DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
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HYPOXIC-ISCHAEMIC ENCEPHALOPATHY IN ASPHYXIATED TERM INFANTS

A prospective clinical, biochemical, ultrasonographical study

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Tartu Ülikooli Kirjanduse trükikoda
Tiigi 78, Tartu 50410
Tellimus nr. 475

To my family

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by Roman numerals:

I. P. Ilves, M. Blennow, E. Kütt, M-L. Mägi, G. Kudrjavtseva, H. Lagercrantz, T. Talvik. Concentrations of magnesium and ionized calcium in umbilical cord blood in distressed term newborn infants with hypoxic-ischemic encephalopathy. Acta Paediatrica 1996; 85: 1348–50.

II. P. Ilves, M. Kiisk, T. Soopõld, T. Talvik. Serum total magnesium and ionized calcium concentrations in asphyxiated term infants with hypoxic-ischaemic encephalopathy. (submitted for publication).

III. P. Ilves, R. Talvik, T. Talvik. Changes in Doppler ultrasonography in asphyxiated term infants with hypoxic-ischaemic encephalopathy. Acta Paediatrica 1998; 87: 680–4.

IV. P. Ilves, M. Thetloff, T. Talvik. Cerebral blood flow velocities in term newborn infants in the first week of life. Eesti Arst 1997; 4: 299–303 (in Estonian).

V. P. Ilves, T. Talvik. The cerebral blood flow velocity in term newborn infants with hypoxic-ischemic encephalopathy. Eesti Arst 1999; 1: 13–7 (in Estonian).

In addition, some previously unpublished data will be presented.

ABBREVIATIONS

AMPA	alpha-amino-hydroxy-5-methyl-4-isoxazole propionic acid
ATP	adenosine triphosphate
Ca^{2+}	calcium ion
CBF	cerebral blood flow
CNS	central nervous system
CP	cerebral palsy
CT	computed tomography
EEG	electroencephalography
HIE	hypoxic-ischaemic encephalopathy
ICAM-1	intercellular adhesion molecule-1
ICU	intensive care unit
IL-1β	interleukin-1(beta)
IL-1ra	interleukin-1 receptor antagonist
IL-6	interleukin-6
K^+	potassium ion
Mg	serum total magnesium
Mg^{2+}	magnesium ion
Na^+	sodium ion
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
O_2	oxygen
OFR	oxygen free radicals
PCD	programmed cell death
PIVH	peri-intraventricular haemorrhage
RI	resistance index
Vd	diastolic cerebral blood flow velocity
VLBW	very low birth weight
Vm	time mean of cerebral blood flow velocity
Vs	systolic cerebral blood flow velocity

1. INTRODUCTION

Perinatal hypoxic-ischaemic brain injury as a result of birth asphyxia remains a major cause of neonatal morbidity and infant mortality (Volpe, 1995). According to World Health Organization data, about 4 million newborn infants worldwide are affected annually, and of these infants about 1 million die, and an approximately equal number develop sequelae such as cerebral palsy (CP), mental retardation and epilepsy (World Health Organization, 1991). Already over a century ago (1862), W. J. Little recognised the association between difficult and premature delivery and neonatal asphyxia, and the later mental and physical condition of the child (Little, 1862). S. Freud (1897) suggested that antenatal factors are superior to intranatal ones in the development of CP. Up to the present day there exists no consensus regarding the importance of prenatal and perinatal events in the development of brain damage in asphyxiated infants (Nelson and Ellenberg, 1985 and 1986; Talvik, 1992; Volpe, 1995; Badawi *et al.*, 1998; Nelson *et al.*, 1998).

During asphyxia the perinatal brain can be deprived of oxygen (O_2) by two major pathogenic mechanisms, by hypoxaemia, which is a diminished amount of O_2 in the blood supply, and ischaemia, which is a diminished amount of blood perfusing the brain (Volpe, 1995). The period of reperfusion has now been clearly demonstrated to be the time of the occurrence of the most deleterious consequences of asphyxia (Pulsinelli *et al.*, 1982; Fellman and Raivio, 1997; Delivoria-Papadopoulos and Mishra, 1998; Shadid *et al.*, 1998). Brain damage following hypoxic-ischaemic insults develops after a delay, being preceded by a free interval or temporary improvement of the clinical condition (Petito *et al.*, 1987; Siesjö *et al.*, 1995; Robertson and Edwards, 1998; Van Bel *et al.*, 1998). The development of successful strategies for the management of asphyxiated neonates will depend on improving the understanding of the mechanisms of neuronal damage after a hypoxic-ischaemic cerebral insult.

The time before the full development of brain damage represents a window of opportunity for therapeutic interventions (Volpe, 1995; Robertson and Edwards, 1998). It is therefore essential to assess the severity of asphyxia in the newborn infant at birth or during the first hours after asphyxia to provide adequate care and treatment before the final damage develops. So far there are no suitable markers of hypoxic-ischaemic injury available during the first 24 hours after asphyxia. There is an urgent need to develop techniques with a very high sensitivity and specificity for the early detection of the development of major neuronal necrosis (Volpe, 1995). The goal of investigators is to identify infants with a serious threat of irreversible neurologic injury in order to commence adequate treatment and early habilitation (Ruth, 1988; Allen and Riviello, 1992; Nelson and Emery, 1993). On the other hand we must avoid an increase in the risk of the adverse effects of diagnostic or therapeutic inter-

ventions for the large majority of infants not at threat from asphyxia (Ruth, 1988; Allen and Riviello, 1992; Nelson and Emery, 1993).

The basis of this series of studies is the realisation that what are required are both objective measures of the severity of asphyxia and also prognostic indicators of perinatal brain damage, which are available during the first hours after a hypoxic-ischaemic insult, providing the opportunity for therapeutic intervention.

2. REVIEW OF THE LITERATURE

2.1. Definition of asphyxia

The word "asphyxia" is of Greek derivation and originally indicated the absence of a pulse. Although the term "asphyxia" is widely used as a clinical diagnosis, there is little consensus as to what it means. Hypoxaemia is defined as low O_2 content in arterial blood, tissue hypoxia implies O_2 deficiency with altered or interrupted cellular energy metabolism and ischaemia is the diminished amount of blood perfusing the organ (Rootwelt, 1995; Volpe, 1995).

The term "birth or perinatal asphyxia" means impaired placental or pulmonary gas exchange during or soon after labour, which results in hypoxaemia and hypercapnia with a respiratory and eventually a mixed respiratory-metabolic acidosis (Rootwelt, 1995). In significant perinatal asphyxia there is always a variable degree of (relative) ischaemia from myocardial depression and redistribution of blood flow with subsequent hypoxic-ischaemic organ damage (Davies and Tweed, 1984; Perlman *et al.,* 1989; Pasternak and Gorey, 1998). On the other hand, ischaemia may be the primary disturbance resulting in impairment of materno-foetal gas exchange (Ruth, 1988). Thus the term "birth asphyxia" is generally used to describe a combination of hypoxia, hypercarbia and ischaemia (Hill and Volpe, 1982). In 1976 Sarnat and Sarnat developed a clinical scoring system for term infants with hypoxic-ischaemic injury and stages of hypoxic-ischaemic encephalopathy (HIE) are now widely used to describe the severity of a hypoxic-ischaemic insult.

2.2. Etiology

Impaired gas exchange may be of foetal, maternal or placental origin. Foetal causes are interruption of umbilical circulation (cord compression or other accidents), anaemia or cardiac decompensation. Maternal causes include abnormal uterine contractions, cardiovascular disease with forward cardiac failure, severe anaemia, and maternal hypotension or hypertension from any cause. Placental causes of asphyxia are abruption, infection and infarction (Rootwelt, 1995; Volpe, 1995). Failure of the neonate to accomplish lung inflation and a successful transiton of circulation from foetal to neonatal life may be the causes of postnatal asphyxia (Ruth, 1988).

In utero, total cessation of gas exchange is rare, but varying degrees of hypoxia and transient disturbances in materno-foetal respiratory exchange are common in all labours (Winkler *et al.,* 1991). An intense insult such as cord prolapse or massive placental abruption causes the foetus to be rapidly compromised. A less intense insult such as intermittent placental insufficiency

associated with hypertonic uterine contractions will need a longer duration to have a similar severe effect on the foetus. It is often not possible to know clinically either the degree of intensity or the duration of intrapartum insult (Volpe, 1995).

2.3. Incidence of asphyxia

Since the clinical definition of birth asphyxia is not uniform, there is a large variation in reported incidence, as well as in outcome. In the developed countries, severe birth asphyxia is a relatively rare event, with an incidence of between 2 to 9 cases per thousand in full term infants (MacDonald *et al.*, 1980; Nelson and Ellenberg, 1981; Finer *et al.*, 1983; Ergander *et al.*, 1983; Levene *et al.*, 1985; Thornberg *et al.*, 1995). Death from birth asphyxia occurs in 0.26 per 1000, and 0.2 per 1000 have neurological impairments (Thornberg *et al.*, 1995). In developing countries the incidence of birth asphyxia is much higher. In Estonia the incidence of asphyxia is about 5–6% (Talvik, 1992). Epidemiological studies use depression of neonatal vitality, mainly low Apgar scores, to define asphyxia.

Table 1. Incidence of asphyxia, evaluated according to different criteria.

Marker	Incidence	Author
An umbilical artery base deficit >12 mmol/l	2.3 %	Low *et al.*, 1995
Apgar score ≤3 at 1 min in infants with birth weight >2500g	4.5%	Nelson and Ellenberg, 1981
Apgar score ≤3 at 5 min in infants with birth weight >2500g	0.9%	Nelson and Ellenberg, 1981
	0.17%	Ergander *et al.*, 1983
Apgar score ≤3 at 1min and/or <7 at 5 min in infants with birth weight >2500g	1.24%	Palme-Kilander, 1992
Apgar score <7 at 5 min	0.69%	Thornberg *et al.*, 1995
Development of HIE	0.36 %	Finer *et al.*, 1983
	0.6%	Levene *et al.*, 1985
	0.18%	Thornberg *et al.*, 1995
Positive pressure ventilation >1min	0.5%	MacDonald *et al.*, 1980

2.4. Diagnostic criteria for perinatal asphyxia

Several symptoms are associated with birth asphyxia, but no single sign is both specific for asphyxia and sufficient to predict CP (Freeman and Nelson, 1988; Nelson and Emery, 1993; Ekert *et al.,* 1997; Badawi *et al.,* 1998). Although many babies who exhibit only one or another sign suggestive of birth asphyxia do not differ much in risk from the total population, there might be some babies who exhibit a whole constellation of these characteristics and are especially likely to have a sufficient hypoxic-ischaemic insult (Nelson and Emery, 1993; Ekert *et al.,* 1997; Badawi *et al.,* 1998). An intervention during labour will also reduce the association between different risk factors and an adverse outcome, as compared with allowing events to take their natural course (Steer *et al.,* 1989). For diagnosis of asphyxia, multiple criteria indicating the concomitant existence of foetal and neonatal distress and the development of neonatal neurological symptoms are required (Volpe, 1995; Blennow, 1995; Ekert *et al.,* 1997; Badawi *et al.,* 1998).

2.4.1. Intrapartum criteria

2.4.1.1 Passage of meconium

Meconium is passed prior to delivery in 7 to 22% of full-term infants (Freeman and Nelson, 1988; Steer *et al.,* 1989; Katz and Bowes, 1992). However, meconium aspiration syndrome develops in only 0.2–1% of all live-born infants and severe meconium aspiration syndrome in one third of these cases (Katz and Bowes, 1992). Therefore the history of meconium-staining in amniotic fluid alone does not prove that a term infant experienced a degree of asphyxia sufficient to account for later neurologic abnormalities (Nelson and Ellenberg, 1984; Freeman and Nelson, 1988). There is some evidence that meconium aspiration syndrome may be a prenatal rather than postnatal event especially in fatal cases (Thureen *et al.,* 1997).

But there may be subgroups of infants with meconium-stained amniotic fluids, in whom meconium-staining of amniotic fluids is a more specific symptom for asphyxia (Nelson and Emery, 1993). Heavy meconium staining may be an indication of more severe or repeated foetal stress (Katz and Bowes, 1992). An early (more than one hour prior to delivery) passage of the meconium is a serious indication of intrapartum distress (Blennow, 1995). Meconium-staining of the amniotic membranes begins 1 to 3 hours after the foetus passes meconium (Katz and Bowes, 1992) and staining of the skin or umbilical cord suggest that the insult occurred at least four to six hours prior to membrane rupture (Freeman and Nelson, 1988). The combination of meconium-staining fluids with changes in acid-base status and foetal heart rate will increase the specificity of this marker (Steer, 1989; Katz and Bowes, 1992).

2.4.1.2. Changes in foetal heart rate

Foetal heart rate monitoring during labour was designed as a screening test to discover early changes in foetal heart rate, which might give evidence of asphyxia in the uterus. This detection would hopefully allow intervention before asphyxia sufficient to produce irreversible encephalopathy or death has occurred. Intrapartum cardiotocography, if properly used, is a sensitive indicator of foetal compromise (Nelson and Emery, 1993; Ekert *et al.,* 1997; Low *et al.,* 1999). Unfortunately, cardiotocography is not a very specific method and an abnormal cardiotocogram occurred at some stage in 41% of all labours (Steer *et al.,* 1989). The poor specificity must be improved by appropriately timed scalp blood sampling in carefully selected cases: at least two-thirds of abnormal cardiotocograms are associated with a normal pH (Steer *et al.,* 1989; Richards *et al.,* 1993, Low *et al.,* 1999). A normal cardiotocogram is highly predictive of normal pH (Steer *et al.,* 1989). It is believed that experience in interpretation of the cardiotocogram is particularly important (Murphy *et al.,* 1990, Low *et al.,* 1999).

2.4.1.3. pH and other acid-base measures

Low pH and changes in other acid-base measurements has been considered the best widely-available measure for the identification of asphyxia during labour and after delivery (Tammpere, 1984; Mälksoo, 1988; Talvik, 1992; Nelson and Emery, 1993; Ekert *et al.,* 1997; Low, 1997; Badawi *et al.,* 1998). Acidosis at birth may give us an indication of the severity and duration of the preceding asphyxia, and reflects the cumulative events that have occurred during labour (Tammpere, 1984; Mälksoo, 1988; Goldaber *et al.,* 1991; Low, 1993). A normal vaginal labour with a clinically normal foetus may, however, also affect foetal blood gas and acid base status (Vannucci and Duffy, 1974). Anaerobic metabolism with the generation of lactic acid is a normal physiological response during episodes of hypoxaemia in normal labour (Winkler *et al.,* 1991). A pH level 7.2 can be taken as evidence that substantial asphyxia has not recently occurred. Neonatal acidaemia is defined as an umbilical artery pH<7.15 or 7.20 because these values are 2 SD below the mean umbilical artery pH of normal infants (Freeman and Nelson, 1988; Winkler *et al.,* 1991).

Yet most acidotic babies do not become neurologically symptomatic, and the majority of neurologically symptomatic infants are not markedly acidotic (Ruth, 1988; Nelson and Emery, 1993). Only a severe decrease in pH and base excess correlates with severity of asphyxia (Levene *et al.,* 1985; Ekert *et al.,* 1997; Badawi *et al.,* 1998). Even most infants with pH as low as <7.0 demonstrate no evidence of brain damage, although the risk of complications is greater in these infants (Gilstrap *et al.,* 1989; Goldaber *et al.,* 1991; Perlman *et*

al., 1993; Ekert *et al.*, 1997; Badawi *et al.*, 1998). In infants whose pH was <7.05 developed seizures in 1% of cases, while 9.2% of infants whose pH was <7.0 had otherwise unexplained neonatal seizures (Nelson and Emery, 1993).

Gilstrap and colleague (1989) suggested that newborn infants are at risk for immediate complications which result from intrapartum asphyxia only when the umbilical artery pH is <7.0 and Apgar scores are <4 at both 1 and 5 minutes. Acidosis alone is not highly specific for asphyxia (Ruth, 1988). The acid-base status of the infant is also influenced by the acid-base status of the mother (Richards *et al.*, 1993). But on the other hand cord blood acid-base status is also a useful tool in excluding intrapartum asphyxia as a cause of low Apgar scores, particularly in premature infants (Richards *et al.*, 1993).

2.4.2. Neonatal criteria at birth

2.4.2.1. Apgar scores

The Apgar score was initially developed to identify quickly the newborn infant in need of resuscitation (Apgar, 1953). Five signs were chosen which could be determined easily and without interfering with the care of the infant: heart rate, respiratory effort, reflexes, muscle tone and colour. The most practical time for judging these signs appeared to be 1 minute after birth (Apgar, 1953). Later, Drage and coworkers (1964) recommended reassessment of the infant 5 minutes after birth.

Birth asphyxia is not the only cause of depressed Apgar scores: such factors include immaturity, muscle disease, central nervous system (CNS) abnormalities and infection, cardiac and respiratory problems, maternal medications, sepsis, trauma, and problems with resuscitation (Freeman and Nelson, 1988; Richards *et al.*, 1993). Low Apgar score may be also a consequence of pre-existing neurological impairment (Nelson and Emery, 1993).

Yet in the case of abnormal intrapartum cardiotocogram, low pH and Apgar score at birth, a strong association exists with poor postnatal outcome (Tammpere, 1984; Mälksoo, 1988; Sööt, 1989; Murphy *et al.*, 1990; Steer *et al.*, 1989; Talvik, 1992; Nelson and Emery, 1993; Ekert *et al.*, 1997; Badawi *et al.*, 1998). The Apgar score apparently predicts the outcome at least as well as pH, but may offer somewhat different information. Studies have shown that only 30–40% of foetuses with low Apgar scores are acidotic at delivery (Richards *et al.*, 1993). A foetus with severe asphyxia just before birth may be born with low Apgar scores, but may improve quickly and probably have a low risk of suffering permanent brain damage. A foetus with severe asphyxia, which may cause permanent brain damage, may be stablized by the time of birth, and his Apgar score may therefore be normal (Levene, 1995).

6–8% of surviving infants have a one-minute Apgar score of 3 or less (Nelson and Ellenberg, 1979). A low one-minute Apgar score indicates an infant in need of resuscitation. If a nonbreathing infant is left to lie unattended then whatever the cause of the nonbreathing, that infant will rapidly become hypoxic. The one- minute Apgar score is closely related to neonatal mortality, but will not influence the condition of the child later (Tammpere, 1984; Mälksoo, 1988; Sööt, 1989; Talvik, 1992). A rapid improvement in Apgar scores after 5 and 10 minutes makes it very unlikely that the insult was sufficiently severe to cause permanent brain damage (Freeman and Nelson, 1988).

A correlation exists between the Apgar score at the 5th minute of life and the severity of HIE and cerebral palsy at the age of 12 months (Tammpere, 1984; Mälksoo, 1988; Sööt, 1989; Talvik, 1992; Ekert *et al.,* 1997). The 5th minute score is of greater importance in estimating the damage of CNS, reflecting the efficiency of resuscitation as well as the infants' condition at birth (Tammpere, 1984; Mälksoo, 1988; Sööt, 1989; Talvik, 1992). Even an Apgar score of 7 at the 5th minute of life characterises asphyxia and is an indication to include the baby in the risk group (Tammpere, 1984; Mälksoo, 1988; Sööt, 1989; Talvik, 1992). There is a relationship between the 5-minute Apgar score and the outcome in children who develop neonatal seizures (Holden *et al.,* 1982; Talvik, 1992).

Infants with prolonged (10 min) very low Apgar scores (≤3) have a dramatically increased mortality (30–50%), and there is a high risk of CP in survivors (Thomson *et al.,* 1977; Ergander *et al.,* 1983; Freeman and Nelson, 1988).

Jain and coworkers (1991) have found that 39% of apparently stillborn infants (1-min Apgar score 0) survived the neonatal period and 61% of those who survived had normal development. If the Apgar score was 0 at 5 minutes, only 15,9% survived and had a 25% chance of having normal findings upon neurological examination. However, if the 5-minute Apgar score had improved to between 4 to 7, the chance of having normal findings increased to 80%. However, only one infant out of 59 (1.7%) with a 10 minute Apgar score of 0 survived, and this infant was also brain damaged.

2.4.2.2. Delayed onset of spontaneous respiration and need for resuscitation

Time until the onset of spontaneous respiration has been used as an indicator of asphyxia, and identifies a group in need of resuscitation (MacDonald *et al.,* 1980; Ekert *et al.,* 1997). There are several causes other than asphyxia which cause a delay in spontaneous respiration, such as trauma, narcotics, immaturity, sepsis, muscle hypotonia, anaemia, congenital anomalies of the lungs, heart and CNS. If the baby needs ventilation for any cause for more than 1 minute, the risk of mortality increases 100 times (MacDonald *et al.,* 1980).

The incidence of severe adverse outcome (death or severe disability) for the delayed onset of breathing of 10–19 min was 56% and >20 minutes 88% (Ekert *et al.,* 1997). In the other study in the case of an absence of respiratory efforts for 20–45 minutes, 75% of the infants died or survived with permanent brain damage, whereas of infants with no spontaneous respiration for 45 minutes, all died (Thornberg *et al.,* 1995).

Most infants need only bag and mask ventilation for resuscitation, a need for epinephrine administration and cardiac massage during resuscitation are associated with a poor prognosis (Perlman and Risser, 1993; Thornberg *et al.,* 1995; Ekert *et al.,* 1997; Badawi *et al.,* 1998). 95% of infants who needed only bag and mask ventilation were later healthy, in contrast to only 1 of 11 infants in whom resuscitation included cardiac massage and adrenaline (Perlman and Risser, 1993; Thornberg *et al.,* 1995).

2.4.3. Hypoxic-ischaemic encephalopathy

Sarnat and Sarnat (1976) introduced an assessment of the severity of asphyxia in term infants on the basis of clinical symptoms developing during the first days after birth. HIE is a consistent pattern of neurological signs which progress in a regular manner (Volpe, 1995). The use of HIE stages in preterm infants was introduced in 1995 by Talvik and coworkers. HIE can be classified as mild with irritability and brisk reflexes during the first days after birth. Infants with a moderate degree of HIE are lethargic, hypotonic with decreased spontaneous movements and primitive reflexes with or without seizures. Flaccid comatose infants with absent primitive reflexes and hardly treatable seizures or without seizures are diagnosed as having the severe stage of HIE (Sarnat and Sarnat, 1976). During recent years a falling incidence of HIE in term infants has been noted in developed countries and is thought to be as low as 1–2 per 1000 (Thornberg *et al.,* 1995). The mortality rate of term infants with HIE is 10% (Levene *et al.,* 1985).

HIE is probably the best diagnostic and predictive tool in asphyxiated term infants (Levene *et al.,* 1986; Nelson and Ellenberg, 1986; Mälksoo, 1988; Sööt, 1989; Tammpere, 1984; Talvik, 1992; Rootwelt, 1995) and even in preterm babies (Sööt, 1989; Talvik, 1992; Talvik *et al.,* 1995). The severity and duration of the perinatal asphyxia are obviously critical in developing HIE (Volpe, 1995; Badawi *et al.,* 1998). It is likely that intrapartum asphyxia severe enough to cause a neurodevelopmental handicap later in life will be associated with clinical neurological dysfunction during the first days of life, and if no abnormalities occur the newborn infant appears to be at little risk (Freeman and Nelson, 1988; Volpe, 1995). It is suggested that prenatal compromise play an important role in developing HIE (Badawi *et al.,* 1998). Compromised babies may have already developed encephalopathy by the onset of labour, and may

have a diminished reserve to cope with hypoxia during labour (Badawi *et al.,* 1998). It is believed that to consider intrapartum insult, the likely cause of neonatal brain injury, the neonate must have some evidence of foetal distress, depression at birth and neonatal neurological syndrome (Volpe, 1995, Blennow, 1995; Badawi *et al.,* 1998).

The severity of HIE correlates directly with the incidence of neurological sequelae (Tammpere, 1984; Mälksoo, 1988; Sööt, 1989; Talvik, 1992; Robertson and Finer, 1993; Low, 1993; Talvik *et al.,* 1995). Asphyxiated infants without the development of signs of HIE or with mild HIE do not have or have a very low risk of permanent neurological sequelae (Robertson and Finer, 1985; Levene *et al.,* 1985; Thornberg *et al.,* 1995). Infants with moderate HIE have a 20–30% possibility of a severe handicap, and almost all infants with severe HIE (75–90%) die, and survivors develop severe neurological damage (Levene et al, 1985; Robertson *et al.,* 1993; Thornberg *et al.,* 1995).

Unfortunately encephalopathy as well as a low Apgar score, is a clinical entity and several conditions apart from birth asphyxia, such as antepartum events, metabolic diseases, cerebral malformations, trauma and infection can also give rise to the condition (Levene *et al.,* 1985). The clinical picture also develops over the first days of life, being the most expressed after 1–2 days, at a time when it is too late to start brain saving treatment (Freeman and Nelson; 1988; Blennow, 1995; Volpe, 1995). The exact clinical assessment is also complicated, especially in infants who need mechanical ventilation and in infants who need sedation or muscle relaxation (Robertson and Finer, 1985; Blennow, 1995; Thornberg *et al.,* 1995). Some technical methods such as ultrasonography, electroencephalography (EEG), evoked potentials may help in the evaluation of correlation between morphological changes and clinical stages of HIE.

2.4.3.1. Seizures

Although seizures are one part of the diagnosis of the severity of HIE (Sarnat and Sarnat, 1976), some authors suggest that seizures may be taken as independent criteria of the severity of hypoxic-ischaemic brain damage (Blennow, 1995; Ekert *et al.,* 1997). The seizures after asphyxia usually occur early, within the first 6 to 12 hours of life, increase in frequency over 24 to 36 hours, and are commonly resistant to anticonvulsant therapy (Finer *et al.,* 1983; Talvik, 1992; Bernes and Kaplan, 1994; Volpe, 1995; Selton and Andre 1997; Ekert *et al.,* 1997). The incidence rate of neonatal seizures, however, is 2.0 per 1000 live births in term neonates and HIE is the presumed cause in 40–65% of all seizures in newborn infants, infection in 20% and metabolic abnormalities in 20% (Bernes and Kaplan, 1994; Ronen *et al.,* 1999). Postasphyxial seizures are associated with a high incidence of severe sequelae (Mulligan *et al.,* 1980;

Talvik, 1992; Ekert *et al.,* 1997). The total mortality among infants with seizures was high, 41% (Thornberg *et al.,* 1995). Neonates with frequent seizures lasting for more than 30 minutes and resistant to treatment are more likely to die and they die earlier than those who have no prolonged seizures (Holden *et al.,* 1982; Finer *et al.,* 1983; Thornberg *et al.,* 1995). The incidence of seizures is greater in those asphyxiated infants who had not achieved spontaneous respiration within 5 minutes of age (Mulligan *et al.,* 1980; Thornberg *et al.,* 1995).

2.4.3.2. Multiorgan involvement

Severe asphyxia is likely to cause disturbances in a number of organ systems other than the brain (Perlman *et al.,* 1989; Volpe, 1995; Saugstad, 1996; Pasternak and Gorey, 1998). The circulatory response to intrauterine asphyxia in experimental animals resulted in the redistribution of cardiac output with a larger proportion of flow distributed to the cerebral, coronary and adrenal circulation and decreased perfusion to the kidneys, gastrointestinal tract and skin (Rudolph, 1984). The consequence of decreased perfusion may be an organ injury. Among severely asphyxiated infants (a 5-minute Apgar score ≤5) in one half of the infants multiple organ failure was noted (Perlman *et al.,* 1989). The severity of the organ injury is determined in part by the duration of the insult and by adaptive ability of the foetus to respond to hypoxic-ischaemic insult (Perlman *et al.* 1989; Pasternak and Gorey, 1998). In a severe, sudden disruption of substrate supply, injury of other organs than the brain may be subtle, but in more prolonged but less severe intrauterine asphyxia, in which shunting of blood flow from nonbrain organs to the brain occurs, nonbrain organs are more vulnerable (Pasternak and Gorey, 1998). Some authors believe that acute and transient compromise to more than one organ prove that an acute hypoxic-ishaemic event during labour was the common pathway by which these organs were affected (Freeman and Nelson, 1988).

The following systemic organ injuries beside brain were identified most often in asphyxiated and consequently intubated term infants (Perlman *et al.,* 1989; Martin-Ancel *et al.,* 1995):

- Renal abnormalities (40–50%);
- Cardiovascular complications (25–29%);
- Pulmonary complications, including persistent pulmonary hypertension (23–26%);
- Gastrointestinal complications (29%).

2.4.4. Other methods for evaluation of severity of hypoxic-ischaemic encephalopathy

Some methods such as electroencephalography (EEG), evoked potentials, brain imaging and methods to evaluate cerebral blood flow (CBF) and metabolism may help to diagnose the severity of HIE during the first day after asphyxia before a full clinical picture of HIE develops, when the damage may be partially reversible (Rootwelt 1995; Volpe, 1995).

The severity of **electroencephalographic abnormalities** and their duration have a prognostic importance. The EEG gives more information when recorded during the first 24–48 hours of life: a normal EEG implies a good prognosis, a very abnormal EEG background 12 hours after asphyxia precedes severe sequelae (Selton and Andre, 1997). An improvement of background activity before 7 days also indicates a good prognosis, whereas identical or worsened activity was noted in all cases with poor outcomes (Selton and Andre, 1997). Burst supression or isoelectric pattern on any day in an asphyxiated infant is virtually always associated with a poor outcome (Volpe, 1995).

Visual and somatosensory evoked potentials provide valuable prognostic information in asphyxiated term infants (Eken *et al.,* 1995). In one study of 20 survivors of asphyxia, all 13 infants with normal outcomes had normal somatosensory evoked responses by 4 days of age, whereas 7 infants with subsequent brain damage had abnormal or absent responses beyond 4 days (Gibson *et al.,* 1992).

Cerebral ultrasonography is widely used as a screening tool in asphyxiated term infants. The most common parenchymal abnormality in asphyxiated infants is diffuse hyperechogenicity. This so called "bright brain" with full development by the second day after birth is characterised by an excess of small high amplitude echoes, scattered diffusely, making some major structures indistinct. There are two elements of increased parenchymal echogenicity an increase in diffuse echogenicity and a consequent partial or complete obliteration of structures normally visible (Skeffington and Pearse, 1983; Siegel *et al.,* 1984). Ultrasonography is highly sensitive in the detection of injury in the basal ganglia and thalamus, and of focal ischemic lesions (Eken *et al.,* 1994; Volpe, 1995). Large periventricular echodensities and other parenchymal echodensities were indicative of the development of major degrees of leucomalacia and carried a bad prognosis: 50% of infants died and 80% of survivors had a residual neurological deficit, but none of the infants with a normal ultrasound died (Siegel *et al.,* 1984). However, the abnormal cranial ultrasound findings develop over the first days and thus may be normal in the first days of life (Stark and Seibert, 1994; Eken *et al.,* 1995). The positive predictive value of cranial sonogram diagnosis is 77% (Adcock *et al.,* 1998). Half of the failures to detect the primary diagnosis found during postmortem

study were the bad timing of the ultrasound in relation to the age of the lesion and/or the microscopic nature of the lesions.

Doppler ultrasonogography is a non-invasive method which allows repeated and safe assessment of neonatal cerebral hemodynamics. Changes in CBF velocity have been found to correlate with changes in CBF measurements in experimetal studies (Rosenberg *et al.,* 1985; Sonesson *et al.,* 1988; Haaland *et al.,* 1994) and in newborn infants (Greisen *et al.,* 1984). Measurements of CBF velocity using the Doppler technique have provided useful prognostic information in full term asphyxiated infants (Archer *et al.,* 1986; Levene *et al.,* 1989; Eken *et al.,* 1995). High CBF velocities and significantly lowered resistance indices at the age of 24–72 hours have been reported in asphyxiated infants with irreversible brain injury (Archer *et al.,* 1986; Levene *et al.,* 1989; Eken *et al.,* 1995). Levene and colleague (1989) have proposed that the high-risk group of patients are those with CBF velocity measurements <2SD or >3SD from the mean with a positive predictive value of 94% for death or severe impairment. Pryds and coworkers (1990) have also described increased CBF in asphyxiated infants with permanent brain damage, indicating that the Doppler measurements of increased CBF velocity in fact reflect increased volemic CBF in asphyxiated infants with poor outcome.

Computed tomography (CT) effectively defines the site and extent of focal ischaemic lesions and allows prediction of the neurological sequelae (Mälksoo, 1988; Sööt, 1989; Talvik, 1992), but CT may be normal on the first days of life, with the optimal time to be performed at 2 to 5 days after birth (Allen and Riviello, 1992; Blennow, 1995).

Magnetic resonance imaging (MRI) is found to be superior to CT and ultrasound scans in the recognition of parenchymal lesions like periventricular leucomalacia, basal ganglia hemorrhage, multicystic encephalomalacia, and focal parenchymal lesions, determining the prognosis well after 2 to 5 weeks (Allen and Riviello, 1992; Rutherford *et al.,* 1998). Newer techniques such as near infrared spectroscopy (van Bel *et al.,* 1993), magnetic resonance spectroscopy (Lorek *et al.,* 1994) and positron emission tomography (Blennow *et al.,* 1995) may prove to be of great value in the evaluation of the severity of hypoxic-ischaemic brain damage in asphyxiated infants, but are not available in all hospitals where asphyxiated children are born.

2.5. Neuropathologic manifestation

Experimental work on brain damage in asphyxiated newborn monkeys demonstrated that two different patterns of damage could be recognised, depending on whether the animals were subjected to either acute total asphyxia or prolonged partial asphyxia (Myers, 1972).

1. In total asphyxia, profound hypoxaemia and hypercapnia with severe acidosis develop quickly. Acute total asphyxia in the monkey was seldom followed by brain swelling, even when the insult produced structural changes in the brain. Neuropathological examination demonstrated neuronal necrosis mainly in the brainstem, thalamus and basal ganglia (Myers, 1972). This distribution of neuropathology in the brainstem and basal ganglia, whereas cerebral hemispheres and cerebellum appear normal, is occasionally observed in severely asphyxiated human foetuses and newborn (Low, 1993; Pasternak and Gorey, 1998). The distribution of injury in these infants reflects the hierarchy of metabolic needs that are unmet after a severe, sudden disruption of substrate supply. A higher metabolic rate of subcortical nuclei compared with the cerebral hemispheres explains the preponderance of subcortical damage, while injury to organs other than the brain may also be subtle (Pasternak and Gorey, 1998). The higher metabolic rate of the brain compared to other organs explains significant neonatal encephalopathy with relative sparing of nonbrain organs (Pasternak and Gorey, 1998). Anoxia in the human foetus is a rare event and usually results in the death of the foetus. The anoxic events must be recognised immediately, with intervention between 10 and 20 minutes to permit the resuscitation and survival of the newborn infant (Low, 1993; Volpe, 1995).

2. The effect of prolonged hypoxia on the foetal brain, with a lesser degree of hypoxaemia and hypercapnia than in anoxia, is different. The neuropathologic lesions in prolonged hypoxia were either a generalised or focal cerebral necrosis and principally involved the parasagittal regions, basal ganglia and thalamus (Myers, 1972). Initially the foetal brain is protected from the asphyxial insult. The key to the protection of the CNS is the adaptation of the foetal cardiovascular system, which by increasing CBF maintains cerebral O_2 metabolism (Low, 1993). However, if asphyxia persists, a point is reached when decompensation occurs. After 1–3 hours of hypoxia, acidosis and hypotension were present to the same extent as that caused by anoxia (Myers, 1972). Even a brief non-lethal ischaemic insult can produce severe neuronal damage in selectively vulnerable regions, when induced repeatedly at a certain interval and the severity of neuronal damage is dependent on the number and interval of ischaemic episodes (Kato and Kogure, 1990).

A striking relationship exists between the severity and duration of asphyxia and the subsequent severity of HIE and the distribution of neuropathologic findings in asphyxiated term newborns (Low, 1993; Volpe, 1995). Five major neuropathologic lesions can be recognised in term infants after birth asphyxia (Hill and Volpe, 1982; Low, 1993; Volpe, 1995):

- selective neuronal necrosis (the most common lesion after hypoxia in term infants, characterised by neuronal injury at specific sites in the cerebral cortex, diencephalon, brain stem, cerebellum, and spinal cord);

- parasagittal cerebral injury (major ischaemic lesion in term infants in "watershed" areas of major cerebral arteries);
- focal ischaemic brain necrosis;
- status marmoratus in the basal ganglia and thalamus (prominence of neuronal loss, astrocyte response, and hypermyelinisation of the basal ganglia);
- periventricular leucomalacia (periventricular white matter necrosis, rare in term neonates).

2.6. Foetal response to asphyxia

Transient hypoxaemia and hypercapnia, variable in severity and duration, are consistent occurrences during vaginal delivery and are associated with biochemical signs of hypoxic insult (Winkler *et al.*, 1991) as shown also in animal experiments with rats (Vannucci and Duffy, 1974). Even a slight "physiological" hypoxia associated with normal vaginal delivery produces a significant hormonal surge, which can be measured as increased plasma concentrations of catecholamines, renin, angiotensin and cortisol (Tammpere, 1984; Ruth, 1988; Mälksoo, 1988; Sööt, 1989; Talvik, 1992; Talvik *et al.*, 1995).

It is well known that newborn animals have relatively greater tolerance to anoxia than the mature at the same species (Fazekas *et al.*, 1941; Himwhich *et al.*, 1942). The minimum CBF necessary to sustain neuronal viability in newborn infants is also considerably lower than in adult humans, measured by positron emission tomography (Altman *et al.*, 1988). The biochemical and physiological bases for this relative resistance are not entirely understood. It is thought to be connected with a lower cerebral rate of energy utilisation (Vannucci and Jager, 1992), a greater ability to utilise lactate and ketone bodies for energy (Young *et al.*, 1991) and the resistance of the cardiovascular system of foetuses related to greater stores of myocardial glycogen (Wells *et al.*, 1972). But certain neuronal groups in the perinatal animal appear to be more vulnerable to asphyxia than similar neurons in the adult. This phenomenon seems to be related primarily to the activation of excitatory amino acid (EAA) receptors in such neuronal groups (Slater *et al.*, 1992; D'Souza *et al.*, 1993).

The key to the protection of the CNS is the adaptation of the foetal cardiovascular system, which by increasing CBF maintains cerebral O_2 metabolism (Koehler *et al.*, 1985; Pasternak and Gorey, 1998). The compensatory cardiovascular response of the foetus to hypoxemia is an increase of arterial pressure caused by increased systemic vascular resistance and initial increase of cardiac output (Koehler *et al.*, 1985; Ruth, 1988; Low, 1993). Redistribution of the cardiac output with reduced blood flow to the pulmonary, renal, and gastrointestinal circulation, but increased flow to the brain, heart, and adrenal glands is a major protective mechanism to maintain the perfusion of vital organs during asphyxia (Davies and Tweed, 1984; Koehler *et al.*, 1985).

The larger proportion of cardiac output is distributed to the brain with an increase of total and regional CBF and a loss of cerebral autoregulation (Cavazzuti and Duffy, 1982; Volpe, 1995, Pasternak and Gorey, 1998). The mechanisms underlying the elevated CBF in hypoxia-induced cerebral vasodilatation secondary to hypoxaemia and hypercapnia remain poorly understood (Leffler *et al.,* 1997). Cerebral vasodilatation may be related to the increased perivascular hydrogen ion and potassium concentration, elevated adenosine and arachidonic acid metabolites, and some other endothelial-dependent components may play a role in this cerebral vasodilatation (Armstead, 1995; Leffler *et al.,* 1997). The initial rise in blood pressure may play a role in the initial increase in CBF during asphyxia (Volpe, 1995). The role of nitric oxide (NO) in vasodilatation during asphyxia is not clear. Leffler and colleague (1997) suggest that endothelial-derived NO appears not to contribute to the hypoxia-induced cerebral vasodilatation. Possibly, NO is more important in the maintenance of cerebral vasodilatation over more prolonged hypoxia, being secondary to the elevation in opioids (Armstead, 1995). As hypoxia is prolonged, additional mechanisms may promote the maintenance of cerebral vasodilatation, maybe even replacing the mechanism that iniatiated the dilatation (Leffler *et al.,* 1997).

Beside the increase of total CBF, regional redistribution also occurs between different brain regions with preferential shunting of the blood to critical brain stem regions (Hernandez *et al.,* 1979; Low, 1993,). The increase in CBF is most marked in brain stem structures and least in cerebral white matter. This mechanism must maintain the integrity of vital brain stem structures (Volpe, 1995). The exact mechanism of such a redistribution of CBF in the brain is unknown. It is suggested that the redistribution of CBF is an important opioid-mediated homeostatic mechanism, which diminishes the metabolic requirements of the newer part of the brain and allows preferential perfusion of the vital structures of the brain stem (Lou *et al.,* 1985; Volpe, 1995).

But experimental data with foetal lambs strongly suggets that foetal CBF may not increase and may even full during severe asphyxia of rapid onset (such as complete occlusion of umbilical cord) (Bennet *et al.,* 1998). In case of near total intrauterine asphyxia in term infants such compensatory shunting of blood flow from nonbrain organs to the brain and from cerebral hemispheres to the thalamus and brainstem may not occur (Pasternak and Gorey, 1998).

If the hypoxaemia is also severe and longlasting with serious metabolic acidosis (pH less than 7.0), diminished cardiac output due to reduced contractility and bradycardia develops. Decompensation of the circulatory responses leads to systemic hypotension and ischaemia in different organs including brain (Perlman *et al.,* 1989). A serious impairment of cerebral vascular autoregulation leads to pressure-passive cerebral blood flow (Lou *et al.,* 1979; Yoshida-Shuto *et al.,* 1992). A deficit in CBF develops, particularly in the parasaggital regions of the cerebral hemispheres (Volpe, 1995).

2.7. Mechanisms of perinatal hypoxic-ischaemic brain damage

2.7.1. Primary cell death

Severe and prolonged hypoxia and ischaemia of any organ will result in cell death and tissue damage (Fellman and Raivio, 1997). The brain is especially sensitive to a decrease in cellular O_2 supply, with neurons in the selectively vulnerable parts of the brain being primarily affected (Siesjö *et al.*, 1995). After longer periods of cellular O_2 shortage, both neuronal populations as well as glial cells and the vascular endothelium are affected, with the development of pannecrosis of the brain (Siesjö *et al.*, 1995).

Glycose and O_2 are the two main substrates of cerebral metabolism. The primary cell death from O_2 deprivation begins with the disturbances of brain glucose and energy metabolism, resulting in the decreased development of adenosine triphosphate (ATP) through oxidative phosphorylation (Ruth, 1988; Volpe, 1995; Fellman and Raivio, 1997). In response to the anaerobic state, glycolysis is accelerated 5 to 10-fold in combination with glycogenolysis and increased uptake of glycose from the blood, with development of hypoglycemia and lactate accumulation (Kaasik *et al.*, 1970; Holowach-Thurston *et al.*, 1973; Fellman and Raivio, 1997). Despite this acceleration, ATP levels begin to fall, because only 2 molecules of ATP are generated for each molecule of glucose metabolised, instead of the 36 molecules generated by oxidative phosphorylation (Ruth, 1988; Gluckman and Williams, 1992; Fellman and Raivio, 1997). Eventually there is energy failure with a loss of ATP and phosphocreatine levels (Kaasik *et al.*, 1970; Lorek *et al.*, 1994; Fellman and Raivio, 1997). Due to energy failure, ionic gradients across the cell membrane cannot be maintained, partly as a result of ATP depletion and a loss of Na^+/K^+ -ATPase activity (Fellman and Raivio, 1997). Membrane depolarisation leads to an influx of Ca^{2+} and Na^+ into the cytoplasma through opened ion channels, accompanied by a passive influx of water and chloride leading to an osmotic swelling of the cells (Gluckman and Williams, 1992; Volpe, 1995; Fellman and Raivio, 1997). During the initial reperfusion phase, lasting 10 to 30 minutes, the primary intracellular oedema may resolve (Gluckman and Williams, 1992; Volpe, 1995). If the hypoxic-ischaemic insult is severe enough, these changes may become irreversible and lead to cell necrosis as a result of hypoxia-ischaemia alone (Fellman and Raivio, 1997). The more severe the hypoxic-ischaemic insult, the greater the "primary neuronal death" will be (Gluckman and Williams, 1992; Fellman and Raivio, 1997).

2.7.2. Reperfusion injury

2.7.2.1. Secondary cell death

Restoration of blood flow and O_2 delivery after asphyxia is necessary for organ survival, but is critical, because damage may progress during this period (Ito *et al.,* 1975; Pulsinelli *et al.,* 1982; Saugstad, 1996; Fellman and Raivio 1997). The onset of ischaemic cell changes during reperfusion is delayed by several days as described in animal experiments (Pulsinelli *et al.,* 1982) and also in humans (Petito *et al.,* 1987; van Bel *et al.,* 1993 and 1998) and is called delayed or "secondary " cell death phenomenon.

Cerebral energy metabolism may recover during reperfusion (within 2 hours) and may be normal during the following 24 hours, as shown in infants (Hanrahan *et al.,* 1996) and in animal models (Lorek *et al.,* 1994). But this improvement seen also in clinical picture may be temporary (Volpe, 1995). If the initial energy failure during asphyxia is sufficiently prolonged and severe, it is capable of triggering a series of additional mechanisms, which initiates a number of intracellular signalling events, such as an accumulation of excitatory amino acids (EAA), an increase in cytosolic Ca^{2+} and the generation of oxygen free radicals (OFR) leading to secondary delayed cell death without continuing energy failure (Volpe, 1995; Saugstad, 1996; Fellman and Raivio, 1997; Delivoria-Papadopoulos and Mishra, 1998; Shadid *et al.,* 1998).

After 24–48 hours a secondary or "delayed" energy failure develops, characterised by a progressive decrease in high energy phosphate levels, in spite of normal substrate supply, normal blood pressure and acid base status as shown in human infants (Hope *et al.,* 1984; Roth *et al.,* 1992) and in newborn piglets (Lorek *et al.,* 1994). In the case of the development of secondary energy failure, survival without permanent brain damage is very unlikely (Hope *et al.,* 1984; Azzopardi *et al.,* 1989; Roth *et al.,* 1992).

The importance of this delayed death phenomenon of neurons, hours after hypoxia-ischaemia, is the possibility that therapeutic intervention after a hypoxic-ischaemic insult can avoid the development of secondary cell death (Volpe, 1995; Robertson and Edwards, 1998). In experiments with rats, neurons remain viable for at least 8 hours after transient ischaemia (Pulsinelli and Cho, 1992). Allopurinol was protective if administered up to 6 hours after hypoxic-ischaemic insult, as shown in the newborn rat model (Palmer *et al.,* 1993; Shadid *et al.,* 1998) and also in severely asphyxiated infants (van Bel *et al.,* 1998). These studies indicate that irreversible mechanisms must be initiated somewhat later and that an "open window" period exists, giving us the possibility for treatment before final damage develops (Robertson and Edwards, 1998).

2.7.2.2. Role of excitatory amino acids

Excitatory amino acids (EAA), such as glutamate and aspartate, are the principal excitatory neurotransmitters in the mammalian brain, and play a key role in chemical signalling in the CNS (Johnston, 1993; Delivoria-Papadopoulos and Mishra, 1998). Glutamate is toxic to neurones in culture (Rothman and Olney, 1986) and when injected into the brain in experiments (McDonald *et al.,* 1988) or applied to the immature brain (Hagberg *et al.,* 1987). Animal experiments strongly suggest that EAA are involved in the pathogenesis of hypoxic-ischaemic brain lesions (Pulsinelli and Cho, 1992; D'Souza *et al.,* 1993; Feet *et al.,* 1998). Neurons that release glutamate are activated during hypoxia by depolarisation and the entrance of calcium into these cells (Siesjö, 1984). During hypoxia, excessive amounts of EAA, the most important of which is probably glutamate, may accumulate in the extracellular space of the brain (Feet et al. 1998). At the same time the energy-dependent uptake of glutamate, is impaired both by astrocytes and presynaptic nerve endings (Levene *et al.,* 1992). Asphyxiated infants had a considerably higher cerebrospinal fluid concentration of EAAs, with a positive correlation between the concentration of EAAs and the degree of HIE (Hagberg *et al.,* 1993).

Excessive activation of N-methyl-D-aspartate (NMDA), kainate and alpha-amino-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors by EAA leads to excitotoxicity in neurons. The stimulation of NMDA receptors produces an opening of voltage-dependent Ca^{2+} channels. Non-NMDA (AMPA and kainate) receptor stimulation increases Na^{+} influx. Metabotrope NMDA receptors activate a G-proteine and trigger the formation of inositol triphosphate, which stimulates intracellular Ca^{2+} release (Rootwelt, 1995).

Two mechanisms of glutamate-induced neuronal death have been identified:

1. Rapid cell death occurs within minutes and is initiated by glutamate receptor activation leading to Na^{+} entry through all three types of ionotropic receptors, with chloride and water following and with subsequent cell swelling and lysis (Levene *et al.,* 1992; Fellman and Raivio, 1997).
2. A delayed cell death, occurring over many hours, is initiated by the activation of NMDA-receptors with an influx of Ca^{2+} and a series of Ca^{2+} mediated events. The concomitant decrease in the uptake of glutamate by the presynaptic membranes during energy failure contributes to the maintenance of an increased extracellular glutamate concentration, allowing prolonged stimulation of its receptors (Levene *et al.,* 1992; Delivoria-Papadopoulos and Mishra, 1998). Subsequent intracellular events include the activation of lipases, proteases, endonucleases, and nitric oxide synthase (NOS) (Levene *et al.,* 1992).

Neurons with high densities of EAA receptors are particularly vulnerable to hypoxic-ischemic injury (Silverstein *et al.,* 1987; Johnston *et al.,* 1995; Volpe, 1995). The topography of NMDA receptors correspond closely to the topo-

graphy of hypoxic-ischaemic neuronal injury (Volpe, 1995). The infant brain has pronounced regional differences in NMDA receptor densities, with a relatively higher density of NMDA receptors in the temporal lobe and hippocampus as compared to the basal ganglia (D'Souza *et al.,* 1993).

The density of NMDA receptors in term newborn infants is higher in human hippocampus and in basal ganglia and thalamus than in comparable areas in the adult brain, and therefore NMDA receptor-mediated neurotoxicity is stronger in the developing brain compared to the adult brain (Slater *et al.,* 1992; D'Souza *et al.,* 1993; Chahal *et al.,* 1998). In addition, it has been found that hypoxia modifies the NMDA receptor in newborn piglets, resulting in an increased affinity of the receptor channel for EAA (Delivoria-Papadopoulos and Mishra, 1998).

The NMDA receptor-ion channel complex can be modulated by at least six pharmacologically distinct compounds: glutamate, glycine, magnesium ions (Mg^{2+}), zinc ions, phencyclidine and spermine (Delivoria-Papadopoulos and Mishra, 1998).

These findings have therapeutic implications. Drugs that reduce NMDA-receptor function would diminish brain damage after hypoxia-ischaemia (McDonald *et al.,* 1987; Hagberg *et al.,* 1993). Neuronal death after hypoxia-ischemia has been prevented by glutamate receptor antagonist, dizocilpine maleate (MK-801) (Hattori *et al.,* 1989; Ford *et al.,* 1989), even when the treatment is instituted 1–2 hours after the insult. Unfortunately a dose-related increase in mortality was found with MK-801, and the substance may cause considerable neurobehavioural abnormalities in exposed rat pups and interfere with learning or behaviour later in development (Ford *et al.,* 1989). Pretreatment with a glutamate release inhibitor also reduced hypoxic-ischaemic injury in neonatal rats (Gilland *et al.,* 1994).

2.7.2.3. Role of accumulation of cytosolic calcium

A large amount of information indicates the major role of cytosolic Ca^{2+} accumulation in mediating cell death during and after hypoxia-ischaemia (Siesjö *et al.,* 1995; Fellman and Raivio, 1997; Delivoria-Papadopoulos and Mishra, 1998). It seems possible that secondary brain damage is caused by a sustained dysregulation of cell calcium metabolism (Siesjö *et al.,* 1995). The intracellular concentration of free Ca^{2+} is normally very low and closely regulated. The opening of voltage-dependent and agonist-operated Ca^{2+} channels leads to a rapid influx of Ca^{2+}, followed by the release of Ca^{2+} from the endoplasmic reticulum and mitochondria (Delivoria-Papadopoulos and Mishra, 1998).

At high intracellular concentrations Ca^{2+} is toxic, but due to cellular energy failure is not removed from the cytoplasm, and free Ca^{2+} may rise to potentially harmful levels (Siesjö, 1984; Chen *et al.*, 1987).

A high level of Ca^{2+} plays a key role in the sequential events that lead to cell death (Nowak *et al.*, 1984; Hoffman *et al.*, 1994; Delivoria-Papadopoulos and Mishra, 1998). Ca^{2+} activates phospholipases, which degradate cellular and membrane lipids. Ca^{2+} also activates other potentially destructive enzymes such as proteases, lipases, endonucleases and mitochondrial dehydrogenases. As Ca^{2+} activates several enzymes that trigger the production of OFR, in this way $Ca^{2+}$and OFR can interact to yield ischaemic brain damage (Siesjö *et al.*, 1995). Ca^{2+} is also involved in the activation of NO synthesis. It can be speculated that the sustained dysregulation of cell calcium metabolism is maintained because the initial production of OFR by calcium-triggered reactions enhances the influx/release of Ca^{2+}. Loss of precise control of cell Ca^{2+} homeostasis and continued production of OFR set the stage for the gradual devitalisation of cells by causing the alteration of membrane function, gene expression, or protein synthesis, possibly leading to the activation of a programmed cell death (Siesjö *et al.*, 1995).

Secondary brain damage can be ameliorated both by drugs that act on membrane Ca^{2+} flux and by those that scavenge OFR (Siesjö *et al.*, 1995). The protection from subsequent neuronal injury by pretreatment with voltage-dependent calcium-channel antagonists has been documented (Siesjö, 1992). The protection from brain injury afforded by antagonists of the NMDA-type of glutamate receptor-channel complex may be mediated primarily by the decreasing accumulation of cytosolic Ca^{2+} (Volpe, 1995). In asphyxiated newborns the use of calcium-channel blocking agents may cause hypotension and in combination with absent cerebral autoregulation, the induced hypotension may exacerbate cerebral injury (Levene *et al.*, 1990).

2.7.2.4. Role of magnesium

Magnesium is a selective, noncompetitive antagonist of the NMDA receptor. Magnesium ions (Mg^{2+}) are essentially intracellular cations and only about 1% of body Mg^{2+} is distributed in the extracellular space (Levine and Coburn, 1984; Quamme, 1993). The concentration of intracellular Mg^{2+} is kept within a relatively narrow range by powerful mechanisms of cellular Mg^{2+} uptake and release (Levine and Coburn, 1984; Quamme, 1993; Romani *et al.*, 1993). Normally the exchange of Mg^{2+} between extra-and intracellular compartments occurs at a very slow rate (Romani *et al.* 1993). A consistently large increase in cytosolic free Mg^{2+} has been observed under relatively drastic conditions such as hypoxia (Romani *et al.*, 1993). The fraction of free Mg^{2+} then rapidly changes in proportion to the extracellular Mg^{2+} (Quamme, 1993).

Mg^{2+} is important in the regulation of a large range of intracellular processes and may stabilise membrane components and alter membrane fluxes of Ca^{2+}, Na^{+} and potassium ions (K^{+}) (Headrick and Willis, 1991). Mg^{2+} is also one of the most important regulators of NMDA channel function. The channel is normally closed by Mg^{2+} ions in a voltage-dependent manner (Morisett *et al.,* 1993; Johnston, 1993; Delivoria-Papadopoulos and Mishra, 1998) and is believed thereby to block excessive Ca^{2+} entry into the cell (Johnston, 1993; Greenamyre and Porter, 1994). The processes impairing the neuron's ability to maintain its membrane potential could reduce Mg^{2+} blockade of the NMDA-receptor and result in increased Ca^{2+} entry (Greenamyre and Porter, 1994; Zhang *et al.,* 1996). Tissue hypoxia results in the modification of the Mg^{2+} sites of the NMDA-receptor (Delivoria-Papadopoulos and Mishra, 1998).

As indicated in experimental studies, the treatment of severely asphyxiated infants with magnesium sulphate after hypoxic-ischemic insult may be neuroprotective (Thordstein *et al.,* 1993; Hoffman *et al.,* 1994). Improved neonatal survival (Grether *et al.,* 1998), a lower risk of cystic periventricular leucomalacia (FineSmith *et al.,* 1997) and cerebral palsy (Schendel *et al.,* 1996; Nelson and Grether, 1995) in VLBW infants with prenatal exposure to magnesium sulphate compared to controls has been described. The combination of Mg^{2+} with OFR scavengers after the hypoxic-ischemic insult offers better protection from perinatal hypoxic-ischemic brain damage in rats than the use of these drugs separately (Thordstein *et al.,* 1993).

Chahal and coworkers (1998) have found that although the infant brain may have excess NMDA receptors which are hyperresponsive to EAA, newborn babies may have NMDA receptors without the normal complement of Mg^{2+}. This may explain the failure of magnesium sulphate treatment to reduce the risk of neonatal brain lesions or CP as described by Paneth and coworkers (1997). These findings suggest that the therapeutic NMDA receptor block in neonates requires higher concentrations of magnesium sulphate in the brain (Chahal *et al.,* 1998). On the other hand, higher concentrations of magnesium sulphate may cause an unacceptable risk of hypotension, as do other Ca-channel blocking agents (Levene *et al.,* 1990 and 1995).

2.7.2.5. Role of oxygen free radicals

One possible mechanism for delayed brain damage during reperfusion /reoxygenation may be an explosive generation of (oxygen free radicals) OFR (Saugstad, 1988; Ruth, 1988; Rootwelt, 1995; Saugstad, 1996; Rosenbaum *et al.,* 1994; Fellman and Raivio, 1997; Van Bel *et al.,* 1998; Shadid *et al.,* 1998). Free radicals have an unpaired electron in the outer orbit that makes them very reactive (Delivoria-Papadopoulos and Mishra, 1998). They have a strong tendency to initiate chain reactions that result in lipid membrane and DNA

peroxidation and cell damage. The excess production of free radicals via the metabolisation of arachidonic acid, xanthine oxidase, and non-protein-bound iron play an important role in secondary cell death (Shadid *et al.,* 1998; van Bel *et al.,* 1998).

Normally over 80% of the O_2 consumed by the cell is reduced by cytochrome oxidase without the production of OFR. The remaining 10–20% follows other oxidation reactions in the cytoplasm and mitochondria that produce the superoxide anion radical (Delivoria-Papadopoulos and Mishra, 1998). The cells have an array of defence mechanisms that include such enzymes as catalases, endoperoxidases, dismutase, and glutathione, cholesterol, ascorbic acid, and tocopherol among scavengers (Palmer *et al.,* 1990; Delivoria-Papadopoulos and Mishra, 1998).

The introduction of O_2 after hypoxia may result in a burst of OFR (Saugstad, 1988, 1996). Experiments with foetal sheep demonstrate that an increased release of OFR from the foetal brain occurs during reperfusion after ischaemia, in spite of the fact that reoxygenation occurs at foetal levels of O_2 tension (Bågenholm *et al.,* 1998). OFR may arise in the brain parenchyma, but the main sites of their generation are endothelial cells (Fellman and Raivio, 1997). These reactive metabolites may directly damage cells, but may promote the expression of adhesion molecules and may lead to the accumulation of granylocytes with further circulatory disturbancies (Fellman and Raivio, 1997). So even the energy status is initially corrected, an OFR attack and derangements in postischaemic microcirculation may lead to secondary cell damage (Rosenberg *et al.,* 1989; Fellmann and Raivio, 1997)

The successful management of hypoxic-ischaemic brain injury includes the prevention of the formation of OFR by supplying extra antioxydants or radical scavengers (Palmer *et al.,* 1990, and 1993; Pourcyrous *et al.,* 1993; Rosenbaum *et al.,* 1994; Van Bel *et al.,* 1998; Shadid *et al.,* 1998). In animal models of cerebral ischaemia, pretreatment with allopurinol or oxypurinol successfully reduced ischaemic brain injury (Palmer *et al.,* 1990; Thordstein *et al.,* 1993, Shadid *et al.,* 1998). Allopurinol was protective also as rescue treatment, administred up to 6 hours after hypoxic insult, as shown in the newborn rats model (Palmer *et al.,* 1993, Shadid *et al.,* 1998) and also in severely asphyxiated infants (Van Bel *et al.,* 1998). Treatment with the cyclooxygenase inhibitor indomethacin or iron chelator deferoxamine after hypoxic-ischemic insult in neonatal lambs also restores electrocortical brain activity and cerebral O_2 metabolism (Shadid *et al.,* 1998). These findings provide evidence to support the role of neuronal free radical formation in cell death secondary to hypoxia (van Bel *et al.,* 1998).

2.7.2.6. Role of nitric oxide

Recent evidence suggests that the newly discovered radical NO (the formerly endothelium derived relaxing factor), is not only a potent vasodilatator, but is also a critical mediator of damage in the developing brain (Ferriero *et al.*, 1995).

NMDA receptors' stimulation by EAA during a hypoxic-ischaemic insult and Ca^{2+} influx lead to the activation of NOS (Dawson *et al.*, 1992, Lipton *et al.*, 1993). This results in large amounts of NO diffusing out of the neuron into the extracellular space, where it can combine with a superoxide anion to form very reactive peroxynitrite (Lipton *et al.*, 1993). Glutamate neurotoxicity can be prevented by L-nitroarginine, an inhibitor of NO production (Dawson *et al.*, 1991). The elimination of NOS from neurons before hypoxic-ischaemic injury protects the immature rat brain from severe damage, which supports the role of glutamate-induced NO release as a mechanism of neuronal loss seen after this form of injury (Ferriero *et al.*, 1995).

On the other hand, as NO is a potent vasodilatator, an inhibition of NO production may worsen the damage by decreasing CBF in marginally perfused areas (Huang *et al.*, 1994).

2.7.2.7. Role of cytokines

Ischaemia and reperfusion induce an inflammatory response in many tissues, including the brain (Fellman and Raivio, 1997). Cytokines may participate in the inflammatory response to hypoxia-ischaemia and act as important neurotransmitters in the biochemical cascade leading to delayed cell death after asphyxia (Martin *et al.*, 1994; Kogure *et al.*, 1996; Hagberg *et al.*, 1996, Fellman and Raivio, 1997; Nelson *et al.*, 1998). Pro-inflammatory cytokines including interleukin-1β (IL-1β) and interleukin-6 (IL-6) are expressed acutely in the injured brain and contribute to progressive neuronal damage in asphyxiated neonates (Hagberg *et al.*, 1996; Martin-Ancel *et al.*, 1997; Savman *et al.*, 1998). The concentration of free IL-6, but not IL-1β has been found to be higher in the cerebrospinal fluid of asphyxiated infants compared to controls, and a significant relationship was discovered between IL-6 and the degree of HIE and outcome (Martin-Ancel *et al.*, 1997; Savman *et al.*, 1998). The expression of IL-6 was also observed more frequently in infants with periventricular leucomalacia than in those infants without periventricular leucomalacia (Yoon *et al.*, 1997). IL-1β is directly neurotoxic if applied to the brain and the IL-1 receptor antagonist (IL-1ra) reduces excitotoxic and ischaemic brain injury in animals (Relton and Rothwell, 1992; Martin *et al.*, 1994). Hata and coworkers (1996) have found that although there was no IL-1β found in the blood serum

after foetal distress, the concentration of IL-1ra was increased in these infants. The anti-inflammatory properties of IL-6 may be due in part to the induction of IL-1ra synthesis and the release of soluble tumor necrosis factor receptors (Tilg *et al.,* 1994).

Cytokines presumably mediate the influx of inflammatory cells into reperfused tissue (Fellman and Raivio, 1997). Neutrophil involvement in reperfusion damage is suggested by indirect evidence. Transgenic mice deficient in intercellular adhesion molecule-1 (ICAM-1) have smaller infarct volumes as well as better survival and neurologic function than wild-type animals after transient cerebral ischaemia and reperfusion (Connolly *et al.,* 1996).

Thus pro-inflammatory cytokines can contribute to the progression of perinatal brain injury, and these mediators are important targets for neuroprotective interventions in the acute post-injury period (Silverstein *et al.,* 1997; Edwards and Nelson 1998; Nelson and al., 1998).

2.7.2.8. Programmed cell death

Programmed cell death (PCD), where cells die due to apoptosis, is well known to occur in neurons during the normal development of the CNS (Weissner *et al.,* 1996; Silverstein, 1998). These neurons fail to develop trophic synapses, and disappear without inflammation (Rootwelt, 1995).

Recent data indicate that a hypoxic-ischaemic insult to the brain can also activate PCD pathways in injured neurons (Weissner *et al.,* 1996; Silverstein, 1998). Edwards and coworkers (1997) have demonstrated that in infants who die after intrauterine insults, a large number of brain cells undergo apoptosis. This mechanism of brain damage may prove to be even more important than the better-known pathways of cell necrosis by EAA, OFR, NO and Ca^{2+} influx (Gluckman and Williams, 1992).

Ischaemia and reperfusion induce both rapid and delayed changes in gene expression (Fellman and Raivio, 1997). Several genes are found to be involved in PCD, among them several immediate early genes such as members of the c-fos and c-jun family (Weissner *et al.,* 1996; Fellman and Raivio, 1997). The resulting pattern of gene activation is an expression of the corresponding proteins, so called "killer proteins", with the end result of PCD (Fellman and Raivio, 1997). Gene induction also accompanies delayed neuronal death via necrosis after ischaemia (Wiessner *et al.,* 1996). Many potential links exist between the apoptotic and necrotic pathways of cell death that may contribute to irreversible neuronal damage after acute brain injury (Weissner *et al.,* 1996; Silverstein, 1998). Such PCD-triggering events may be disturbances of Ca^{2+} homeostasis, OFR, and impairments of protein synthesis, all of which are known to occur after cerebral ischaemia (Weissner *et al.,* 1996; Silverstein, 1998).

Whether a particular cell undergoes necrosis or apoptosis may depend upon many factors: the severity of the cellular insult, cellular energy reserves, the events associated with delayed energy failure, the ability of the mitochondria to recover function upon resuscitation or trophic support from neighbouring cells (Edwards *et al.,* 1997). PCD may be more readily activated in pathological conditions that occur during the period of brain development (Edwards *et al.,* 1997; Silverstein, 1998).

It is suggested that as PCD is a relatively slow and multistep process, therapeutic intervention with inhibitors of apoptosis can rescue the ischaemic brain (Silverstein, 1998). The identification of crucial genes of PCD activated by cerebral ischaemia may allow us to develop therapeutic strategies to rescue cells at a very late stage after the initial ischaemic insult (Weissner *et al.,* 1996). Additional trophic support with growth factors may overcome the inappropriate activation of the PCD (Gluckman and Williams, 1992). Treatment with a protein synthesis inhibitor gave almost complete protection against delayed neuronal death in gerbils (Shigeno *et al.,* 1990). Several factors limit the clinical utility of anti-apoptotic therapies. Nonspecific inhibition of PCD may have deleterious effects, particularly in the neonate in whom apoptosis inhibitors can disrupt critical developmental events (Silverstein, 1998).

2.7.2.9. Initial cerebral hyperaemia and "no reflow" phenomenon

Changes in CBF and metabolism after hypoxic-ischaemic insult are potential mechanisms of subsequent damage during reperfusion (Rosenberg *et al.,* 1986; Fellman and Raivio, 1997). CNS damage has been minimised by posthypoxic-ischaemic pharmacologic interventions designed to improve CBF (Steen *et al.,* 1983).

In human infants the transition from foetal to newborn life is normally associated with an immediate decrease in CBF velocity, followed by an increase above the foetal level by the age of 24 hours (Sonesson *et al.,* 1987; Fenton *et al.,* 1990; Connors *et al.,* 1992).

In animal models, resuscitation after *mild to moderate ischaemia* is accompanied by a period of initial hyperaemia (Rosenberg, 1986). In newborn pigs the duration of reactive hyperemia is short, peaking by 5 min and subsiding by 20 min of reperfusion after total brain ischaemia (Leffler *et al.,* 1989). The early increase in CBF is related to the local elevation in vasodilatator factors, such as H^+, K^+, adenosine, prostaglandins and NO (Volpe, 1995; Leffler *et al.,* 1997). Evidence exists that the initial hyperaemia is a prerequisite for functional recovery, at least following long periods of ischaemia (Kångström *et al.,* 1983).

Severe hypoxic-ischaemic insult is followed by a failure to reperfuse ischaemic regions even when partial pressures of arterial CO_2 and O_2 have returned to

baseline values and blood pressure is within the autoregulatory range (Pourcyrous *et al.*, 1997). Failure to reperfuse ichaemic regions after restoration of circulation is called the "no reflow" phenomenon and is one potential mechanism of extended injury during the period after ischaemia. The "no-reflow" phenomenon was first described in an adult rabbit cerebral ischemia model (Ames *et al.*, 1968), but has been observed also in other species including nonhuman primates (Ginsberg *et al.*, 1972). It is unknown whether a "no reflow phenomenon" exists in asphyxiated infants, as global CBF measurements do not reveal possible flow changes in critical regions (Fellman and Raivio, 1997).

It is possible that not all structures of the brain are affected by the "no reflow" phenomenon. In some structures there may be evidence of "reactive hyperaemia", whereas others may show perfusion defects of the "no-reflow" type (Kångstöm *et al.*, 1983). Typically these defects affect striatum, the thalamus, and hippocampus, as well as frontal and parietal cortices after complete global ischaemia (Kångström *et al.*, 1983). Perhaps the duration of ischaemia influences the conditions of flow restoration in "no-reflow" areas: the longer the period of ischaemia, the more structures were affected by the "no-reflow" phenomenon in rats (Kångström *et al.*, 1983). The causes of such "no-reflow" may also be endothelial injury and swelling (Petito *et al.*, 1987), the granulocyte plugging of microvessels (Zhang *et al.*, 1994), or intravascular clotting (Thomas *et al.*, 1993). The discovery of markedly higher CBF after reperfusion in transgenic mice deficient in ICAM-1 supports the role of granulocytes (Connolly *et al.*, 1996). Among factors that influence the occurrence of a "no-reflow" phenomenon, postischaemic perfusion pressure may be of special importance (Kångström *et al.*, 1983).

2.7.2.10. Delayed cerebral hypoperfusion

Initial cerebral hyperaemia after hypoxic-ischaemic insult is followed by delayed cerebral hypoperfusion. A steady decrease of CBF 12 hours after asphyxia has been described in infants (van Bel *et al.*, 1993 and 1998; Shadid *et al.*, 1998) and also in different animal models (Kångström *et al.*, 1983; Rosenberg *et al.*, 1989; Karlsson *et al.*, 1994). It has been speculated that the delayed hypoperfusion may add a secondary ischaemic insult to the tissue, especially if the reduction in CBF is not matched to the metabolic rate (Ito *et al.*, 1975; Pulsinelli *et al.*, 1982; Kångström *et al.*, 1983). As has been shown in animal models, the severity of posthypoxic hypoperfusion increases with the increasing duration of a preceding cerebral ischaemia (Ito *et al.*, 1975; Kångström *et al.*, 1983; Karlsson *et al.*, 1994). Among infants with severe birth asphyxia, babies with the severest stage of HIE demonstrated the greatest decrease in CBF (Van Bel *et al.*, 1998).

This delayed postasphyxial cerebral hypoperfusion could be related to an excess of vasoconstrictor molecules (tromboxanes and leukotrienes) or OFR-induced vascular injury (Rosenberg *et al.,* 1989; Fellman and Raivio, 1997; van Bel *et al.,* 1998) and probably an accumulation of granulocytes in the reperfused tissue, leading to further circulatory disturbances (Fellman and Raivio, 1997). Reduced perfusion because of oedema seems unlikely, since the highest water content occurs in the brain during the first 15–20 minutes of reperfusion and then gradually returns toward normal values (Williams *et al.,* 1991). A persistent defect in cerebrovascular autoregulation also plays a role in this situation (Lou *et al.,* 1979).

2.7.2.11. Delayed cerebral hyperaemia

Several authors have reported high CBF velocities and significantly lowered resistance indices (RI) in infants with severe HIE at the age of 24–72 hours (Archer *et al.,* 1986; Levene *et al.,* 1989; Stark and Seibert, 1994). CBF has also been shown to be higher in infants with permanent brain damage during the first days of life (Pryds *et al.,* 1990). Several animal models have also shown initial posthypoxic hypoperfusion followed by delayed vasodilatation, increased CBF and cerebral energy failure 24–72 hours after a hypoxic-ischemic insult (Lorek *et al.,* 1994; Marks *et al.,* 1996).

Lassen (1966) first described the over-abundant CBF relative to the metabolic needs of the brain tissue, so-called "luxury-perfusion". In this situation dissociation exists between cerebral metabolic rate and CBF (Levene *et al.,* 1989). Markedly high CBF despite isolectric EEG activity and thereby a presumably low cerebral metabolism is found in these infants (Pryds, 1991). The CBF was to be higher, but cross-brain oxygen extraction was lower in asphyxiated infants who died, compared to the neonates who survived with a normal neurologic outcome (Frewen *et al.,* 1991).

Delayed hyperaemia often occurs as a focal phenomenon, but may also be generalised (Levene *et al.,* 1989). The reason for this cerebral hyperaemia and low RI may be severe vasoparalysis, a form of irreversible cerebral vascular injury (Levene *et al.,* 1989; Pryds *et al.,* 1990). In infants with birth asphyxia hyperperfusion is thought to be a sign of permanent brain damage with poor neurodevelopmental prognosis. The severity of vasoparalysis and cerebral hyperemia is thought to correlate with the degree of brain injury and presumably the severity of the asphyxial insult (Levene *et al.,* 1989; Pryds *et al.,* 1990; Baenziger *et al.,* 1999). Infants with the poorest neurologic outcome (isoelectric EEG and death) had the highest values for CBF and no cerebrovascular autoregulation or CO_2 reactivity. Infants with burst suppression EEG and moderate to severe brain injury had slightly elevated values of CBF and impaired autoregulation, but retained reactivity to $PaCO_2$. Infants without evi-

dence of brain injury had normal values for CBF, intact autoregulation and reactivity to $PaCO_2$ (Pryds *et al.,* 1990).

The mechanisms of such "luxury cerebral perfusion" following ischaemic brain injury are still unclear. Neuronal disruption causing a release of vasoactive substances such as adenosine and lactate, or of EAA (Pryds, 1991), irreversible cerebral vascular injury with complete loss of tone in resistance vessels may play a role in the pathogenesis of delayed hyperaemia (Levene *et al.,* 1989; Pryds *et al.,* 1990; Fellman and Raivio, 1997).

2.7.2.12. Regional redistribution of cerebral blood flow

It is proposed that variations in regional CBF and metabolism after asphyxia may be intimately related to the pathogenesis of HIE in asphyxiated infants (Younkin *et al.,* 1988).

A preferential localisation of CBF changes and cell damage to certain selectively vulnerable regions after cerebral ischaemia has been shown in different animal models (Kångstöm *et al.,* 1983; Radovsky *et al.,* 1995). The hierarchy of the CBF reduction has been found to correlate closely with the distribution and extent of ischaemic neuronal necrosis, the fronto-parietal region being the most vulnerable after a hypoxic-ischaemic insult (Vannucci *et al.,* 1988; Radovsky *et al.,* 1995). Multiple cystic lesions of the brain parenchyma, supplied by the anterior cerebral circulation and relative preservation of the cerebellum, brainstem and cerebral structures supplied by the vertebrobasilar circulation is a recognised pattern of cerebral injury associated with HIE in term infants (Sheth *et al.,* 1995; Frigieri *et al.,* 1996).

The exact mechanism of the redistribution of CBF in asphyxiated infants is not conclusively clear. The role of the sympathetic nervous system has been suggested. The anterior cerebral artery in the term infant has a dense sympathetic innervation (Auer *et al.,* 1981) and asphyxia, a potent sympathetic stimulator, is more likely to induce vasoconstriction in the anterior circulation, which would differentially accentuate the effects of hypoxic-ischemic insult in this part of the brain (Sheth *et al.,* 1995).

Prostaglandins are suggested to be responsible in part for the control of local CBF. During hypoxia, significant decrease of frontal, parietal and temporal CBF with an increase in vasoconstrictor prostaglandin concentrations was found in the beagle pup model (Ment *et al.,* 1987). The role of NO as an endothelium-derived relaxing factor may also be greater in vessels of the posterior than those of the anterior circulation (Kajita *et al.,* 1995).

It is unknown whether a regional "no reflow phenomenon" exists in asphyxiated infants, as global CBF measurements do not reveal possible flow changes in critical regions (Fellman and Raivio, 1997). Data are lacking concerning CBF velocity changes in different cerebral arteries. Only Bennhagen and co-

workers (1998) have reported on the subnormal distribution of CBF velocities of infants suffering from HIE with higher values in the internal carotid than in the anterior cerebral artery.

2.8. Consequences of perinatal asphyxia

Neonatal asphyxia causing HIE in early postnatal life is associated with a high risk of irreversible brain injury (Volpe, 1995). Perinatal hypoxic-ischaemic injury has been the most common of the recognised causes of neurological morbidity (Hill and Volpe, 1982). The development of brain damage after asphyxia depends on the duration and severity of the insult, and also on intervention during and after the hypoxic-ischaemic insult (Low, 1993). Foetal asphyxia may cause the major neuropathologic lesions responsible for CP, mental retardation, seizures, hearing loss and blindness (Volpe, 1995). CP is a disorder of a motor function with an abnormal control of movement or posture and with the absence of any signs of progressive course, which is caused by brain lesions from the ante-or perinatal period and is mainly of hypoxic-ischemic origin (Talvik, 1992). Minor neurological defects after perinatal asphyxia include a variety of abnormalities in muscle tone, impaired balance, poor fine or gross motor coordination (Low, 1993). These neurological defects may impair function to some extent, but impose no significant handicap and in some cases these impairments are transitory (D'Souza *et al.*, 1981). Cognitive development may be normal or impaired in infants with CP and the severity of the impairment is not necessarily related to the severity of the motor disability (Ruth, 1988). It is believed that asphyxia is not a cause of isolated mental retardation (Freeman and Nelson, 1988).

A long-term evaluation of survivors of HIE suggests that a significant percentage of survivors without neurologic sequelae may have disorders of learning and behaviour in later childhood (D'Souza *et al.*, 1981; Robertson and Finer, 1985; Sööt *et al.*, 1998).

There is increasing evidence that antepartum hypoxic-ischaemic events may be important factors in the development of brain damage in children (Low, 1993; Badawi *et al.*, 1998). To attribute CP to birth asphyxia with reasonable certainty, there must be signs of hypoxic-ischaemic insult around the birth with subsequent development of a clinical picture of HIE during the first days of life (Volpe, 1995). Obstetric complications were not associated with a higher rate of CP unless signs of HIE were also present (Nelson and Ellenberg, 1986; Nelson and Emery, 1993). For the term newborn free from congenital or genetic abnormalities, an adverse long-term outcome of perinatal asphyxia does not occur unless signs of neurologic dysfunction are present in the newborn period (Robertson and Finer, 1993; Volpe, 1995).

3. AIMS OF STUDY

1. to estimate the prognostic value of perinatal risk factors, early neonatal symptoms and severity of HIE in asphyxiated term newborn infants (unpublished data)
2. to estimate the prognostic value of total magnesium and ionised calcium concentrations in umbilical cord blood and venous blood serum in asphyxiated term newborn infants (Paper I–II)
3. to estimate changes in brain ultrasonography in asphyxiated term newborn infants and to evaluate their prognostic value (unpublished data)
4. to establish a normal range of CBF velocities in different cerebral arteries for full-term infants during the first week of life (Paper IV)
5. to look at the prognostic value of changes in CBF velocities in asphyxiated term infants during the first week of life (Paper III, V)
6. to identify the pattern of changes in CBF velocities in different cerebral arteries in asphyxiated term infants with HIE during the first week of life (Paper V)

4. PATIENTS AND METHODS

This longitudinal prospective study includes a group of asphyxiated term newborn infants (n=90) and selected control infants (n=35). The asphyxia cohort consisted of 77 asphyxiated term infants born at the Women's Clinic of Tartu University and of 13 asphyxiated infants born in different county hospitals and treated in the Intensive Care Unit (ICU) of the Children's Hospital of the University of Tartu from March 1994 to March 1997.

4.1. Study population

From March 1994 to March 1997 5142 term newborns were born in the Women's Clinic of Tartu University. Based on foetal signs of asphyxia and/or low Apgar scores, asphyxia was diagnosed clinically in 337 infants (6.55% of all alive-born term infants). HIE developed in 153 term infants: a mild stage of HIE in 65.4%, moderate in 32% and severe in 2.6% of infants with HIE. Based on inclusion and exclusion criteria, 77 infants born in the Women's Clinic of Tartu University were enrolled in the study. Fifteen of 77 infants born in the Women's Clinic of Tartu University were also later treated in the intensive care unit (ICU) of the Children's Hospital of the University of Tartu.

During the study period 163 term newborn infants were treated in the ICU of the Children's Hospital of Tartu University: 28 asphyxiated term infants were enrolled in the study and 7 infants were excluded due to late hospitalisation from county hospitals during the years 1994–95.

4.1.1. Study group

The study group consisted of 90 infants with signs of foetal distress defined as continuous cardiotocographic changes in foetal heart rate (baseline heart rate <100, >170 beats per minute or late decelerations) and/or neonatal distress (Apgar score ≤7 at the 5th minute of life). The asphyxiated infant was included in the study if the baby was less than 36 hours of age at the first examination of CBF velocities. Infants with major or multiple minor malformations, systemic infections or a haemolytic disease were excluded from the study. After the first week of life 4 severely asphyxiated infants, treated in the ICU of the Children's Hospital of Tartu University, were excluded from the study: 2 due to chromosomal anomalies, 1 infant due to Werdnig-Hoffman disease and 1 due to lung anomaly. Thus the final analysis for the thesis includes 86 asphyxiated infants.

Gestational age, birth weight, sex and mode of delivery, mother's age and number of deliveries were similar in study and control group infants (Table 2).

4.1.2. Control group

The control group (n=35) consisted of the first healthy infant born during a 12 hour period after a given study group infant at the Women's Clinic of the University of Tartu and fulfilling all of the following criteria: 1) no maternal illness, 2) no complications or medication during pregnancy, 3) singleton pregnancy, 4) gestational age between 37 and 42 weeks, 5) birth weight appropriate for gestational age 6) uneventful vaginal delivery or elective caesarean section without signs of foetal distress, 7) Apgar score of 7 or more at 1 minute and 8 or more at 5 minutes, 8) no neonatal disorders such as HIE, meconium aspiration syndrome, respiratory distress syndrome, clinically significant patient ductus arteriosus, pneumothorax, hypoglycaemia, anaemia, haemoconcentration, sepsis, haemolytic disease.

The main characteristics of the study and control group infants are shown in Table 2.

Table 2. Main characteristics of asphyxiated and control group infants, values are given as mean ±SD or median (min-max).

	Control group N=35	Study group N=86
Gestational age (weeks)	39.8±1.2	40.0±1.2
Birth weight (g)	3611±454	3576±599
Male/female	17/18	53/33
Vaginal delivery/of these ventous delivery	18/0	52/16
Caesarean section	17	34
Mother's age (y)	27.9±6.3	26.2±5.5
Number of deliveries	1(1–4)	1(1–8)

4.2. Methods

The study included questionnaires, clinical, biochemical, ultrasonographical, Doppler-sonographical and electroencephalographical investigations. In all cases mothers' (parents') informed consent for investigation and follow-up study was obtained.

Questionnaires included family socio-economic conditions, education, occupation, history of pregnancy, delivery and neonatal course.

Apgar scores at birth were assigned to all infants according to clinical routine by the attending pediatrician or midwife. Gestational age was verified

by a combination of last menstrual date and an ultrasonography performed before the 20th week of gestation.

The stage of HIE according to Sarnat and Sarnat (1976) were registered during the first 5 days of life, and the grading was based on the worst degree of HIE. Based on the clinical signs of the severity of HIE, asphyxiated infants were divided into 4 groups. The first group consisted of nine infants with intranatal distress as an indication for emergency caesarean section or ventous delivery in eight infants, without the later development of any signs of HIE. The second group (n=40) had mild signs of HIE during the first day of life, infants in the third group (n=25) had a moderate stage of HIE and 12 newborns in the fourth group were diagnosed as having a severe stage of HIE. Four infants with a severe stage of HIE died during the neonatal period. The groups with different development of HIE were similar in gestational age, birth weight, sex and mode of delivery (Table 3).

Two infants (5%) with mild, 10 infants (40%) with moderate and all 12 with a severe stage of HIE were treated in the ICU. All but 1 infant with severe and 4 infants with a moderate stage of HIE needed mechanical ventilation for more than 24 hours.

Table 3. Main characteristics of infants with different stages of HIE, data given as mean±SD or median (min-max).

	Controls N=35	HIE=0 N=9	HIE=I N=40	HIE=II N=25	HIE=III N=12
Birth weight (g)	3611±454	3484±475	3527±568	3677±848	3681±577
Gestational age	39.8±1.2	40.2±1.2	40.0±1.1	40.4±1.4	40.7±1.4
Male/female/	17/18	5/4	24/16	12/8	7/5
Apgar score at 1st min	8 (7–9)	7 (4–9)	3.5 (1–8)	1 (1–8)	1* (0–7)
at 5th min	9 (8–9)	8 (7–9)	7 (4–8.5)	6** (3–8)	2* (0–8)
at 10th min	9 (8–9)	9 (8–9)	8 (7–8.5)	7** (6–8)	4* (2–8)
Vaginal delivery/of these instrumental deliveries	18/0	2/1	29/8	13/5	8/2
Caesarean section	17	7	11	12	4

* $p<0.005$ HIE III vs control group and HIE I–II

** $p<0.05$ HIE II vs HIE I

The renal failure was defined as any episode of oliguria (urine output of less than 1ml/kg/h) in the acute period of disease after the age of 24 hours and/or increased urea concentration in venous blood serum >15mmol/l.

4.2.1. Biochemical measurements

Mixed umbilical cord blood was collected after umbilical cord clamping and centrifuged after clotting for 15 minutes. For the electrolyte and urea assay 1 ml of venous blood was drawn at the age of 24–48 hours. Ca^{2+}, Na^{+}, K^{+} were analysed with an ion-selective analyser Microlyte (Kone, Finland) within an hour. Serum samples for Mg determination were stored at –20°C until analysis. Mg was analysed colorimetrically using the xylidyl blue method with a Technicon RA-100 Analyzer (USA) according to the instruction manual.

Venous blood serum urea was measured by kinetic method and blood glucose by enzymatic-colorimetric method with a FP 401 Incubator (Labsystem, Finland). Acid-base status measurements were performed with an IL 1420 BG3 Analyzer (Instrumentation Laboratory, Italy). Haemoglobin and haematocrit was measured with Sysmex K-1000 (TOA-Medical Electronics, Japan). Acid-base status, blood glucose and haemoglobin measurements were performed simulataneously with CBF velocity investigations in asphyxiated infants.

4.2.2 Cerebral ultrasonography and CBF velocity measurements

In asphyxiated infants, early cerebral ultrasonography and CBF velocity measurements were performed at least 3 times between 10 hours and 5 days of age. CBF velocity measurements were grouped into four postnatal age-periods: 12±2, 24–35.9, 36–71.9, 72–120 hours and the mean and median values were calculated, together with 2 and 3 standard deviations (SD) from the mean. Late cerebral ultrasonography was performed at least once a week until discharge and during follow-up examinations at the age of 1, 3, 6 and 9 months.

Cerebral ultrasonography and CBF velocity recordings were made using a colour Doppler ultrasound scanner (Hitachi Medical Corporation, Model EUB-515 with 3.5MHz pulsed wave and 5MHz imaging crystal) or using a duplex Doppler scanner (Siemens, Sonoline SL 2 with 7.5MHz pulsed wave and 7.5 and 5MHz imaging crystal) and a Fast Fourier real-time frequency analyser.

4.2.2.1. Cerebral ultrasonography

Cerebral ultrasonography scanning was performed through the anterior fontanelle in both coronal and parasagittal planes (Pidcock *et al.*, 1990). Ultrasonographic images were interpreted during scanning. The right and left cerebral hemispheres were described separately.

On early ultrasonographic examination during the first week of life the size and configuration of the ventricles, interhemispheric fissure and subarachno-

ideal space, the visualisation of the normal structures (including sulci), existence of peri-intraventricular haemorrhages (PIVH) and parenchymal echodensities, and the presence, location and size of cystic degeneration were examined (Rutherford *et al.,* 1994).

PIVH were classified according to Papile and coworkers (1978). Ventricular size was measured as the greatest distance perpendicular to the ventricular axis (the greatest axis of the lateral ventricle on the coronal scan), and maximal interhemispheric distance and maximal width of the subarachnoideal space on the coronal scan were also measured (Liao *et al.,* 1986).

The periventricular regions were examined for abnormal echodensities. A homogeneous striated echogenic triangle posterior to the atrial region, less echodense than the choroid plexus and no larger than the size of the atrial region, was considered to be normal posttrigonal "blush". Pidcock and coworkers (1990) classified abnormal periventricular echodensities as follows:

- Mild periventricular echodensities were less echogenic than the chorioid plexus but larger than the atrial region of the lateral ventricles.
- Moderate periventricular echodensities were heterogeneous periventricular echodensities equal in brightness to the chorioid plexus
- Severe periventricular echodensities were heterogeneous echodensities brighter than the chorioid plexus or extending into the brain for a distance at least twice the diameter of the ventricles.

Abnormalities in parenchymal echogenicity were graded according to a modified Eken scale (Eken *et al.,* 1994):

1. diffuse
   - mild parenchymal echogenicity with mildly increased echogenicity and clearly visible normal structures
   - moderate parenchymal echogenicity with moderately increased echogenicity, and poorly visible normal structures
   - severe parenchymal echogenicity with severe increase in echogenicity and indistinguishable normal structures
2. focal area of parenchymal echogenicity in the region of the anterior, middle or posterior cerebral artery.

A parenchymal cyst was an umbrella term used for any parenchymal echolucency suggestive of a cavity. These lesions were further described as porencephalic, where there was a single large echolucent cavity and multicystic encephalomalacia, where multiple cavities of variable size were found (Sheth *et al.,* 1995).

On late ultrasonographic examination after the 1st week of age the size and configuration of the ventricles, interhemispheric fissure and subarachnoideal space, brain parenchymal echogenicity, visualization of the normal structures (including sulci), periventricular densities, PIVH and cystic degeneration were evaluated (Rutherford *et al.,* 1994). The persistent ventricular dilatation and

cystic degeneration of the brain, both definite and questionable, comprised the category of major abnormal findings (Boal *et al.*, 1995).

Cortical atrophy was diagnosed if the surface of the brain was easily visible with prominence of the cortical sulci and widening of the interhemispheric fissure and/or subarachnoideal space (Rutherford *et al.*, 1994).

4.2.2.2. CBF velocity measurements

The anterior cerebral artery was visualised by a hand-held transducer in the sagittal plane through the anterior fontanel and the signals recorded from the point midway between the inferior-most border of the corpus callosum and the vessel's origin from the circle of Willisi. The middle cerebral artery was visualised through the temporal bone in the region above the zygomatic arch in the fold of the temporal lobe from the straight mid-portion of the artery (Evans *et al.*, 1988). The basilar artery was visualized in the sagittal plane just before the pons. The internal carotid artery was found in the coronar plane in the basis of the scull just below the corner of the sella in the pars petrosa, where the vessel's orientation is toward the scanner (Hayashi *et al.*, 1992).

During CBF velocity recordings the high pass filter, used to remove low frequency noise, i.e. vessel wall movements, was set at a level of 100 Hz. Guided by the velocity signal displayed on an oscilloscope and an audio-signal, the highest possible velocities were used in the range-gated mode. After a stable velocity recording over 20–30 consecutive beats was received, the angle correction was performed and 5 consecutive beats with the highest amplitude recorded were analysed by hand. Systolic peak flow velocity (Vs), mean flow velocity (Vm) (time-mean of the maximum velocity envelope curve) and end diastolic peak flow velocity (Vd) were recorded as a mean of 5 consecutive beats from both sides of the skull (Evans *et al.*, 1989). As no statistical differences were found between the measurements from different sides of the skull, the data reported represent the average of two determinations. The resistance index (RI) was calculated according to the formula RI=(Vs–Vd)/Vs.

The time of ultrasound exposure of the artery on each side of the skull was never more than 60 sec and the output intensity did not exceed the suggested safety limit of 100mW/cm^2.

Heart rate was calculated from the velocity recordings. Arterial blood pressure in asphyxiated infants was measured noninvasively by a cuff on the right upper arm and using a BP 107 Nippon Colin sphygmomanometer. The average of two blood pressure measurements made simultaneously with the Doppler recordings was used.

Recordings of CBF velocities and arterial pressure were performed in a supine position 30–60 minutes after feeding. Observations were made when the infant was quiet, with eyes closed and performing no gross body movements.

4.2.3. Electroencephalography

EEG was performed using digital equipment of Nihon-Kohden (Japan) with software "Neurofile II" in 49 asphyxiated infants during the first 2 weeks of life: in 19 infants with mild or lacking signs of HIE, in 18 infants with moderate and in all 12 with a severe stage of HIE. In 27 asphyxiated infants a second EEG was performed in the case of abnormal neonatal EEG, abnormal development or seizures manifesting later during infancy. The neurophysiologist, who did not know the clinical picture and the data of history of the infant, interpreted the EEG. The duration of EEG recording was 30 minutes. EEG abnormalities were defined as severe with burst supression, low voltage or isoelectric EEG, moderate abnormalities included a moderate decrease of voltage and/or paroxysmal discharges on the EEG and a mild decrease in voltage was defined as a mild change in EEG (Volpe, 1995; Selton and Andre, 1997).

4.2.4. Computed tomography

Brain computed tomography (CT) was performed using Siemens "Somatom ARHP Spiral" equipment in infants with abnormal development or if seizures were present. All infants were investigated under general anesthesia with barbiturates. Cranial CT scans were evaluated for ventricular size and configuration, size of extra-axial fluid spaces, overall density of the brain parenchyma and grey-white matter differentiation, and the presence of multicystic encephalopathy (Allen and Riviello, 1992).

4.2.5. Infant follow-up

Of the families of 117 surviving infants, who initially agreed to participate, 3 (2.6%) were lost for follow-up: 1 in the group of infants without HIE and 2 with a mild stage of HIE. The reason for dropout reason was moving out of the country.

The psychomotor development of infants was followed at the age of 1, 3, 6, 9, 12 and 18 months of age and motor, mental and prelanguage speech development was examined. The standardised test for neurological examinations of infants in Estonia and Caput scale (CAT/CLAMS) for cognitive development were used to follow the neurodevelopment of the infants. In infants with CP its severity was estimated (I–IV) according to T. Talvik (1992).

The children were divided into 3 groups: 1) normal, 2) mild impairments with I–II stage of CP (abnormalities in tone and increased deep tendon reflexes and positive Babinski sign, but with minimal or no functional defect), 3) severe

disability with III–IV stage of CP (CP with definite physical abnormality with significant interference in independent motion) and/or delay in cognitive development and/or epilepsy; blindness or deafness.

4.2.6. Statistical methods

The study analyses were performed using the statistical package SAS Version 6.11.

Non-parametric tests (Kruskal-Wallis, Mann-Whitney and Wilcoxon tests) were used for statistical analysis to compare differences between groups of HIE. Spearman Rank test was used to find correlations between HIE groups. Values are given as mean ±SD and median (min-max). The accepted level for statistical significance was $p<0.05$.

5. MAIN RESULTS AND DISCUSSION

There is increasing evidence that a "therapeutic window" exists in the early hours following birth asphyxia, when intervention can attenuate activation of the neurotoxic cascade that leads to delayed cell death hours, days or months later (Pulsinelli *et al.*, 1982; Petito *et al.*, 1987; Volpe, 1995; Van Bel *et al.*, 1998; Robertson and Edwards, 1998). We need good early indicators of the severity of asphyxia to assess the need for treatment before the full development of hypoxic-ischemic brain damage.

The severity of HIE is a good indicator of the severity of asphyxia, successfully predicting the permanent brain damage of asphyxiated infants. In the present study group, only 4 of 38 infants (10.5%) with a mild stage of HIE developed mild impairments (Table 4). The development of moderate or severe signs of HIE after birth asphyxia worsens the prognosis considerably. 6 of 25 infants (24%) with a moderate stage of HIE were disabled at the age of 18 months and another 24% were mildly impaired. 11 of 12 infants (92%) with a severe stage of HIE had a poor outcome: 4 infants died (on the 8th, 13th and two infants on the 28th day of life) and in 7 infants developed a severe disability. Thus intrapartum asphyxia, severe enough to cause permanent brain damage or death, will be associated with moderate — severe signs of HIE during the first days after asphyxia (Robertson and Finer, 1993; Volpe, 1995).

Table 4. Development of infants with different stages of HIE.

	Controls N=35	HIE=0 N=9	HIE=I N=40	HIE=II N=25	HIE=III N=12
Normal development	35	8	34	13	0
Mild impairment	0	0	4	6	1
Severe disability	0	0	0	6	7
Death	0	0	0	0	4
Lost for follow-up	0	1	2	0	0

But unfortunately symptoms of HIE develop over the first days of life and exact clinical assessment is complicated, especially in infants who are mechanically ventilated or sedated (Robertson and Finer, 1985; Blennow, 1995; Thornberg *et al.*, 1995). Simple predictors of poor outcome are therefore needed, because refined tools (e.g. cerebral function monitoring, evoked potentials) which are highly predictive for poor outcomes, are not available in the hospitals, where most infants with severe birth asphyxia are born (Ekert *et al.*, 1997).

5.1. Perinatal risk factors

5.1.1. Ante- and intranatal risk factors

Since the investigations of Little (1862) and Freud (1897) many studies have been performed to identify the significance of ante-and intranatal risk factors for the development of asphyxia (MacDonald *et al.*, 1980; Mulligan *et al.*, 1980; Nelson and Ellenberg, 1985 and 1986; Talvik, 1992; Low, 1997; Badawi *et al.*, 1998; Nelson and al., 1998). Several criteria have been suggested as risk factors for the development of asphyxia and HIE.

We investigated such **maternal risk factors** as maternal age, the marital status of the mother, the education of the mother, the mother's incidence of chronical diseases, the incidence of previous neonatal deaths or spontaneous abortions, the number of previous pregnancies and deliveries and found no differences between asphyxiated or control group infants. Nor was any correlation found between maternal risk factors and the severity of HIE and psychomotor development at the age of 18 months in spite of other studies indicating the importance of maternal risk factors (MacDonald *et al.*, 1980; Talvik, 1992).

The material of the present study does not allow one to say much about the significance of different antenatal risk factors for the development of birth asphyxia. As we excluded from the control group all infants with previously well documented ante-or intranatal risk fators (maternal chronical illness, infection, multiple pregnancy, preeclampsia, diabetes, anaemia, growth retardation, abnormal delivery) we were only able to find the correlations between the ante- and intranatal risk factors and severity of HIE and also outcome at the age of 18 months among asphyxiated infants.

Analysing the data of the present study regarding **antenatal risk factors** in asphyxiated infants, no correlation was found between such risk factors as growth retardation, preeclampsia, anaemia, infection, or threatening disruption of the pregnancy and severity of HIE and the outcome of infants at the age of 18 months. This is somewhat controdictory to the result of other studies, which have found a correlation between some antenatal risk factors (mothers infection, growth retardation) and development of HIE, although the risk is small (MacDonald *et al.*, 1980; Talvik, 1992; Badawi *et al.*, 1998; Nelson *et al.*, 1998). Antenatally compromised babies may have a diminished reserve to cope with hypoxia during labour and are more likely to experience hypoxic-ischaemic injury during the intrapartum period (Volpe, 1995; Badawi *et al.*, 1998).

Different **intranatal factors** have been suggested as risks for asphyxia (MacDonald *et al.*, 1980; Talvik, 1992; Badawi *et al.*, 1998). But some authors (Nelson and Ellenberg, 1984; Low *et al.*, 1984) have found that late obstetric complications are common, one or more occurring in about 60% of pregnan-

cies. They found that the risk of different obstetric complications for CP did not exceed the risk of CP in uncomplicated deliveries. In the present study also no correlation was found between such intranatal risk factors as meconium-stained amniotic fluids, shoulder dystocia, degeneraton of the placenta or cord complications with severity of HIE or outcome of infants at the age of 18 months (Table 5).

Table 5. Number of infants with ante-and intranatal risk factors among asphyxiated infants with HIE.

	HIE=0 n=9	HIE=I N=40	HIE=II N=25	HIE=III N=12
Growth retardation	1	2	4	1
Preeclampsia	2	15	10	2
Anaemia <100g/l	2	4	3	2
Infection	4	12	3	3
Bleeding during pregnancy	0	3	3	2
Abruption of amniotic fluids >12 h	2	6	5	3
Stimulation	2	9	10	5
Shoulder dystocia	1	10	4	0
Malpresentation	2	5	6	1
Abruption of placenta	1	1	4	5*

* $p<0.05$ HIE III vs HIE I–II

We found a correlation between severe intranatal events such as the abruption of placenta ($r=0.26$, $p=0.01$), persistent bradycardia <100 beats per minute ($r=-0.43$, $p=0.03$) and the severity of HIE. In the present study abruption of the placenta was found in 11 of 86 infants (13%) with asphyxia, more frequently with a severe stage of HIE compared to a mild or moderate stage of HIE ($p<0.05$). Earlier reports (Mulligan *et al.*, 1980; Badawi *et al.*, 1998) have also found that acute intrapartum events as abruption of the placenta and severe bradycardia were associated with a significantly increased risk for neonatal encephalopathy. The other risk factors as cord pathology, meconuim stained waters etc. somewhat increased the risk (Talvik, 1992; Nelson and Ellenberg, 1984; Badawi *et al.*, 1998).

The identification of the importance of different risk factors for birth asphyxia and poor outcome is difficult. An intervention during pregnancy and labour will also reduce the association between different risk factors and an adverse outcome, as compared with allowing events to take their natural course (Steer *et al.*, 1989). The early identification of possible risk factors and intense

ante-and intranatal care with a timely performed caesarean section reduce the incidence of birth asphyxia. The infants with a severe stage of HIE had significantly more frequently signs of acute intrapartum distress as abruption of placenta and persistent bradycardia compared to infants with a mild or moderate stage of HIE (Table 5). This means that even in the case of low antenatal risk, the delivery must be followed carefully to detect acute distress in time.

The study showed the correlation between the duration of probable distress ($r=0.27$, $p=0.03$) and the development of the severity of HIE. As the duration of possible asphyxia plays an important role in the severity of HIE, a close follow-up of all parturitians is especially needed. It is believed that experience in the interpretation of the cardiotocogram and other signs of foetal distress is particularly important (Murphy *et al.*, 1990; Low *et al.*, 1998).

Conclusion. The severity and duration of signs indicating acute foetal distress are of great importance in predicting the severity of HIE.

5.1.2. Apgar scores and duration of resuscitation

In the present study, in 46 of 86 infants (53%) the 1st minute Apgar score was ≤3, indicating an acute need for the resuscitation of the infant (Table 6). In most infants with an Apgar score ≤3 at the 1st minute, this improved quickly. Several authors have also found that the 1st minute Apgar score was closely related to neonatal mortality and indicated infants who may need resuscitation, and have a risk for disability (Freeman and Nelson, 1988; Talvik, 1992; Nelson and Emery, 1993). In the present study the 1st minute Apgar score of asphyxiated correlated with the development of HIE or psychomotor development at the age of 18 months (respectively $r=0.42$, $p<0.0001$ and $r=0.4$, $p<0.0001$) and this once again shows that Apgar score at birth is a simple marker indicating infants with risk for the developmental delay and need for close follow-up during infancy.

By the age of 5 minutes Apgar scores have improved considerably in most of our infants. Only 2 infants with a moderate, but 10 of 12 infants with a severe stage of HIE still had an Apgar score ≤3 (Table 6). Out of 33 infants whose Apgar score was ≤3 at the 1st min, but improved by the 5th minute, only 4 infants (12%) were disabled, compared to an 84% death and disability rate with a continuing low Apgar score. Thus the 5th minute score is of greater importance in estimating the damage of CNS, reflecting the efficiency of resuscitation as well as babies' condition at birth. The improvement of a low Apgar score by the age of 5 minutes indicates that the risk of death or disability due to birth asphyxia is small (Freeman and Nelson, 1988; Talvik, 1992).

Table 6. Apgar scores at the age of 1, 5 and 10 minutes in term asphyxiated newborn infants.

	HIE =0	HIE=I	HIE=II	HIE=III
1st min Apgar 0–3	0	20	14	12
4–5	2	7	7	0
6	2	7	1	0
7	4	2	0	0
8–9	3	4	3	0
5th min Apgar 0–3	0	0	2	10
4–5	0	8	10	0
6	0	8	6	1
7	1	15	3	0
8–9	8	9	4	0
10th min Apgar 0–3	0	0	0	4
4–5	0	0	0	5
6	0	1	4	3
7	0	9	15	0
8–9	9	30	6	0

Apgar score at the 10th min among asphyxiated newborns has a strong correlation between development of HIE ($r=0.72$, $p<0.0001$) or psychomotor development at the age of 18 months ($r=0.56$, $p=0.0001$). By the age of 10 minutes all infants with a mild or moderate stage of HIE had Apgar scores of at least 6. Only 3 of 12 infants with a severe stage of HIE had an Apgar score of 6 or more at the age of 10 minutes (Table 6). It has been suggested that prolonged low Apgar scores are good predictors of the development of HIE and the long-term outcome of asphyxiated infants at earlier times (Freeman and Nelson, 1988; Talvik, 1992).

Fifty-four of 86 infants (63%) in our study group required bag and mask ventilation. 65% of infants who needed ventilation were successfully resuscitated with only bag and mask ventilation (Table 7). In 24 of 54 infants (44%) ventilated at birth developed only mild signs of HIE with good prognoses for development. In this study the duration of artificial ventilation for resuscitation was considerably shorter in neonates with a mild or moderate stage of HIE compared to infants with a severe stage of HIE (Table 7). A correlation also existed between the duration of initial resuscitation and the severity of HIE ($r=0.58$, $p<0.001$) and the outcome of infants at the age of 18 months ($r=0.53$, $p<0.001$).

36 infants needed bag and mask ventilation for 3 min or more and 41% of them were normal at follow-up. But 9 of 13 newborn infants, who needed

ventilation for 10 min or longer died during the neonatal period or were disabled at the age of 18 months.

Eight newborn infants needed mechanical ventilation for 20 minutes or longer and all developed a severe stage of HIE. Such prolonged resuscitation was associated with an extremely poor prognosis. None of these infants developed normally: 4 infants died (all ventilated for 45 min or longer), 3 infants were severely disabled and only one infant had mild cerebral palsy and a delay in speech development at the age of 18 months. Other investigators have also suggested that the delayed onset of breathing is one of the early indicators of the severity of asphyxia and HIE and also poor outcome (Palmer and Risser, 1993; Thornberg *et al.*, 1995; Ekert *et al.*, 1997). The death of infants, from whom no spontaneous ventilation was recorded before 45 min of age, have also been reported in other studies (Thornberg *et al.*, 1995).

Table 7. Duration of resuscitation in infants with HIE.

Number of infants	Controls N=35	HIE=0 n=9	HIE=I N=40	HIE=II N=25	HIE=III N=12
Infants requiring bag and mask ventilation ≥3min	0	0	9	16	11*♦
Infants requiring intubation at birth	0	0	1	6	12*♦
Infants requiring cardiac massage	0	0	11	12	10*
Infants requiring adrenaline	0	0	0	3	9*♦
Infants requiring intubation	0	0	1	6	12*♦
Duration of ventilation (min)	0	0	3.96±2.6	5.21±2.51	37.5±24.6*♦
Duration of cardiac massage (min)	0	0	1.1±1.14	1.91±1.44	9.8±11.66*♦

* $p<0.05$ HIE III vs HIE I; ♦ $p<0.05$ HIE III vs HIE II

Nineteen of 54 newborn infants (35%) were intubated immediately after birth. The need for intubation was associated with the development of a moderate-severe stage of HIE. Only one of 24 infants who needed bag and mask ventilation required intubation among infants with a mild stage of HIE, compared to 6 of 17 infants (35%) with moderate and all of those with a severe stage of HIE. Only 4 of 19 infants (21%) intubated at birth developed normally: 3 infants with a moderate stage and 1 with a mild stage of HIE, all with meconium aspiration syndrome. The need for intubation at birth was highly correlated

with the severity of HIE (r=0.65, p<0.0001) and psychomotor development at the age of 18 months (r=0.65, p<0.0001). The duration of resuscitation and need for intubation are clinical signs of possible development of a moderate-severe signs of HIE.

The duration of cardiac massage may be an indicator of the severity of asphyxia. In the present study group 33 infants needed cardiac massage to establish a normal heart rate (Table 7). The duration of cardiac massage in infants with a mild and moderate stage of HIE was significantly shorter than in infants with a severe stage of HIE (p<0.005). 17 of 33 newborns (52%) requiring short-term cardiac massage (<5 min) were normal at follow-up. But only 1 of 12 infants requiring cardiac massage for more than 5 minutes developed normally at the age of 18 months.

Only 12 of 33 neonates (36% of infants who required cardiac massage at birth to establish a normal heart rate) also received adrenaline. 9 of 12 newborn infants who required adrenaline at birth developed a severe stage of HIE (Table 7). The outcome of infants who required adrenaline use for resuscitation was poor: 2 infants with a moderate stage of HIE developed normally and one had mild impairments, but of infants with a severe stage of HIE, 4 infants died or were disabled and only one infant was mildly impaired. Successful resuscitation with only ventilation, oxygen and closed chest massage was associated with a favourable outcome, the poor outcome of infants requiring adrenaline for resuscitation has also been reported by several authors (Thornberg *et al.*, 1995; Ekert *et al.*, 1997). Ekert and co-workers (1997) suggested that those chest compressions, presumably a surrogate for the poor recovery of heart rate, were an early indicator of the severity of asphyxia and were associated with a poor prognosis. The need for long-term cardiac massage and adrenaline use to establish a normal heart rate after birth asphyxia indicates that asphyxia was significantly severe and long-term and probably indicates that the decompensation of the circulatory system had occurred.

Conclusions. Simple criteria of asphyxia as low Apgar scores at 5^{th} and 10^{th} minutes of life, long resuscitation time and the need for intubation and adrenaline use are valuable criteria of the severity of HIE and good predictors of brain damage in asphyxiated infants.

5.1.3. Seizures

In the present study, seizures developed after birth in 17 asphyxiated newborn infants. Seizures developed in all 12 infants with a severe stage of HIE. In the group with a moderate stage of HIE (n=25), 5 newborn infants had clinically diagnosed seizures and 5 infants without clinically visible seizures had paroxysmal discharges on the EEG. No infants with a mild stage of HIE also had paroxysmal discharges on EEG investigation, indicating that, as demonstrated

above, clinical stages of HIE are valuable in the evaluation of the severity of asphyxia and brain damage in asphyxiated infants (Talvik, 1992). Our investigation also stresses the need for early EEG investigation in asphyxiated term newborns, especially in asphyxiated infants with a moderate-severe stage of HIE, in order to detect all cases of seizure activity and to provide adequate treatment. Previous research has also shown that about 10–50% of infants lack clinical seizures, and that EEG helps to identify subclinical seizures in asphyxiated infants (Bernes and Kaplan, 1994; Volpe, 1995).

In infants with long resuscitation times and who developed seizures later, the development of seizures was a sign of an extremely poor prognosis. In 10 out of 13 infants with a resuscitation time of 10 min or more, seizures developed during the first day of life, indicating that severe asphyxia with brain damage had occurred. The prognosis of neonates was poor after long resuscitation time and seizures: 4 newborn infants died, 5 were disabled and only 1 was normal at the age of 18 months. Earlier Thornberg and coworkers (1995) had also found that of infants with no respiration efforts at 5 minutes who developed seizures, 50% died and of the survivors 50% had mental retardation, 50% had CP and 33% had epilepsy.

Seizures after asphyxia develop soon after birth. In the present group of asphyxiated newborn infants with seizures, in 14 of 17 infants (82%) seizures developed within 12 hours of age and 11 of 17 (65%) had seizures before 4 hours of age. Earlier studies have also found that 60% of seizures after birth asphyxia develop within 6–12 hours after birth (Holden *et al.,* 1992; Bernes and Kaplan, 1994; Volpe, 1995, Ekert *et al.,* 1997). As the seizures occur so early after asphyxia that secondary brain damage during the reperfusion period is probably not fully developed and is at least partly irreversible, seizures may be an early indicator of severe hypoxic-ischaemic insult indicating infants, who may likely need brain protection treatment in addition to anticonvulsants. It has also previously been suggested that seizures provides an earlier measure of the severity of HIE than the clinical staging of HIE itself, which may develop up to approximately 24 to 36 hours of age (Ekert et al. 1997).

In this study the prognosis in 76% of asphyxiated infants with the development of seizures was poor. In our study group 4 of 17 infants (24%) with clinically visible seizures died, 9 infants (52%) were severely disabled and only 4 (24%) asphyxiated infants with seizures developed normally. The development of seizures among asphyxiated infants bore a close correlation with the development of infants at the age of 18 months ($r=0.56$, $p<0.0001$). The sensitivity of seizures for death or disability was 76%, but specificity was high, 94%. The poor outcome of asphyxiated infants with seizures has been reported earlier, and some authors have found that mortality in asphyxiated infants with seizures is even higher: 40% (Mulligan *et al.,* 1980; Thornberg *et al.,* 1995; Ekert *et al.,* 1997). As seizures after asphyxia indicate severe brain damage,

which are usually not well controlled by anticonvulsants, all infants with seizures after birth asphyxia need treatment in a high-level ICU.

Earlier studies have found that seizures appearing early are associated with more severe brain damage and poorer prognoses than seizures developing by the second day after asphyxia (Holden *et al.*, 1982; Freeman and Nelson, 1988, Ekert *et al.*, 1997). In the present study, 10 of 14 infants (71%) with an early (within 12 hours of age) onset of seizures were disabled or died, compared to the 76% disability rate of all asphyxiated neonates with seizures. We believe that not only the early beginning of seizures, but also the duration of seizures and effectiveness of treatment are important in predicting outcome in infants with seizures after severe birth asphyxia. We found that all 7 infants with seizures beginning before 2 hours of age and whose seizures were frequent and resistant to treatment, had a poor outcome: 4 died and 3 were severely disabled at the age of 18 months. All of these 7 infants with seizures that were difficult to control were severely asphyxiated at birth with 1st min Apgar scores of 3 or less and 5 or less at the 5th min of life, and all needed at least 5 minutes of resuscitation. At the same time, of 3 infants with a short resuscitation time (<5 minutes) had short-term seizures before 2 hours of age, which were well controlled by phenobarbital treatment. Of these infants with very early yet short-term seizures, 2 developed normally and 1 was clumsy at the age of 18 months. The more difficult seizures are to control, the more likely it is that they are indicators of severe brain damage and are associated with death or subsequent CP (Holden *et al.*, 1982; Freeman and Nelson, 1988, Thornberg *et al.*, 1995). We must also remember that seizures which appear early may be an indicator of severe birth asphyxia, but may also be a sign of the antenatal beginning of hypoxic-ischaemic brain damage.

A normal or slightly abnormal EEG during the first 14 days was observed with normal outcome or minor sequelae: of 23 infants with normal EEG background, 1 infant had mild impairments and 1 infant had epilepsy on follow-up. Severe EEG abnormalities during the first 7 days of life (markedly decreased voltage or burst-supression patterns) were found in 8 newborn infants. All but 1 of them had a severe stage of HIE. Newborn infants with severe EEG abnormalities had a poor prognosis: two infants died on the 28th day of life, 5 infants were disabled and only 1 infant with a moderate stage of HIE was normal at the age of 18 months. Two neonates had isoelectric EEG during the first week of life and died on the 8th and 13th day of life. The finding of poor outcome in infants with severe changes in EEG has been noted by others (Archbald *et al.*, 1984; Bernes and Kaplan, 1994; Volpe, 1995; Selton and Andre, 1997).

Conclusions. The development of seizures is a good early indicator of the severity of asphyxia and brain damage. Frequent and long-lasting seizures resistant to therapy and seizures developing after resuscitation lasting 10 min or more, are connected with an extremely poor prognosis.

A normal EEG background in asphyxiated infants is associated with a good prognosis. Severely depressed EEG or burst-suppression pattern are associated with severe sequelae, and neonates with isoelectric EEG died.

5.2. Changes in electrolyte concentrations (Paper I–II)

5.2.1. Changes in umbilical cord blood serum electrolyte concentrations

The data about changes in serum total magnesium (Mg) concentration in asphyxiated term newborn infants described by different authors are controversial. Several authors have found that birth asphyxia causes hypomagnesemia (Tsang *et al.*, 1977; Geven *et al.*, 1993), while at the same time others describe hypermagnesemia after birth asphyxia (Engel and Elin, 1970; Broner *et al.*, 1990; Handwerker *et al.*, 1993). The nature of the causes of changes in serum Mg concentrations in asphyxiated term newborns is also not clear.

In the present study distressed newborn infants without HIE or with the development of mild signs of HIE had significantly higher ($p<0.05$) median concentrations of Mg in umbilical cord blood compared to the control group (Table 8). In infants with the development of moderate signs of HIE, the median concentrations of Mg in umbilical cord blood was not different from the control group, although some infants with a moderate yet not severe stage of HIE were also hypermagnesemic at birth. Infants with the development of a severe stage of HIE had significantly lower ($p<0.05$) median umbilical cord blood Mg concentrations compared to the control group infants. No differences in mean concentrations of Ca^{2+}, K^+ and Na^+ were found in the umbilical cord blood of control and HIE groups.

Table 8. Electrolyte concentrations in umbilical cord blood serum: mean±SD and median (min-max).

	Controls N=35	HIE=0+I N=12	HIE =II N=12	HIE=III N=8
Magnesium	0.72±0.1 0.71 (0.53–1.0)	0.81±0.11* 0.81 (0.61–1.01)	0.78±0.17 0.75 (0.6–1.1)	0.59±0.12* 0.59 (0.5–0.8)
Ionised calcium	1.08±0.17 1.1 (0.77–1.44)	1.08±0.26 1.1 (0.51–1.69)	0.98±0.26 0.95 (0.52–1.38)	1.06±0.21 1.05 (0.8–1.33)
Ionised sodium	141.9±6.35 140.2 (127–168)	141.8±5.2 142.5 (129.7–147.4)	143.0±5.61 144.6 (133.4–152.9)	139.72±3.19 139.35 (136–144.6)
Ionised potassium	5.1±0.61 5.0 (4.2–6.97)	5.4±0.78 5.2 (4.03–6.57)	5.42±1.17 5.04 (4.74–6.37)	4.65±0.6 4.32 (4.2–5.44)

$p<0.05$ HIE I–III vs. control group

Four asphyxiated infants (12.5%) had Mg concentrations above 2SD in umbilical cord blood. In 3 infants with a mild stage of HIE, Mg normalised by the second day of life, but one infant with a moderate stage of HIE also had an increased concentration of Mg (above 2SD) at the age of 34 h. Deviations in the other electrolytes occurred only in single cases (Table 9).

Table 9. Alterations in electrolyte concentrations in asphyxiated infants and these infants' outcome.

	Umbilical cord blood serum n=32			Venous blood serum (24–48h) n=39		
	Total (%)	Death or disability	Mild impairement	Total (%)	Death or disability	Mild impairement
Mg >2SD (n)	4 (12.5%)	0	1	14 (36%)	6	2
Mg <2SD (n)	1 (3%)	1	0	0	0	0
Ca^{2+} <2SD (n)	3 (9%)	0	0	9 (23%)	6	0
Na^{+} <2SD (n)	1 (3%)	0	0	15 (38%)	8	1
K^{+} >2SD	3 (9%)	0	0	1 (3)	1	0

Handwerker and coworkers (1993) described hypermagnesemia in infants born by forceps delivery. Mg was increased in serum 0–2 hours after birth in newborn infants with foetal distress associated with birth asphyxia (Jukarinen *et al.*, 1971). It has been suggested that the cause of such hypermagnesemia is acidosis, which depolarises the cell membrane (Bachman *et al.*, 1976). In normal newborn infants less than 1% of body Mg is distributed extracellularly and therefore a minor loss of intracellular cations could account for the observed rise in Mg concentrations (Engel and Elin, 1970; Broner *et al.*, 1990).

We also discovered a stepwise decrease of Mg in umbilical cord blood in proportion with the developing severity of HIE (Table 8). Several authors have found that birth asphyxia causes hypomagnesemia (Tsang *et al.*, 1977; Geven *et al.*, 1993). It is possible that different changes in Mg concentration are seen following prolonged or versus acute hypoxia, explaining also hypomagnesemia found in newborns with intrauterine growth retardation (Geven *et al.*, 1993). In the present study the newborn infants with growth retardation had normal umbilical cord blood Mg concentrations.

The decreased concentrations of Mg may be involved in the development of HIE as Mg takes part in the biochemical cascade after hypoxic-ischaemic insult. Asphyxia can disturb the neuron's ability to maintain its membrane potential and reduce Mg^{2+} blockade of the NMDA receptor and result in increased Ca^{2+} entry (Greenamyre *et al.*, 1994).

We can speculate that foetal distress with development of mild signs of HIE causes hypermagnesemia, but whether this is a result of the mild hypoxic-

ischaemic insult or a physiological defense mechanism remains to be explained. No changes in Ca^{2+}, Na^{+} and K^{+} levels were found in the umbilical cord blood serum of asphyxiated term infants, indicating that changes of Mg may precede changes in Ca^{2+} and Na^{+} concentrations in the extracellular compartment of asphyxiated infants.

Conclusions. Hypermagnesemia in the umbilical cord is an indicator of the development of a mild stage of HIE and hypomagnesemia of a severe stage of HIE.

5.2.2. Changes in venous blood serum electrolyte concentrations on the second day of life (Paper II)

At the age of 24–48 hours infants with a severe stage of HIE had significantly higher serum concentrations of Mg and infants with a severe stage of HIE also lower concentrations of Ca^{2+} and Na^{+} ($p<0.05$) compared to the control group (Table 10).

Table 10. Electrolyte concentrations in venous blood serum at the age of 24–48 hours (mmol/l): mean (mean±SD) and median (min-max).

	Controls n=35	HIE=0+I n=15	HIE=II n=14	HIE=III n=10
Magnesium	0.86±0.008 0.86 (0.66–0.99)	0.88±0.13 0.86 (0.69–1.15)	1.01±0.22 1.01 (0.7–1.4)	0.97±2.6* 0.97 (0.77–1.23)
Ionised calcium	0.89±0.18 1.0 (0.43–1.1)	0.79±0.15* 0.74 (0.56–1.01)	0.78±0.21 0.83 (0.51–1.06)	0.6±0.2* 0.68 (0.26–0.79)
Ionised sodium	149.5±5.8 148.5 (141–161)	145.48±5.8 146.5 (138.6–154)	143.04±8.63 144 (132–159)	141.8±8.4* 139.4 (129–141)
Ionised potassium	5.1±0.9 4.9 (4.0–6.0)	4.5±0.68 4.6 (3.67–6.48)	4.97±0.58 4.79 (4.28–5.9)	4.9±1.1 4.8 (2.28–7.21)

* $p<0,05$, HIE I–III vs. controls

In the present study, however, we found a high incidence of severe hypermagnesemia and hyponatremia among asphyxiated infants on the second day of life. On the second day of life 36% of asphyxiated infants demonstrated an increase in Mg concentration of more than 2SD, 38% a decrease in Na^{+} of more than 2SD and 23% had a decrease in Ca^{2+} of more than 2SD (Table 9). The incidence of severe hypermagnesemia and hyponatremia among asphyxiated infants on the second day of life was even higher than the incidence of hypocalcemia. Hypocalcemia is a widely known feature of asphyxiated infants, with lower values developing in infants with more severe signs of asphyxia (Petersen *et al.*, 1981). Hyponatremia has also been described in asphyxiated infants (Feldman *et al.*, 1970). A secondary increase in total Mg on the second day

after birth asphyxia in spite of normal acid-base status has not previously been described. A significant correlation was found between the severity of HIE and the concentration of Mg ($r=0.3$, $p<0.05$), Ca^{2+} ($r=-0.4$, $p<0.05$) and Na^{2+} ($r=-0.5$, $p<0.00005$) at the age of 24–48 hours. The more severe the signs of asphyxia, the more severe hypermagnesemia, hypocalcemia and hyponatremia developed by the second day of life.

Acute renal failure may decrease the renal excretion of magnesium and reduce resorption of sodium and calcium in asphyxiated infants (Broner *et al.*, 1990). In the present study acute renal failure during the first days of life was diagnosed in 14 of 41 asphyxiated infants (34%): in all 11 infants with a severe stage of HIE and in 3 infants with a moderate stage of HIE. Acute renal failure correlated with decreased serum Na^{+} concentration on the second day of life ($r=0.49$, $p=0.0003$) but not with Mg and Ca^{2+} concentration. So our data support only the possible role of renal function disturbances in the development of serum Na^{+}concentration derangement, but we must also admit the relatively small number of patients, studied.

But also the changes in the cell membrane integrity may be the causes of these changes in serum electrolyte concentrations in asphyxiated newborns. The plasma membrane is relatively impermeable to divalent ions such as Ca^{2+} and Mg^{2+}, but becomes permeable to them after reoxygenation (Hayasi *et al.*, 1986). Mg^{2+} is mainly an intracellular cation and would be expected to be released with cellular injury or death, thus hypermagnesemia may be an indicator of serious tissue damage (Broner *et al.*, 1990). Alterations in intra-and extracellular Mg concentrations after asphyxia may potentially affect cell function through the interactions of Mg with ionised Ca^{2+} by binding competitively to the same sites as Ca^{2+}, and by altering the distribution of Ca^{2+} due to changes in the influx of Ca^{2+} across cell membranes (Levine and Coburn, 1984). The hypoxia-induced modification of the NMDA receptor-ion channel complex decreases the blocking effect of Mg and leads to increased intracellular Ca^{2+}, which is the key mechanism in the biochemical cascade leading to secondary cell damage after asphyxia (Hoffman *et al.*, 1994; Delivoria-Papadopoulos *et al.*, 1998).

A deviation of at least two electrolytes for more than 2SD from the control group values on the second day of life had a high sensitivity (89%) and specificity (93%) for severe death and disability at the age of 18 months (Table 11). Four infants with a severe stage of HIE had alterations in the concentrations of 3 electrolytes (Mg, Ca^{2+}, and Na^{+}), one of them also in K^{+}, all died. Significant correlation was found between the concentration of Mg ($r=0.34$, $p<0.01$), Ca^{2+} ($r=-0.4$, $p<0.05$) and Na^{+} ($r=-0.47$ and $p<0.005$) at the age of 24–48 hours and the development of severe disability or death.

Table 11. Sensitivity and specificity of changes in electrolyte concentrations (mmol/l) in asphyxiated infants in the venous blood serum at the age of 24–48 hours.

	Sensitivity for death or severe disability	Specificity for death or severe disability	Sensitivity for mild impairment or severe disability	Specificity for mild impairment or severe disability
Magnesium >2SD	66%	70%	53%	72%
Ionised calcium <2SD	66%	92%	46%	90%
Ionised sodium <2SD	88%	78%	64%	78%
One electrolyte deviated >2SD	100%	41%	88%	45%
Two electrolytes deviated >2SD	89%	93%	57%	92%

According to our data, high serum Mg and low Ca^{2+} and Na^{+} concentrations on the second day of life correlated with the severity of HIE and a poor outcome. Both hypermagnesemia and hypocalcemia in critically ill children have been found to be associated with a poor outcome as measured by survival or length of stay in ICU (Broner *et al.*, 1990). Magnesium, calcium and sodium are biologically important minerals involved in maintaining the stability of membranes, and derangements in their homeostasis can precipitate serious and life-threatening problems in critically ill patients (Broner *et al.*, 1990). According to present data, the severe derangement of at least two electrolytes had a high sensitivity and specificity for death or disability, and severe changes in 3 electrolytes was associated with a fatal outcome in all cases, indicating severe cell damage in asphyxiated infants.

Data about improved neonatal survival (Grether *et al.*, 1998), cystic periventricular leucomalacia (FineSmith *et al.*, 1997) and a lower risk of CP (Nelson and Grether, 1995; Schendel *et al.*, 1996) in VLBW infants with prenatal exposure to magnesium sulphate compared to controls, show that treatment with magnesium sulphate may be neuroprotective in severely asphyxiated infants. Data about lower concentrations of umbilical cord blood serum Mg in asphyxiated newborns with a severe stage of HIE may support the suggestion of a possible neuroprotective effect of magnesium sulphate in asphyxiated newborns. The present study, however, showed also a secondary increase in serum Mg concentrations by the second day of life in infants with a moderate-severe stage of HIE and poor outcome. Timing is probably very important in neuroprotective treatment with Mg in asphyxiated newborn infants. Mg may be neuroprotective only during a short time after birth, when the mechanisms leading to secondary cell damage are not completely developed. Later serum Mg may increase due to acute renal failure and due to cell damage. In the case of severe cell damage, which may be indicated by severe derangements in

serum electrolyte concentrations, it is too late to start brain saving procedures including Mg treatment.

Conclusions. Hypermagnesemia, hypocalcemia and hyponatremia developing by the second day of life in asphyxiated infants are a frequent finding in asphyxiated infants and are correlated with the severity of HIE. Severe derangements in at least two electrolytes by the second day of life are connected with severe brain damage and a poor prognosis. We recommend a routine determination of Mg in addition to other electrolytes in asphyxiated infants to evaluate the prognosis more exactly.

5.3. Cerebral ultrasonography

5.3.1. Ventricular size

In the present study the mean size of the lateral ventricles of the brain in control neonates at the age of 12±2 h was 1.19±0.8 mm and did not change significantly during the first 5 days. None of the control infants had a ventricular size of greater than 3 mm. Such a small size of normal lateral ventricles (1.8mm with range 1.3–2.3mm) has been found earlier (Liao *et al.*, 1986) based on the data of 533 infants between 48–96 hours of age. Perry and coworkers (1987) have found that after 26 weeks of gestational age, ventricular size did not vary and only 2.8% of neonates have a ventricular size of greater than 3 mm. No differences in ventricular size between normal infants born by elective caesarean section or vaginal delivery were found in the present study.

Table 12. Incidence of slitlike ventricles in newborn infants.

Newborns with slitlike ventricles/investigated infants (%)	Controls	HIE=0	HIE=I	HIE=II	HIE=III
12±2h	19/30 (63%)	4/8 (50%)	22/24 (92%)	10/14 (71%)	4/10 (40%)
24–35.9h	14/25 (56%)	5/8 (63%)	20/24 (83%)	9/16 (56%)	5/11 (45%)
36–71.9h	13/22 (25%)	5/6 (83%)	20/24 (83%)	12/20 (60%)	6/12 (50%)
72–120h	18/31 (58%)	4/9 (44%)	23/34 (68%)	8/23 (35%)*	3/11 (27%)*
No. of infants with normal ventricle size throughout 5 days	5/35 (14%)	3/9 (33%)	0/40 (0)	4/25 (16%)	2/12 (16%)

*$p<0.05$ HIE II–III vs control group

We found that small or nonvisualised lateral ventricles (≤1mm) were present in 71% of asphyxiated infants with HIE (Figure 1a,b) and also in 63% of control infants at the age of 12±2h (Table 12). No differences in ventricular size between infants with different stages of HIE and the control group were found until 72 hours of age. Furthermore, 10.4% of asphyxiated infants and also 14% of control infants never showed slitlike ventricles during the first 5 days, among them 2 severely asphyxiated infants with acute intrapartum events who later died. No correlation was found between ventricular size during the first 3 days of life and HIE or disability at the age of 18 months.

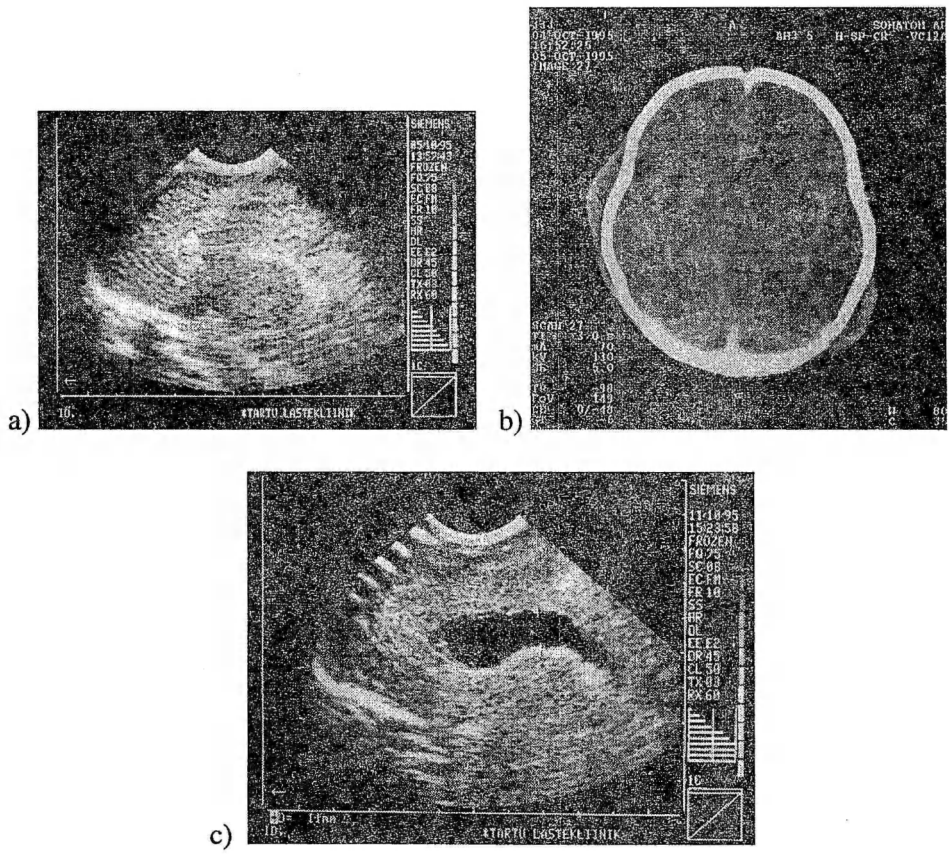


Figure 1. Ultrasound and CT scans of a severely asphyxiated newborn infant with a severe stage of HIE, who died at the age of 13 days.

a) Parasagittal ultrasound scan with severely increased parenchymal echogenicity, indistinguishable normal structures and slitlike lateral ventricle at the age of 30 hours;

b) CT scan of the same infant 2 hours later with loss of cortical grey-white matter differentiation: featureless brain;

c) Parasagittal ultrasound scan with severely increased parenchymal echogenicity and enlarged lateral ventricle at the age of 6 days.

The cause of slitlike ventricles in newborn infants remains unknown. Earlier small ventricles have been suggested to be the consequence of brain oedema, based of the experiments of Myers (1972). The present study indicated that compressed ventricles were not a specific symptom for brain damaged infants, but also occur in infants who are healthy in follow-up as described also by Siegel and coworkers (1984). Winchester and coworkers (1986) suggest that vaginal delivery has a significant association with compressed lateral ventricles, but we were unable to identify any differences in ventricle size during the first 5 days of life among infants born by vaginal delivery or elective caesarean section.

A significantly larger mean ventricle size was found in infants with a moderate and severe stage of HIE compared to the control group and infants with a mild stage of HIE at the age of 72–120h. A correlation was found between ventricular size at the age of 72–120 hours and outcome ($r=0.31$, $p=0.005$). Two infants with a moderate stage of HIE and subsequent serious disability and all 4 infants who died during the neonatal period had enlarged ventricles (more than 4 mm) by the age of 5 days, not found in earlier investigations. The anterior horns of the lateral ventricles were most often involved in this enlargement (Figure 1c). In the most severe cases, diffuse brain atrophy and areas of cystic degeneration take at least 3–7 days to develop (Babcock and Ball, 1983). This phenomenon of some ventriculomegaly related to widely distributed white matter damage, has been mentioned earlier in preterm infants (Eken and coworkers, 1994; Leviton and Gilles, 1996). We suggest that some venrticulomegaly found in some asphyxiated term newborns may also reflect widely distributed white matter damage and is an early sign of severe brain damage.

Eken and coworkers (1994) showed that early white matter necrosis is in most cases connected with antenatal compromise of the infant. In the present study all these infants were severely asphyxiated with severe signs of foetal heart rate changes in CTG, with a 5 minute Apgar score of 5 or below, with a resuscitation time of more than 5 minutes and in all but 1 case, seizures developed within 2 hours of age. All but 1 infant had no risk factors indicating foetal compromise during pregnancy. As none of these infants with early enlargement of the ventricles and poor prognosis showed ventricular enlargement during the first 3 days of life, yet had an acute intranatal event and were born severely asphyxiated, we suggest that the white matter necrosis was a sequelae of severe intanatal rather than antenatal insult in all but 1 of these infants.

Conclusion. As the slitlike ventricles occur in normal newborns with normal development at 18 months, this phenomenon cannot have a predictive value for asphyxiated infants during the first days of life. The development of the enlargement of the ventricles by the 5th day of life may be an early sign of brain necrosis due to widely distributed white matter damage and is a predictive indicator of poor outcome.

5.3.2. Peri-intraventricular haemorrhages

Peri-intraventricular haemorrhage (PIVH) originating from the subependymal germinal matrix is common in asphyxiated premature newborn infants (Volpe, 1995). In term newborn infants PIVH arises most often from the chorioid plexus, but much less frequently (Bernes and Kaplan, 1994).

In healthy term newborn infants a small incidence of PIVH (1.1%) has been discovered at the age of 48–96 h (Perry *et al.*, 1987). The PIVH are usually small (I–II grades) and not associated with any abnormal neurological signs in the neonatal period (Perry *et al.*, 1987). PIVH was not found among newborn infants in our control group, but the incidence (15%) of PIVH among asphyxiated infants was high. Among infants with a moderate stage of HIE PIVH was significantly more frequent (32%) than in infants with a mild (5%) or severe stage of HIE (16%) ($p<0.05$). All our newborn infants had PIVH of grade I–II according to Papile and coworkers (1978). The considerably high incidence of small PIVH among asphyxiated term newborn infants was not connected with poor outcome, and the sensitivity of PIVH for death or disability was poor (31%). It has also earlier been suggested that the presence or absence of PIVH in asphyxiated infants did not worsen the prognosis of asphyxiated term infants and did not help in the prediction of outcome in asphyxiated infants (Pidcock *et al.*, 1990).

Some authors have described only a small proportion of newborn infants (6 of 53 infants) as possessing asymmetric lateral ventricles of the brain (Winchester *et al.*, 1986). The other authors have found that a high proportion of full-term newborn infants have asymmetry of lateral ventricles, with a similar frequency in boys (47%) and girls (41%) (Shen and Huang, 1989). We did not find any newborn infants in the control group with asymmetric ventricles, although we found that 15 of the asphyxiated infants had ventricle asymmetry. In 13 of them the asymmetry of lateral ventricles was connected with a previous unilateral I–II stage of PIVH.

Conclusion. Ischaemia is probably more important than I–II grade PIVH in developing brain damage in the asphyxiated term newborn infant. We suggest that asymmetry in ventricle size in term infants is associated first of all with I–II grade PIVH.

5.3.3. Diffuse parenchymal hyperechogenicity

The parenchymal hyperechogenicity is a common finding in asphyxiated term newborn infants (Siegel *et al.*, 1984).

In the present study parenchymal echogenicity was normal in control group infants and in distressed newborn infants without the development of HIE during the first 5 days of life. In 4 of 24 newborn infants (17%) with a mild

stage of HIE and in 4 of 14 infants (29%) with a moderate stage of HIE, parenchymal hyperechogenicity was visible at the age of 12±2h, but the increase in echogenicity was mild in all but one of these newborns, and normal structures were clearly visible (Table 13). But 9 of 10 investigated infants with a severe stage of HIE at the age of 12±2 hours had a moderate to severe increase in parenchymal echogenicity with a partial or total obliteration of normally visible structures. A correlation was found between increased parenchymal echogenicity at the age of 12±2 hours and the severity of HIE ($r=0.62$, $p<0.0001$) and outcome at the age of 18 months ($r=0.4$ and $p=0.04$). The moderate-severe increase of parenchymal echogenicity at the age of 12±2h has a high sensitivity (88%) for death or severe disability. We believe that a severe increase of parenchymal echogenicity on the first day of life is a good early predictor of permanent brain damage in asphyxiated infants and is available before the full development of the clinical picture of HIE.

Table 13. Incidence of increased parenchymal echogenicity in asphyxiated term newborn infants.

Newborns with parenchymal hyperechogenicity/ investigated infants	Controls	HIE=0	HIE=I	HIE=II	HIE=III
12±2 h	0/30	0/8	Mild: 4/24	Mild: 3/14 Moderate: 1/14	Mild: 1/10 Moderate: 5/10 Severe: 4/10
24–35.9 h	0/25	0/8	Mild 3/24	Mild: 4/16 Moderate: 3/16	Mild: 1/11 Moderate: 3/11 Severe: 7/11
36–71.9 h	0/22	0/6	0/24	Mild: 5/20 Moderate: 4/20	Mild: 1/12 Moderate: 3/12 Severe: 8/12
72–120h	0/31	0/9	Mild: 1/34	Mild: 5/23	Mild: 1/11 Moderate: 5/11 Severe: 6/11

The "bright brain" phenomenon was fully developed by the second-third day of life (Figure 1a,b). Parenchymal hyperechogenicity was found in 4 of 40 newborn infants (10%) with mild HIE, in 9 of 25 infants (36%) with moderate HIE and in all infants with severe HIE during the first 3 days after birth. The sensitivity of the "bright brain" phenomenon at the second day of life for death or disability was high (87%) and a significant correlation was discovered with the severity of HIE ($r=0.54$, $p<0.0001$). Some authors suggest that the predic-

tive value of parenchymal hyperechogenicity is as much as 90–100% (Babcock and Bal, 1983; Siegel *et al.,* 1984).

By the age of 72–120 hours parenchymal echogenicity normalised in all but 1 infant with a mild stage of HIE. In 5 of 23 infants (22%) with a moderate stage of HIE investigated at the age of 72–120 hours still had mildly increased parenchymal echogenicity, 3 of these infants were disabled at the age of 18 months. In 11 of 12 newborn infants with a severe stage of HIE, a moderate-severe increase in parenchymal echogenicity was still visible at the age of 72–120 hours and all these infants died or were disabled at the age of 18 months (Figure 1c). A strong correlation exists between increased parenchymal echogenicity at the age of 72–120 h and HIE (r=0.77 and, 0.0001) and also outcome at the age of 18 months (r=0.66, p<0.0001). Skeffington and Pearse (1983) have also found that if parenchymal hyperechogenicity resolves over a few days, the finding will be non fatal. We believe that increased parenchymal echogenicity at the age of 72–120 h is a better indicator for permanent brain damage and a poor prognosis than the increase of parenchymal echogenicity echogenicity at the of 2 days as suggested earlier (Babcock and Bal, 1983; Siegel *et al.,* 1984).

The neuropathological basis of the phenomenon of increased parenchymal echogenicity is not clear. Upon late ultrasound examinations over the first week of life in the present study in infants with a severe increase of parenchymal echogenicity, the enlargement of the ventricles and multicystic encephalopathy were found, suggestive to be the consequence of extensive hypoxic-ischaemic brain damage. Based on earlier neuropathological examinations, the cause of such increased parenchymal echogenicity in asphyxiated term newborns has been suggested to be a widespread severe anoxic-ischaemic injury of grey and white matter (Siegel *et al.,* 1984) or cerebral oedema (Skeffington and Pearse, 1983). In recent years cerebral oedema as a only cause of brain damage in most term newborn infants has been questioned (Volpe, 1995). Thus it is difficult to say what is the cause of this ultrasonographical phenomenon in different newborn infants, whether cerebral oedema or widespread cell necrosis, as the ultrasonographical picture is the same. In different newborn infants brain oedema or widespread necrosis as a cause of hyperechogenicity may dominate. But as the outcome of infants with long-lasting increased parenchymal echogenicity is so poor, the final morphological bases of this finding may be widespread cell necrosis in the brain. Machine artefact has also been excluded by repeating the investigation with the same settings on subsequent examinations (Skeffington and Pearse, 1983).

An increase of parenchymal echogenicity was not, however, found during the first days of life in all infants with later poor outcome. Skeffington and Pearse (1983) have also found, that 11% of patients with normal parenchyma seen on ultrasonograms had a neurological deficit on follow-up examinations. Siegel and coworkers (1984) have found that if parenchymal hyperechogenicity was not found through ultrasonography, in the autopsy only grey matter without white matter damage was found.

We found 3 asphyxiated infants with a moderate stage of HIE and normal brain parenchyma according to ultrasonography during the first 5 days of life, but with severe disability on follow-up. All 3 infants had signs of acute foetal distress, an Apgar score <6 at the 5th minute and required resuscitation for more than 3 minutes. Yet all 3 newborn infants had different risk factors for hypoxic-ischaemic insult even during pregnancy. It is difficult to say what may have been the cause of encephalopathy in these cases: birth asphyxia and/or something else. The babies might really have had a hypoxic-ischaemic insult during the prenatal period, with further compromise during delivery, as suggested by Badawi and coworkers (1998). The normal-looking brain in the case of moderate-severe signs of encephalopathy through ultrasonography may also be due to the bad sensitivity of the machine. In the case of moderate-severe signs of encephalopathy without ultrasound pathology, we suggest the selection of some other brain-imaging method (MRI, CT scanning), to exclude the possibility of intracranial haemorrhage and other causes of encephalopathy. In the case of normal brain imaging findings, some other cause of encephalopathy must be considered, such as metabolic diseases, chromosomal anomalies etc. We must remember that if in newborns with clinical signs of a moderate-severe stage of HIE, the signs of the morphological damage of the brain are lacking, asphyxia may be not the cause of HIE, but the result of an underling disorder. We have done screening tests for metabolic diseases from urine and chromosomal investigation in all infants with a poor outcome. Four infants were excluded from the study due to a later diagnosis of Werdnig-Hoffman disease, chromosomal anomaly or pulmonary anomaly. In these infants the brain parenchyma was also normal, but moderate-severe signs of HIE developed.

Conclusion. The increase of parenchymal echogenicity is a valuable indicator for the detection of infants with the later development of severe stage of HIE and poor prognosis already at as early as 12±2h from birth. Long-term increased parenchymal echogenicity in asphyxiated term newborn infants is a sign of a poor prognosis.

5.3.4. Focal parenchymal hyperechogenicity

We found increased focal echogenicity in different structures of the brain: the periventricular white matter, brain parenchyma as well as thalamic and cortical regions. The focal increase of parenchymal echogenicity is correlated with pathology discovered later in infancy or with postmortem studies as found also by other authors (Siegel *et al.,* 1984; Eken *et al.,* 1994).

Focal parenchymal echogenicity is generally related to ischaemia in the distribution area of one of the major cerebral vessels, most frequently of the middle cerebral artery (Eken *et al.,* 1994). We found 2 infants with such pathology. A wedge-shaped area of increased echogenicity in the region of the left

capsula interna including a significant decrease of CBF velocity in the left-side middle cerebral artery was found from the second day of life in 1 infant with a moderate stage of HIE (Figure 2a). Paroxysmal discharges from the same region were found on EEG. Later a large porencephalic cyst was found in this region, and clinically this infant developed hemiparesis (Figure 2b). In the other infant with a severe stage of HIE, born after the abruption of the placenta, focal hyperechogenicity in the region of the anterior cerebral artery was found from the age of 10 hours. Cystic degeneration in the same region was seen at the age of 1 month, and hemiparesis also.

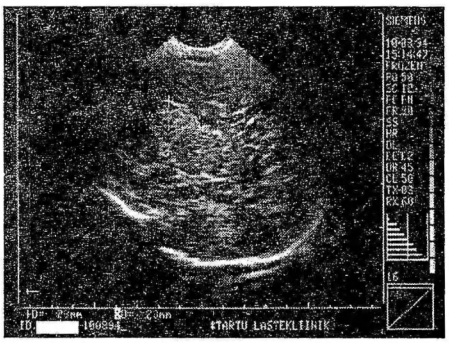
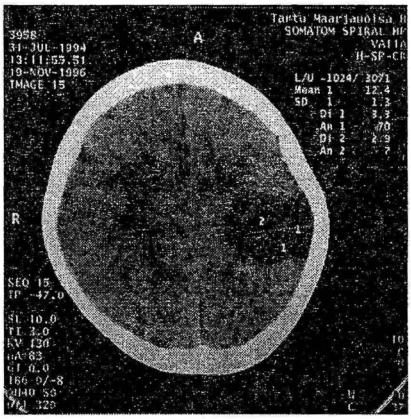
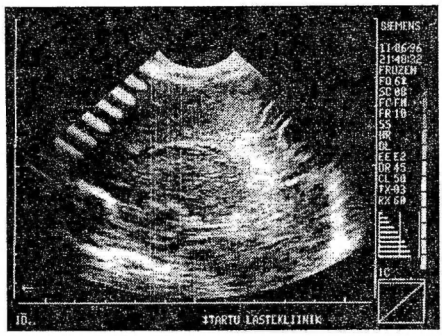
a)  b) 

Figure 2. Ultrasound and CT scans of a severely asphyxiated newborn infant with a moderate stage of HIE, with hemiparesis on follow-up.

a) Parasagittal ultrasound scan with large focal increase in echogenicity in the region of basal ganglia at the age of 2 days.

b) CT scan of the same infant with a porencephalic cyst in the region of capsula interna at the age of 11 months.

Increased periventricular echogenicity (Figure 3a) was found in 1 control infant, in 16 of 40 infants (40%) with mild HIE and in 19 of 25 infants (76%) with a moderate stage of HIE. In infants with a severe stage of HIE the diffuse parenchymal hyperechogenicity was so severe that the increased periventricular echogenicity was hardly distinguishable at the age of 2 days. The cause of a mild periventricular echogenic halo around the posterior part of the lateral ventricles visible in almost all normal infants is controversial and may be a scanning artefact (Siegel *et al.,* 1984). Moderate-severe and long-lasting increased periventricular echogenicity may be caused by hypoperfusion of the periventricular area (Figure 3b). Widespread white matter damage and/or periventricular leucomalacia is found on autopsy in these infants (Siegel *et al.,* 1984; Eken *et al.,* 1994).

a) 

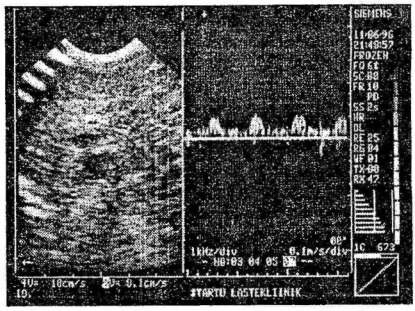
b) 

Figure 3. Ultrasound scans of a severely asphyxiated newborn infant with a severe stage of HIE with spastic diplegia at the age of 18 months.

a) Parasagittal ultrasound scan with moderately increased parenchymal echogenicity, moderately increased periventricular echogenicity and slitlike lateral ventricles at the age of 9 hours.

b) Parasagittal ultrasound scan of the same infant with moderately increased parenchymal echogenicity, and severely decreased CBF velocity in anterior cerebral artery at the age of 9 hours.

The thalamic image with high echogenicity compared to the surrounding brain parenchyma so-called "bright thalamus" may be a sign of severe hypoxic-ischaemic brain damage with adverse outcome (Siegel *et al.,* 1984; Shen *et al.,* 1986; Eken *et al.,* 1994). Hyperechogenicity in the thalamic region take time to develop, and a correct ultrasound diagnosis will not always be possible during the first 24 hours of age unless there is an antenatal onset of the lesion (Eken *et al.,* 1994).

We found 7 infants with a moderate-severe stage of HIE who had a bilateral increase of echogenicity in the thalamus with full development by 2–5 days of life. Four infants with a severe stage of HIE and a severe increase in echogencity in the thalamic region had poor prognoses. Multicystic encephalopathy developed in these infants and 2 infants died and 2 were severely disabled at the age of 18 months. Three infants with a moderate stage of HIE developed a mild increase of echogenicity in the thalamic region: 1 infant with cystic degeneration in the thalamic region is disabled and the other 2 are mildly impaired.

An increase in **cortical and/or subcortical echogenicity** was seen in 11 of 12 with severe HIE and in 2 infants with a moderate stage of HIE, fully developed by the second day of life and still visible by the 5th day of life. All infants had poor outcome.

If not done by 10 MHz transducer ultrasonography probably underestimates the extent of cortical ischaemic damage (Skeffington *et al.,* 1983). Therefore we believe the incidence of cortical damage was actually also higher in the present study group.

Normal findings through ultrasonography during the first days of life were observed in 29 of 40 infants with a mild stage of HIE, but in only 3 of 25 with moderate and in none of the infants with a severe stage of HIE. Only 9% of infants with a normal ultrasound scan developed mild impairments. Thus the present study showed that ultrasound is a suitable screening procedure for the examination of term infants with HIE.

Conclusions. Focal parenchymal hyperechogenicities are frequent findings in asphyxiated term infants. The clinical form of the neurological syndrome of CP depends of the localisation and severity of the damage.

Abnormalities in cerebral ultrasonography are a frequent finding in asphyxiated infants and normal ultrasonography predicts them a favourable outcome.

5.3.5. Late ultrasonography

According to late ultrasonography over the first week of life, multicystic degeneration was found in 2 infants with moderate and in 9 infants with a severe stage of HIE, and all died or were disabled. Cystic degeneration was found in the frontal and parietal regions of the brain, the regions supplied by the anterior and medial cerebral arteries (Figure 4). Cystic degeneration became visible from the second week of life, and all cases could be diagnosed by the age of 1 month. Small cysts of <3 mm were no longer visible by the age of 3 months. Therefore it seems reasonable to perform ultrasonography in infants with a moderate or severe stage of HIE at the age of 1 month in order to detect all infants with cystic degeneration. In 2 infants, who died on the 8th and 13th day of life, enlargement of the anterior horns of the side ventricles without cystic degeneration was found (Figure 1c). We suggest that cystic degeneration had not by the time of the infants' death progressed to the size visible on ultrasonography. But as the enlargement of the ventricles was seen before death, we suggest widespread white matter damage in these infants.

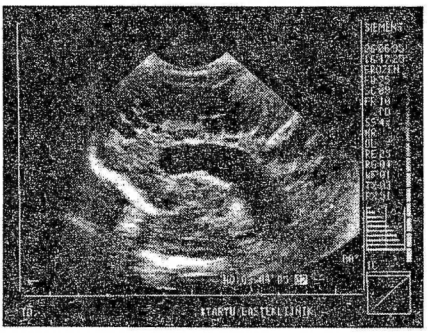


Figure 4. Parasagittal ultrasound scan of a severely asphyxiated newborn infant with a severe stage of HIE with multicystic encephalopathy and enlargement of lateral ventricle at the age of 26 days. The infant died at the age of 28 days.

During repeated ultrasonogragraphical investigations, we found that the signs of brain atrophy develop in asphyxiated infants over the first months of life (Figure 5). By the end of the 1st week 6 infants, by the age of 1 month 18 infants, by the age of 3 months 30 infants and by the age of 6 months 36 infants exhibited enlargement of the ventricles, subarachnoideal space and/or interhemispheric fissure. No infants were added by the age of 9 months (Figure 5). All of these signs were interpreted as signs of brain atrophy, as has also been described earlier (Pidcock *et al.*, 1990; Leviton and Gilles, 1996).

The signs of brain atrophy seen on ultrasonography are valuable predictors of the outcome of asphyxiated infants at the age of 18 months. Of 36 infants with signs of brain atrophy 12 infants with moderate-severe signs of brain atrophy were disabled at the age of 18 months and 12 infants with mild ventricular enlargements were normal at follow-up. In only 1 of 13 infants with later disability at the age of 18 months were the signs of brain atrophy not visible on the late ultrasonography. The signs of brain atrophy on late ultrasonography were highly sensitive (92%), but with lower specificity (65%) for the detection of severe disability.

Earlier studies have also found that major abnormalities (cystic degeneration of the brain and/or moderate-severe enlargement of the ventricles) in asphyxiated infants on late ultrasonography were highly predictive (90%) of a poor prognosis (Pidcock et al. 1990; Leviton and Gilles, 1996). Ventriculomegaly with an increased gyral pattern and without accompanying macrocephaly reflects diffuse white matter damage resulting in diminished white matter or an inadequate density of axons (Leviton and Gilles, 1996). Some occurrences might be due to an impairment of cerebrospinal fluid flow or absorption (Leviton and Gilles, 1996).

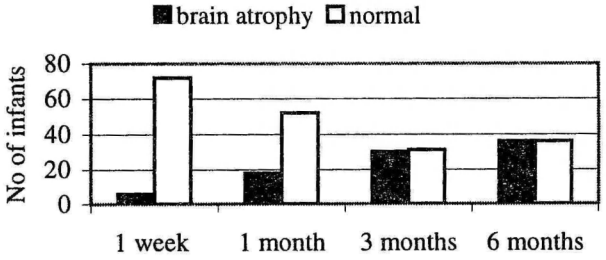


Figure 5. Incidence of signs of brain atrophy among asphyxiated infants with HIE during the first year of life.

Thus, based on our material on asphyxiated infants, we can show that the development of signs of brain atrophy may be delayed for several months. A delayed mechanism of activation of the neurotoxic cascade leading to delayed

cell death days and months later has been suggested in asphyxiated infants (Robertson and Edwards, 1998; Edwards and Nelson, 1998). Pro-inflammatory cytokines and inflammation can contribute to the delayed progression of perinatal brain injury, and these mediators are important targets for neuroprotective interventions in the acute post-injury period (Silverstein *et al.,* 1997; Edwards and Nelson 1998; Nelson and *al.,* 1998). Recent data also indicate that hypoxic-ischaemic insult to the brain can activate PCD pathways in injured neurons, where cells die due to apoptosis (Weissner *et al.,* 1996; Silverstein, 1998). It is suggested that these mechanisms of brain damage may prove to be even more important than the better known pathways of cell necrosis by EAA, OFR, NO and Ca^{2+} influx (Gluckman and Williams, 1992). It is suggested that as PCD is a relatively slow and multistep process, late therapeutic intervention with inhibitors of apoptosis can rescue the ischaemic brain (Silverstein, 1998, Edwards and Nelson, 1998). The finding of the development of brain atrophy during the first months after birth may be an indirect sign of apoptosis is the developing brain. The severity of apoptotic cell death may depend on the severity of asphyxial insult. By the age of one month signs of brain atrophy were well developed in infants with a severe stage of HIE, whereas in infants with a mild stage of HIE the signs of brain atrophy were in most cases visible by the age of 3 months. Our finding of the delayed development of signs of brain atrophy several months after birth asphyxia may prove that rescue therapy with inhibitors of apoptotic pathways of delayed cell death may be effective even days and months after the initial asphyxial insult.

The signs of brain atrophy developed in 83% of infants with brain atrophy by the age of 3 months and only in individual cases were the signs of atrophy first visible at the age of 6 months. In such very late development of signs of brain atrophy, the cause of brain atrophy may not be only widespread hypoxic-ischaemic brain damage. These infants must be followed very correctly and additional investigations are needed to exclude other causes of brain atrophy.

Conclusions. The cystic degeneration of the brain in asphyxiated infants is a sign of an extremely poor prognosis, with the best time to identify being 1 month of age. The signs of brain atrophy develop over the first months after hypoxic-ischaemic insult and the finding is sensitive for poor psychomotor development.

5.4. Changes in CBF velocities in normal infants (Paper IV)

During the transition of the human organism from foetal to neonatal life, extreme changes take place in blood circulation, including that of the brain (Sonesson *et al.,* 1987). After investigation of normal newborn infants we found that no differences exist in CBF velocities (Vs, Vm, Vd) and the resistance index

(RI) between female and male infants during the first week of life. Neither were differences in CBF velocities found between right and left hemisphere arteries. The asymmetry of CBF between arteries is not normal and is associated with the development of PIVH or a stroke (Mullart *et al.*, 1995).

A significantly higher Vs in the internal carotid artery (Vs=45.4±6.2 cm/s) was found in infants born by elective caesarean section compared to infants born by normal vaginal delivery (Vs=39,4±6.2 cm/s) at the age of 12 hours. In the other investigated arteries and during later investigation times such differences between normal vaginal delivery and elective caesarean section was no longer found. The cause of the increased CBF velocity and increased RI during the first hours after elective caesarean section may be differences in the closure in the ductus arteriosus after caesarean and vaginal delivery. Although clinically significant open *ductus arteriosus* was not diagnosed in any of our control group infants, we suggest that the changes in CBF velocity in the internal carotid artery in infants born after elective caesarean section may be caused by the different cardiovascular adaptation of infants born after caesarean section, as suggested earlier by Sonesson and coworkers (1987).

We found that in normal infants, CBF velocities in the medial cerebral and internal carotid arteries were significantly higher than in the anterior cerebral and basilar arteries. Similar lower CBF velocities in the anterior cerebral and basilar arteries and higher velocities in the medial cerebral and internal carotid arteries has also been described earlier (Evans *et al.*, 1988; Hayashi *et al.*, 1992).

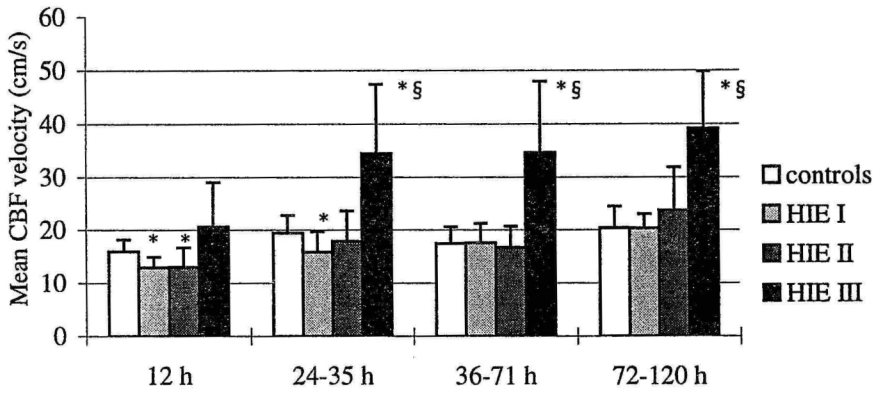
We found, that Vs, Vd and Vm increased significantly by the second day of life compared to the age of 12±2 hours in the anterior, medial cerebral and basilar arteries and in infants with vaginal delivery also in the internal carotid artery ($p<0.05$). By the end of the first week a further increase in CBF velocities takes place compared to the second day of life. Other investigators have also found that the transition from foetal to newborn life is normally associated with an immediate decrease in CBF velocity during the first hours after birth, followed by an increase above the foetal level by the age of 24 hours (Sonesson *et al.*, 1987; Fenton *et al.*, 1990; Connors *et al.*, 1992).

Conclusion. Significant changes in CBF velocities take place during the first week of life in normal newborn infants, being most severe during the first day of life.

5.5. Changes in CBF velocities in asphyxiated infants (Paper III)

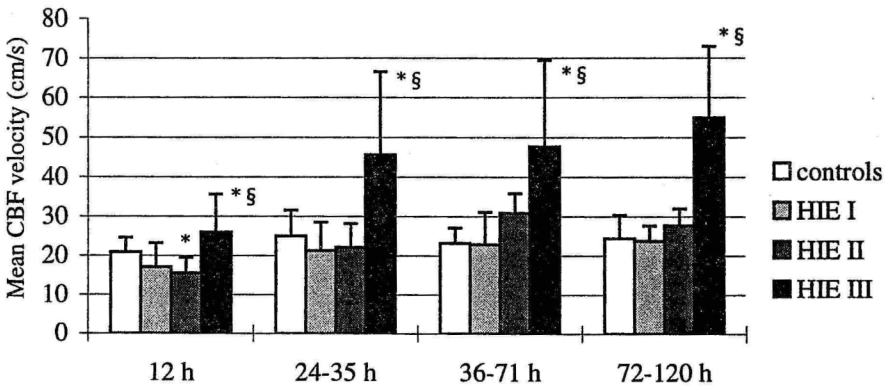
In the group of severely asphyxiated infants with an Apgar score ≤3 at 1st and <7 at the 5th minute of life, infants with a moderate stage of HIE, investigated at the age of 12±2 hours (n=7), had a significantly lower ($p<0.05$) mean CBF

velocity in both the medial and anterior cerebral arteries compared to the control group infants, investigated at the age of 12±2 hours (n=27). From the age of 24–35.9 hours onwards no differences in mean CBF velocity were found between infants with a moderate stage of HIE compared to normal infants (Figure 6–7). In infants with a mild stage of HIE, investigated at the age of 12±2 hours (n=6), a significant decrease ($p<0.05$) of mean CBF velocity was found only in the anterior cerebral artery, and no differences were found from the age of 36–71.9 hours (Figure 6–7).



p<0.05, HIE I–III vs controls; § p<0.05, HIE III vs HIE I–II

Figure 6. Mean CBF velocity in anterior cerebral artery in asphyxiated infants with different development of HIE and infants in the control group during the first 120h of life.



* p<0.05, HIE I–III vs. controls; § p<0.05, HIE III vs. HIE I–II

Figure 7. Mean CBF velocity in medial cerebral artery in asphyxiated infants with HIE and in control group infants during the first 120h of life.

Earlier low CBF velocity (2SD below the mean for normal infants) is associated with poor outcome (Levene *et al.,* 1989). We found that almost half of the investigated infants with birth asphyxia (9 of 21) had mean CBF velocity 2SD below the mean for normal infants at the age of 12±2 hours (Figure 3b). Only 3 of these with CBF velocity 3SD below the mean for normal infants (1 infant with a moderate and 2 with a severe stage of HIE) were disabled by the age of 18 months.

By the age of 24–35 hours only 2 infants with a mild stage of HIE still had a mean CBF velocity 2SD below the mean for normal infants, and both were normal at follow-up. 2 infants with a moderate stage of HIE with a low first day CBF developed increased (more than 2SD, but less than 3SD of the mean for normal infants) mean CBF velocity (1 infant is normal and 1 mildly impaired). Two infants with a severe stage of HIE had extremely low CBF velocities (3SD below the mean for normal infants) at the age of 12±2 hours, in 1 of them CBF normalised later, the other developed extremely high CBF velocities (3SD above the mean for normal infants) by the age of 24–35 hours and cystic degeneration of the brain was observed in this patient at the age of 21 days. Both infants with a severe stage of HIE were later disabled.

The steady decrease of CBF at the age of 12 hours after asphyxia has been described in different animal models (Rosenberg *et al.,* 1989; Kångstöm *et al.,* 1983; Karlsson *et al.,* 1994). A steady decrease in CBF has been described 12 hours after asphyxia in asphyxiated infants (Van Bel., 1993 and 1998; Shadid *et al.,* 1998), indicating that a decrease in CBF velocities probably indicates the real hypoperfusion of the brain at the age of 12 hours. The drop in CBF in asphyxiated infants during the first 12 hours of life was related with an adverse outcome and may possibly cause additional damage (Van Bel., 1993 and 1998; Shadid *et al.,* 1998).

In the present study the most severe hypoperfusion was found in infants with the poorest prognosis, so the hypoperfusion may cause some additional damage in asphyxiated infants, as has been previously suggested by experiments (Ito *et al.,* 1975; Pulsinelli *et al.,* 1982; Karlsson *et al.,* 1994). Although the treatment of asphyxiated infants with free radical scavengers improved CBF velocity and also the outcome of asphyxiated infants in 2 studies (Van Bel., 1998; Shadid *et al.,* 1998), so far we do not have good treatment strategies used everywhere in asphyxiated infants with brain hypoperfusion. Meantime we emphasise that care must be taken not to decrease CBF during diagnostic or therapeutic procedures or with medicaments during the first day of life in asphyxiated infants.

We found that in most infants with a severe stage of HIE (6 out of 8 investigated infants) CBF velocities were significantly increased (3SD above the mean for normal infants) already by the age of 12±2 hours (Figure 8a). In asphyxiated infants with a severe stage of HIE, investigated at the age of 12±2 hours (n=8), the mean CBF velocity in the medial cerebral artery was

significantly increased ($p<0.05$) at the age of 12±2 hours compared to the control group and also to infants with a moderate stage of HIE (Figure 6–7). By the age of 24–35 hours, mean CBF velocity was significantly increased both in the anterior (mean±SD 34.4±13cm/s and median (max-min) 40.0 (52.2–18.1cm/s)) and medial cerebral arteries (45.6±21cm/s and 42.5 (82.0–21.0cm/s) respectively) compared to control group infants (19.5±3.3cm/s and 20.1 (26.6–13cm/s respectively) in the anterior and (25.0±6.6cm/s and 24.5 (45.2–15cm/s respectively) in the medial cerebral arteries ($p<0.001$) and to the infants with a mild or moderate stage of HIE ($p<0.05$).

a) b)

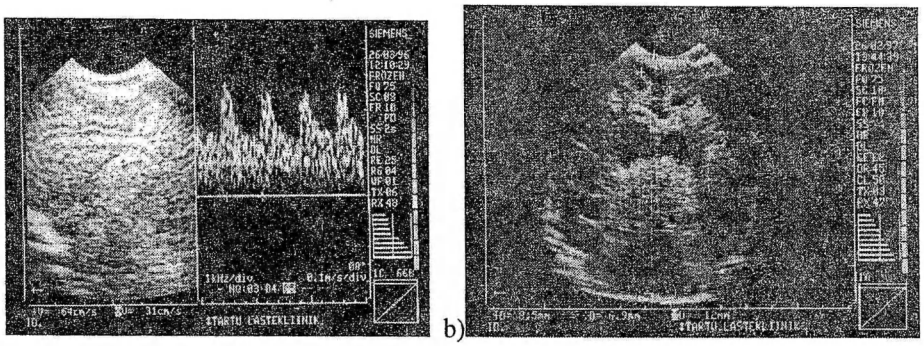


Figure 8. Ultrasound scan of a severely asphyxiated newborn infant with a severe stage of HIE and the IV stage of CP on follow-up.

a) Parasagittal ultrasound scan with severely increased parenchymal echogenicity, severely increased systolic, diastolic and mean CBF velocity and decreased resistance index at the age of 14 hours.

b) Coronal ultrasound scan of the same infant with multicyctic encephalopathy and signs of brain atrophy at the age of 11 months.

High CBF at the mean age of 9 hours has been described by Pryds and coworkers (1990) in infants who subsequently died. Most previous Doppler ultrasonography studies have shown that high CBF velocities develop relatively late (usually after 24 hours after asphyxia) (Archer *et al.*, 1986; Levene *et al.*, 1989; Stark and Seibert, 1994). In the present study six out of eight infants with a severe stage of HIE had an extremely high mean CBF velocity already at the age of 12±2 hours (3SD above mean for normal infants), whereas by the age of 24–35 hours a further increase of CBF velocities was observed in all of them.

Four of these infants with extremely high CBF velocities died, and two survivors developed multicystic encephalopathy and were severely disabled at follow-up (Figure 4; 8b). In infants with birth asphyxia hyperperfusion is thought to be a sign of permanent brain damage with poor neurodevelopmental

prognosis (Levene *et al.,* 1989; Pryds *et al.,* 1990; Stark and Seibert, 1994). The mechanisms of such luxury cerebral perfusion following ischaemic brain injury are still unclear. Neuronal disruption causing a release of vasoactive substances such as adenosine and lactate, or the liberation of EAA (Pryds, 1991), irreversible cerebral vascular injury and complete loss of tone in resistance vessels may play a role in pathogenesis (Levene *et al.,* 1989; Pryds *et al.,* 1990). Extremely high CBF velocities found in asphyxiated infants are a sign of severe brain damage and are associated with death or severe disability. As this phenomenon was discovered already at the age of 12 hours in severe asphyxia, treatment must begin considerably earlier in severely asphyxiated newborn infants.

Several animal models have shown initial hypoperfusion followed by delayed vasodilatation, increased CBF and cerebral energy failure 24–72 hours after hypoxic-ischaemic insult (Lorek *et al.,* 1994; Marks *et al.,* 1996). Although such a schedule of CBF velocity changes has not previously been described in asphyxiated term newborns, we can observe this phenomenon in some infants with a poor prognosis. In our study group we found 6 infants with an initial severe decrease of CBF velocity and the development of a severe increase in CBF velocities. In 2 infants who died, the low CBF velocity was found at the age of 4 and 6 hours respectively, with a development of high CBF velocity by the age of 14 hours. In 4 newborn infants, a CBF velocity at least 2SD below normal at the age of 12 hours was followed by an increase of CBF velocity above 2SD by the second day of life. Only 1 of these 6 infants with an initial low and later high CBF velocity is normal at follow-up, indicating again that the hypoperfusion may cause some additional damage to the brain and need treatment before permanent brain damage develops.

Eleven of 39 infants had decreased RI (<0.56 according to Archer *et al.,* 1986) during the first 35 hours of life. Low RI was combined with low CBF velocities in 3 infants at the age of 12±2 hours and in 2 infants during the period between 24–35 hours from birth. Only 2 infants with a severe stage of HIE had an RI below 0.56 in combination with high CBF velocities at the age of 12±2 hours (Figure 8a), but in 4 other infants with severe HIE, low RI developed by the age of 24 hours.

Our data confirm the finding of other authors (Archer *et al.,* 1986; Stark and Seibert, 1994; Eken *et al.,* 1995) that high CBF velocities are more precise than low RI for the prediction of abnormal outcome. Although in 6 infants with a severe stage of HIE, CBF velocities were increased 3SD above the mean for normal infants at the age of 12 hours, the lowering of RI was seen in only two of them at this early age. Decreased RI was seen in combination with high velocities mainly from the age of 24 hours. Thus lowered RI alone may not predict poor outcome.

No differences were found in the frequency of ante- and intranatal risk factors (infectious complications, bleedings, and degenerative changes in the

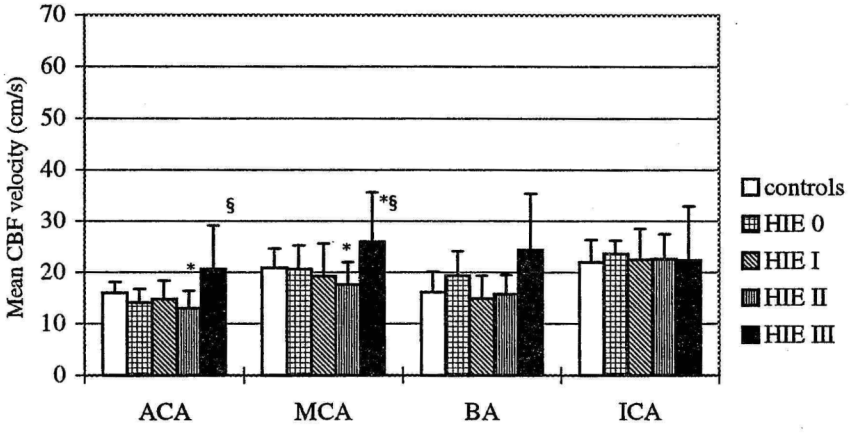
placenta, umbilical cord pathology, foetal growth retardation) between infants with or without a severe increase in CBF velocities. Cystic degeneration of the brain was found in infants with a severe stage of HIE after the first week of life by ultrasonography. As in most severe cases, diffuse brain atrophy and areas of cystic encephalopathy take at least 3–7 days to develop (Babcock and Ball, 1983), the antenatal origin of the insult is also unlikely.

No differences in blood pressure, oxygen or carbon dioxide tension, blood sugar or hematocrit, which could explain the differences in CBF velocities, were found between the groups of infants with different development of HIE or between the infants with normal, high or low CBF velocities. Care was also taken to avoid the investigation of CBF velocities during seizure activity, which can change CBF velocity.

Conclusions. Doppler CBF velocity investigations during the first 12 hours may help to evaluate the severity of brain damage and the long-term prognosis of the infants after birth asphyxia before the full development of the clinical picture of HIE. Hypoperfusion during the first day after asphyxia may cause additional damage to the brain and further decrease of CBF velocity must be avoid. Development of CBF velocity above 3SD was always a sign of severe brain damage and severe disability or even death.

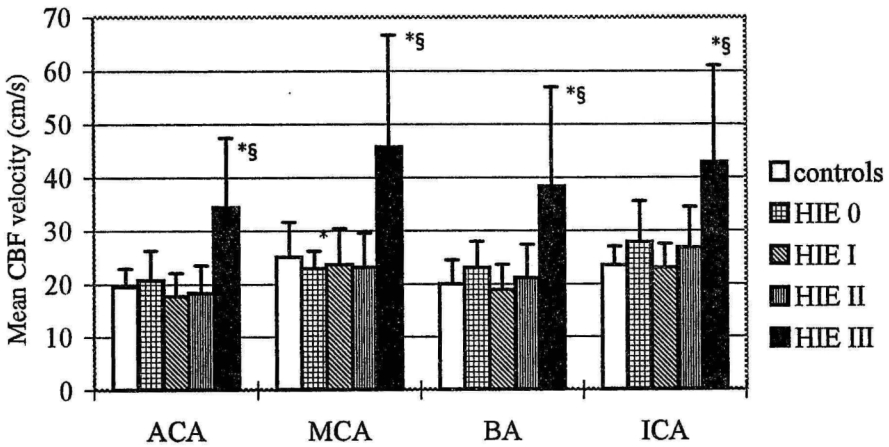
5.6. Regional differences in CBF velocity changes (Paper V)

The present study group of infants with a moderate stage of HIE, investigated at the age of 12±2 hours, had a significantly decreased mean CBF velocity in the anterior (13.0±3.3cm/s, $p<0.005$) and medial cerebral arteries (17.6±4.3cm/s, $p<0.05$), compared to the control group infants (16.0±2.2cm/s and 20.9±3.7cm/s respectively) at the age of 12±2 hours (Figure 9). No statistically significant changes in mean CBF velocities were found in the basilar and internal carotid artery in distressed infants with a mild or moderate stage of HIE or without the development of HIE compared to the control group infants. At the age of 24–35.9 hours the mean CBF velocity was still statistically significantly lower in the medial cerebral artery in distressed infants without the development of symptoms of HIE. In infants with a mild or moderate stage of HIE, statistically significant changes in mean CBF velocities were not found at the age of 24–35.9 hours compared to the control group infants (Figure 10).



* p<0.05, HIE I–III vs. controls, § p<0.05, HIE III vs. HIE II.

Figure 9. Mean CBF velocity (cm/s) in cerebral arteries in control group and asphyxiated infants with HIE at the age of 12±2 hours.



* p<0.05, HIE I–III vs. controls, § p<0.05, HIE III vs HIE II.

Figure 10. Mean CBF velocity (cm/s) in cerebral arteries in control group and asphyxiated infants with HIE at the age of 24–35.9 hours.

Infants with a severe stage of HIE had a significantly increased (p<0.05) mean CBF velocity in the anterior (20.5±9.1cm/s) and medial cerebral artery (26.0±9.6cm/s) at the mean age of 12±2 hours compared to the infants with a moderate stage of HIE and to the control group (Figure 9). Only 8 infants with

a severe stage of HIE were investigated at the age of 12±2 hours and the range was high in this group: 2 of them had a mean CBF velocity in the medial or anterior cerebral arteries 3 SD below the mean for normal infants and 6 infants 3 SD above the mean for normal infants. Although mean CBF in the basilar artery was not statistically significantly different from that of the control group ($p=0.07$), 4 of 6 infants with a mean CBF velocity 3 SD above the mean for normal infants in the anterior and medial cerebral arteries also had a mean CBF velocity 3SD above the mean for normal infants in the basilar artery. No statistically significant changes in the mean CBF velocity was found in the internal carotid artery in infants with a severe stage of HIE. We propose that the increase in CBF velocity in infants with a severe stage of HIE is a sign of vasoparalysis, which also seems to start from the anterior circulation, but in severe cases basilar artery is also involved by the age of 12±2 hours.

At the age of 24–35.9 hours, 9 out of 12 investigated infants with a severe stage of HIE had significantly increased mean CBF velocity in all investigated arteries compared to the control group ($p<0.001$) and to the infants with a mild or moderate stage of HIE ($p<0.05$) (Figure 10). All these infants were dead or were disabled at the age of 18 months. Severe hyperperfusion found also in internal carotid artery indicates a widespread vasoparalysis of the cerebral arteries and is a sign of severe brain damage and extremely poor prognosis.

The present study shows that differences exist between arteries in the development of CBF velocity changes in distressed term infants with HIE during reperfusion. We found that CBF velocity is more easily disturbed in the anterior and medial cerebral arteries and is more preserved in the basilar and internal carotid arteries. We suggest that redistribution of the CBF may be the possible cause of these changes in CBF velocities in different cerebral arteries in asphyxiated infants with HIE. Several animal studies have shown the preferential localisation of CBF changes and cell damage to certain vulnerable regions after cerebral ischaemia (Kangström *et al.*, 1983; Radovsky *et al.*, 1995). This CBF reduction has been shown to correlate with the distribution and extent of hypoxic-ischaemic injury, with the most vulnerable region being the fronto-parietal region (Vannucci *et al.*, 1988; Radovsky *et al.*, 1995). The CBF redistribution of the blood during reperfusion is not described in asphyxiated newborn infants, as is not possible to reveal regional flow changes in infants using global CBF measurements. Yet multiple cystic lesions of the brain parenchyma, supplied by the anterior cerebral circulation, are a recognised pattern in asphyxiated term infants (Sheth *et al.*, 1995; Frigieri *et al.*, 1996). We found multicystic degeneration in 11 asphyxiated infants with a moderate-severe stage of HIE and poor outcome. The multicystic degeneration was more pronounced in the frontal and parietal regions of the brain, supplied by the anterior and medial cerebral arteries (Figure 4; 8b).

Our data show that the preferential location of brain damage in term infants correlates with the findings of the most severe disturbances in CBF velocities in the anterior and medial cerebral arteries. Two infants who died and 4 survivors with a severe increase in CBF velocities in the anterior and medial cerebral arteries developed multicystic encephalopathy mainly in the frontal and parietal regions during the first month of life. Multiple cystic lesions of the brain parenchyma supplied by the anterior cerebral circulation and relative preservation of the cerebellum, brainstem and cerebral structures supplied by the vertebrobasilar circulation is a recognised pattern of severe cerebral injury associated with HIE in term infants (Frigieri *et al.,* 1996; Sheth *et al.,* 1995; Volpe, 1995).

As the mean CBF velocities are disturbed preferentially in the anterior and medial cerebral arteries during the first days after hypoxic-ischaemic insult in term infants and the mean CBF velocities are preserved in the basilar and internal carotid artery in distressed infants at the age of 12±2 hours, we suggest that during Doppler-ultrasonography investigations of asphyxiated infants, the CBF velocities in these arteries should first of all be assessed. Severe increase in CBF velocities also in the basilar and internal carotid arteries indicate a more severe injury of the brain and the development of a severe stage of HIE.

No differences in blood pressure, oxygen or carbon dioxide tension, blood sugar or hematocrit which could explain the differences of CBF velocities between the groups of infants with different stages of HIE or between infants with normal, high or low mean CBF velocities. Thus local mechanisms must be involved in the regulation of CBF velocity in different cerebral arteries in asphyxiated infants.

Conclusions. Regional differences exist in CBF velocity changes in distressed term infants. The brain blood supply from the anterior and medial cerebral arteries will be disturbed more easily compared to the arteries supplying the brainstem and occipital regions of the brain. Regions supplied by the anterior and medial cerebral arteries are the most vulnerable in term infants.

5.7. Psychomotor development of asphyxiated infants

The present study shows, that the stages of severity of HIE are good indicators in the evaluation of the severity of asphyxia, successfully predicting psychomotor developmental disorders.

In the present study asphyxiated term infants had significantly delayed development throughout the first year compared to control group infants. Even distressed infants with no evidence of HIE during the first days of life begin to walk significantly later compared to control group infants (Table 14), indicating that infants with foetal signs of hypoxia without development of signs of HIE

during the first days of life form a risk group in later pschyhomotor development. Previous studies have shown that if the intrapartum asphyxia is severe enough to cause permanent brain damage and disability, there must develop signs moderate-severe stage of HIE during the first days after birth (Nelson and Ellenberg, 1986; Nelson and Emery, 1993; Robertson and Finer, 1993; Volpe, 1995). But in infants with mild signs of HIE minor neurological defects may impair function to some extent and cause delay in motor skills (D'Souza *et al.*, 1981; Robertson and Finer, 1985). These infants with minor neurological defects and mild delay in development during the first months of life need careful follow-up during infancy and early intervention with special care by a habilitation team (physiotherapist, speech therapist, and mother to handle the child). Although significant delay in psychomotor development was seen in some asphyxiated infants without signs of HIE or with a mild stage of HIE during the first year of life, after intervention most of them were normal according to their age at 18 months in present study.

Studies of asphyxiated infants show that language disorders and behavioural problems may arise later and disturb school attendance of asphyxiated infants (D'Souza *et al.*, 1981; Robertson and Finer, 1993; Sööt *et al.*, 1998). In this study the delay in prelanguage development was found in 5 infants with a moderate and in 7 of 8 survivors with a severe stage of HIE. Sööt and co-workers (1998) show that 29% of premature asphyxiated infants failed to start school on time, and that 30% of asphyxiated premature infants had behavioural disturbances at the age of 8 years. This indicates to the need to follow-up language development and discoves behavioural disturbances of asphyxiated infants before the problems arise at school.

Table 14. Achievement of developmental milestones in asphyxiated infants (median (min-max)).

	Controls N=35	HIE=0 n=8	HIE=I N=38	HIE=II N=25	HIE=III N=8
Head control	1(1–2)	1(1–2)	1(1–3)*	1(1–3)*	1.5(1–3)*
Smiling	1(1–2)	1(1–2)	1(1–2)	2(1–2)*	2(2–3)*§
Turning around	5(3–6)	5(4–6)	5(2–9)*	5(2–9)	6(4–9)*§
Sitting	7(5–8)	8(6–10)	7(6–11)	7(6–11)	7(7–12)*§
Crawling	7(6–9)	7(6–8)	7(6–10)*	7(6–10)*	10(8–12)*§
Standing	10(7–12)	11(9.5–12)*	11(8–14)*	11(8–14)*	13(10–16)*§
Walking	11(8.5–13)	12.25(10–13)*	12(9–15)*	12(9–15)	13(10–18)*§

$p<0.05$ HIE 0–III vs. controls, § $p<0.05$ HIE I–II vs. HIE III

There was a significant retardation in head growth in asphyxiated infants with HIE compared to the control group (Table 15). Infants with a mild stage of HIE catch up to normal infants in head growth by the age of 12 months, but infants with a moderate-severe stage of HIE had significantly lower head growth throughout the first year. There were no differences between controls and asphyxiated infants in increases in weight and length during the first 18 months of life in spite of delay in head growth. A correlation was found between the severity of HIE and head growth at the age of 12 months ($r=0.37$, $p<0.001$), indicating that delay in head growth in spite of normal increase in weight and length may be an indicator of brain damage in asphyxiated infants and must be followed in the asphyxiated infant.

Table 15. Growth of head circumference (median (min-max)).

	Controls N=35	HIE=0 n=8	HIE=I N=38	HIE=II N=25	HIE=III N=8
At birth (cm)	35 (33–37)	35 (34–38)	35 (32–37)	35 (33–39)	36 (33–40)
1 month (cm)	38 (35–40)	38 (36–40)	37.5 (35–40.5)	38 (33–41)	36 (34–41)
3 months (cm)	41 (39–43)	41 (40–44)	41 (38–43.5)	40.5 (37–43)*	40 (34–42)*
6 months (cm)	44 (42.5–47)	44 (43–46.5)	44 (41–46.5)	43 (40–46)*	43 (35–45.5)*
9 months (cm)	46 (44–49)	45 (45–49)	45 (42–49)*	45.5 (42–49)*	43 (36–47)*
12 months (cm)	47.75 (44.5–49.5)	48.5 (46–50)	46.5 (43.5–50)*	46.5 (43.5–49)*	45 (38–49)*
18 months (cm)	48.5 (48–50)	49 (47–50.5)	48.5 (46–50)	48 (44–50)*	46.5 (39–51)*

*$p<0.05$HIE I–III vs. controls

The severity of HIE after birth significantly correlates with the outcome of asphyxiated infants at the age of 18 months ($r=0.65$, $p<0.0001$). Although some delay in psychomotor development during the first year of life was found in distressed infants without HIE, by the age of 18 months all infants were normal according to their age. Only 4 of 38 infants (10.5%) with a mild stage of HIE developed mild impairments. But 6 of 25 infants (24%) with a moderate stage of HIE developed a severe disability, and 6 developed a mild impairment by the age of 18 months, but none in this group died. Eleven of 12 infants (92%) with a severe stage of HIE had a poor outcome: 4 infants died and 7 were severely disabled at the age of 18 months (Figure 11).

The study confirms earlier reports that asphyxiated infants without signs of HIE or with mild HIE do not have an increased risk of death or severe disability, but asphyxiated term newborn infants with moderate–severe signs of HIE indicate considerable risk for a poor outcome (Finer *et al.*, 1983; Robertson and Finer, 1993; Thornberg *et al.*, 1995).

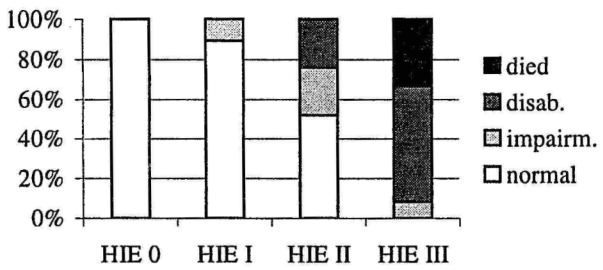


Figure 11. Outcome of infants with HIE at the age of 18 months.

Multiple major neuropathological sequelae were found in asphyxiated infants with a moderate-severe stage of HIE. Epilepsy developed in 4 infants: in 1 infant with a moderate and in 3 of 8 survivors with a severe stage of HIE. Two infants with a severe stage of HIE were blind and deaf. The III–IV stage of CP with definite physical abnormality (significant interference in independent motion) was found in 4 of 25 infants with a moderate stage of HIE and in 7 of 8 survivors with a severe stage of HIE. The delay in prelanguage development was found in 5 infants with a moderate and in 6 of 8 survivors with a severe stage of HIE. Due to severe multiple neurological sequlae in infants with a moderate-severe stage of HIE, these infants are required to undergo follow-up with different specialists: neurologist, physiotherapist, speech therapist, oculist, otologist, neurophysiologist.

Conclusions. Severity stages of HIE after birth correlate with the psychomotor development of asphyxiated infants at the age of 18 months. A delay in development among asphyxiated infants with HIE during the first year of life indicates the need to follow infants with HIE closely during the first year of life and if needed start early habilitation.

6. SUMMARY

The present study of asphyxiated term newborn infants shows that the Sarnat score of the severity of signs of HIE (Sarnat and Sarnat, 1976) is a good indicator of the severity of asphyxia, successfully predicting the brain damage and psychomotor development of asphyxiated infants at the age of 18 months. But as has been suggested by other authors (Volpe, 1995; Blennow, 1995), signs of HIE develop over the first days of life.

The present study shows that several simple markers of the severity of asphyxia in term newborns are available already during the first day of life, accurately predicting the development of the severity of HIE, brain damage and psychomotor developement at the age of 18 months. These simple markers available during the first days of life give us the possibility to commence the treatment of asphyxiated infants if needed, and decrease the full development of HIE and probably also permanent brain damage.

We found that severe intranatal events such as the abruption of the placenta and persistent bradycardia <100 beats are of great importance in predicting the severity of HIE and the outcome of asphyxiated infants at the age of 18 months.

Simple criteria of asphyxia such as low Apgar scores, a long resuscitation time and the need for intubation and adrenaline use are valuable predictors of the severity of HIE and brain damage in asphyxiated infants. In the present study, mechanical ventilation for more than 20 minutes and/or cardiac massage for at least 5 minutes or the use of adrenaline during resuscitation were associated with a high risk of severe HIE and a poor prognosis.

We confirm the finding of earlier investigators (Ekert *et al.*, 1997) that the development of seizures is an early indicator of the severity of asphyxia and brain damage. In the present study, seizures began in 82% of asphyxiated infants with seizures during the first 12 hours after birth. Seizures developing in asphyxiated infants were connected with a poor prognosis: only 24% of asphyxiated infants with seizures after birth developed normally. But this study did not confirm the suggestion by Ekert and coworkers (1997) that the earlier the seizures begin, the greater is the likelihood that they will be connected with a poor prognosis. In the present study, only frequent and long-lasting early seizures resistant to therapy, and seizures developing after resuscitation lasting 10 min or more are connected with an extremely poor prognosis. As seizures after asphyxia indicate severe brain damage and are usually not well controlled by anticonvulsants, all infants with seizures after birth asphyxia need treatment in a high level ICU. In 5 of 25 infants with a moderate stage of HIE but without clinical seizures, paroxysmal discharges were visible on the EEG. Therefore all infants with a moderate stage of HIE, in addition to infants with a severe stage of HIE, will need EEG during the first days of life to assess the existence of seizure activity and the commencement of treatment, if needed.

The data described above concerning changes in serum magnesium concentration in asphyxiated infants are controversial. In the present study hypermagnesemia in the umbilical cord was an indicator of the development of a mild stage of HIE and hypomagnesemia of a severe stage of HIE. Hypermagnesemia found in asphyxiated neonates with mild signs of HIE may be a result of a foetal hypoxic-ischaemic insult or a physiological defence mechanism. Experimental studies and data regarding improved neonatal survival (Grether *et al.,* 1998) and a lower risk of CP (Nelson and Grether, 1995; Schendel *et al.,* 1996) in infants with prenatal exposure to magnesium sulphate show that treatment with magnesium sulphate may be neuroprotective in severely asphyxiated infants. Data about lower concentrations of serum magnesium in asphyxiated newborns with a severe stage of HIE may support the suggestion of possible neuroprotection with magnesium sulphate in asphyxiated newborns.

In the present study, however, hypermagnesemia developed by the second day of life in 36% of asphyxiated infants with HIE. Treatment with magnesium must likely take place very early, before secondary cell damage has developed. Hypermagnesemia, hypocalcemia and hyponatremia developing by the second day of life are frequent findings in asphyxiated infants, and are correlated with the severity of HIE. Severe derangements in at least two electrolytes by the second day of life are connected with severe brain damage and a poor prognosis. We recommend the routine determination of Mg in asphyxiated infants, in addition to other electrolytes, in order to evaluate the prognosis more precisely.

Cerebral ultrasonography is a good screening method to detect the extent of brain damage after birth asphyxia in term newborn infants. Normal brain ultrasonography during the first 5 days after birth predicts a favourable outcome. Abnormalities in early cerebral ultrasonography, however, are a frequent finding in asphyxiated infants. The increase of parenchymal echogenicity is a good early marker to detect infants with the later development of a severe stage of HIE and brain damage. In newborns with a severe stage of HIE the moderate-severe parenchymal hyperechogenicity is visible already at the age of 12±2h. Long-term increased parenchymal echogenicity in asphyxiated infants is a sign of a poor prognosis. Focal parenchymal hyperechogenicity in asphyxiated infants is a frequent finding in asphyxiated infants, but is usually fully developed by the second day after asphyxia.

Slitlike side ventricles were visible in 71% of asphyxiated infants during the first day of life. As the slitlike ventricles also occur among 63% of control group newborns with normal development at 18 months, this phenomenon cannot have a predictive value among asphyxiated infants during the first days of life. The development of the enlargement of the ventricles by the 5th day of life may be an early sign of brain necrosis due to widely distributed white matter damage, and is a predictive indicator of poor outcome. An early beginning of signs of brain atrophy may be an indicator of the severe hypoxic-ischaemic insult or antenatal origin of a hypoxic-ischaemic insult.

During repeated ultrasonographical investigations we found that the signs of brain atrophy in asphyxiated infants develop over a first months of life. The development of cystic degeneration of the brain in asphyxiated infants is a sign of extremely poor prognosis.

Doppler CBF velocity investigations during the first 12 hours may help to evaluate the severity of brain damage and the long-term prognosis of the infants after birth asphyxia before the full development of the clinical picture of HIE. Almost half of asphyxiated infants have decreased CBF velocities during the first day of life, which may cause additional damage to the brain tissue. Care must be taken to avoid a further decrease in CBF velocity during treatment procedures among asphyxiated infants on the first day of life.

The development of CBF velocity above 3SD was always a sign of severe brain damage and severe disability or even death. CBF velocities above 3SD at the age of 12±2 hours in newborn infants with a severe stage of HIE indicate an early beginning of severe vasoparalysis of the brain and are associated with the development of a severe stage of HIE and an extremely poor prognosis. Severe hyperperfusion seen in asphyxiated infants with a poor prognosis already by the age of 12 hours indicates the need to start medical brain protection during the first hours after asphyxia before the signs of permanent brain damage have developed.

Regional differences exist in CBF velocity changes in distressed term infants. CBF in the anterior and medial cerebral arteries will be more easily disturbed in comparison to the arteries supplying the brainstem and occipital regions of the brain, indicating that local mechanisms are involved in CBF velocity regulation in asphyxiated infants. Regions supplied by the anterior and medial cerebral artery are the most vulnerable in term newborn infants.

Stages of HIE correlated with the psychomotor development of asphyxiated infants at the age of 18 months. Although distressed newborn infants without HIE or with mild stage of HIE experienced delayed psychomotor and prelanguage development compared to control group infants during the first year of life, after early intervention by a habilitation team (neurologist, speech-and physiotherapist, mother) normal development according to their age was found at the age of 18 months in all asphyxiated infants without HIE and in 89.5% of infants with a mild stage of HIE. Infants with a moderate or severe stage of HIE had more poor prognosis. Only half of infants with a moderate stage of HIE were normally developed by the age of 18 months and no normal infants were found in the group of infants with a severe stage of HIE at the age of 18 months.

Much scientific work needs to be done in order to achieve a full understanding of what happens with newborn infants during and after a hypoxic–ischaemic insult. Although there is a period of time before the full development of final hypoxic-ischaemic damage, giving us the possibilities at least partly to stop the processes leading to permanent brain damage, probably the best choice is to avoid birth asphyxia.

7. CONCLUSIONS

1. Hypoxic-ischaemic encephalopathy with its severity stages is of great value in characterising babies' condition after birth, in measuring the severity of asphyxia and in predicting the development of brain damage in asphyxiated infants.
2. Low Apgar scores, a long duration of neonatal resuscitation and the need for intubation and adrenaline use are valuable indicators of the severity of perinatal asphyxia and are predictive of long-term outcome in asphyxiated term infants.
3. The development of seizures is an early indicator of the severity of asphyxia, developing in most cases during the first 12 hours after asphyxia. Early, frequent and long-lasting seizures poorly controlled by medications are signs of severe brain damage and are connected with a poor prognosis.
4. Umbilical cord blood magnesium concentration is a useful indicator of the severity of HIE. Hypermagnesemia in the umbilical cord was an indicator of the development of a mild stage of HIE and hypomagnesemia of severe stage of HIE.
5. Hypermagnesemia, hypocalcemia and hyponatremia developing by the second day of life are frequent findings in asphyxiated infants. Severe derangements in two electrolyte concentrations are signs of severe brain damage and are associated with a poor prognosis. We recommend the routine measurement of serum magnesium concentration in asphyxiated infants, as well as those of other electrolytes.
6. Cerebral ultrasonography is a helpful screening tool in the early determination of brain damage and prognosis in asphyxiated term infants. Normal ultrasonography predicts a favourable outcome in asphyxiated term neonates. The early appearance and/or a long-lasting severe increase in parenchymal echogenicity is a good marker of the severity of an asphyxial insult and is a good predictor of outcome in asphyxiated infants. Ventricular size at the age of 5 days, but not during the first days of life, has a value in predicting outcome in asphyxiated infants.
7. The development of cystic degeneration and signs of severe brain atrophy during the first months of life in asphyxiated term infants are connected with a poor outcome.
8. In term healthy infants a significant increase in CBF velocity in different cerebral arteries occurred during the first week of life, with the most severe changes found during the first day of life.
9. Changes in CBF velocities at the age of 12 hours may be indicators of the severity of asphyxia and have a great prognostic value. A decrease in CBF velocity during the first day of life is a frequent finding in asphyxiated term newborn infants and may cause additional damage to the brain. It is impor-

tant not to decrease CBF velocity during diagnostic and therapeutic procedures or with medicaments during the first day of life in asphyxiated infants. The development of an extreme increase in CBF velocity is an indicator of severe brain damage and is connected with a very poor prognosis.

10. CBF velocities in the anterior and medial cerebral arteries are disturbed more easily and the regions supplied by the anterior and medial cerebral arteries are the most vulnerable in asphyxiated term infants.

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Hüpoksilis-isheemiline entsefalopaatia ajalistel asfüksias sündinud vastsündinutel. Prospektiivne kliiniline, biokeemiline ja ultrasonograafiline uuring

Kokkuvõte

Perinataalse asfüksia järel tekkinud hüpoksilis-isheemiline ajukahjustus on endiselt peamine ajaliste vastsündinute haigestumuse ja suremuse põhjus (Volpe, 1995). Et asfüksias sündinud vastsündinuid ravida, on oluline aru saada mehhanismidest, kuidas hüpoksilis-isheemiline ajukahjustus kujuneb (Ruth, 1988; Volpe, 1995). Asfüksia korral tekivad häired organite hapnikuga varustamises, millele võib lisanduda isheemia (Hill ja Volpe, 1982). Kuigi asfüksia korral jaotub verevool ümber, et säilitada aju, koronaararterite ja neerupealiste verevarustus, tekib raske ja pikaajalise asfüksia tagajärjel organite hüpoksilis-isheemiline kahjustus (Fellman ja Raivio, 1997). Aju on hüpoksilis-isheemilisele kahjustusele eriti tundlik (Siesjö jt., 1995). Organi ellujäämiseks on vajalik vere ja hapniku juurdevoolu taastumine, kuid viimaste aastate uurimused on näidanud, et reperfusiooniperioodil toimuvad olulised muutused, mis viivad rakkude lõpliku hukkumiseni (Fellman and Raivio, 1997). Seega kujuneb lõplik ajukahjustus välja mõne aja jooksul pärast asfüksiat (Siesjö jt., 1995; Robertson and Edwards, 1998).

Asjaolu, et lõplik ajukahjustus ei kujune asfüksia ajal, vaid pärast seda, annab võimaluse alustada ravi ka pärast asfüksia lõppu (Volpe, 1995; Robertson and Edwards, 1998). Meie eesmärk on võimalikult vara pärast sündi välja selgitada lapsed, kellel on raske ajukahjustuse oht, et alustada ravi ja varase habilitatsiooniga (Allen ja Riviello, 1992; Nelson ja Emery, 1993; Volpe, 1995).

Kahjuks pole leitud häid meetodeid, millega saaks vahetult pärast sündi objektiivselt hinnata läbi tehtud asfüksia raskust ning mis oleksid piisavalt tundlikud ja spetsiifilised püsiva ajukahjustuse tekke prognoosimiseks (Volpe, 1995).

Uuringu eesmärgid

1. Hinnata perinataalsete riskifaktorite, varaste neonataalsete sümptomite ja hüpoksilis-isheemilise entsefalopaatia (HIE) raskuse prognostilist väärtust (publitseerimata andmed).

2. Hinnata magneesiumi ja ioniseeritud kaltsiumi konsentratsiooni prognostilist väärtust nabaväädi- ja venoosses veres asfüksias sündinud ajalistel vastsündinutel (artiklid I–II).

3. Hinnata aju ultrasonograafiliste muutuste prognostilist väärtust asfüksias sündinud ajalistel vastsündinutel (publitseerimata andmed).

4. Määrata aju verevoolu kiiruse normväärtused erinevates ajuarterites tervetel ajalistel vastsündinutel esimesel elunädalal (artikkel IV).

5. Hinnata esimesel elunädalal esinevate aju verevoolu kiiruse muutuste võimalikku seost hilisema ajukahjustuse ulatusega asfüksias sündinud ajalistel vastsündinutel (artikkel III, V).

6. Leida aju verevoolu kiiruse muutuste erinevusi asfüksias sündinud ajaliste vastsündinute erinevates ajuarterites esimesel elunädalal (artikkel V).

Uurimisgrupid ja metoodika

Uurimisgrupid

Uurimisgrupi moodustasid TÜ Kliinikumi Naistekliinikus ja TÜ Kliinikumi Lastehaiglas aastatel 1994–1997 ravil olnud 90 asfüksias sündinud ajalist vastsündinut (gestatsiooniaeg 37–42 rasedusnädalat), kellel oli diagnoositud loote hüpoksia koos loote südamelöökide sageduse kardiotokograafiliste muutustega ja/või vastsündinu asfüksia (Apgari hinne 5. eluminutiks ≤7). Neli uuritud last lülitati uuringust välja pärast esimese elunädala lõppu, sest neil diagnoositi kromosoomianomaalia (n=2), Werdnig-Hoffmani haigus (n=1) ja kaasasündinud kopsuanomaalia (n=1). Kontrollgrupi moodustasid normaalse sünnikaaluga komplikatsioonideta üksikrasedusest sündinud 35 tervet ajalist vastsündinut. Kontrollgruppi ei võetud vastsündinuid, kellel sünnituse käigus ilmnes loote hüpoksia tunnuseid või kellel vastsündinueas kujunes välja HIE. Väärarendite, süsteemse infektsiooni või hemolüütilise tõvega lapsi ei uuritud.

Uurimismeetodid

1. HIE raskust esimestel elupäevadel hinnati Sarnat, Sarnati järgi (1976), mille alusel jaotati asfüksias sündinud vastsündinud nelja gruppi: 1) lapsed, kellel HIE-t ei kujunenud (n=9); 2) kerge astme HIE-ga (n=40); 3) keskmise astme HIE-ga (n=25); 4) raske HIE-ga (n=12).

2. Nabaväädivere ja 24.–48. elutunnil võetud venoosse vere seerumis määrati kolorimeetriliselt magneesiumi ning ioonselektiivselt ioniseeritud kaltsiumi, naatriumi ja kaaliumi sisaldus.

3. Elektroentsefalograafilisel uuringul hinnati aju bioelektrilist aktiivsust.

4. Aju ultraheliuuringul hinnati aju külgvatsakeste ja teiste liikvoriruumide suurust, ajukoe üldist ehhotihedust, aju ehhotiheduse koldelisi muutusi, verevalumite ja tsüstilise degeneratsiooni olemasolu.

5. Dopplersonograafiliselt uuriti aju verevoolu kiirust eesmises ja keskmises ajuarteris, basilaararteris ning sisemises unearteris esimesel elunädalal.

6. Hinnati laste motoorset, vaimset ja kõne arengut 18 kuu jooksul. Vastavalt tulemustele jaotati asfüksias sündinud kolme gruppi: 1) terved (n=58), 2) kerge arenguhäirega (n=11); 3) raske puudega lapsed (n=13). Neli last suri vastsündinuperioodil.

Uuringu peamised tulemused

Käesolev töö asfüksias sündinud ajaliste vastsündinute uurimisel on näidanud, et HIE oma raskusastmetega on hea kriteerium asfüksia raskuse hindamiseks. HIE kliiniline sümptomatoloogia pärast asfüksiat korreleerub kujuneva ajukahjustuse ulatusega ja laste psühhomotoose arenguga 18 elukuul. HIE kujuneb aga välja esimeste elupäevade jooksul asfüksia järel (Blennow, 1995; Volpe, 1995).

Käesoleva töö alusel võib öelda, et mitmed lihtsad kriteeriumid asfüksia raskuse hindamiseks on olemas juba esimesel elupäeval. Need lihtsad kriteeriumid annavad meile võimaluse vajadusel alustada vastsündinute raviga enne HIE sümptomatoloogia täielikku väljakujunemist ning arvatavasti ka enne püsiva ajukahjustuse teket.

Käesolev töö näitab, et sellised rasked olukorrad sünnituse käigus nagu platsenta irdumine ja püsiv bradükardia <100 korra minutis on olulised kriteeriumid HIE raskuse ja laste arengu prognoosimisel.

HIE raskuse ja ajukahjustuse ulatuse prognoosimisel pärast lapse sündi on olulised madal Apgari hinne, pikk elustamise aeg ning vajadus intubeerida ja kasutada elustamisel adrenaliini. Elustamist vajanud vastsündinutest elustus 65% pärast lühiaegset maskiga ventileerimist. Intubeerida tuli lapsi, kellel hiljem kujunes keskmise või raske astme HIE. Kõigil lastel, kes vajasid elustamiseks kunstlikku hingamist üle 20 minuti ja/või kaudset südamemassaaži üle 5 minuti, kujunes raske astme HIE ja nad surid või neil kujunes püsiv ajukahjustus. Kui vastsündinu vajas elustamisel südametegevuse taastamiseks peale kaudse südamemassaaži ka adrenaliini, oli suur oht raske HIE ja püsiva ajukahjustuse tekkimiseks.

Krampide teke asfüksias sündinud lastel on seotud raske ajukahjustusega ja halva prognoosiga. Ainult 24% asfüksias sündinud lastest, kellel tekkisid sünnijärgsed krambid, arenes normaalselt. Sarnaselt eelnevate töödega (Ekert jt., 1997) näitas käesolev uuring, et krampide teke on varane sümptom asfüksia raskuse ja ajukahjustuse ulatuse hindamiseks. Antud uuringus algasid 82%-l asfüksias vastsündinutest, kellel esinesid krambid, need esimese 12 elutunni

jooksul. Kuid käesolev töö ei kinnitanud varasemat arvamust, et mida varem asfüksia järel tekivad krambid, seda halvem on prognoos. Uurimus näitas, et ainult sagedased, kauakestvad, ravile allumatud ja need krambid, mis tekkisid pärast rohkem kui 10-minutist elustamist, olid seotud raske ajukahjustuse kujunemise ja väga halva prognoosiga. Et asfüksiajärgsed krambid viitavad raskele ajukahjustusele, mis alluvad sageli halvasti ravile, vajavad kõik vastsündinud, kellel need tekivad, ravi kõrgema astme intensiivravi osakonnas. Viiel 25-st mõõduka HIE-ga vastsündinust ei olnud kliiniliselt nähtavaid krampe, kuid esinesid krambiavaldused EEG-l. Seetõttu vajavad kõik mõõduka ja raske HIE-ga lapsed esimestel elupäevadel EEG-uuringut, et hinnata krambiavalduste olemasolu ja vajadusel alustada raviga.

Varasemad andmed vereseerumi magneesiumisisalduse muutustest asfüksias sündinud vastsündinutel on vasturääkivad. Antud töös kujunes hüpermagneseemia korral nabaväädiveres kerge ja hüpomagneseemia korral raske HIE. Asfüksias sündinute magneesiumisisalduse muutuste põhjused pole selged. Asfüksias sündinutel kerge astme HIE-ga võib hüpermagneseemia olla ägeda lühiajalise loote hüpoksia tagajärg või vastsündinu füsioloogiline kaitsemehhanism. Hüpomagneseemia raske astme HIE-ga vastsündinutel võib olla pikemaajalise ja/või kroonilise asfüksia tagajärg. Raske astme HIE-ga vastsündinute nabaväädivere väiksem magneesiumisisaldus kinnitab, et magneesiumil võib olla neuroprotektiivne toime. Magneesiumsulfaadi kaitsvat toimet kinnitavad ka uuringud, mis on näidanud, et vastsündinute suremus (Grether *et al.*, 1998) ja tserebraalparalüüsi tekke oht (Nelson ja Grether, 1995; Schendel jt., 1996) on väiksem vastsündinutel, kes said prenataalselt magneesiumsulfaati.

Ilmselt on oluline, et magneesiumsulfaatraviga alustataks varakult. Teiseks elupäevaks kujuneb 36%-l asfüksias sündinud lastest hüpermagneseemia, 28%-l hüpokaltseemia ja 38%-l hüponatreemia. Elektrolüütide kontsentratsiooni muutuste tugevus teisel elupäeval korrelleerus asfüksias sündinud ajalistel vastsündinutel HIE raskusega. Tugevad (üle 2 SD) vähemalt kahe elektrolüüdi kontsentratsiooni häired, mille põhjuseks võib olla tugev rakukahjustus, olid väga halva prognoosiga. Asfüksias sündinud vastsündinutel soovitatakse vereseerumis lisaks teistele elektrolüütidele määrata rutiinselt magneesiumisisaldus, et hinnata lapse prognoosi täpsemalt.

Aju ultrasonograafia on hea skriiningmeetod ajukahjustuse ulatuse hindamiseks asfüksias sündinud ajalistel vastsündinutel. Normaalne ajusonograafia leid esimesel viiel elupäeval on seotud hea prognoosiga.

Aju parenhüümi ehhogeensuse tõus on varane sümptom ajalistel vastsündinutel, kellel kujuneb raske astme HIE ja ajukahjustus. Raske astme HIE-ga vastsündinutel oli mõõdukas kuni raske üldine aju parenhüümi ehhogeensuse tõus märgatav juba 12.±2. elutunnil. Üle viie päeva kestev aju parenhüümi hüperehhogeensus oli samuti seotud halva prognoosiga. Koldeline aju parenhüümi ehhogeensuse tõus kujuneb asfüksias sündinud ajalistel vastsündinutel välja hiljem: teiseks-kolmandaks elupäevaks.

Aju külgvatsakeste mõõtmetel esimestel elupäevadel ei olnud seost kujuneva ajukahjustuse raskusega. Kitsad aju külgvatsakesed olid 71%-l asfüksias sündinud, kuid samuti 63%-l kontrollgrupi lastest, kes arenesid hiljem normaalselt. Viiendaks päevaks kujunenud külgvatsakeste laienemine >4 mm oli algava ajuatroofia tunnuseks ja seotud halva prognoosiga.

Korduvate ultraheliuuringute käigus selgus, et aju atroofia tunnused kujunevad välja esimeste elukuude jooksul. Aju tsüstilise degeneratsiooni tekkimine on seotud väga halva prognoosiga.

Esimesel elunädalal tõuseb ajalistel tervetel vastsündinutel statistiliselt oluliselt verevoolu kiirus suurtes ajuarterites. Kõige kiiremini muutub aju verevoolu kiirus esimese elupäeva jooksul.

Asfüksias vastsündinu aju verevoolu kiiruse hindamine Doppler-sonograafiliselt esimesel elupäeval on oluline asfüksia raskuse üle otsustamisel enne HIE sümptomatoloogia lõplikku väljakujunemist ja lapse edasise arengu prognoosimisel. Peaaegu pooltel (9 vastsündinul 21-st) raske asfüksiaga vastsündinuist ilmnes 12.±2. elutunnil aju verevoolu kiiruse langus rohkem kui 2 SD, mis võib omakorda kahjustada aju. Seega on oluline esimesel elupäeval vältida asfüksias sündinute aju verevoolu vähendamist raviprotseduuride käigus.

Aju verevoolu kiirenemine üle 3 SD juba 12.±2. elutunnil viitab varasele raske vasoparalüüsi kujunemisele ning tähendab raske astme HIE-t ja väga halba prognoosi. Varane hüperperfusiooni algus raskes asfüksias sündinutel 12. ±2. elutunniks näitab, et aega aju kaitsva ravi rakendamiseks enne püsiva ajukahjustue kujunemist on rasketel juhtudel ainult mõni tund pärast asfüksiat.

Asfüksias sündinud vastsündinutel muutus verevoolu kiirus erinevates aju arterites eri moodi, eelkõige häirub verevoolu kiirus eesmises ja keskmises ajuarteris. Asfüksias sündinud lastel tuleks uurida aju verevoolu kiirust eelkõige nendes arterites. Eesmise ja keskmise ajuarteri varustatavad piirkonnad kahjustuvad ajalisel vastsündinul asfüksia korral kõige kergemini.

HIE raskusastmed korrelleerusid asfüksias sündinud ajaliste vastündinute arenguga 18. elukuul. Isegi asfüksias sündinud, kellel ei kujunenud üldse või kujunesid kerged HIE nähud, jäid esimesel eluaastal oma psühhomotoorse ja keelelise arengu poolest eakaaslastest maha, kuid pärast varajast taastusravi oli 89,5% nende laste areng eakohane 18. elukuuks. Samas olid ainult pooled mõõduka HIE-ga lapsed 18. elukuuks normaalselt arenenud. Ükski raske astme HIE-ga laps ei olnud 18. elukuul normaalselt arenenud.

Täielik arusaamine, mis juhtub vastsündinud lapsega asfüksia ajal ja järel, nõuab veel palju teaduslikku uurimistööd. Kuigi lõplik ajukahjustus kujuneb välja mõninga aja jooksul pärast asfüksiat, on ajukahjustuse täieliku ravi võimalused pärast asfüksiat praegu kasinad. Parim oleks vastsündinu asfüksiat vältida, nii palju kui võimalik.

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PUBLICATIONS

I

P. Ilves, M. Blennow, E. Kütt, M-L. Mägi, G. Kudrjavtseva, H. Lagercrantz, T. Talvik.
Concentrations of magnesium and ionized calcium in umbilical cord blood
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Concentrations of magnesium and ionized calcium in umbilical cord blood in distressed term newborn infants with hypoxic-ischemic encephalopathy

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Ilves P, Blennow M, Kütt E, Mägi M-L, Kudrjavtseva G, Lagercrantz H, Talvik T. Concentrations of magnesium and ionized calcium in umbilical cord blood in distressed term newborn infants with hypoxic-ischemic encephalopathy. Acta Pædiatr 1996: 85: 1348–50. Stockholm. ISSN 0803-5253

Magnesium and ionized calcium in mixed umbilical cord blood was assessed colorimetrically in 38 distressed and 21 healthy term newborn infants. Distressed infants with a severe or moderate degree of hypoxic-ischemic encephalopathy (HIE) ($n = 8$) had significantly lower ($p < 0.001$) concentrations of magnesium (0.52 ± 0.08 mmol/L) compared to the control group (0.69 ± 0.06 mmol/L). No differences in concentrations of ionized calcium between distressed and control infants were detected. □ *Calcium, hypoxic-ischemic encephalopathy, magnesium, newborn infant*

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The pathogenesis of hypoxic-ischemic encephalopathy (HIE) after perinatal asphyxia in term infants is not completely understood (1). At the cellular level, hypoxic-ischemic insult causes a cascade of biochemical events leading to accumulation of intracellular calcium (Ca^{2+}) (1, 2). The syndrome of HIE can be considered as a reflection of the pathological alterations in neuronal function (2).

Magnesium ions (Mg^{2+}) have a binding site inside the NMDA channel and are thereby believed to block excessive Ca^{2+} entry into the cell in a voltage-dependent manner (2, 3). The processes impairing the neuron's ability to maintain its membrane potential could reduce Mg^{2+} blockade of the NMDA receptor and result in increased Ca^{2+} entry (3).

Magnesium sulphate has been used for many years in obstetrics for the treatment of eclampsia and prenatal magnesium sulphate has been associated with improved neonatal survival with a lower risk of cerebral palsy in very low birthweight infants (4). Several experimental studies (5, 6) have indicated a neuroprotective effect of post-ischemic Mg^{2+} against NMDA-mediated brain injury.

Data about normal levels of Mg^{2+} and Ca^{2+} in umbilical cord blood have been published (7–10), but changes in magnesium and calcium levels described in asphyxiated newborn infants are controversial.

The aim of this study was to investigate the concentration of total magnesium (Mg) and ionized calcium (Ca^{2+}) in umbilical cord blood of distressed term newborn infants with HIE.

Methods

Mg was analysed colorimetrically in mixed umbilical cord blood serum by xylidyl blue method with a Technicon Analyzer RA-100 (USA) and Ca^{2+} with an ion-selective analyser Microlyte (Kone, Finland). Apgar scores at birth and the degree of HIE by Sarnat and Sarnat (11) during the first 5 days of life were registered.

Patients

The study group consisted of 38 term newborn infants with fetal distress defined as continuous cardiotocographic changes of fetal heart rate (baseline heart rate < 100, > 170 beats per min or late or prolonged decelerations more than 1 min) and/or neonatal distress (Apgar scores ≤ 7 at the 5th min of life). Newborn infants with major or multiple minor malformations, systemic infections, haemolytic disease and infants of mothers with diabetes or receiving magnesium sulphate were excluded. Infants without any signs of perinatal distress or HIE ($n = 21$) served as controls.

The investigation was approved by the Ethical Committee of the University of Tartu and written informed consent from the mother was obtained in every case. Non-parametric tests were used for statistical analysis. Values are given as mean ± SD and the accepted level for significance was $p < 0.05$.

Table 1. Main characteristics of the groups.

	Controls (mean ± SD) $n = 21$	HIE = 0 (mean ± SD) $n = 8$	HIE = I (mean ± SD) $n = 22$	HIE = II + III (mean ± SD) $n = 8$
Gestation age (weeks)	39.4 ± 1.2	40.1 ± 0.8	39.4 ± 1.1	39.1 ± 1.9
Birth weight (g)	3501.2 ± 0.5	3820.0 ± 0.3	3594.3 ± 0.5	3270.1 ± 0.7
Apgar score at 1 min	8.1 ± 0.5	7.8 ± 0.7	4.6 ± 1.8**	3.5 ± 1.9**
Apgar score at 5 min	8.7 ± 0.4	8.6 ± 0.7	6.7 ± 1.0**	6.0 ± 1.5**
Duration of positive pressure ventil. (min)	0	0	0.8 ± 1.7*	4.0 ± 5.0**
Magnesium in umbilical cord blood serum (mmol/L)	0.69 ± 0.06	0.81 ± 0.11	0.74 ± 0.20	0.52 ± 0.08**
Ionized calcium in umbilical cord blood serum (mmol/L)	1.06 ± 0.14	1.01 ± 0.09	1.04 ± 0.21	1.07 ± 0.19

*$p < 0.05$, **$p < 0.001$: HIE I vs controls or HIE II-III vs controls (Mann–Whitney test).

Results

Severely-distressed newborn infants (Apgar score ≤5 at 5th min or ≤7 at the 10th min of life) had lower mean concentrations of Mg (0.58 ± 0.15 mmol/L or 0.59 ± 0.06 mmol/L) ($p < 0.05$), respectively, compared with the control group (0.69 ± 0.06 mmol/L). There were no changes in mean Ca^{2+} concentrations between these groups.

Based on the clinical signs of the development of HIE, infants were divided into three groups. Main characteristics of the newborn infants are shown in the Table 1. The first group consisted of eight infants with perinatal distress, which was the indication for emergency caesarean section or ventouse delivery in five newborn infants, none displayed signs of HIE later in this group. The second group ($n = 22$) had mild signs of HIE with irritability and brisk reflexes during the first day of life. Eight infants developed moderate or severe HIE with hypotonia, seizures and severe disturbances in consciousness (third group).

The level of Mg in umbilical cord blood correlated with the severity of HIE ($p < 0.005$, Kruskal–Wallis test) (Fig. 1). Distressed newborn infants without any signs ($n = 8$) or with mild HIE ($n = 22$) had slightly higher mean umbilical cord blood Mg concentrations (0.81 ± 0.11 mmol/L and 0.74 ± 0.2 mmol/L, respectively) compared to the control group, but the differences were not significant (Table 1). The mean level of Mg (0.52 ± 0.08 mmol/L) in distressed newborn infants with later evolution of moderate or severe HIE was remarkably lower ($p < 0.001$). No differences were found in mean concentrations of Ca^{2+} between control and HIE groups (Table 1).

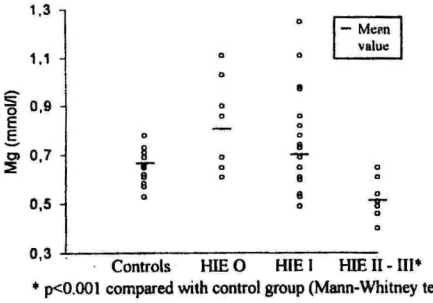

Fig. 1. Individual values of total magnesium (Mg) in umbilical cord blood according to degree of hypoxic-ischemic encephalopahy (HIE) compared with control group.

Discussion

Our results demonstrate that Mg was lower in umbilical cord blood in infants after severe distress and in those with moderate or severe HIE. A stepwise decrease of Mg in umbilical cord blood was seen with developing HIE. Birth asphyxia causes hypomagnesemia (8) and Caddell (12) describes decreased blood pH and normal plasma Mg values in severely asphyxiated neonates, although some newborns have hypomagnesemia. He shows that the true hypomagnesemia will be evident only after the correction of acid–base balance.

The duration and severity of hypoxic-ischemic insult seems to influence the concentrations of Mg in umbilical cord blood. It is possible that different changes in Mg concentrations are seen following chronic vs acute hypoxia. In infants with intrauterine growth retardation, hypomagnesemia has been described (8, 9). Our material included no infants with intrauterine growth retardation.

The infants with changes in Mg concentrations, showed no changes in Ca^{2+} levels. This may indicate that the increase or later decrease of magnesium concentration may precede the changes in Ca^{2+} concentrations.

The decreased levels of magnesium may be involved in the development of HIE as Mg takes part in the

biochemical cascade after hypoxic-ischemic insult. A lower incidence of CP was recently reported in VLBW infants with prenatal exposure to magnesium sulphate, compared to controls (4). Treatment of severely asphyxiated infants with Mg after hypoxic-ischemic insult may limit neuronal injury as indicated in experimental studies (5, 6) and may prevent the development of HIE. The first multicentre study on acute effects of magnesium sulphate in infants with birth asphyxia has indicated that the safe-dosing regimen begin a study evaluating the effectiveness of magnesium sulphate as cerebral protection (13).

We conclude that hypomagnesemia is seen in umbilical cord blood after severe asphyxia and with developing moderate or severe signs of hypoxic-ischemic encephalopathy. We hypothesize that magnesium sulphate might prove to be beneficial by correcting the hypomagnesemia following birth asphyxia.

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II

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Serum total magnesium and ionized calcium concentrations
in asphyxiated term infants with hypoxic-ischaemic encephalopathy.
(submitted for publication).

Serum total magnesium and ionised calcium concentrations in asphyxiated term infants with hypoxic-ischaemic encephalopathy

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Short title: Magnesium and ionised calcium in term infants with HIE.

Abstract

Magnesium, ionised calcium, potassium and sodium concentrations in mixed umbilical cord blood and venous blood serum at the age of 24–48 hours were colorimetrically assessed in 41 asphyxiated and 35 healthy term infants. Asphyxiated infants without any signs or with signs of mild hypoxic-ischaemic encephalopathy (HIE) (n=15) had significantly increased, and infants with a severe stage of HIE (n=11) decreased umbilical cord blood serum magnesium (median (max-min) 0.81 (1.07–0.61mmol/l) and 0.59 (1–0.53mmol/l) respectively, $p<0.05$) compared to the control group (0.71(1–0.53mmol/l)). A secondary increase of magnesium in infants suffering from a severe stage of HIE compared to the control group infants was found by the second day of life (0.97(1.23–0.77mmol/l) and 0.86(0.99–0.66mmol/l) respectively, $p<0.05$). At the age of 24–48 hours hypermagnesemia (>2SD) was discovered in 36%, hyponatremia (<2SD) in 38%, and hypocalcemia (<2SD) in 23% of asphyxiated infants. Derangements (>2SD) in at least two electrolytes were significantly associated with poor outcome. *Calcium, magnesium, hypoxic-ischaemic encephalopathy, newborn infant.*

At the cellular level, a hypoxic-ischaemic insult causes a cascade of biochemical events leading to cell death (1, 2). The syndrome of hypoxic-ischaemic encephalopathy (HIE) can be considered to be a reflection of these alterations in neuronal function (2).

Magnesium ions (Mg^{2+}) are essentially intracellular cations (3) and only about 1% of the body total (Mg) is distributed in the extracellular space (4). Concentration of Mg^{2+} is kept within a relatively narrow range by powerful mechanisms of cellular Mg^{2+} uptake and release located on the plasma membrane as well in the cytosol (3–5). Normally the exchange of Mg^{2+} between extra- and intracellular compartments occurs at a very slow rate (5). A consistently large increase in cytosolic free Mg^{2+} has been observed under relati-

vely drastic conditions such as hypoxia (5), and in such cases a rapid exchange between intra- and extracellular Mg^{2+} compartments occurs (3).

The Mg^{2+} cation is important in the regulation of a large range of intracellular processes and may act to stabilise membrane components. Mg^{2+} is one of the most important regulators of N-methyl-D-aspartate (NMDA) channel function (1, 6). The NMDA-operated channel permits the entry of ionised calcium (Ca^{2+}) and sodium (Na^{+}) and the exit of potassium (K^{+}) ions (6). The channel is normally closed by Mg^{2+} ions in a voltage-dependent manner (2, 6, 7). The processes impairing the neuron's ability to maintain its membrane potential could reduce the Mg^{2+} blockade of the NMDA receptor and result in increased Ca^{2+} entry into the cell (2, 7). The hypoxia-induced modification of the NMDA receptor-ion channel complex decreases the blocking effect of Mg and leads to increased intracellular Ca^{2+} concentrations (1, 8). Increased intracellular Ca^{2+} induces events leading to secondary cell death, such as the synthesis of oxygen free radicals, protease activation, nuclear enzyme activation and DNA fragmentation (1, 8).

Data about normal concentrations of Mg and Ca^{2+} in umbilical cord blood and venous blood have been published (9–13), but data about changes of Mg and Ca^{2+} concentrations described in asphyxiated infants are controversial.

The aim of this study was to investigate the concentration of Mg, Ca^{2+}, Na^{+} and K^{+} in umbilical cord and venous blood serum in asphyxiated infants with different stages of hypoxic-ischaemic encephalopathy (HIE) during the first days of life, and to examine their prognostic value in predicting outcome.

Methods

Mixed umbilical cord blood and venous blood serum collected at the age of 24–48 hours were used for the analysis. Total Mg was analysed colorimetrically in blood serum using the xylidyl blue method with a Technicon Analyzer RA-100 (USA) and Ca^{2+}, Na^{+} and K^{+} with an ion selective analyser Microlyte (Kone, Finland). The person who performed the measurements of electrolytes was blinded to the clinical data of the patient. Venous blood serum urea was measured by kinetic method with a FP 401 Incubator (Labsystem, Finland). Acid-base status measurements were performed with an IL 1420 BG3 Analyzer (Instrumentation Laboratory, Italy).

Apgar scores at birth and the degree of HIE according to Sarnat and Sarnat (14) during the first five days of life were registered by the same examiner and the severest degree was used as a basis for statistical calculations. The renal failure was defined as any episode of oliguria (urine output of less than

1ml/kg/h) in the acute period of disease after the age of 24 hours and/or increased urea concentration in venous blood serum >15mmol/l. The development of infants was followed by a paediatrician, a child neurologist, a physiotherapist and a speech therapist at the ages of 1, 3, 6, 9, 12 and 18 months and classified as normal, mild impairment (abnormalities in tone and reflexes) or severe disability (death or disabling abnormalities in tone and reflexes, seizures, or blindness or deafness). The standardised tests for the neurological examination of infants in Estonia were used to follow the neurodevelopment of the infants. Information on control infants was also collected from the responsible well-baby clinic.

Patients

The study group consisted of 41 infants born during the period from March 1994 to March 1997 and treated at the paediatric intensive care or neonatal care units at the Children's and Maternity Hospitals of the University of Tartu. Term infants (gestation age 37–42 weeks) with signs of foetal distress (meconium-stained amniotic fluids, changes in cardiotocogram) and Apgar scores <4 at the 1^{st} and <7 at the 5^{th} min of life were studied. Infants with major or multiple minor malformations, systemic infections, haemolytic disease and infants of mothers with diabetes or receiving magnesium sulphate before delivery were excluded.

The study protocol did not affect the clinical decision making and treatment of asphyxiated newborn infants. For clinical indications blood serum electrolyte measurements were performed in 3 newborn infants with mild, in 10 infants with moderate and in 8 infants with severe stage of HIE during the first 24 hours of age. Based on these results 1 of 10 infants with moderate and 1 infant with severe stage of HIE needed calcium supplementation due to low Ca^{2+}concentration in blood serum. In these 2 newborn infants only umbilical cord blood serum values were used for analysis.

Term infants without any signs of perinatal distress or HIE (n=35) born at the Maternity Hospital of the University of Tartu served as controls. Eleven asphyxiated infants were born in different county hospitals and the umbilical cord blood of these infants was not available. The data concerning the umbilical cord magnesium and ionised calcium concentrations of 19 asphyxiated and 21 control group infants have been published in our preliminary study on distressed infants (13).

Based on the clinical signs of the development of HIE, infants were divided into 3 groups. The first group (n=15) consisted of 2 infants with perinatal distress without signs of HIE later and 13 infants with mild signs of HIE during the first days of life. Infants with a moderate degree of HIE (n=15) were lethargic, hypotonic, with decreased spontaneous movements and primitive

reflexes with or without seizures. Flaccid infants with coma, absent primitive reflexes and frequent seizures were diagnosed as having a severe stage of HIE (n=11). The main characteristics of the infants are shown in Table 1.

The Ethical Committee of the University of Tartu approved the study and written informed consent from mother/parents was obtained in every case. Non-parametric tests were used for statistical analysis. Values are given as mean ±SD and median with range (max–min). The accepted level for significance was $p<0.05$.

Results

Distressed infants without any signs or with mild signs of HIE had significantly higher median umbilical cord blood Mg concentrations compared to control group infants (median (max–min) 0.81 (1.07–0.61mmol/l) and 0.71 (1–0.53mmol/l) respectively, $p<0.05$). In infants with the development of moderate signs of HIE, median concentrations of Mg in umbilical cord blood (respectively 0.75 (1.1–0.6mmol/l) did not differ from the control group. Infants with the development of a severe stage of HIE had significantly lower median umbilical cord blood Mg concentrations (0.59 (0.8–0.5mmol/l, $p<0.05$) compared to control group infants. No differences in median concentrations of Ca^{2+}, K^+ and Na^+ were found in umbilical cord blood between control and HIE groups.

In the group of normal infants there was a significant increase ($p<0.05$) in serum Mg and Na^+ concentrations and a decrease in Ca^{2+} concentrations by the second day of life compared to umbilical cord blood values (Figure 1).

At the age of 24–48 hours infants with a severe stage of HIE had significantly higher median serum concentrations of Mg and lower concentrations of Ca^{2+} and Na^+ compared to control group infants (Table 1). Individual values of electrolyte concentrations in umbilical cord and venous blood are shown in Figure 1.

Four asphyxiated infants (12.5%) had Mg concentrations >2SD in umbilical cord blood. In 3 infants with a mild stage of HIE, Mg normalised by the second day of life, but one infant with a moderate stage of HIE had increased concentrations of Mg (>2SD) even at the age of 34 h. On the second day of life 36% of asphyxiated infants had increased Mg concentrations for more than 2SD, 38% had decreased sodium for more than 2SD and 28% had decreased calcium for more than 2SD (Table 2).

A weak correlation was found between Apgar scores at 1, 5 and 10 minutes of age, the duration of resuscitation procedures, the severity of HIE and the level of Mg (Spearman $r=0.3$, $p<0.05$), Ca^+ ($r=-0.4$ and $p<0.05$) and Na^+ ($r=-0.5$, $p<0.00005$) at the age of 24–48 hours.

Acute renal failure during the first days of life was diagnosed in 14 of 41 asphyxiated infants (34%): in all 11 infants with a severe stage of HIE and in 3 infants with a moderate stage of HIE. Acute renal failure correlated with decreased serum Na^+ concentration on the second day of life ($r=0.49$, $p=0.0003$) but not with Mg, and Ca^{2+} concentration.

Four out of 11 infants with a severe stage of HIE died during the neonatal period, 6 infants were severely disabled and 1 infants had mild impairments at the age of 18 months. Of 15 infants with a moderate stage of HIE, 1 infant had severe disability and 4 had mild impairments at the age of 18 months. Two infants with mild impairments at the age of 18 months were also found among infants with mild HIE. A significant correlation was found between abnormal outcome (death and severe disability development) and concentrations of Mg ($r=0.34$, $p<0.01$), Ca^+ ($r=-0.4$, $p<0.05$) and Na^+ ($r=-0.47$ and $p<0.005$) (Spearman Rank Correlation) at the age of 24–48 hours.

A deviation of at least two electrolytes for more than 2SD from the control group values on the second day of life had a high sensitivity (89%) and specificity (93%) for severe disability or death (Table 3). Four infants with a severe stage of HIE had alterations in the concentrations of 3 electrolytes (Mg, Ca^{2+}, and Na^+), one of them also in K^+, and all died during the neonatal period.

Discussion

Our results demonstrate that distressed infants without HIE or with the development of mild signs of HIE had higher median concentrations of Mg without changes in Ca^{2+}, K^+ and Na^+ concentrations in umbilical cord blood compared to the control group. Some infants with a moderate but not severe stage of HIE were also hypermagnesemic at birth. Handwerker *et al.* (1993) showed hypermagnesemia in infants born through forceps delivery. Mg was increased in serum 0–2 hours after birth in newborn infants with foetal distress associated with birth asphyxia (15). The cause of such hypermagnesemia may be acidosis, which depolarises the cell membrane (12). In normal newborn infants less than 1% of body Mg is distributed extracellularly and therefore a minor loss of intracellular cations could account for the observed rise in Mg concentrations (16, 17).

In infants with a severe stage of HIE, we found decreased median concentrations of Mg in umbilical cord blood. Hypomagnesemia after birth asphyxia has also been described earlier (10, 13, 18). The decreased concentrations of Mg may be involved in the development of HIE, as Mg takes part in the biochemical cascade after hypoxic-ischaemic insult. Asphyxia can disturb the neuron's ability to maintain its membrane potential and reduce Mg^{2+} blockade of the NMDA receptor and result in increased Ca^{2+} entry. A lower risk of cerebral palsy (19, 21) and cystic periventricular leucomalacia (20) in VLBW

infants with prenatal exposure to magnesium sulphate, has been described in comparison to controls. The treatment of severely asphyxiated infants with Mg after hypoxic-ischaemic insult may be neuroprotective as suggested in experimental studies (8, 22). We can speculate that foetal distress with the development of mild signs of HIE causes hypermagnesemia, but whether this is a result of the insult or a physiological defense mechanism remains to be explained.

Recent data show that decreased concentrations of total Mg in infants do not reflect actual concentrations of ionised magnesium (Mg^{2+}). Newborn infants with low total concentrations of Mg may have normal concentrations of Mg^{2+}, and the treatment of hypomagnesemia in such cases may result in abnormally high Mg^{2+} (23). According to our data, increase in total Mg by 24–48 h of life showed a high specificity and sensitivity in predicting abnormal development or death. As the method of Mg^{2+} measurement is not readily available in all centres, further studies are required to understand the Mg/Mg^{2+} relationship in asphyxiated infants with HIE.

As described in our preliminary study and also earlier (13, 16), asphyxiated infants demonstrate changes in Mg concentrations without differences in Ca^{2+}, Na^{+} and K^{+} concentrations in umbilical cord blood. This may indicate that changes in Mg may precede changes in Ca^{2+} and Na^{+} concentrations in the extracellular compartment in asphyxiated infants.

By the second day of life Mg and Na^{+} concentration has increased and Ca^{2+} decreased in venous blood serum compared to the first day in normal infants, possibly due to low urinary Mg and Na^{+} excretion and reduced Ca resorption during the first 48 hours of life (10, 18).

Similar but more profound changes in median serum Mg, Ca^{2+} and Na^{+} concentrations were observed in asphyxiated infants with a severe stage of HIE at the age of 24–48 hours compared to the control group. A secondary increase in Mg on the second day after asphyxia in spite of normal acid base status has not yet been described. We discovered a high incidence of severe hypermagnesemia and hyponatremia in asphyxiated infants on the second day of life. Derangements of Mg, and Na^{+} were even more frequent than the occurrence of hypocalcemia. Hypocalcemia is a widely known feature of asphyxiated infants with lower values, developing in infants with more severe signs of asphyxia (24). Hyponatremia has also been also described in asphyxiated infants (25).

Acute renal failure may decrease the renal excretion of magnesium and reduce resorption of sodium and calcium in asphyxiated infants (17). Our data support only the possible role of renal function disturbances in the development of serum Na^{+}concentration derangement, but we must also admit the relatively small number of patients, studied.

According to our data, high serum Mg and low Ca^{2+} and Na^{+} concentrations on the second day of life correlated with the severity of HIE and poor outcome. Also according to our data, the severe derangement of at least two electrolytes had high sensitivity and specificity for death or abnormal development, and

severe changes in 3 electrolytes were associated with a fatal outcome in all cases. Both hypermagnesemia and hypocalcemia in critically ill children have been found to be associated with a poor outcome as measured by survival or length of stay in ICU (17). The plasma membrane is relatively impermeable to divalent ions such as Ca^{2+} and Mg^{2+} but becomes permeable to them after re-oxygenation (26). Mg^{2+} is mainly an intracellular cation and would be expected to be released with cellular injury or death, and thus hypermagnesemia may be an indicator of serious tissue damage (17). Alterations in intra- and extra-cellular Mg concentrations after asphyxia may potentially affect cell function through its interactions with ionised Ca^{2+} by binding competitively to the same sites as Ca^{2+}, and by altering the distribution of Ca^{2+} due to changes in the influx of Ca^{2+} across cell membranes (4). The hypoxia-induced modification of the NMDA receptor-ion channel complex decreases the blocking effect of Mg and leads to increased intracellular Ca^{2+}, which is the key mechanism in the biochemical cascade leading to secondary cell damage after asphyxia (1, 8).

Conclusions. Magnesium, calcium and sodium derangements are a frequent finding in asphyxiated infants, and these abnormalities are significantly associated with a poor outcome. Hypermagnesemia in umbilical cord blood is associated with the development of mild signs of HIE and hypomagnesemia with a severe stage of HIE. Hypermagnesemia, hypocalcemia and hyponatremia developing by the second day of life in asphyxiated infants are associated with severe signs of HIE and a poor prognosis. For a better outcome prediction we recommend the routine determination of magnesium in addition to other electrolytes in asphyxiated infants.

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Table 1. Main characteristics of the groups (mean±SD), Apgar scores and concentrations of electrolytes in venous blood serum at the age of 24–48 hours (mmol/l) (median and max–min).

	Controls	HIE=0+I	HIE =II	HIE=III
GA (weeks)	39.8±1.2	39.9±1.0	40.7±1.1	40.8±1.8
Birth weight (g)	3611±454	3723±647	3686±882	3621±591
Apgar score				
1 min	8(7–9)	3(1–3)	2(1–3)**	1(0–3)*
5 min	9(8–9)	6(4–6)	5(1–6)**	2(0–6)*
10 min	9(8–9)	8(6–9)	7(6–8)**	4(2–7)*
Magnesium (mmol/l)	0.86(0.66–0.99)	0.84(0.69–1.15)	1.01(0.7–1.4)	0.97(0.77–1.23)♦
Ionised calcium (mmol/l)	1.0(0.43–1.1)	0.74(0.56–1.01)♦	0.83(0.51–1.06)	0.68(0.26–0.79)♦
Ionised sodium (mmol/l)	148.5(141–161)	146(138–154)	144(132–159)	139(129–141)♦
Ionised potassium (mmol/l)	4.9(4.0–6.0)	4.53(3.67–6.48)	4.79(4.28–5.9)	4.8(2.28–7.21)

*p<0.05; HIE III vs controls and HIE I–II (Kruskal Wallis test).
**p<0.05; HIE II vs HIE I (Kruskal Wallis test).
♦p<0.05: HIE I–III vs controls (Kruskal Wallis test)

Table 2. Alterations in electrolyte concentrations in asphyxiated infants and the outcome of these infants.

	Umbilical cord blood serum n=32			Venous blood serum (24–48h) n=39		
	Total (%)	Death or severe disability	Mild impairment	Total (%)	Death or severe disability	Mild impairment
Mg >2SD (n)	4 (12.5%)	0	1	14 (36%)	6	2
Mg <2SD (n)	1 (3%)	1	0	0	0	0
Ca^{2+} <2SD (n)	3 (9%)	0	0	9 (23%)	6	0
Na^{+} <2SD (n)	1 (3%)	0	0	15 (38%)	8	1
Ca^{+} >2SD	3 (9%)	0	0	1 (3)	1	0

Table 3. Sensitivity and specificity of electrolyte concentrations in asphyxiated infants in venous blood serum at the age of 24–48 hours (mmol/l).

	Sensitivity for death or abnormal development	Specificity for death or abnormal development
Magnesium >2SD	66%	70%
Ionised calcium <2SD	66%	92%
Ionised sodium <2SD	88%	78%
One elctrolyte deviated >2SD	100%	41%
Two electrolytes deviated >2SD	89%	93%

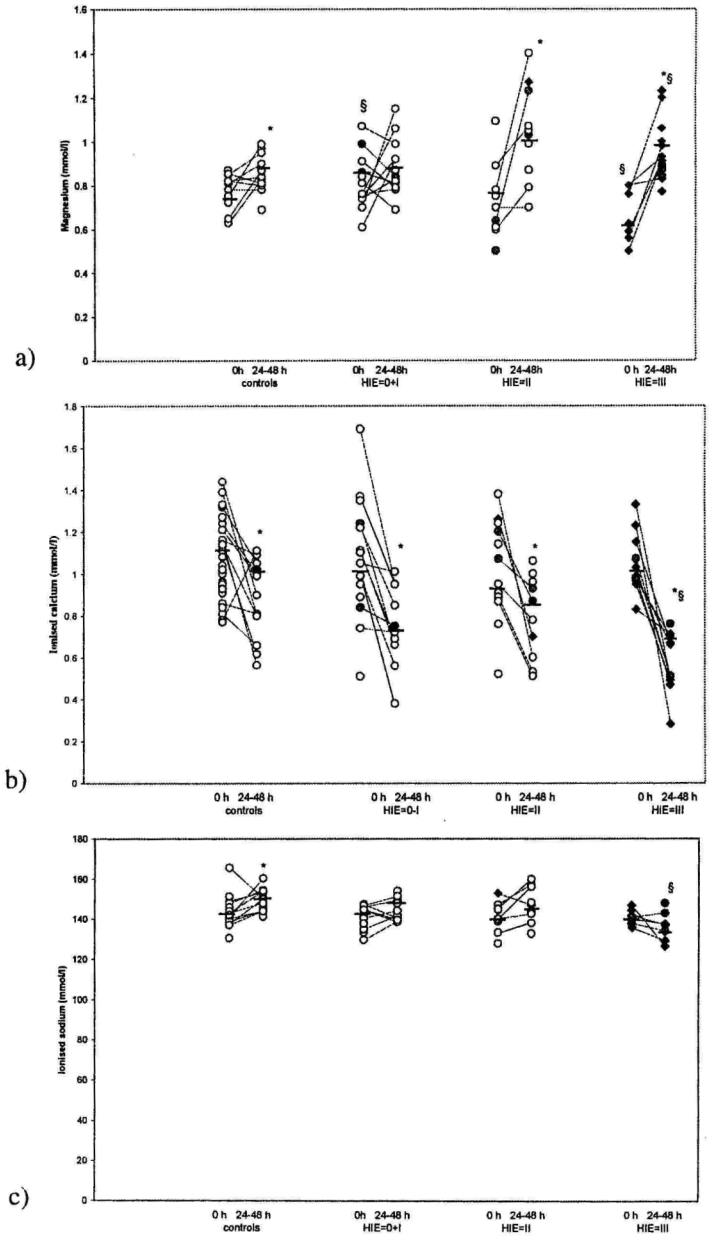

Figure 1. Individual values of a) total magnesium, b) ionised calcium and c) ionised sodium in umbilical cord blood (0 h) and venous blood serum (24–48 h) according to degree of hypoxic-ischaemic encephalopahy (HIE) indicated as: o-infants with normal development, •-infants with mild impairment, ♦-infants with disability or who died subsequently *$p<0.05$ electrolyte values at the age 24–48 h vs umbilical cord values of the same group; §$p<0.05$ HIE I–III vs controls at the same age.

III

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Changes in Doppler ultrasonography in asphyxiated term infants with hypoxic–ischaemic encephalopathy

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Ilves P, Talvik R, Talvik T. Changes in Doppler ultrasonography in asphyxiated term infants with hypoxic–ischaemic encephalopathy. Acta Pædiatr 1998; 87: 680–4. Stockholm. ISSN 0803–5253

Cerebral blood flow velocity was assessed by pulsed-Doppler ultrasonography in 39 asphyxiated and 35 healthy term newborn infants during the first days of life. Asphyxiated infants, investigated at the age of 12 ± 2 h, with moderate stage hypoxic–ischaemic encephalopathy (HIE) ($n = 7$) had decreased (15.6 ± 3.9 cm/s) and infants with severe stage of HIE ($n = 8$) increased (26.5 ± 9.6 cm/s) mean cerebral blood flow velocity in medial cerebral artery compared to the control group (20.9 ± 3.7 cm/s). Four out of six infants with severe stage of HIE and mean cerebral blood flow velocity of 3 SD above the mean for normal infants at the age of 12 h died and two developed multicystic encephalopathy during the neonatal period. We conclude that severe post-hypoxic increase of mean cerebral blood flow velocity at the age of 12 ± 2 h is connected with development of severe stage HIE and poor prognosis. □ *Asphyxia, cerebral blood flow velocity, infant, hypoxic–ischaemic encephalopathy*

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Neonatal asphyxia causing hypoxic–ischaemic encephalopathy (HIE) frequently results in the chronic handicapping conditions of cerebral palsy, mental retardation, learning disabilities and epilepsy (1). The pathogenesis of HIE after perinatal asphyxia in term infants is not completely understood (2), but impaired energy metabolism and disturbed cerebral circulation seem to play a key role in causing brain damage (2).

The period following termination of the asphyxial insult is critical as progression to brain injury occurs (1). Early recognition of the hypoxic–ischaemic injury is important in guiding management during those critical first days (3). The symptoms of HIE develop over the first days of life and exact clinical assessment is complicated as the babies are usually mechanically ventilated and/or sedated. Imaging findings on ultrasonography (3, 4), computed tomography scan (5) or magnetic resonance spectroscopy (7) may be normal despite anoxic injury, if performed on the first day after injury. Neurophysiological methods (somatosensory evoked potentials, visual evoked potentials, cerebral function monitoring) (4) and near-infrared spectroscopy (8) have proved predictive also during the first hours after asphyxic insult, but are not available in all centres. Doppler ultrasonography is a non-invasive method, which allows repeated and safe assessment of neonatal cerebral hemodynamics and shows consistent changes in cerebral blood flow (CBF) velocities in infants with intrapartum asphyxia (6, 9, 10).

The initial cerebral hyperaemia after hypoxic–ischaemic insult is followed by delayed post-asphyxial cerebral hypoperfusion related to an excess of vasoconstrictor molecules or free-radical induced vascular injury (11–13) and probable accumulation of granulocytes in the reperfused tissue leading to further circulatory disturbances (12).

Several authors have reported high CBF velocities and significantly lowered resistance indices (RI) in infants with severe HIE at the age of 24–72 h (3, 6, 9). The reason for this cerebral hyperaemia and low RI may be severe vasoparalysis, a form of irreversible cerebral vascular injury (9, 10). Levene et al. have proposed that high-risk groups of patients are those with CBF velocity measurements <2 SD or >3 SD from the mean with positive predictive value of 94% for death or severe impairment (9).

CBF has been shown to be increased in infants with permanent brain damage also during the first day of life (12), but Doppler CBF velocity investigations have shown an increase in CBF velocities in severely asphyxiated infants by the second day of life (6, 9).

The aim of the study was to investigate CBF velocities in asphyxiated infants with different stages of HIE during the first days of life.

Subjects and methods

CBF velocities were investigated in 39 term infants (gestational age 37–42 weeks) with perinatal asphyxia, defined as Apgar scores <4 at the 1 and <7 at 5 min of life. Infants with major or multiple minor malformations, systemic infections or haemolytic disease were excluded. Apgar scores at birth and the degree of HIE by Sarnat and

Sarnat (14) during the first 5 d of life were registered; the grading is based on the worst degree of HIE.

In asphyxiated infants, cerebral ultrasonography and CBF velocity measurements were made at least three times between 10 h and 5 d of life and then at least once a week until discharge. In order to determine the normal range for CBF velocities 35 healthy term infants without any signs of perinatal asphyxia, symptoms of HIE or clinically significant patent ductus arteriosus were studied at least three times during the first 5 d of life. CBFV measurements were grouped into four postnatal age periods: 12 ± 2, 24–35.9, 36–71.9, 72–120 h and the mean values were calculated, together with 2 and 3 SD from the mean.

Anterior cerebral artery was visualized in the sagittal plane through the anterior fontanel and the signals were recorded from the point midway between the inferior-most border of the corpus callosum and the vessel's origin from the circle of Willisi. Middle cerebral artery was visualized through the temporal bone in the region above the zygomatic arch in the fold of the temporal lobe from the straight mid-portion of the artery (15). CBF velocity recordings were made by color Doppler ultrasound scanner (Model EUB-515 with 3.5 MHz pulsed wave and 5 MHz imaging crystal, Hitachi Medical Corporation, Japan) or by duplex Doppler scanner (Sonoline SL 2 with 7.5 MHz pulsed wave and 7.5 and 5 MHz imaging crystal, Siemens, Germany) and a Fast Fourier real time frequency analyser.

The high-pass filter, used to remove low frequency noise (i.e. vessel wall movement) was set at the level of 100 Hz. Guided by the velocity signal displayed on an oscilloscope and an audio-signal, the highest possible velocities were used in the range-gated mode. After a stable velocity recording over 20–30 consecutive beats was received, the angle correction was performed and 5 consecutive beats with the highest amplitude recorded were analysed by hand. Systolic peak flow velocity, mean flow velocity (time–mean of the maximum velocity envelope curve) and end diastolic peak flow velocity were recorded as a mean of 5 consecutive beats from both sides of the skull (16). As no statistical differences were found between the measurement from different sides of the skull, the data reported represent the average of two determinations. The resistance index (RI) was calculated according to the formula $RI = (S-D)/S$, where S and D are systolic and end-diastolic peak flow velocities respectively.

Heart rate was calculated from the velocity recordings. Arterial blood pressure in asphyxiated infants was measured non-invasively by cuff on the right upper arm by using a BP 107 Nippon Colin sphygmomanometer. The average of two measurements made simultaneously with the Doppler recordings was used. Recordings of CBF velocities and arterial pressure were made in the supine position 30–60 min after feeding. Observations were made when the infant was in the quiet state, eyes closed and with no gross body movements. Haemoglobin, blood sugar, PaO_2 and $PaCO_2$ measurements were performed simultaneously in asphyxiated infants.

The Ethical Committee of the University of Tartu approved the investigation and written informed consent from mother/parents was obtained in every case. At least one of the parents, mainly mothers, was present during the investigations, with the exception of the first day investigation in case of Caesarean section delivery. Non-parametric tests (Kruskal–Wallis and Wilcoxon tests) were used for statistical analysis to compare differences between groups of infants with different stage of HIE. Values are given as mean ± SD and median with range (max–min). The accepted level for significance was $p < 0.05$.

Results

Based on the clinical signs of the development of HIE, infants were divided into three groups. The main characteristics of the newborn infants are given in Table 1. The first group of infants ($n = 14$) had mild signs of HIE with irritability and brisk reflexes during the first days of life. Infants with a moderate degree of HIE ($n = 14$) were lethargic, hypotonic, with decreased spontaneous movements and primitive reflexes with or without seizures. Flaccid infants with stupor, absent primitive reflexes and frequent hardly treatable seizures were diagnosed as having severe stage HIE ($n = 11$). Infants with severe stage HIE had significantly lower Apgar scores and they needed more vigorous resuscitation compared to the infants with mild or moderate stage HIE (Table 1).

Asphyxiated infants with moderate stage HIE, investigated at the age of 12 ± 2 h ($n = 7$), had significantly lower ($p < 0.05$) mean CBF velocity in both medial and anterior cerebral arteries, compared to the control group infants investigated at the age of 12 ± 2 h ($n = 27$) (Table 2). From the age of 24 to 35.9 h onwards, no differences in mean CBF velocity between infants with moderate stage HIE, compared to normal infants, were found (Figs 1 and 2). In infants with mild stage HIE, investigated at the age of 12 ± 2 h ($n = 6$), a significant decrease ($p < 0.05$) of mean CBF velocity was found only in the anterior cerebral artery (Table 2), no differences were found from the ages of 36–71.9 h (Figs 1 and 2).

In asphyxiated infants with severe stage of HIE, investigated at the age of 12 ± 2 h ($n = 8$), the mean CBF velocity in medial cerebral artery was significantly increased ($p < 0.05$) at the age of 12 ± 2 h compared to the control group and to the infants with moderate stage of HIE (Table 2). By the age of 24–35 h, the mean CBF velocity was significantly increased both in anterior [mean ± SD 34.4 ± 13 cm/s and median (max – min) 40.0 (52.2 – 18.1 cm/s)] and in medial cerebral artery [45.6 ± 21 cm/s and 42.5 (82.0–21.0 cm/s) respectively] compared to the control group infants (19.5 ± 3.3 cm/s and 20.1 (26.6–13 cm/s) respectively in anterior and 25.0 ± 6.6 cm/s and 24.5 (45.2–15 cm/s) in medial cerebral artery) ($p < 0.001$) and to the infants with mild or moderate stage of HIE ($p < 0.05$).

Nine out of 21 investigated infants with birth asphyxia

Table 1. Main characteristics of the groups (mean ± SD), including Apgar scores (median and max–min).

	Control group infants ($n = 35$)	Mild stage of HIE ($n = 14$)	Moderate stage of HIE ($n = 14$)	Severe stage of HIE ($n = 11$)
GA (weeks)	39.8 ± 1.2	39.8 ± 1.4	39.8 ± 1.2	40.8 ± 1.8
Birthweight (g)	3611 ± 454	3374 ± 689	3314 ± 889	3729 ± 582
Apgar score				
1 min	8 (9–7)	2 (3-1)	1** (2–0)	1* (3–0)
5 min	9 (9–8)	5.5 (6–4)	5 (6–1)	2* (6–0)
10 min	9 (9–8)	7 (8–6)	7** (7–6)	4* (6–2)
No. of infants who needed bag and mask ventilation ≥3 min	0	10	13	11
No. of infants who needed cardiac massage	0	9	11	10
No. of infants who needed epinephrine	0	0	3	9***
No. of infants who needed long-term ventilation >24 h	0	1	3	11***

*$p < 0.05$: HIE III vs controls and HIE I–II (Kruskal–Wallis test).
**$p < 0.05$: HIE II vs HIE I (Kruskal–Wallis test).
***$p < 0.05$: HIE III vs controls and HIE I–II (Tukey's Studentized Range).

had decreased mean CBF velocity 2 SD below the mean for normal infants at the age of 12 ± 2 h (Table 3). By the age of 24–35 h only two of them had still decreased mean CBF velocity 2 SD below the mean for normal infants, but in two infants moderate stage of HIE had developed increased (>2 SD, but <3 SD of the mean for normal infants) mean CBF velocities (Fig. 3), all of these infants had normal ultrasonography of the brain by discharge. Two infants with severe stage of HIE had extremely low CBF velocities (3 SD below the mean for normal infants) at the age of 12 ± 2 h. One of them developed extremely high CBF velocities (3 SD above the mean for normal infants) by the age of 24–35 h and cystic degeneration of the brain was seen in this patient at the age of 21 d. In the other infant, CBF velocities normalized by the age of 24–35 h and ultrasonography revealed no abnormalities by the age of 1 month (Fig. 3).

Six out of eight infants with severe stage HIE had, at the age of 12 ± 2 h, extremely high mean CBF velocity (3 SD above mean for normal infants) (Table 3); by the age of 24–35 h a further increase of CBF velocities was seen in all of them. Four of these infants died and two survivors developed multicystic encephalopathy (Fig. 3).

Eleven of 39 infants had decreased RI [<0.56 according to Archer et al. 1986 (6)] during the first 35 h of life and this number did not change thereafter. Low RI was combined with low CBF velocities in three infants at the age of 12 ± 2 h and in two infants during the period 24–35 h. Only two infants with severe stage of HIE had decreased RI <0.56 in combination with high CBF velocities at the age of 12 ± 2 h, but in four other infants with severe HIE, low RI developed by the age of 24 h.

No differences in frequency of prenatal risk factors (infectious complications, bleedings, degenerative changes in placenta, umbilical cord pathology, foetal growth retardation) were found between infants with or without severe increase of CBF velocities, but infants with high CBF velocity at the age of 12 ± 2 h had significantly longer stage I of delivery and in this group stimulation was used more frequently.

Cystic degeneration of the brain was found in infants with severe stage HIE after the first week of life by ultrasonography.

No statistically significant differences in blood pressure, haemoglobin, haemotocrit, arterial oxygen or carbon dioxide tension were found in infants with different stage of HIE or between infants with low or high CBF velocities in no time points.

Table 2. Mean cerebral blood flow velocity (Vm) in anterior (ACA) and medial (MCA) cerebral artery at the age of 12 ± 2 h.

	Mean ± SD (ACA)	median (max–min) (ACA)	Mean ±SD (MCA)	Median (max–min) (MCA)
Controls	16.0 ± 2.2	15.8 (19.6–10)	20.9 ± 3.7	21.2 (26.6–12.5)
HIE I	13.0 ± 2.0*	12.5 (16.9–11)*	17.1 ± 6.09	18.4 (22.6–9.1)
HIE II	13.1 ± 3.7*	12 (19.1–7.9)*	15.6 ± 3.9*	16 (19.8–10.7)*
HIE III	20.6 ± 8.5§	20 (32.0–10.0)§	26.5 ± 9.6*§	27 (45.0–12.0)*§

*$p < 0.05$, HIE I–III vs controls.
§$p < 0.05$, HIE III vs HIE I–II (Kruskal–Wallis and Wilcoxon tests).

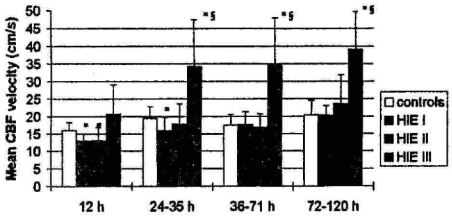

Fig. 1. Mean cerebral blood flow velocity in anterior cerebral artery in asphyxiated infants with different development of HIE and infants in the control group during the first 120 h of life. $*p < 0.05$, HIE I–III vs controls; § $p < 0.05$, HIE III vs HIE I–II (Kruskal–Wallis and Wilcoxon tests).

Discussion

Our results demonstrate that at the age of 12 ± 2 h the mean CBF velocity in anterior and medial cerebral arteries is decreased in asphyxiated infants developing mild or moderate stage HIE and increased in most of the infants with severe stage of HIE.

After an initial short-term reperfusion phase, CBF gradually declines again. The steady decrease of CBF at the age of 12 h after asphyxia has been described in infants (8) and also in different animal models (13, 17–18). This delayed post-asphyxial cerebral hypoperfusion could be related to an excess of vasoconstrictor molecules (tromboxanes, leukotrienes) or free-radical induced vascular injury (11–13) and probable accumulation of granulocytes in the reperfused tissue leading to further circulatory disturbances (12).

Previous works have shown that low CBF velocity (2 SD below the mean for normal infants) is associated with poor outcome (9). It is shown in animal models that the severity of post-hypoxic hypoperfusion increases with duration of cerebral ischaemia (17, 18). We found decreased CBF velocity 2 SD below the mean for normal infants in almost half of asphyxiated infants investigated at the age of 12 h; nobody died in this group. Decreased CBF velocity was associated with severe stage of HIE in two cases. Only one of them, with extremely high CBF velocities (>3 SD) by the age of 24–35 h, developed multicystic degeneration of the brain. According to our data, decreased CBF velocity at the age of 12 h is not necessarily associated with bad prognosis.

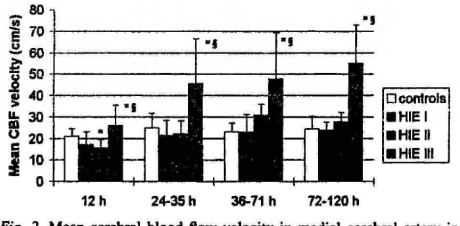

Fig. 2. Mean cerebral blood flow velocity in medial cerebral artery in asphyxiated infants with different development of HIE and infants in the control group during the first 120 h of life. $*p < 0.05$, HIE I–III vs controls; § $p < 0.05$, HIE III vs HIE I–II (Kruskal–Wallis and Wilcoxon tests).

Table 3. Mean cerebral blood flow velocity (Vm) at the age of 12 ± 2 h.

	Controls	HIE I	HIE II	HIE III
Investigated infants (*n*)	27	6	7	8
Vm <2 SD/died (*n*)	0/0	3/0	4/0	2/0
Vm <3 SD/died (*n*)	0/0	1/0	1/0	2/0
Vm >3 SD/died (*n*)	0/0	0/0	0/0	6/4
RI <0.56/died (*n*)	0/0	0/0	1/0	4/2

By the age of 24–35.9 h only two out of nine infants with low CBF velocity at the age of 12 h, had still decreased mean CBF velocity, indicating that the hypoperfusion of the brain is probably most severe during the first day after hypoxic–ischaemic insult and cerebral perfusion improves thereafter.

We found that in most infants with severe stage HIE (six out of eight investigated infants) CBF velocities were significantly increased (3 SD above the mean for normal infants) at the age of 12 ± 2 h, indicating very early beginning of severe vasoparalysis. High CBF at the mean age of 9 h has been described by Pryds et al. (10) in infants, who died subsequently. Most of previous Doppler ultrasonography works have shown that high CBF velocities develop relatively late (usually after 24 h after asphyxia) (6, 9), although Levene et al. have found high CBF velocity in 8/17 asphyxiated infants before that age (9). A similar pattern of later increase of CBF velocity was seen in our series in some patients with mild or moderate stage HIE.

The overabundant cerebral blood-flow relative to the

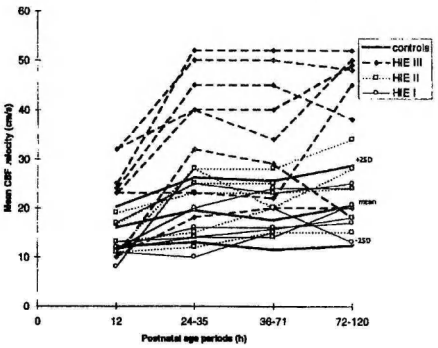

Fig. 3. Individual changes of mean cerebral blood flow velocity in anterior cerebral artery in asphyxiated infants investigated from the age of 12 ± 2 h compared to the control group infants.

metabolic needs of the brain tissue, so called "luxury-perfusion", was first described by Lassen et al. (19). In infants with birth asphyxia, hyperfusion is thought to be a sign of permanent brain damage with poor neurodevelopmental prognosis (9, 10). The mechanisms of such luxury cerebral perfusion following ischaemic brain injury are still unclear, although in foetal sheep it is mediated in part by NO production (20). Neuronal disruption causing a release of vasoactive substances, such as adenosine and lactate, or liberation of excitatory amino acids, such as glutamate and aspartate (21), irreversible cerebral vascular injury and complete loss of tone in resistance vessels, may play a role in the pathogenesis (9, 10).

Our data confirm the findings of other authors (3, 4, 6) that high CBF velocities are more precise than low RI in predicting abnormal outcome. Although in six infants with severe stage HIE CBF velocities were increased 3 SD above the mean for normal infants at the age of 12 h, the lowering of RI was seen in only two of them at this early age. Decreased RI was seen in combination with high velocities mainly from the age of 24 h. So lowered RI alone may not predict poor outcome.

Although severe increase of the CBF velocities in infants with poor prognosis was seen early after asphyxia, no risk factors indicating prenatal cause of hypoxic–ischaemic insult was found in our infants with high CBF velocity. As in most severe cases, diffuse brain atrophy and areas of cystic encephalopathy take at least 3–7 d to develop (22), prenatal beginning of the insult is also unlikely.

No differences in blood pressure, oxygen or carbon dioxide tension, blood sugar or haematocrit, which could explain the differences of CBF velocities, were found between the groups of infants with different development of HIE or between the infants with normal, high or low CBF velocities. Care was also taken to avoid the investigation of CBF velocities during seizure activity, which can change the CBF velocity.

Doppler CBF velocity investigations at the age of 12 h may help to evaluate the brain damage and long-term prognosis of the infants after birth asphyxia. As four of six infants died and survivors developed multicystic degeneration of the brain during the neonatal period, we propose that very early onset of high CBF is connected with extremely bad prognosis. Severe hyperperfusion seen in asphyxiated infants with poor prognosis already by the age of 12 h indicates the need to start medical brain protection before that time when the signs of permanent brain damage have already developed.

In conclusion, increased CBF velocities >3 SD at the age of 12 ± 2 h indicate an early beginning of severe vasoparalysis of the brain and are associated with the development of severe stage HIE and poor prognosis. CBF velocities <3 SD are associated with bad prognosis, if severe hyperperfusion develops during following days.

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IV

P. Ilves, M. Thetloff, T. Talvik.
Cerebral blood flow velocities in term newborn infants in the first week of life.
Eesti Arst 1997; 4: 299–303 (in Estonian).

Eesti Arst, 1997, 4, 296–299

Aju verevoolu kiirus ajalistel vastsündinutel esimesel elunädalal

Pilvi Ilves Maie Thetloff Tiina Talvik

aju verevoolu kiirus, ajaline vastsündinu

Sünd toob endaga kaasa suuri muutusi vastsündinu hemodünaamikas: katkeb platsentaarne tsirkulatsioon, avaneb kopsuvereringe ja muutub vere gaasisisaldus (3). Aju verevoolu häiretel sünni ajal ja vahetult pärast sündi arvatakse olevat oluline osa ajuverevalumite, periventrikulaarsete leukomalaatsiate ja hüpoksilis-isheemilise entsefalopaatia kujunemises vastsündinutel (5, 6, 10). Sellised perinataalsed ajukahjustused kujunevad välja esimeste päevade jooksul pärast sündi. Aju verevoolu uurimine võimaldab hinnata ajukahjustuse kujunemise riski ja alustada ravi õigeaegselt (6, 10).

Oma lihtsuse tõttu kasutatakse vastsündinu aju hemodünaamika hindamiseks enamasti aju verevoolu kiiruse määramist dopplersonograafia abil. Täiskasvanutel, vastsündinutel ja katseloomadel tehtud uuringute varal on leitud, et dopplersonograafial saadud aju verevoolu kiirus korreleerub aju verevooluga mõõdetuna teiste meetoditega (^{133}ksenooni kliirensi ja mikrosfääride meetod, venoosne pletüsmograafia-5).

Vastsündinute uurimine on omakorda näidanud, et verevoolu kiirust suurtes ajuarterites mõjutavad paljud tegurid: sünnikaal (2), vanus (4, 6), vererõhk (3, 7), vere gaasisisaldus (3, 7) ja muu. Osa neid tegureid mõjutab verevoolu kiirust otseselt, teine osa aga kaudselt, avaldades toimet vererõhusse, intrakraniaalrõhusse või vere gaasisisaldusesse.

Pilvi Ilves, Maie Thetloff, Tiina Talvik — Tartu Ülikooli Lastekliinik

Aju verevoolu kiirus sõltub otseselt lapse kaalust (2). Vastsündinute üsasisese kasvu kõverad on näidanud, et Eestis on ajalised vastsündinud keskmiselt 200–300 g raskemad kui Lääne-Euroopa riikides (1). Seega on patoloogiliste muutuste avastamiseks vaja teada Eesti tervete vastsündinute aju verevoolu kiirust esimestel elupäevadel.

Töö eesmärk oli uurida verevoolu kiirust tervetel ajalistel vastsündinutel erinevates ajuarterites esimesel elunädalal.

Uuritavad ja uurimismeetodid. Uuritavate rühma moodustasid 34 ajalist (gestatsiooniaeg 37–42 rasedusnädalat) normaalse sünnikaaluga komplikatsioonideta üksikrasedusest sündinud vastsündinut Tartu Ülikooli Naistekliinikus aastail 1994–1996 (1). Laste kliinilised andmed (keskmine ± SD) leiate tabelist.

Tabel. Vastsündinute kliinilised andmed (keskmine ± SD)

Keskmine gestatsiooniaeg (rasedusnädalates)	39,9±1,25
Keskmine sünnikaal (g)	3624,8±453,5
Apgari hinne: 1. minutil	8±0,5
5. minutil	8,7±0,5
Sünnitus: vaginaalne/plaaniline keisrilõige	17/17
Sugu: poeglapsed/tütarlapsed	16/18

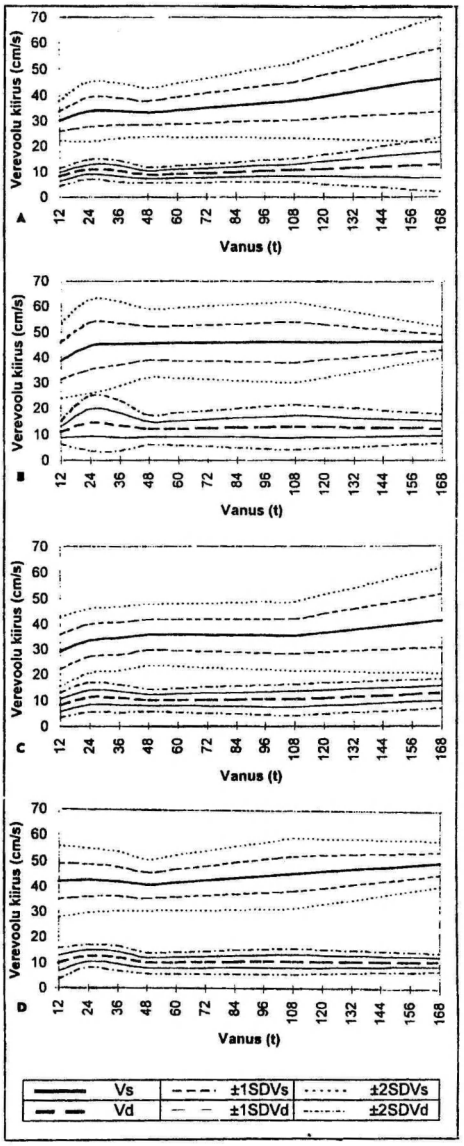
Uuringust jäeti välja vastsündinud, kellel sünni ajal esinesid hüpoksiale viitavad sümptomid või kellel vastsündinueas kujunesid välja hüpoksilis-isheemiline entsefalopaatia, aneemia, hemokontsentratsioon, vastsündinu hemolüütiline tõbi ja sepsis, samuti suurte väärarendite või üle 3 mikroanomaaliaga vastsündinud. Uuringu ajal laps magas. Uuring toimus umbes 0,5–1 tund pärast söömist.

Aju verevoolu kiirust määrati eesmises, keskmises ja tagumises ajuarteris, sisemises unearteris ja basilaararteris esimese elunädala jooksul. Ajuarterites hinnati maksimaalset süstoolse (Vs), lõpp-diastoolse (Vd) ja keskmist verevoolu kiirust (Vm). Uurimiseks kasutati *Hitachi Medical Corporation*'i 3,5 MHz-se sektoranduriga ultraheliaparaati (mudel

EUB-515), millel on ka pulss- ja värvusdopplerуuringu võimalus. Pärast kahemõõtmelist aju sonograafilist uuringut otsiti värvusdopplerimeetodil üles uuritavad arterid. Eesmise ja tagumise ajuarteri, basilaararteri ning sisemise unearteri kulg visualiseeriti sagitaal- ja koronaarlõikes läbi eesmise lõgeme kohas, kus nende kulg on väikseima nurga all ultraheli lainete leviku sihiga. Keskmine ajuarter visualiseeriti läbi eesmis-külgmise lõgeme või õhukese temporaalluu aksiaallõikes. Pärast nurgakorrektsiooni määrati uuritavad verevoolu kiirused parimas saadud verevoolu kiiruste spektri osas, juhindudes audiosignaalist ja kiiruse signaalist monitoril. Verevoolu kiirusi mõõdeti viies südametsüklis järjestikku, hiljem arvutati mõõtmistulemuste keskmine.

Uuringu tegemiseks oli luba saadud Tartu Ülikooli Eetikakomiteelt. Kõik vanemad olid uuringu metoodikast ja eesmärgist teadlikud, andsid uuringuks kirjaliku loa ja vähemalt üks vanematest viibis uuringu juures (välja arvatud keisrilõigesünnitusest sündinud laste emad esimesel elupäeval). Statistiliseks analüüsiks rühmitati andmed viide klassi: vanuses 12±2 tundi, 24 – 35,9 tundi, 36 – 48 tundi, 5 ja 7 päeva. Analüüsiks kasutati SAS-programmi t-testi ja Spearmani korrelatsioone, $P<0,05$ peeti statistiliselt oluliseks.

Uurimistulemused. Uuringute tulemuste analüüs näitas, et maksimaalne süstoolse, lõpp-diastoolse ja keskmine verevoolu kiirus olid esimestel elupäevadel poeg- või tütarlastel sarnased ($P>0,05$). Uuritav verevoolu kiirus ei erinenud ka vasakut ega paremat hemisfääri varustavates arterites. Analüüsides tulemusi vaginaalselt ja plaanilise keisrilõike teel sündinuil, selgus, et plaanilise keisrilõike teel sündinud 12 tunni vanustel lastel on sisemises unearteris maksimaalne süstoolse verevoolu kiirus (Vs=45,4±6,2 cm/s) statistiliselt oluliselt ($P<0,05$) suurem kui vaginaalsel teel sündinuil (Vs=39,4±6,2 cm/s). Teistes ajuarterites ja hilisematel uuringuperioodidel erinevused keisrilõike



Joonis. Maksimaalne süstoolse (Vs) ja lõppdiastoolse (Vd) verevoolu kiirus (keskmine ±2SD) vastsündinuil esimesel elunädalal; A — eesmises ajuarteris, B — keskmises ajuarteris, C — basilaararteris ja D — sisemises unearteris.

ja vaginaalsel teel sündinud laste aju verevoolu kiiruses puudusid.

Maksimaalne süstoolse verevoolu kiirus keskmises ajuarteris ja sisemises unearteris oli esimesel elunädalal suurem kui eesmises ajuarteris ja basilaararteris (vt. joonis).

Teiseks elupäevaks toimus kiire statistiliselt oluline ($P<0,05$) maksimaalse süstoolse, lõpp-diastoolse ja keskmise verevoolu kiiruse tõus 12. elutunniga võrreldes eesmises ja keskmises ajuarteris, basilaararteris ning vaginaalsel teel sündinud lastel ka sisemises unearteris (vt. joonis). Esimese elunädala lõpuks aju verevool jätkuvalt kiirenes võrreldes teise elupäeva seisuga, statistiliselt oluline oli maksimaalne süstoolse verevoolu kiirenemine eesmises ajuarteris ja sisemises unearteris. Verevoolu kiirenemine tagumises ajuarteris ei olnud esimesel elunädalal statistiliselt oluline.

Arutelu. Verevoolu kiiruse määramine vastsündinute ajuarterites näitas, et intrakraniaalne hemodünaamika muutub esimesel elunädalal oluliselt. Alates 12. elutunnist kiireneb aju verevool pidevalt. Eriti suured muutused toimuvad vastsündinu aju hemodünaamikas 1.–2. elupäeval, sellele on viidanud ka teised autorid (2, 4). Osa autoreid (3, 6) on kirjeldanud ajutist aju verevoolu kiiruse aeglustumist 4–8 tunni vanustel, mida on seostatud nn. verevoolu varguse sündroomiga läbi avatud *ductus arteriosus*'e (6). G. Connors ja kaasautorid peavad aju verevoolu kiiruse aeglustumise põhjuseks ajutist aju veresoonte resistentsuse tõusu ja aju verevoolu kiiruse vähenemist vastuseks arteriaalse oksügenisatsiooni paranemisele vahetult pärast sündi (3). Meie vastsündinute uuring algas alles 12. elutunnil.

Muutused maksimaalses süstoolse ja lõpp-diastoolse verevoolu kiiruses erinevates ajuarterites on esimesel elunädalal sarnased: verevoolu kiirus suureneb tunduvalt teiseks elupäevaks võrreldes 12. elutunniga, edasine aju verevoolu kiirenemine esimese elunädala jooksul on aeglasem. Seejuures on verevool kiirem sisemises unearteris ja tema suurimas harus — keskmises suurajuarteris.

Uurides aju verevoolu kiirust vaginaalselt ja plaanilise keisrilõike teel sündinuil, on leitud erinevusi esimestel tundidel pärast sündi (8). Suuremat verevoolu kiirust ja suuremat resistentsusindeksit esimestel tundidel pärast sündi võib plaanilise keisrilõike teel sündinuil seostada erinevusega *ductus arteriosus*'e sulgumises keisrilõigesünnitusest ja vaginaalselt sündinud lastel (8). Meie andmed näitasid keisrilõike teel sündinuil suuremat süstoolse verevoolu kiirust sisemises unearteris 12. elutunnil. Teiseks elupäevaks olid erinevused aju hemodünaamikas plaanilise keisrilõike ja vaginaalsel teel sündinud lastel kadunud. Kuigi kliiniliselt ega hemodünaamiliselt olulist *ductus arteriosus*'t ei diagnoositud ühelgi lapsel, võib keisrilõike teel sündinuil esinevat verevoolu kiiruse tõusu sisemises unearteris esimesel elupäeval seostada erineva hemodünaamika adaptatsiooniga keisrilõike ja vaginaalsel teel sündinud lastel, mille põhjuseks võib olla *ductus arteriosus*'e hilisem sulgumine keisrilõike teel sündinud lastel.

Meie andmetest nähtus, et aju verevoolu kiirus vasak- ja parempoolsetes arterites tervetel vastsündinutel oluliselt ei erine. Verevoolu asümmeetria eri ajupoolkerades ei ole normaalne ja tema olemasolu seostatakse periventrikulaarsete hemorraagiate ja infarktide kujunemisega ajus (9).

Järeldused.

1. Esimese elunädala jooksul toimub statistiliselt oluline verevoolu kiiruse tõus suurtes ajuarterites. Kõige kiiremad muutused aju hemodünaamikas toimuvad esimese elupäeva jooksul.

2. Verevoolu kiirus mõlemat hemisfääri varustavates arterites statistiliselt ei erine.

3. Aju verevoolu kiirus poeg- ja tütarlastel ei erine esimesel nädalal pärast sündi statistiliselt oluliselt.

4. Keisrilõike teel ja vaginaalselt sündinud vastsündinute aju hemodünaamika adaptatsioon esimesel elupäeval on erinev, hiljem erinevused kaovad.

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Summary

Cerebral blood flow velocities in term newborn infants in the first week of life. A study of 34 healthy full-term infants was made using colour flow mapping and pulsed Doppler ultrasonography in order to establish a normal range for cerebral blood flow velocity (CBFV) in the first week of life. Recordings were made from the anterior, middle and posterior cerebral artery, basilar artery and internal carotid artery. There was a statistically significant increase in CBFV in cerebral arteries over the first week of life. There is a close relationship between measurements of CBFV from different cerebral arteries, although the velocity tends to be higher in the middle cerebral artery and internal carotid artery. The results suggest that the mode of delivery has a transitory effect on infants' cerebral circulation.

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V

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Eesti Arst 1999; 1: 13–7 (in Estonian).

Eesti Arst, 1999, 1, 13–17

TEOORIA JA PRAKTIKA

Aju verevoolu kiirus asfüksias sündinud vastsündinutel

Pilvi Ilves Tiina Talvik

aju verevoolu kiirus, asfüksia, ajaline vastsündinu

Asfüksias sündinud vastsündinutel võib esimestel elupäevadel areneda hüpoksilis-isheemiline entsefalopaatia (HIE), mis on seotud püsiva ajukahjustuse kujunemise suure riskiga. Asfüksia raskuse varajane teadasaamine on oluline, et alustada adekvaatse raviga, sest lõplik ajukahjustus kujuneb välja esimeste päevade jooksul pärast asfüksiat. HIE sümptomid aga kujunevad välja esimeste päevade jooksul pärast asfüksiat ja sageli on lapse kliinilist seisundit hinnata raske, sest laps on juhitaval hingamisel ja ravinarkoosis. Aju sonograafilise ja kompuutertomograafilise uuringu tulemused võivad esimesel elupäeval samuti olla normaalsed, vaatamata raskele anoksilisele kahjustusele (3).

Loomkatsete varal on näidatud, et aju verevoolu muutused ja rakukahjustus tekivad pärast asfüksiat selektiivselt kindlates kohtades: aju verevool jaotub ümber eesmärgiga säilitada ajutüve piirkond. Aju verevoolu vähenemine frontoparietaalpiirkonnas korreleerub kujuneva neuronite nekroosiga (5). Ajalistel vastsündinutel kahjustub asfüksiajärgselt samuti eelkõige frontoparietaalpiirkond, mida varustab eesmine ja keskmine ajuarter, ning vähem ajutüvi ja väikeaju, mida varustab tserebrobasilaarne tsirkulatsioon. Rasketel juhtudel kujuneb välja multitsüstiline entsefalopaatia, mis võtab enda alla eelkõige frontoparietaalpiirkonna (11). Regionaalseid muutusi aju verevoolus asfüksias sündinutel on vähe uuritud.

Vastsündinu aju hemodünaamika hindamiseks kasutatakse enamasti aju verevoolu kiiruse määramist dopplersonograafia abil, mis on lihtne, ohutu ja korduvalt kasutatav meetod. Täiskasvanutel, vastsündinutel ja katseloomadel tehtud uuringutega on leitud, et dopplersonograafial saadud aju verevoolu kiirus korreleerub aju verevooluga mõõdetuna teistel meetoditel (^{133}ksenooni kliirensi ja mikrosfääride meetod, venoosne pletüsmograafia) (3).

Töö eesmärk oli võrrelda aju verevoolu kiirust erinevates ajuarterites asfüksias sündinud ajalistel vastsündinutel esimesel elunädalal ja teada saada ajukahjustuse ulatus.

Uuritavad patsiendid ja uurimismetoodika. Uurimisrühma moodustasid Tartu Ülikooli Naistekliinikus ja Tartu Ülikooli Kliinikumi Lastehaiglas aastail 1994–1997 ravil viibinud 86 asfüksias sündinud ajalist (gestatsiooniaeg 37–42 rasedusnädalat) vastsündinut, kellel oli diagnoositud loote hüpoksia koos kardiotokograafiliste muutustega loote südamelöökide sageduses (pidev bradükardia <100 või tahhükardia >170 korra minutis või hilisdetseleratsioonid kestusega üle 1 minuti) ja/või vastsündinu asfüksia (Apgari hinne ≤7 viiendaks eluminutiks).

Et teada saada aju verevoolu kiiruse normaalväärtusi esimesel elunädalal, uuriti aastail 1994–1997 Tartu Ülikooli Naistekliinikus normaalse sünnikaaluga

Pilvi Ilves, Tiina Talvik — Tartu Ülikooli Lastekliinik

Tabel. Vastsündinute kliinilised andmed (keskmine ±SD); Apgari hinne (*median+min.* ja *max.*)

	Kontrollrühm (n=35)	Vastsündinud ilma HIE-ta (n=9)	Kerge astme HIE (n=40)	Keskmise astme HIE (n=25)	Raske astme HIE (n=12)
Gestatsiooniiga (rasedusnädalates)	39,8±1,2	40,2±1,2	40,0±1,1	40,4±1,4	40,7±1,4
Sünnikaal (g)	3611±454	3484±475	3527±568	3677±848	3681±577
Apgari hinne					
1. minutil	8	7	3,5	2	1*
	(7-9)	(4-9)	(1-8)	(1-8)	(0-7)
5. minutil	9	8	7	6**	2*
	(8-9)	(7-9)	(4-8,5)	(3-8)	(0-8)
10. minutil	9	9	8	7**	4*
	(8-9)	(8-9)	(7-8,5)	(6-8)	(2-8)
Laste arv, kes vajasid					
maskiga ventilatsiooni ≥3 min.	0	0	9	16	11‡◊
kaudset südamemassaaži	0	0	11	12	10◊
adrenaliini	0	0	0	3	9‡◊
kopsude juhitavat hingamist >24 tunni	0	0	1	6	12‡◊

*P<0,005 HIE III *versus* kontrollrühm ja HIE I-II (Kruskali-Wallise ja Wilcoxoni test); **P<0,05 HIE II *versus* HIE I (Kruskali-Wallise ja Wilcoxoni test); ◊P<0,05 HIE III *versus* HIE I (Turkey test); ‡P<0,05 HIE III *versus* HIE II (Turkey test).

komplikatsioonideta üksikrasedusest sündinud 35 ajalist vastsündinut (kontrollrühm) (4). Kontrollrühma ei arvatud vastsündinuid, kelle puhul olid sünnituse käigus esinenud loote hüpoksia tunnused või kellel vastsündinueas kujunesid välja HIE, aneemia, hemokontsentratsioon. Väärarendite, süsteemse infektsiooni ja hemolüütilise tõvega lapsi ei uuritud.

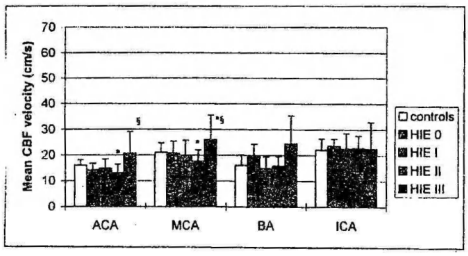
HIE raskust hinnati Sarnati järgi (10). Uuriti verevoolu kiirust eesmises ja keskmises ajuarteris, sisemises unearteris ning basilaararteris esimese elunädala jooksul. Ajuarterites hinnati maksimaalset süstoolset (Vs), lõpp-diastoolset (Vd) ja keskmist verevoolu kiirust (Vm). Uurimiseks kasutati *Hitach*'i *Medical Corporation*'i 3,5 ja 5 MHz anduriga ultraheliaparaati (mudel EUB-515) koos pulss- ja värvusdoppleruuringu võimalusega ning *Siemens*'i ultraheliaparaati *Sonoline* SL 2 (7,5 ja 5 MHz anduriga) koos pulssdoppleruuringu võimalusega. Eesmise ajuarteri, basilaararteri ning sisemise unearteri kulg visualiseeriti sagitaal- ja koronaarlõikes läbi eesmise lõgeme kohas, kus nende kulg on ultrahelilainete leviku sihiga väikseima nurga all. Keskmine ajuarter visualiseeriti läbi eesmis-külgmise lõgeme või õhukese temporaalluu aksiaallõikes. Pärast nurgakorrektsiooni määrati uuritavad verevoolu kiirused parimas saadud verevoolu kiiruste spektri osas, juhindudes audiosignaalist ja kiiruse signaalist monitoril. Verevoolu kiirust mõõdeti viies südametsüklis järjestikku, hiljem arvutati mõõtmistulemuste keskmine. Uuring toimus umbes 0,5–1 tund pärast söötmist, uuringu ajal laps magas.

Uuringu tegemiseks oli luba saadud Tartu Ülikooli arstiteaduskonna eetikakomiteelt. Kõik vanemad olid uuringu metoodikast ja eesmärgist teadlikud, andsid uuringuks kirjaliku loa ja vähemalt üks vanemaist viibis uuringu juures, välja arvatud keisrilõigesünnitusest sündinute emad lapse esimesel elupäeval. Statistiliseks analüüsiks rühmitati andmed nelja klassi: vanuses 12±2 tundi, 24–35,9 tundi, 36–71,9 ja 72–120 tundi. Analüüsiks kasutati SAS-programmi ja rühmade võrdlemisel mitteparameetrilisi meetodeid (Kruskali-Wallise ja Wilcoxoni test). P<0,05 peeti statistiliselt oluliseks.

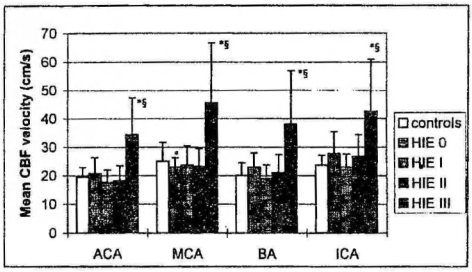
Uurimistulemused. Kontrollrühma lapsed ja asfüksias sündinud vastsündinud olid sarnased gestatsioonieralt, sünnikaalult, soolt ja sünniviisilt. Arvestades HIE kujunemise kliinilist sümptomatoloogiat, jaotati asfüksias sündinud lapsed nelja rühma. Esimese rühma moodustasid 9 last loote hüpoksiaga, mis oli erakorralise keisrilõike tegemise või sünnituse instrumentaalse lõpetamise näidustuseks 8 sünnituse korral, kuid kellel ei kujunenud HIE sümptomatoloogiat. Teise rühma (n=40) moodustasid kergete ja kolmanda (n=25) mõõdukate HIE nähtudega vastsündinud. Raske HIE diagnoositi 12 lapsel, kes olid stuporoosses seisundis või koomas, kellel puudusid lihastoonus, primitiivsed refleksid ja esinesid raskesti ravile alluvad sagedad krambid. Vastsündinutel, kellel ilmnesid rasked HIE nähud, olid Apgari hinded esimese 10 eluminuti jooksul tunduvalt madalamad. Nad vajasid elustamist tunduvalt kauem võrreldes lastega, kellel kujunes kerge või keskmise raskusega HIE, ning nad vajasid pikaajalist juhitavat hingamist lasteintensiivravi osakonnas (vt. tabel).

Uuringu tulemuste analüüs näitas, et keskmise raskusega HIE-ga vastsündinutel esines oluliselt väiksem keskmine aju verevoolu kiirus eesmises (13,0±3,3 cm/s, P<0,05) ja keskmises ajuarteris (17,6±4,3 cm/s, P<0,05) kontrollrühma lastega võrreldes (vastavalt 16,0±2,2 cm/s ja 20,9±3,7 cm/s) 12±2 tunni vanuses (vt. joonis 1), verevoolu kiirus normaliseerus neil 24.–34,9. elutunniks (vt. joonis 2). Kuigi verevoolu kiiruse langustendents esimesel elupäeval esines keskmises ajuarteris ka nendel lastel, kellel ei kujunenud üldse või kujunesid ainult kerged HIE nähud, ei saavutanud verevoolu kiiruse langus neil statistiliselt olulist taset (0,1<P<0,05). Lastel, kellel esines ilma HIE kliiniliste nähtudeta loote hüpoksia, püsis statistiliselt madalam keskmine aju verevoolu kiirus keskmises ajuarteris ka 24.–34,9. elutunnil. Statistiliselt olulisi erinevusi keskmises aju verevoolu kiiruses basilaararteris ning sisemises unearteris kerge ja keskmise raskusega HIE-ga ning ilma HIE nähtudeta lastel ei leidunud.

Raskete HIE nähtude korral esines lastel 12.±2. elutunnil oluline (P<0,05) keskmise aju verevoolu kiiruse tõus keskmises ajuarteris (26,0±9,6 cm/s) võrreldes kontrollrühma lastega (vt. joonis 1.). 24–35,9 tunni vanusest 12 uuritust 9-l oli tugevalt tõusnud keskmine aju verevoolu kiirus (>3SD) kõigis uuritud arterites võrreldes



Joonis 1. Keskmine aju verevoolu kiirus (Vm) 12±2 tunni vanustel vastsündinutel eesmises ajuarteris, keskmises ajuarteris, basilaararteris ja sisemises unearteris.
***P<0,05, HIE I-III *versus* kontrollrühm; §P<0,05, HIE III *versus* HIE II (Kruskali-Wallise ja Wilcoxoni testid).**



Joonis 2. Keskmine aju verevoolu kiirus (Vm) 24–35,9 tunni vanustel vastsündinutel eesmises ajuarteris, keskmises ajuarteris, basilaararteris ja sisemises unearteris.
***P<0,05, HIE 0-III *versus* kontrollrühm; §P<0,05, HIE III *versus* HIE II (Kruskali-Wallise ja Wilcoxoni testid).**

kiirusega kontrollrühma ja kergemate HIE nähtudega lastel ($P<0,05$) (vt. joonis 2). 9 lapsest, kellel aju verevoolu kiirus oli ülikõrge, suri neli esimesel elukuul, kahel neist oli jõudnud areneda ka multitsüstiline entsefalopaatia. Viiest ülikõrge aju verevoolu kiirusega ellujäänud lapsest kujunesid neljal rasked multitsüstilise entsefalopaatia nähud eelkõige frontoparietaalpiirkonnas koos raske psühhomotoorse arengu peetusega.

Erinevusi keskmises vererõhus, veresuhkru- ja hemoglobiinisisalduses, hematokriti näidus, vere hapniku- ja CO_2-sisalduses erineva astmega HIE-ga laste vahel ja madala või kõrge verevoolu kiirusega lastel ei esinenud.

Arutelu. Meie andmed näitavad, et pärast asfüksiat häirub ajalistel vastsündinutel verevool eelkõige eesmises ja keskmises ajuarteris ning säilib paremini basilaararteris ja sisemises unearteris. Selle põhjuseks võib olla verevoolu ümberjaotumine, millega püütakse asfüksias sündinutel säilitada ajutüve piirkonda.

Aju verevoolu kiiruse languse põhjusteks esimesel elupäeval pärast asfüksiat võivad mõõdukate HIE nähtudega lastel olla vasokonstriktoorsete ainete liigne produktsioon ning vabadest hapnikuradikaalidest ja võimalik, et ka granulotsüütide akumulatsioonist tingitud veresoontekahjustus (2). Olulist osa aju hüpoperfusiooni kujunemises võivad etendada ka ajuveresoonte autoregulatsiooni häire ja asfüksiajärgsest südame löögimahu langusest tingitud vererõhu langus (6).

Täpne mehhanism aju verevoolu ümberjaotumiseks asfüksiajärgselt pole selge. See võib olla opioidide vahendatud homöostaatiline mehhanism, millega vähendatakse uuemate ajuosade metaboolseid vajadusi, et säilitada ajutüve paremat perfusiooni (7). Vastsündinu eesmisel ajuarteril on tihe sümpaatiline innervatsioon ja asfüksia tugeva sümpaatilise stimulaatorina võib põhjustada tugevamat vasokonstriktsiooni eesmises ajuarteris (11). Frontaal- ja temporaalpiirkonnas esineb vastsündinul võrreldes vastavate täiskasvanu aju osadega rohkem N-metüül-D-aspartaat-retseptoreid. Retseptorite ülestimulatsioon ekstsitatoorsete aminohapete poolt pärast asfüksiat võib vastsündinul põhjustada tugevamat ajukahjustust frontotemporaalpiirkonnas (1). Loomkatsetes on ka vasokonstriktoorsete prostaglandiinide taseme tõusu leitud rohkem frontaal-, parietaal- ja temporaalpiirkonnas (8).

Raske HIE korral tekkis 12.±2. tunnil keskmises ja eesmises ajuarteris ning hiljem ka basilaar- ja sisemises unearteris tugev verevoolu kiiruse tõus koos resistentsuse langusega. Liigse aju perfusiooni põhjuseks pärast asfüksiat peetakse neuronite lagunemisel vabanevaid aineid, NO-d ja ekstsitatoorseid aminohappeid, mille toimel kujuneb välja pöördumatu veresoonte kahjustus. Ajuveresooned kaotavad oma toonuse ja aju verevool kiireneb nii, et see ei ole enam vastavuses aju metaboolsete vajadustega. Sel juhul on välja kujunenud raske, pöördumatu vasoparalüüs, mille korral ajuveresooned on maksimaalselt laiad ja neil puudub autoregulatsioon vererõhu ja vere CO_2-sisalduse suhtes. Selline ajuveresoonte kahjustus on seotud äärmiselt halva prognoosiga (9).

Kuuel vasoparalüüsiga lapsel kujunes välja aju multitsüstiline degeneratsioon eeskätt frontaal- ja parietaalpiirkonnas. Sellega on seletatav ka tserebraalparalüüsi atoonilis-astaatilise ja bilateraalse hemipleegia kujunemine (11). Aju tsüstiline kahjustus piirkondades, mida varustatakse eesmise ja keskmise ajuarteri poolt, ja suhteliselt hästi säilinud ajutüve ja väikeaju piirkond on iseloomulikud ajalistele vastsündinutele, kellel kujuneb HIE (10).

Järeldused.

1. Asfüksias sündinud vastsündinutel esinevad aju verevoolu kiiruse muutustes regionaalsed erinevused.

2. Aju verevoolu kiirus muutub ajalistel vastsündinutel eelkõige eesmises ja keskmises ajuarteris, seega asfüksias sündinud lastel tuleks dopplersonograafiliselt aju verevoolu kiiruse muutusi uurida eelkõige nendes arterites.

3. Tugev aju verevoolu kiiruse tõus ka basilaararteris ja sisemises unearteris viitab raskete HIE nähtude kujunemisele ja on seotud väga halva prognoosiga.

4. Piirkonnad, mida varustavad eesmine ja keskmine ajuarter, on ajalisel vastsündinul kõige kergemini kahjustatavad.

5. Et asfüksias sündinud raske HIE-ga lastel on pöördumatud muutused välja kujunenud juba 12. elutunniks, tuleb nende laste ravi alustada võimalikult varakult, juba esimestel tundidel pärast asfüksiat.

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Summary

The cerebral blood flow velocity in term newborn infants with hypoxic-ischemic encephalopathy. Cerebral blood flow (CBF) velocity assassed by pulsed-Doppler ultrasonography in 86 distressed and 35 healthy term newborn infants. Distressed infants with moderate stage of hypoxic-ischemic encephalopathy (HIE) had decreased ($p<0.05$) mean CBF velocity in anterior and medial cerebral artery compared to the control group infants at the age of 12±2 hours. Infants with severe stage of HIE had increased ($p<0.05$) mean CBF velocity in medial cerebral artery at the age of 12±2 hours compared to the control group infants. Statistically significant changes in mean CBF velocities in basilar and internal carotid artery were not found in distressed term infants with hypoxic-ischemic encephalopathy compared to the control group infants at the age of 12±2 hours. Two who died and four surviviours with severe stage of HIE developed multicystic encephalopathy mainly in frontal and parietal regions of the brain.

Conclusions. CBF velocities in anterior and medial cerebral arteries will be disturbed in term distressed infants more easily compared to the arteries supplying the brainstem and occipital regions of the brain. Regions supplied by anterior and medial cerebral artery are the most vulnerable in term infants.

Uurimistööd on toetanud Eesti Teadusfond (grandid 65 ja 314) ning ARLA projekt (grant 001SO)

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Teadustegevus

Põhiliseks uurimisvaldkonnaks on olnud vastsündinute asfüksia, enneaegsete laste ajuverevalumite kujunemine, vastsündinute meningiit, aju ultraheliuuringud lastel, pikaajaline juhitav ventilatsioon vastsündinutel. 30 teaduspublikatsiooni, 16 ettekannet rahvusvahelistel konverentsidel ja kongressidel.

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1997	neljakuune uurimistööpraktika Oslo rahvuslikus hospidalis
1997	ühenädalane täienduskursus pediaatrias Salzburgi seminaris

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