivity of abnormal VEPs in term asphyxiated infants in predicting abnormal outcome has varied from 78% to 91% and
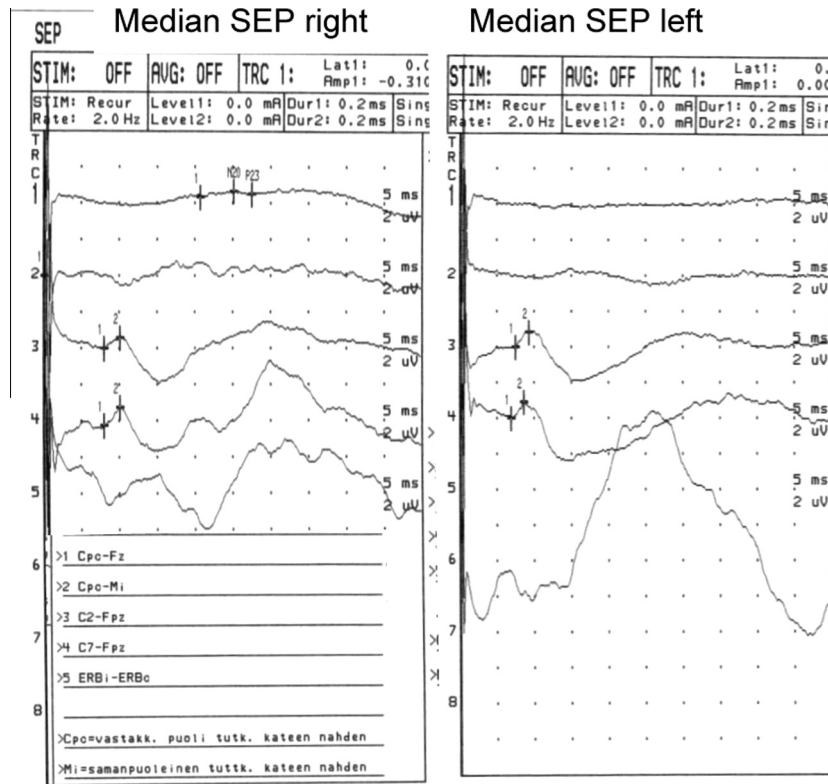
Fig. 2. Right and left median nerve SEP responses in asphyxiated term infant. Channels from the top: 1: Cpc (contralateral to stimulation)- Fz, 2: Cpc- Mastoideus (ipsilateral), 3: C2 – Fpz, 4: C7 – Fpz, 5: ERB ipsilateral – ERB contralateral. For the right median nerve stimulation was small, but repeatable cortical response was achieved with normal cervical responses. For the left median nerve stimulation only cervical responses were obtained. The finding is abnormal.

Table 3
Predictive value of EEG, BAEP, SEP, VEP, HIE and Doppler findings to predict poor and good outcome of asphyxiated infants.[a]

Ability to predict poor outcome	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
EEG-3/EEG-4, ($n = 27$)	67 (30–90)	81 (60–92)	50 (22–79)	89 (69–97)
BAEP-4/BAEP-5, ($n = 26$)	40 (12–77)	95 (77–99)	67 (21–94)	87 (68–96)
SEP-2/SEP-3, ($n = 23$)	75 (30–95)	79 (57–91)	43 (12–80)	94 (68–100)
SEP-3, ($n = 23$)	25 (5–70)	100 (83–100)	100 (21–100)	86 (67–95)
Abnormal VEP, ($n = 26$)	0%	100%		
EEG-3/EEG-4 and SEP-2/SEP-3, ($n = 23$)	50 (15–83)	89 (69–97)	50 (15–85)	89 (69–97)
HIE grade 2–3, ($n = 27$)	83 (44–97)	76 (55–89)	50 (24–76)	94 (73–99)
sCBFV > mean 3SD in the ACA or BA at 24 h, ($n = 27$)	83 (44–97)	95 (77–99)	83 (44–97)	95 (77–99)
EEG-3/EEG-4 and sCBFV > mean 3SD in the ACA or BA at 24 h, ($n = 27$)	50 (19–81)	100 (85–100)	100 (44–100)	88 (69–96)
SEP-2/SEP-3 and sCBFV > mean 3SD in the ACA or BA at 24 h, ($n = 23$)	75 (30–95)	100 (83–100)	100 (31–100)	95 (73–100)
EEG-3/EEG-4 and HIE grade 2–3, ($n = 27$)	67 (30–90)	90 (71–97)	67 (30–90)	90 (72–97)
SEP-2/SEP-3 and HIE grade 2–3, ($n = 23$)	75 (30–95)	95 (75–99)	75 (22–99)	95 (72–100)
sCBFV > mean 3SD in the ACA or BA at 24 h and HIE grade 2–3, ($n = 27$)	100 (57–100)	95 (78–99)	83 (44–97)	100 (85–100)
Ability to predict good outcome	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Normal EEG, ($n = 27$)	81 (60–92)	67 (30–90)	89 (69–97)	50 (21–79)
Normal BAEP, ($n = 26$)	86 (65–95)	40 (12–77)	86 (63–96)	67 (12–98)
Normal SEP, ($n = 23$)	79 (57–91)	75 (30–95)	94 (72–99)	43 (16–75)
sCBFV < mean 3SD in the ACA or BA at 24 h, ($n = 27$)	95 (77–99)	83 (44–97)	95 (77–99)	83 (44–97)
Normal EEG and sCBFV < mean 3SD, ($n = 27$)	71 (50–86)	100 (61–100)	100 (80–100)	50 (25–75)
Normal SEP and sCBFV < mean 3SD, ($n = 23$)	74 (51–88)	75 (30–95)	93 (66–100)	38 (10–74)
Normal EEG, normal SEP and sCBFV < mean 3SD in the ACA or BA at 24 h, ($n = 23$)	74 (51–88)	100 (51–100)	100 (73–100)	44 (15–77)
Normal EEG, HIE 0–1 and sCBFV < mean 3SD in the ACA or BA at 24 h, ($n = 27$)	67 (45–83)	100 (61–100)	100 (78–100)	46 (23–71)

BAEP-4, missing responses bilaterally; BAEP-5, lengthened cervical latencies unilaterally;
EEG-3, abnormal EEG background and no epileptiform discharges; EEG-4, abnormal EEG background with epileptiform discharges;
HIE, hypoxic ischemic encephalopathy; SEP-2, unilaterally lengthened cortical latencies with normal cervical responses;
SEP-3, bilaterally lengthened cortical latencies with normal cervical responses; sCBFV in the ACA or BA, systolic cerebral blood flow velocity in the artery cerebral anterior or basilaris.
[a] 3 of the 30 asphyxiated infants were lost to follow-up.

specificity from 67% to 100% (Muttitt et al., 1991; Taylor et al., 1992; Eken et al., 1995; Kato and Watanabe, 2006), but in this study VEP assessment gave no additional information. In a recent study VEP was likewise reported to be a poor tool in neurodevelopmental prognostication (Suppiej et al., 2010).

The utility of neonatal BAEP assessments in prognostication is limited. A normal BAEP does not ensure a normal outcome, but severely abnormal BAEPs would appear to predict an abnormal outcome at 12–24 months of age (Majnermer et al., 1988; Scalais et al., 1998). Abnormal BAEP has predicted an adverse outcome

with a sensitivity of 40.5% and a specificity of 87.8% (Jiang et al., 2008), similarly to our findings.

There were limitations in this study. Numbers of infants in the study groups were small and the follow-up at 1 year of age was not complete. The time range of EEG and evoked potential recordings after asphyxia was considerable. The strength of the study was a healthy sex- and age-matched control group.

It may be concluded that predictive values of BAEP and VEP were poor among asphyxiated infants with adverse outcome at 1 year of age. SEP might add information relevant to prognostication. In contrast, a normal EEG and a normal SEP might be useful in identifying asphyxiated infants with good outcome. Combining systolic CBFV < 3SD at around one day of life with a normal EEG and/or SEP improves specificity in the prediction of a normal 1-year outcome.

5. Funding

6. Conflict of interest

None declared.

References

Alin-Åkerman B, Nordberg L. Griffith's utvecklingsskalor I och II. Manual för administering och utvärdering. Göteborg: PsykologiFörlaget AB; 1991.

Biagioni E, Bartalena L, Boldrini A, Pieri R, Cioni G. Constantly discontinuous EEG patterns in full-term neonates with hypoxic-ischaemic encephalopathy. Clin Neurophysiol 1999;110:1510–5.

Deeg KH, Rupprecht Th, Zeilinger G. Dopplersonigraphic classification of brain edema in infants. Pediatr Radiol 1990;20:509–14.

Eken P. Toet MC, Groenendaal, de Vries LS. Predictive value of early neuroimaging, pulsed Doppler and neurophysiology in full term infants with hypoxic-ischaemic encephalopathy. Arch Dis Child 1995;73:F75–80.

El-Ayouty M, Abdel-Hady H, El-Mogy S, Zaghlol H, El-Beltagy M, Aly H. Relationship between electroencephalography and magnetic resonance imaging findings after hypoxic-ischemic encephalopathy at term. Am J Perinatol 2007;24:467–73.

Finer NN, Robertson CM, Richards RT, Pinnell LE, Peters KL. Hypoxic-ischemic encephalopathy in term neonates: Perinatal factors and outcome. J Pediatr 1981;98:112–7.

Finer NN, Robertson CM, Peters KL, Coward JH. Factors affecting in hypoxic-ischemic encephalopathy in term infants. Am J Dis Child 1983;137:21–5.

Gibson NA, Graham M, Levene MI. Somatosensory evoked potentials and outcome in perinatal asphyxia. Arch Dis Child 1992;67:393–8.

Goodwin SR, Freidman WA, Bellefleur M. Is it time to use evoked potentials to predict outcome in comatose children and adults? Crit Care Med 1991;19:518–24.

Hagberg B, Hagberg G, Beckung E, Uvebrant P. Changing panorama of cerebral palsy in Sweden: VIII. Prevalence and origin in the birth year period 1991–1994. Acta Paediatr 2001;90:271–7.

Jiang ZD, Liu XY, Shi BP, Lin L, Bu CF, Wilkinson AR. Brainstem auditory outcomes and correlation with neurodevelopment after perinatal asphyxia. Pediatr Neurol 2008;39:189–95.

Julkunen MK, Uotila J, Eriksson K, Luukkaala T, Tammela O. Obstetric parameters and Doppler findings in cerebral circulation as predictors of 1 year neurodevelopmental outcome in asphyxiated infants. J Perinatol 2012;32:631–8.

Kato T, Watanabe K. Visual evoked potential in the newborn. Does it have predictive value? Seminars in Fetal Neonatal Med 2006; 11: 459–463.1.

Leijser LM, Vein AA, Liauw L, Strauss T, Veen S, van Wezel-Meijler G. Prediction of short-term neurological outcome in full-term neonates with hypoxic-ischemic encephalopathy based on combined use of electroencephalogram and neuroimaging. Neuroped 2007;38:219–27.

Levene MI, Sands C, Grindulis H, Moore JR. Comparison of two methods of predicting outcome in perinatal asphyxia. Lancet 1986;1:67–9.

Levene MI, Fenton AC, Evans DH, Archer LNJ, Shortland DB, Gibson NA. Severe birth asphyxia and abnormal cerebral blood-flow velocity. Dev Med Child Neurol 1989;31:427–34.

Liveson JA, Ma DM. Auditory evoked responses. In: Laboratory Reference for Clinical Neurophysiology. Oxford: Oxford University Press; 1992a. p. 324–31.

Liveson JA, Ma DM. Somatosensory evoked responses. In: Laboratory Reference for Clinical Neurophysiology. Oxford: Oxford University Press; 1992b. p. 278–323.

Majnermer A, Rosenblatt B, Riley P. Prognostic significance of the auditory brainstem evoked response in high-risk neonates. Dev Med Child Neurol 1988;40:43–52.

Menache CC, Bourgeois BF, Volpe JJ. Prognostic value of neonatal discontinuous EEG. Pediatr Neurol 2002;27:93–101.

Murray DM, Boylan GB, Ryan CA, Connolly S. Early EEG findings in hypoxic-ischemic encephalopathy predict outcomes at 2 years. Pediatrics 2009;124:e459–67.

Muttitt SC, Taylor MJ, Kobayashi JS, MacMillan L, Whyte HE. Serial visual evoked potentials and outcome in term birth asphyxia. Pediatr Neurol 1991;7:86–90.

Odom JV, Bach M, Brigell M, Holder GE, McCulloch DL, Tormene AP, et al. ISCEV standard for clinical visual potentials (update 2009). Doc Ophthalmol 2010;120:111–9.

Pierrat V, Haouari N, Liska A, Thomas D, Subtil D, Truffert P. Prevalence, causes, and outcome at 2 years of age of newborn encephalopathy: population based study. Arch Dis Child 2005;90:F257–61.

Robinson LR, Micklesen PJ, Tirschwell DL, Lew HL. Predictive value of somatosensory evoked potentials for awakening from coma. Crit Care Med 2003;31:960–7.

Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. Arch Neurol 1976;36:308–13.

Scalais E, Francois-Adant A, Nuttin C, Bachy A, Guerit JM. Multimodality evoked potentials as a prognostic tool in term asphyxiated newborns. Electroencephalogr Clin Neurophysiol 1998;108:199–207.

Selton D, Andre M. Prognosis of hypoxic ischaemic encephalopathy in full-term newborns – value of neonatal electroencephalography. Neuropediatrics 1997;239:839–80.

Sinclair DB, Campbell M, Byrne P, Prasertsom W, Robertson CMT. EEG and long-term outcome of term infant with neonatal hypoxic-ischemic encephalopathy. Clin Neurophysiol 1999;110:655–9.

Stark JE, Seibert JJ. Cerebral artery Doppler ultrasonography for prediction of outcome after perinatal asphyxia. J Ultrasound Med 1994;13:595–600.

Suppiej A, Cappellari A, Franzoi M, Traverso A, Ermani M, Zanardo V. Bilateral loss of cortical somatosensory evoked potential at birth predicts cerebral palsy in term and near-term newborns. Early Hum Develop 2010;86:93–8.

Swarte RMC, Cherian PJ, Lequin M, Visser GH, Govaert P. Somatosensory evoked potentials are of additional prognostic value in certain patterns of brain injury in term birth asphyxia. Clin Neurophysiol 2012;123:1631–8.

Taylor MJ, Murphy WJ, Whyte HE. Prognostic reliability of somatosensory and visual evoked potentials of asphyxiated term infants. Develop Med Child Neurol 1992;34:507–15.

Volpe JJ. Neurology of the newborn. Philadelphia: Saunders; 2008.

Wertheim D, Mercuri E, Faundez JC, Rutherford M, Acolet D, Dubowitz L. Prognostic value of continuous electroencephalographic recording in full term infants with hypoxic ischaemic encephalopathy. Arch Dis Child 1994;71:F97–F102.

Wolrab G, Boltshauser E, Schmitt B. Neurological outcome in comatose children with bilateral loss of cortical somatosensory evoked potentials. Neuropediatrics 2001;32:271–4.

Zeinstra E, Fock JM, Begeer JH, van Weerden TW, Maurits NM, Zweens MJ. The prognostic value of serial EEG recordings following acute neonatal asphyxia in full-term infants. Eur J Paeditr Neurol 2001;5:155–60.

HYPOXIC ISCHEMIC ENCEPHALOPATHY AND NEURODEVELOPMENTAL ASSESSMENTS IN THE PREDICTION OF 1-YEAR OUTCOME IN ASPHYXIATED INFANTS

Mia K Julkunen[1,2]*, Kai Eriksson[3], Ulla Lehto[4], Veikko Kähärä[4], Tiina Luukkaala[5], Outi Tammela[1,2]

[1]Pediatric Research Centre, University of Tampere, [2]Department of Pediatrics, Tampere University Hospital, [3]Pediatric Neurology Unit, Tampere University Hospital, [4]Department of Radiology, Tampere University Hospital, [5]Science Center, Pirkanmaa Hospital District and Tampere School of Public Health, University of Tampere, Tampere, Finland

*Correspondence to: Dr Mia Julkunen, Department of Pediatrics, Tampere University Hospital, P.O. Box 2000, FIN- 33521, Tampere, Finland (email mia.julkunen@fimnet.fi, tel + 358 3 311 64057, fax + 358 3 311 64394)

Abstract

Purpose: To evaluate whether hypoxic ischemic encephalopathy (HIE) predicts the 1-year outcome better than the neurodevelopmental assessments.

Methods: Follow-up was monitored in 30 asphyxiated and 30 healthy term infants by Neuromotor scoring (NMS), General Movements (GMs) and the Alberta Infant Motor Scale (AIMS).

Results: The sensitivity of HIE grade from 2 to 3 to predict poor outcome in the asphyxia group was 83% and specificity 76%. Abnormal GMs at 3 months predicted poor outcome with a sensitivity of 75% and 95% specificity. The sensitivity of abnormal AIMS was 60 % and abnormal NMS 40% at three months, respectively. AIMS at 6 to 12 months predicted a poor 1-year neuromotor outcome with a sensitivity of 100%. Normal fidgety GMs at 3 months seemed to predict good outcome with a sensitivity of 95%.

Conclusion: HIE has a prognostic value during the neonatal period. GMs at 3 months of age is a valuable assessment, and AIMS at 6 and 12 months of age, predicting both a good and a poor one-year outcome.

Keywords: Asphyxia; hypoxic ischemic encephalopathy; neurodevelopmental assessments; term

Introduction

Hypoxic-ischemic encephalopathy (HIE) caused by perinatal asphyxia is a major contributor to neonatal death and disability in full-term infants. The incidence of HIE has been estimated to be 1.5 per 1000 live births (1). HIE in the perinatal period is an important cause of cerebral palsy in 29 % and associated with cognitive, general developmental delay or learning difficulties even in 45 % among term infants with HIE (2) The degree of subsequent neurological problems has been found to be related to the severity of HIE (3)

Prediction of long-term neurodevelopmental outcome in asphyxiated infants during the neonatal period is needed with an eye to informing parents of the infant's prognosis and planning follow-up. The prediction of the outcome in infants with HIE is based on clinical, neuroimaging and neurophysiological parameters. The severity of HIE as assessed on Sarnat criteria has proved a useful determinant of outcome (4, 5). General movements (GMs) can be used in the assessment during the first three months of age. The quality of GMs at three months of age has been shown to predict the development of disorders such as cerebral palsy (CP) (6, 7). Neuromotor scoring (NMS) at 3 months of age has correlated strongly with results at 12-month neurological examination (8). The Alberta Infant Motor Scale (AIMS) has proved a viable mode of clinical assessment in identifying abnormal motor development especially from 4 to 8 months of age (9). It remains, however, to devise reliable means of assessment during the first days of life to identify asphyxiated infants who will later evince neurological impairment.

The present aim was to predict the 1-year neurodevelopmental outcome of asphyxiated term infants by means of the grading of HIE and three different neurological assessments (GMs, AIMS, NMS) at 0 to 12 months of age. Our hypothesis was that severity of HIE will predict the 1-year outcome among the asphyxiated term infants better than the neurodevelopmental assessments.

Patients and methods
Patients

This longitudinal prospective follow-up study was carried out in the neonatal intensive care unit. The Ethics Committee had approved the study protocol. Informed consent was obtained from the parents.

Thirty full-term (gestational age 37 weeks or more) infants with asphyxia and thirty healthy gestational age- and sex-matched infants were enrolled. Infants were included in the asphyxia group, when Apgar scores were 5 or less at the 5th minute of life and at least one of following criteria for asphyxia was fulfilled: (1) signs of fetal distress such as abnormal cardiotocography (CTG) or meconium-stained amniotic fluid, (2) cord arterial pH < 7.10 or (3) symptoms of HIE within 48 hours of life.

The overall neurological outcome was assessed clinically at 1 year of age by a pediatric neurologist who was blinded to the history of the infants. Griffith's Developmental Scales (10) were used to structure the assessment but only raw scores were recorded. According to these raw scores and neuropediatric clinical evaluation the neurological outcome at the age of 1 year was classified as normal, motor disability or motor and mental

disability. Abnormal outcome was defined as any neurological impairment or death. One asphyxiated infant with grade 2 HIE, in whom Kabuki syndrome was diagnosed according to characteristic features at 2 years and 9 months of age, was included in the analysis.

HIE grading
The severity of HIE was graded from 1 to 3 according to modified Sarnat criteria (5). Grading was based on clinical observations (level of consciousness, muscle tone, posture, reflexes, autonomic functions and seizures) during the first 48 hours of life. Infants with grade 1 HIE had normal muscle tone and usually recovered well within 12 to 24 hours without seizure activity. Infants who developed grade 2–3 HIE had altered levels of consciousness and seizure activity 12–4 hours after the hypoxic-ischemic event. Loss of reflex activity, flaccid muscle tone, respiratory failure and coma were connected with grade 3 HIE.

Neurodevelopmental assessments
Alberta Infant Motor Scale (AIMS)
The AIMS is an observational measure of motor development from birth to 18 months of age (11). A 58-item scale is designed to assess gross motor skills in four positions: prone, supine, sitting, and standing. Total raw scores were calculated and converted into an age-related percentile ranking. The risk of abnormal motor developmental performance is defined as a score on AIMS below the 10th percentile at 4 months or below the 5th percentile at 8 months of age (12). The assessments were performed by physiotherapists with between 2 and 15 years' clinical experience.

General movements (GMs)
The GMs of infants were videotaped before hospital discharge and at 3 months of age. All recordings were made during active wakefulness, the infants lying supine and partially dressed. The recordings were assessed by the same physiotherapist, who was certified by the General Movement Trust (GMT) in performing the Prechtl's methodology and was not aware of the medical history of the infants.

Two patterns of normal GMs are observed: writhing and fidgety movements. Writhing movements, observing at term age to the first two months post-term, are small- to moderate-amplitude movements. Normal fidgety movements are defined as circular movements of small amplitude, moderate speed and variable acceleration of neck, trunk and limbs in all directions from 6 weeks to 20 post-term weeks (7). Abnormal movements are defined as having (1) poor repertoire general movements (the sequence of successive movement components is monotonous and movements of different body parts do not occur in complex (7, 13), (2) cramped-synchronised general movements (all limb and trunk muscles contract and relax almost simultaneously or are chaotic (7) or (3) absent fidgety movements. Accordingly, we classified the movements of the child as abnormal if the fidgety GMs were absent at 3 months of age.

Neuromotor scoring system (NMS)
The neuromotor scoring system was applied at 3 months of age by a pediatric neurologist who was blinded to the perinatal history of the infants. The NMS at 3 months (NMS-3) included an assessment of mental status

(adaptive capacity to sound, light and the examiner, alertness, vocalisation, curiosity and consolability), cranial nerve II–XII function, active and passive tone and power in the trunk and extremities, deep tendon reflexes, primitive reflexes (asymmetric tonic neck, Moro's reflex, palmar and plantar grasp, automatic walking, placing, rooting, sucking, cortical thumbing and Babinski's reflex). The summary findings were scored from 0 to 5 as follows: 0 = normal; 1 = mild abnormality of tone or reflexes; 2 = mild abnormality of tone and reflexes; 3 = abnormality of tone or reflexes or both and decreased power in the trunk and extremities; 4 = any motor abnormality and cranial nerve involvement; 5 = spastic quadriparesis (8).

Neuroimaging

Pulsed Doppler and cranial ultrasound examinations were performed with ultrasonography equipment (PowerVision 6000, Toshiba, Nasu, Japan) at around 24 hours of age in all cases.

Brain magnetic resonance imaging (MRI) was performed using 1.5T MR (Siemens Avanto, Erlangen, Germany), when asphyxiated infants were at 1 year of age. MRI were evaluated by two neuroradiologists blinded to the clinical data of the asphyxiated infants.

Statistical analysis

Statistical analyses were performed using SPSS 11.0 and 17.0 for Windows (SPSS, Chicago, IL, USA). Differences between infants with asphyxia and healthy control infants were analysed for statistical significance by independent t test if continuous variables were normally distributed. Mann-Whitney U-test or Kruskal-Wallis test were used for skew-distributed continuous variables and categorical variables were tested by Pearson's chi-square or Fisher's exact test if expected values were too small. A p value below 0.05 was considered significant.

Sensitivity, specificity and positive and negative predictive values for an abnormal neurological outcome at 1 year of age or death were calculated for HIE grading, GMs at discharge and at age 3 months, NMS at 3 months and AIMS assessments at 3 months, 6 months and 12 months of age in asphyxiated infants. Predictive values with 95% confidence intervals were calculated by Confidence Intervals Analysis - program version 2.1.2 (University of Southampton, UK).

Results

Clinical data and outcome

Clinical data in the study groups are presented in Table 1. Altogether six (22%) had an abnormal outcome in the asphyxia group. One infant with grade 1 HIE and two with grade 2 HIE had delay in motor development at 1 year of age, one infant with grade 2 HIE had spastic diplegia and 1 with grade 3 HIE had mental and motor developmental delay at 1 year of age. One infant with grade 3 HIE died at the age of 9 days. Three asphyxiated infants were lost to the follow-up and 27 (90%) asphyxiated infants were followed up to 1 year of age. A total of 22 (73%) of the 30 controls were followed up to 1 year of age and one of them had delayed motor development at age of 1 year.

4

No congenital brain abnormalities during the first day of life were detected in cranial ultrasound imaging in the study groups. One infant with grade 3 HIE developed ventricular dilation after 14 days of severe asphyxia. 20% of the asphyxiated infants but none in the control group had increased systolic cerebral blood flow velocities (CBFV) (mean +3SD) in anterior or basilar arteries by Doppler examination at age of 24 hours. Twenty-three (77%) of thirty asphyxiated infants underwent MRI examinations at 1 year of age. Three (13%) of these had abnormal MRI findings. One with grade 2 HIE had minor white matter lesions and one white matter lesions with thin corpus callosum on MRI. One infant with grade 3 HIE had reduction and lesions in white matter, ventricular dilatation and a thin corpus callosum. Five infants with grade 2 HIE, eight with grade 1 HIE and seven without any HIE symptoms had a normal MRI scanning at 1 year of age.

Neurodevelopmental assessments

GMs, AIMS and NMS findings in the study groups are seen in Table 2. Summary of neurodevelopmental assessment, Doppler and MRI findings in asphyxiated infants with poor 1-year outcome is seen in Table 3. Five of the six asphyxiated term infants with increased systolic CBFV had abnormal outcome at 1 year of age or died in the neonatal period. Twenty of the 23 asphyxiated infants had normal brain MRI scanned at 1 year of age and 19 (95%) of these 20 infants had normal GMs at 3 months and normal 1-year outcome.

Prediction

The predictive values of AIMS, GMs, NMS and HIE in predicting poor and good outcome at 1 year of age in the asphyxia group are presented in Table 4.

Discussion

In the present study only the severity of HIE had a prognostic value during the neonatal period for predicting the one-year outcome of asphyxiated term infants. GMs seemed to be a suitable assessment at 3 months of age. Normal fidgety GMs at 3 months predicted good outcome in the high-risk infants, confirming the results of previous studies (7, 14). AIMS had good predictive value at 6 to 12 months of age. NMS seemed to be a low value as a follow-up method of asphyxiated infants.

Earlier studies suggest that infants with HIE grade 2 have a 30–50% risk of major deficits (15, 16). Our risk percentage was 38%, supporting this conception. The HIE grading from 2 to 3 predicted the poor outcome among the asphyxiated infants with a sensitivity of 83% in this study. Assessment of HIE is subjective and therefore prone to inter observer variability, which might explain the lower predictive value of HIE symptoms. Thompson scores would have been valuable in grading HIE in this study. Thompson scoring is a numeric system for the assessment of HIE during the neonatal period and for prognosis of neurodevelopmental outcome (17). An early Thompson score at age of 3–5 hours seems to be useful in selecting the asphyxiated infants for hypothermia (18). Increased CBFV from 24 to 72 hours by Doppler has been taken as a sign of permanent brain injury with poor outcome in asphyxiated

infants (19, 20), and have been found to have predictive value (21).

Poor repertoire GMs are the most frequently observed abnormal GMs during the writhing age, but their predictive value for neurological outcome is low (7, 22, 23). Poor repertoire GMs constitute a sign of brain immaturity, since almost half of the healthy control infants in the present study displayed a narrow range during the neonatal period. In a recent study, normal MRI or a normal GMS predicted a normal outcome with a negative predictive value of 100 %, but in contrast the pattern of central grey matter damages by early MRI were correlated with cramped-synchronized GMs and poor motor outcome among term infants with HIE (14). In the present study, the MRI was scanned at 1 year of age. It has been reported that the different patterns of injury seen on neonatal MRI after neonatal encephalopathy were still recognised on childhood MRI (24).

In this study, the sensitivity of 3-month AIMS was low (60%) in high-risk infants, but at 6 months and 12 months matched well to our reference neurological examination at 1 year of age. AIMS appeared to measure the infants` ability best from three to nine months of age also in one recent study (25). One explanation here could be that the infants are still "neurologically silent" at 3 months of age and the gross motor development at 3 months of age is somewhat difficult to estimate by observation as even the rate of motor development in normally developing infants is variable (13).

NMS at 3 months used as a scoring system has been found to correlate strongly with the outcome among term infants at 12 months of age, having a low sensitivity, 53% but a specificity of 89% (8). In our study the sensitivity and specificity were fairly similar within the same score limits. NMS would not appear to offer a solid basis for assessment of infants born asphyxiated. Combination with other assessment modes is needed to achieve better prediction of outcome in high-risk infants.

There were limitations in this study. Numbers of infants in the study group were small and the follow-up was not complete. The strength of the study was the healthy term control group as a reference group in evaluating the different neuromotor development assessments.

Conclusion

It may be concluded that in predicting the 1-year outcome only the severity of HIE has a prognostic value during the neonatal period in the asphyxiated infants. GMs is a valuable neurodevelopmental assessment at 3 months of age, predicting both a good and a poor one year outcome. AIMS is a good predictor later, at 6 to 12 months of age. NMS seems to be a low value as an independent assessment tool in infants born asphyxiated.

References

1. Kurinczuk JJ, White-Koning M. Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. Early Hum Develop 2010; 86:329-338.

2. Mwaniki MK, Atieno M, Lawn JE, Newton CRJC. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet 2012; 379:445-52.

3. Hagberg B, Hagberg G, Beckung E, Uvebrant P. Changing panorama of cerebral palsy in Sweden: VIII. Prevalence and origin in the birth year period 1991–1994. Acta Paediatr 2001; 90:271-277.

4. Finer NN, Robertson CM, Richards RT, Pinnell LE, Peters KL. Hypoxic-ischemic encephalopathy in term neonates: Perinatal factors and outcome. J Pediatr 1981; 98:112-17.

5. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. Arch Neurol 1976; 36:308-13.

6. Adde L, Rygg M, Lossius K, Oberg GK, Stoen R. General movements assessment: Predicting cerebral palsy in clinical practise. Early Hum Dev 2007; 83:13-18.

7. Prechtl HFR, Einspieler C, Cioni G, Bos AF, Ferrari F, Sontheimer D. An early marker for neurological deficits after perinatal brain lesions. Lancet 1997; 349:1361-63.

8. Hajnal BL, Sahebkar-Moghaddam F, Barnwell AJ, Barkovich AJ, Ferriero DM. Early prediction of neurological outcome after perinatal depression. Pediatr Neurol 1999; 21:788-93.

9. Darrah J, Redfern L, Maguire TO, Beaulne P, Watt J. Intra-individual stability of rate of gross motor development in full-term infants. Early Hum Dev 1998; 52:169-179.

10. Alin-Åkerman B, Nordberg L. Griffith's utvecklingsskalor I och II. Manual för administering och utvärdering. Göteborg: PsykologiFörlaget AB, 1991.

11. Piper MC, Darrah J. Motor Assessment of the Developing Infant. Philadelphia: WB Saunders, 1994.

12. Darrah J, Piper M, Watt M. Assessment of gross motor skills of at risk infants: predictive validity of the Alberta Infant Motor Scale. Dev Med Child Neurol 1998; 40:485-491.

13. Einspieler C, Prechtl HFR. Prechlt's assessment of general movements: a diagnostic tool for the functional assessment of young nervous system. Ment Retard Dev Disabil Res Rev 2005; 11:61-7.

14. Ferrari F, Todeschini A, Guidotti I, Martinez-Biarge M, Roversi MF, Berardi A et al. General movements in full-term infants with perinatal asphyxia are related to basal ganglia and thalamic lesions. J Pediatr 2011; 158: 904-911.

15. Dilenge M, Majnemer A, Shevell MI. Long-term developmental outcome of asphyxiated term neonates. J Child Neurol 2001; 16:781-792.

16. van Schie PEM, Becher JG, Dallmeijer AJ, Barkhof F, van Weissenbruch MM, Vermeulen RJ. Motor outcome at the age one after perinatal hypoxic-ischemic encephalopathy. Neuropediatrics 2007; 38:71-77.

17. Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, Malan AF. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. Acta Paediatr 1997; 86: 757-761.

18. Horn AR, Swingler GH, Myer L, Linley LL, Raban MS, Joolay Y, Harrison MC, Chandrasekaran M, Rhoda NR, Robertson NJ. Early clinical signs in neonates with hypoxic ischemic encephalopathy predict an abnormal amplitude-integrated electroencephalopathy at age 6 hours. BMC Pediatrics 2013; 13: 52

19. Levene KH, Fenton AC, Evans DH, Archer LNJ, Shortland BD, Gibson NA. Severe birth asphyxia and abnormal cerebral blood-flow velocity. Dev Med Child Neurol 1989; 31.427-434.

20. Stark JE, Seibert JJ. Cerebral artery Doppler ultrasonography for prediction of outcomes after perinatal asphyxia. J Ultrasound Med 1994; 13:595-600.

21. Julkunen MK, Uotila J, Eriksson K, Janas M, Luukkaala T, Tammela O. Obstetric parameters and Doppler findings in cerebral circulation as predictors of 1 year neurodevelopmental outcome in asphyxiated infants. J Perinatol 2012; 32: 631-8.

22. Cioni G, Prechtl HF, Ferrari F, Paolicelli PB, Einspieler C, Roversi MF. Which better predicts later outcome in full-term infants: quality of general movements or neurological examination. Early Hum Dev 1997; 50:71-85.

23. Nakajima Y, Einspieler C, Marschik PB, Bos AF, Prechtl HFR. Does a detailed assessment of poor repertoire general movements help to identify those infants who will develop normally? Early Hum Dev 2006; 82:53-59.

24. van Kojii BJM, van Handel M, Nievelstein RAJ, Groenendaal F, Jongmans MJ, de Vries LS. Serial MRI and neurodevelopmental outcome in 9-to 10-year-old children with neonatal encephalopathy. J Pediatr 2010; 157: 221-7.

25. Pai-jun ML, Campbell SK. Examination of the item structure of the Alberta Infant Motor Scale. Pediatr Phys Ther 2004; 16:31-38.

Table 1. Clinical characteristics of the asphyxiated and control infants.

	Asphyxia group (n = 30)	Control group (n = 30)	p value*
Birth weight (g), median (range)	3325 (2295–4240)	3676 (2920–4440)	0.004
SGA, (n) (%)	1 (3.3)	0 (0)	0.317
Gestational age (wk), median (range)	39 (37–42)	40 (37–42)	0.311
Male/female ratio	26/4	27/3	0.690
Delivery route			< 0.001
Vaginal, (n) (%)	13 (43.3)	29 (96.7)	
Cesarean section, (n) (%)	17 (56.7)	1 (3.3)	
Apgar scores at 5 min, median (range)	4 (1–5)	9 (8–10)	< 0.001
Cord blood pH 7.05 or less, (n) (%)**	8 (27.6)	0 (0)	0.012
Cord blood base deficit 10 or more, (n) (%)***	8 (32.0)	1 (3.8)	0.021
HIE			
No HIE, (n) (%)	9 (30.0)		
Grade 1, (n) (%)	11 (36.7)		
Grade 2–3, (n) (%)	10 (33.3)		

Abbreviations: HIE, hypoxic-ischemic encephalopathy; SGA, small for gestational age.

* Asphyxia group vs control group

** One pH was not available in the asphyxia group and two in the control group.

*** Five cord blood base deficits were not available in both study groups.

Table 2. Neurodevelopmental assessment findings in the study groups.

	Asphyxia group (n = 30)	Control group (n = 30)
GMs at 0 months*		
Normal, (n) (%)	4 (16)	14 (58)
PR, (n) (%)	21 (84)	10 (42)
GMs at 3 months**		
Normal, (n) (%)	24 (88.9)	19 (95)
Abnormal, (n) (%)	3 (11.1)	1 (5)
AIMS at 3 months***		
Normal, (n) (%)	24 (85.7)	20 (100)
≤ 10th centile, (n) (%)	4 (14.3)	0 (0)
AIMS at 6 months****		
Normal, (n) (%)	19 (76)	19 (95)
≤ 10th centile, (n) (%)	6 (24)	1 (5)
AIMS at 12 months*****		
Normal, (n) (%)	19 (79.2)	22 (95.7)
≤ 5th centile, (n) (%)	5 (20.8)	1 (4.3)
NMS at 3 months******		
Normal, (n) (%)	18 (75)	15 (93.7)
≥ 3 scores, (n) (%)	6 (25)	1 (6.3)

* Five missing data in the asphyxia group and six in the control group.

** Three missing data in the asphyxia group and ten in the control group.

*** Two missing data in the asphyxia group and ten in the control group.

**** Five missing data in the asphyxia group and ten in the control group.

***** Six missing data in the asphyxia group and seven in the control group.

****** Six missing data in the asphyxia group and fourteen in the control group.

PR, poor repertoire

GMs

Table 3. Summary of GMs, AIMS, NMS, Doppler and MRI findings in asphyxiated infants with poor 1-year outcome

Cases	HIE grade	GMs at 0 month	GMs at 3 months	AIMS at 3 months ≤ 10th centile	AIMS at 6 months ≤10th centile	AIMS at 12 months ≤5th centile	NMS at 3 months ≥ 3 scores	High sCBFV* by Doppler at 24 hours	MRI at 1 year	Outcome at 1 year of age
1	2	PR*	Abnormal	Yes		Yes	Yes	Yes	Normal	Motor disability
2	2	PR	Abnormal	Yes	Yes	Yes	No	Yes	Normal	Motor disability
3	2	PR	Abnormal	No	Yes	Yes	No	Yes	Abnormal	Motor disability
4	3	PR	Abnormal	Yes	Yes	Yes	Yes	Yes	Abnormal	Mental and motor disability
5		PR	Normal	No	Yes		No	No	Normal	Motor disability

* PR, Poor repertoire GMs; sCBFV, systolic cerebral blood flow velocity

Table 4. Predictive values of neurodevelopmental assessments (GMs, AIMS, NMS) and HIE in predicting poor and good 1-year outcome in asphyxiated infants[a].

	Sensitivity [95 %CI]	Specificity [95 %CI]	Positive predictive value [95 %CI]	Negative predictive value 95 %CI
Ability to predict poor outcome				
GMs at 0 month (PR/normal)[b], (n=23)	100% [51–100]	16% [6–38]	20% [8–42]	100% [44-100]
GMs at 3 months (abnormal/normal), (n=25)	75% [30–95]	95% [77–99]	75% [30–95]	95% [77–99]
AIMS at 3 months ≤ 10th centile, (n=26)	60% [23–88]	100% [84–100]	100% [44–100]	91% [73–98]
AIMS at 6 months ≤ 10th centile, (n=23)	100% [51–100]	89% [69–97]	67% [30–90]	100% [82–100]
AIMS at 12 monts ≤ 5th centile, (n=24)	100% [51–100]	95% [76–99]	80% [38–96]	100% [83–100]
NMS at 3 months ≥ 3 scores, (n=23)	40% [12–77]	78% [55–91]	33% [10–70]	82% [59–94]
HIE (grade 2–3), (n=27)	83% [44–97]	76% [55–89]	50% [24–76]	94% [73–99]
Ability to predict good outcome				
GMs at 3 months (normal/abnormal)	95% [77–99]	75% [30–95]	95% [77–99]	75% [30–99]
AIMS at 3 months > 10th centile, (n=26)	100% [84–100]	60% [23–88]	91% [73–98]	100% [44–100]
AIMS at 6 months > 10th centile, (n = 23)	89% [69–97]	100% [51–100]	100% [82–100]	67% [30–90]
AIMS at 12 monts ≤ 5th centile, (n=24)				
HIE grade 0–1, (n= 27)	76% [55–89]	83% [44–97]	94% [73–99]	50% [38–96]

[a] Three of the 30 asphyxiated infants were lost to follow-up.
[b] PR, poor repertoire

12