Department of Obstetrics and Gynecology

University of Helsinki and Helsinki University Hospital

Finland

BIOMARKERS OF INTRAUTERINE HYPOXIA AND PERINATAL ASPHYXIA, AND GESTATIONAL AGE AS PREDICTORS OF NEONATAL OUTCOME

Laura Seikku

ACADEMIC DISSERTATION

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Supervised by

Adjunct Professor Vedran Stefanovic
Department of Obstetrics and Gynecology
University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Adjunct Professor Leena Rahkonen
Department of Obstetrics and Gynecology
University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Reviewed by

Adjunct Professor Outi Tammela
Division of Neonatology, Department of Pediatrics
Tampere University Hospital and Tampere University, Finland

Adjunct Professor Pekka Taipale
University of Helsinki and Helsinki University Hospital, Helsinki, Finland
Mehiläinen Länsi-Pohja Oy, Kemi, Finland

Official Opponent

Adjunct Professor Susanna Timonen
Department of Obstetrics and Gynecology
Turku University Hospital and Turku University, Finland

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To my family

ABSTRACT

Fetal life occurs in a relatively hypoxic environment. During normal pregnancy, several compensatory mechanisms secure fetal oxygenation and wellbeing. In complicated pregnancies, however, intrauterine hypoxia predisposes the fetus to growth restriction, stillbirth, neurodevelopmental sequelae such as cognitive dysfunction and cerebral palsy (CP), and adverse long-term health impacts. Impairment of respiratory gas exchange—during either pregnancy or delivery—leads to tissue hypoxia, and, if prolonged, to metabolic acidosis and asphyxia. Worldwide, such asphyxia, diagnosed at birth, annually accounts for a million neonatal deaths. Furthermore, neonatal hypoxic ischemic encephalopathy (HIE) originating from perinatal asphyxia may lead to a variety of neurodevelopmental impairments. Therapeutic neuroprotective interventions such as hypothermia have significantly improved the prognosis of severe neonatal encephalopathy.

Increased risk for intrauterine fetal hypoxia and perinatal asphyxia occur in various circumstances and pregnancy complications—such as intrauterine growth restriction (IUGR), which affects up to 10% of pregnancies. Timing the delivery in preterm pregnancy with severe IUGR is challenging, owing to balancing between risks related to prematurity and to fetal hypoxia. Another obstetric challenge concerns timing of delivery as well: Neonatal outcomes vary by gestational age also among term pregnancies. In pregnancies beyond 41 gestational weeks, the risk for perinatal morbidity and mortality increases, probably due to the relative insufficiency of the aging placenta.

Numerous methods such as fetal Doppler assessments and computerized cardiotocography help in monitoring placental function and fetal wellbeing. These methods, however, are not unequivocally efficient in predicting adverse neonatal outcomes in IUGR or in prolonged pregnancies. Furthermore, the time window for neuroprotective treatment in birth asphyxia is narrow, and additional methods for identifying those neonates who would benefit from neuroprotective actions are essential. We thus searched for biomarkers identifying those fetuses at risk for hypoxia-caused complications, and for predicting outcome after birth asphyxia. Erythropoietin (EPO) is a biomarker of chronic hypoxia, with high levels of EPO associating with increased risk for adverse outcome. S100B is a biomarker of brain- cell damage, and its levels rise in early phases of acute asphyxia. Copeptin, a by-product of arginine vasopressin (AVP) production, is a potential biomarker of birth asphyxia and HIE. Additionally, we aimed to evaluate the association of gestational age with perinatal asphyctic complications and long-term neurologic morbidity.

The biomarker studies (I-III) were conducted in the University Hospital of Helsinki, Finland. Data on maternal pregnancy and delivery characteristics, and short-term neonatal outcomes such as Apgar score, originated from hospital charts. The study populations comprised 66 pregnancies complicated by preterm IUGR, 93 low-risk term and prolonged pregnancies, and 140 term neonates with birth asphyxia. Amniotic fluid samples for EPO evaluations we obtained by amniocentesis, at cesarean section, or vaginally at amniotomy. Umbilical serum

plasma samples for EPO, copeptin, and S100B assessments we collected at birth. Biomarker levels in amniotic fluid and umbilical plasma samples we measured by immunoassays. Normal amniotic fluid EPO levels we defined as < 3 IU/L, with abnormal levels exceeding 27 IU/L. We considered as normal umbilical plasma EPO levels below 40 IU/L.

The register-based cohort study on asphyxia and neurologic morbidity (IV) comprised 1 138 109 women with singleton pregnancies and their newborns between 1989 and 2008 in Finland. The Finnish Medical Birth Register (MBR), maintained by the National Institute for Health and Welfare (THL), provided data for this study. Statistical analyses we performed with the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA), GraphPad Prism 6 and SAS version 9.3 (SAS Institute, Inc, Cary, NC, USA). All tests were two-sided, with probability (p) values of < 0.05 as statistically significant.

In IUGR pregnancies, abnormal amniotic fluid EPO levels were associated with decreased umbilical artery pH and base excess (BE) values, abnormal biophysical profile, and reversed end-diastolic flow in the umbilical artery. Most importantly, such abnormal EPO levels were associated with composite adverse neonatal outcomes defined as intraventricular hemorrhage, periventricular leukomalacia, cerebral infarction, or necrotizing enterocolitis ($p < 0.001$). In low-risk term and postterm pregnancies, EPO levels in amniotic fluid and in umbilical serum correlated with gestational age. Furthermore, EPO levels in amniotic fluid correlated with the levels in umbilical serum, even after vaginal delivery. Among low-risk pregnancies, however, EPO levels correlated with neither umbilical artery pH or BE, nor with other adverse pregnancy outcomes. In our study on biomarkers in birth asphyxia, only copeptin correlated with arterial pH. Its correlation with umbilical artery BE was significantly stronger than were the correlations of S100B or of EPO. Copeptin levels, significantly higher among neonates with birth asphyxia, we demonstrated to increase as a function of labor duration.

In the cohort study, multivariate analysis demonstrated an increased risk for low (< 4) one- and five-minute Apgar score, CP, intellectual disability, sensorineural defects, and perinatal mortality among early-term births. Postterm birth resulted in increased risk for low one- and five-minute Apgar scores (< 4), low umbilical artery pH ≤ 7.10, and intellectual disability, whereas risks for CP, epilepsy, sensorineural defects, and perinatal mortality showed no increase.

In conclusion, among preterm IUGR pregnancies, high amniotic fluid EPO levels were associated with decreased umbilical artery pH and BE, and with adverse neonatal outcomes. In selected risk-pregnancies, determining amniotic fluid EPO may thus be a useful additional tool in fetal surveillance and in optimizing delivery timing. In term pregnancies, EPO levels correlated with gestational age, probably explained by advancing gestation resulting in weakening placental function and relative hypoxemia. Among low-risk populations, however, EPO was not related to adverse delivery outcomes, and thus may not prove clinically useful in such populations. Furthermore, in cases of acute birth asphyxia, S100B and EPO as biomarkers may not prove valid. In contrast, copeptin has potential for routine use as a biomarker for acute birth asphyxia and neonatal distress. Future studies should

determine the correlation of biomarker levels at birth with severity of HIE and with long-term neurological outcome following birth asphyxia.

Concerning gestational age at birth, we found an increased risk for low Apgar score, increased neurologic morbidity, and perinatal mortality among early-term neonates. Among postterm births, the risk for birth asphyxia was increased. The long-term neurologic health impacts of postterm birth, however, were less important than previously expected, meaning that further studies on the optimal management of pregnancies beyond 41 gestational weeks are essential.

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications, referred to in the text by their Roman numerals I-IV.

I Amniotic fluid erythropoietin and neonatal outcome in pregnancies complicated by intrauterine growth restriction before 34 gestational weeks. Seikku L, Rahkonen L, Tikkanen M, Hämäläinen E, Rahkonen P, Andersson S, Teramo K, Paavonen J, Stefanovic V. Acta Obstet Gynecol Scand. 2015 Mar;94(3):288- 94. doi: 10.1111/aogs.12553 Epub 2015 Jan 6. IF 2.741

II Amniotic fluid and umbilical cord serum erythropoietin in term and prolonged pregnancies. Seikku L, Stefanovic V, Rahkonen P, Teramo K, Paavonen J, Tikkanen M, Rahkonen L. Eur J Obstet Gynecol Reprod Biol. 2019 Feb;233:1-5. doi: 10.1016/j.ejogrb.2018.11.022. Epub 2018 Dec 3. PMID: 30529256. IF 1.809

III Comparison of Umbilical Serum Copeptin Relative to Erythropoietin and S100B as Asphyxia Biomarkers at Birth. Summanen M, Seikku L, Rahkonen P, Stefanovic V, Teramo K, Andersson S, Kaila K, Rahkonen L. Neonatology. 2017;112(1):60-66. doi: 10.1159/000456063. Epub 2017 Mar 29. IF 2.554

IV Asphyxia, Neurologic Morbidity, and Perinatal Mortality in Early-Term and Postterm Birth. Seikku L, Gissler M, Andersson S, Rahkonen P, Stefanovic V, Tikkanen M, Paavonen J, Rahkonen L. Pediatrics. 2016 May 27. pii: e20153334. IF 4.22

ABBREVIATIONS

ACTH	adrenocorticotropic hormone
aEEG	amplitude integrated electroencephalography
am-EPO	amniotic fluid erythropoietin
AUC	area under the curve
AVP	arginine vasopressin
BE	base excess
BMI	body mass index
BPM	beats per minute
BPP	biophysical profile scoring
cCTG	computerized cardiotocography
CI	confidence interval
CP	cerebral palsy
CPR	cerebroplacental ratio
CTG	cardiotocography
CV	coefficient of variation
EEG	electroencephalography
EPO	erythropoietin
FBS	fetal scalp blood analysis
GW	gestational weeks
HDR	Hospital Discharge Register
HIE	hypoxic ischemic encephalopathy
HIF	hypoxia-inducible transcription factor
HPA	hypothalamic-pituitary-adrenal

ICD	International Classification of Diseases
IQ	intelligence quotient
IU	international unit
IUGR	intrauterine growth restriction
IVH	intraventricular hemorrhage
MAS	meconium aspiration syndrome
MBR	Finnish Medical Birth Register
MCA	middle cerebral artery
MRI	magnetic resonance imaging
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
NSE	neuron-specific enolase
OR	odds ratio
PVL	periventricular leukomalacia
RCT	randomized controlled trial
rEPO	recombinant erythropoietin
ROC	receiver operating characteristics
SD	standard deviation
SGA	small for gestational age
SPSS	Statistical Package for Social Sciences
THL	National Institute for Health and Welfare
us-EPO	umbilical serum erythropoietin
WHO	World Health Organization

INTRODUCTION

Fetal hypoxia occurs in a wide range of complicated pregnancies such as those involving maternal malnutrition, cardiovascular diseases, diabetes, obesity, hypertensive disorders, and inflammatory conditions (1,2). Fetuses have numerous adaptive mechanisms to compensate for hypoxic conditions (2-4). Prolonged fetal hypoxia, however, leads to metabolic acidosis (5). The effects of intrauterine hypoxia on the fetus depend on the severity and duration of hypoxia, on gestational age at time of the hypoxic event, and on the etiology of the insult (1). Intrauterine hypoxia contributes to stillbirths, fetal growth restriction, and neurodevelopmental sequelae, and it influences long-term metabolic and cardiovascular health (3,6). Additionally, antepartum intrauterine hypoxia predisposes to hypoxic ischemic adverse events during birth (7,8).

Worldwide, up to four million neonates are estimated to suffer from birth asphyxia annually (9). The majority will recover without developing long-term sequelae. However, continuous severe metabolic acidosis may eventually lead to vital organ and cerebral injury cascades, leading to neonatal hypoxic ischemic encephalopathy (HIE) or to death (10,11). The second most frequent cause of neonatal mortality, after preterm birth complications, is perinatal asphyxia, which contributes up to 25% of deaths within the first 28 days (12). Furthermore, perinatal asphyxia is estimated to account for millions of antepartum and intrapartum stillbirths, and contribute to equal numbers of permanent neurologic sequelae such as cerebral palsy (CP), epilepsy, intellectual disability, and sensorineural impairments (9,12). Neuroprotective treatments such as therapeutic hypothermia, have, in perinatal asphyxia among near-term and term neonates, substantially improved prognosis (13,14).

The possibilities to influence intrauterine fetal well-being are scarce. The most important opportunity in preventing adverse outcomes is therefore timely delivery. This is a remarkable challenge, since unnecessary interventions can lead to excessive obstetric procedures and neonatal morbidity from prematurity. On the other hand, delayed responses to fetal distress may lead to stillbirth or severe neonatal asphyctic injury. Numerous methods such as fetal Doppler flow assessment and computerized cardiotocography (cCTG) serve to monitor placental function and fetal wellbeing in risk-pregnancies and help in delivery timing. The time-scale and sequence of alterations in these monitored parameters vary by gestational age and underlying pathologies (15-17). Identifying the specific pregnancies at risk for intrapartum fetal compromise and adverse neonatal outcomes is, however, challenging. New methods are called for in detecting individual high-risk pregnancies and optimizing delivery timing.

Despite significant progress in perinatology and neonatology over the decades, neurologic morbidity after HIE remains significant (14,18-20). Furthermore, identifying those neonates who could benefit from neuroprotective treatments such as therapeutic hypothermia constitutes an additional challenge, owing to the restricted time window for these therapies. Risk for HIE increases with deepening acidemia (21,22), and five-minute Apgar scores

correlate with risk for neurological disability (23). However, the sensitivity and specificity of umbilical artery pH values and Apgar scores regarding outcomes following HIE are low (11). Clinical neurological signs during the immediate neonatal period indicate initiation of therapies and are somewhat better predictors of outcomes (24). Reliable and swift additional methods in selecting neonates for treatment and predicting outcome after birth asphyxia are urgently needed, with several neonatal biomarkers under study in recent years (25,26).

Approximately 10% of neonates are born small for gestational age (SGA) (27,28). The subject of fetal growth is critical, since, in fetal growth restriction, risks for perinatal asphyxia and HIE, intracranial hemorrhage, meconium aspiration, immune dysfunction, hypoglycemia, and other metabolic abnormalities are increased (29,30). Moreover, suboptimal fetal growth is accountable for 25% to 43% of all stillbirths (31-33).

Pregnancy outcomes vary by gestational age at birth, even among term pregnancies. The optimal gestational length appears to be reached between the 39^{+0} and 41^{+6} gestational weeks (GW), with risk for adverse outcomes increased outside this period (34). Post-term pregnancies, at or beyond 42^{+0} GW, account for approximately 14% of stillbirths worldwide (12,35). The underlying mechanism is assumed to be aging of the placenta and subsequent relative placental insufficiency, thus predisposing the fetus to hypoxia. Gestational age-associated risks, however, form a biological continuum. Consequently, a U-shaped relationship occurs between adverse pregnancy outcomes and gestational age (34). Risks for perinatal mortality and morbidity increase in pregnancies beyond 41^{+0} GW (36,37). Recently, increased short- and long-term morbidity such as excessive neonatal admissions, prolonged hospitalizations, developmental delay, and CP have been related to early-term births between 37^{+0} and 38^{+6} GW (34,38). In European and North American countries, the proportion of postterm birth is decreasing and that of early-term birth increasing (39,40). In Finland, however, these rates have remained relatively stable until recent years (41). Some questions as to the significance of gestational age on birth asphyxia, long-term neurologic morbidity, and perinatal mortality among term pregnancies in Finland have, however, arisen.

Levels of erythropoietin (EPO), a primary hormone regulating erythropoiesis, rise in response to hypoxia in adults, newborns, and fetuses (42). EPO level in amniotic fluid correlates with its level in fetal plasma during pregnancy (25). Increased EPO levels in amniotic fluid and in umbilical cord serum occur in various complicated pregnancies and are associated with an increased risk for adverse neonatal outcomes such as decreased umbilical artery pH and base excess (BE), and need for neonatal intensive care unit admission (25). Amniotic fluid EPO (am-EPO) may be considered as a biomarker of chronic fetal hypoxia. Data on am-EPO in relation to umbilical artery Doppler flow abnormalities and neonatal outcome in pregnancies complicated by intrauterine growth restriction are lacking. Such data might assist in identifying fetuses suffering from aggravating hypoxia and assist in timing of delivery. Furthermore, identifying those specific postterm pregnancies at increased risk for adverse outcome is especially challenging. Whether am-EPO can identify these risk pregnancies is unknown.

Protein S100B, mainly produced in the central nervous system, is considered a biomarker of brain distress and neuronal cell injury (19,43). It is associated with various conditions including traumatic brain injury and stroke, as well as with neurodegenerative, congenital, and psychiatric diseases (43,44). High S100B concentrations in amniotic fluid may predict intrauterine death (45). Elevated cord plasma S100B concentrations are related to acidemia and asphyxia at birth (46,47). Furthermore, in birth asphyxia, elevated umbilical cord S100B concentrations may predict development of moderate to severe HIE, and predict risk for neurological damage at age six (47).

Copeptin, a stable by-product of arginine vasopressin (AVP), is a novel biomarker serving as a diagnostic and prognostic marker for adult emergency departments (48-50). AVP has crucial effects on cardiac function and physiologic homeostasis in various stress conditions. During normal birth, the fetal hypothalamic-pituitary-adrenal (HPA) axis activates and results in AVP release. This is further accelerated by numerous stress factors such as infections, intrauterine growth restriction (IUGR), and acidosis or birth asphyxia (51-53). The utility of copeptin in comparison with that of other asphyxia biomarkers and the relationship of copeptin to delivery modes in planned vaginal births and duration of delivery both demand study.

This study evaluated the significance of EPO in management of pregnancies complicated by IUGR, and of prolonged pregnancies. Additionally, we evaluated and compared umbilical cord blood biomarkers—EPO, S100B, and copeptin—in neonates with birth asphyxia to test these potential biomarkers in patient selection for therapeutic interventions. Moreover, we assessed the association of gestational age with perinatal asphyxia and long-term neurologic morbidity in term and post-term pregnancies.

REVIEW OF THE LITERATURE

CHARACTERISTICS OF FETAL PHYSIOLOGY

The intrauterine environment is relatively hypoxic as compared to the environment after birth. The partial oxygen-pressure gradients between maternal blood, placenta, and fetal blood and tissues determine fetal oxygenation (54). Maternal arterial partial oxygen pressure is from 80 to 90 mmHg, but in the fetal compartment, pressure ranges from 25 to 30 mmHg (54). The highest fetal oxygen saturation—found in the umbilical vein—ranges between 70% and 80% (4). Fetal tissue oxygenation, however, is comparable to oxygenation during postnatal life, as a result of several delicate compensation mechanisms (54).

Hematologic adaptations

The relatively low oxygen environment induces fetal erythropoiesis via hypoxia-inducible factors and EPO synthesis, thus increasing oxygen transferal capacity (4). Furthermore, fetal hemoglobin has high oxygen affinity which facilitates the uptake of oxygen from the placenta to the fetal circulation (4,54). Local factors such as tissue acidity modulate oxygen delivery from fetal blood to peripheral tissues (4).

Circulation

Oxygenated blood flow from the placenta is directed to the left ventricle and towards the developing brain and heart through anatomic shunts (4). High cardiac output ensures high blood flow to the fetal tissues (54). Adaptations of cardiac output occur mostly by modulations in heart rate. These modifications are limited, however, due to the immaturity of cardiac sympathetic innervation and myocardial contractility (4). The fetal pulmonary circulation consists mostly of deoxygenated blood with a low saturation of 55%. Initially, pulmonary blood flow covers approximately 10% of cardiac output, but its proportion, with advancing gestation, increases up to 50% (4).

INTRAUTERINE HYPOXIA

Fetal hypoxia occurs in a wide range of pathologic circumstances during pregnancy, ones such as maternal malnutrition, smoking, cardiovascular diseases, diabetes, obesity, and pre-eclampsia, as well as in inflammatory conditions like chorioamnionitis (1,2). Intrauterine hypoxia is a major obstetrical challenge contributing to stillbirths, fetal growth restriction, neurodevelopmental complications, and long-term metabolic and cardiovascular health effects (3,6). However, the exact prevalences of antepartum hypoxia and asphyxia are unknown (55).

Definitions

Hypoxia occurs when oxygen demands of the tissue exceed the available supply (56). Normal oxygen tension in fetal arterial blood is close to the hypoxia level in adult tissues. This relative hypoxemia predisposes the fetus to a lower threshold of oxygen insufficiency (3). In uteroplacental hypoxia, maternal oxygenation is normal, but the uteroplacental circulation is impaired, leading to inadequate fetal oxygenation causing, first, decreased oxygen concentration in arterial blood, and ultimately in fetal tissues (57). Oxygen demands of the placenta and the fetus increase along with advancing pregnancy (6).

Etiology of fetal hypoxia

Placental dysfunction is most commonly due to suboptimal remodeling of maternal vessels and superficial trophoblast invasion during early stages of placentation (58). Deficient spiral artery remodeling leads to malperfusion of the placenta, inducing oxidative cell stress, selective suppression of protein synthesis, and reduced cell proliferation (59). Subsequently, reduction in placental volume and surface area for transport ensues. Secondary alterations involving fetal arteries cause increased resistance within the umbilical circulation, resulting in further impairment of placental function (59). Additionally, inflammatory conditions may lead to increased placental vasculature resistance and to oxygen deprivation (2). Regardless of the mechanism initiating placental pathology, abnormalities in placental development or in metabolism result in placental and fetal hypoxia (3,6). Prolonged fetal tissue hypoxia leads to accumulation of lactic acid and results in metabolic acidosis (5). In placental insufficiency, respiratory gas exchange is impaired, thus exposing the fetus to hypercapnia and respiratory acidosis in addition to hypoxia and metabolic acidosis, leading to fetal asphyxia (60).

Fetal compensatory mechanisms in intrauterine hypoxia

The fetus has several adaptive mechanisms to compensate for hypoxic conditions. Hemodynamic responses conserve the oxygenation of organs critical for survival. Energy metabolism turns towards increased glycolysis and utilization of anaerobic energy sources, and genes necessary to alter cell metabolism accordingly are activated during hypoxia (3). The fetus can impede many oxygen-consuming functions (2). For instance, fetal movements are reduced as an adaptation to impaired oxygenation (61). The affinity of fetal hemoglobin for oxygen decreases during fetal acidosis, thus enhancing tissue oxygenation (4). If the hypoxia persists, and the compensation mechanisms become insufficient, fetal distress and eventually demise ensues.

Cardiovascular adaptations. During periods of impaired oxygen delivery, fetal chemoreflex activation rises fetal blood pressure. This mechanism directs more blood flow to the umbilical-placental circulation, thus facilitating oxygen uptake from the placenta (62). At the same time, deceleration of the fetal heart rate occurs, leading to more efficient gas exchange owing to the slower blood flow (2). Additionally, cardiovascular changes can reduce the

adverse effects of tissue hypoxia via redistribution of blood flow to the brain, the coronary arteries, the adrenals, and the upper body. As cerebral vasodilatation occurs and vascular resistance increases in the lower body, cardiac output shifts towards left ventricle outflow tract (63,64).

These responses are regulated by vagal reflexes, by vasoactive hormones such as catecholamines and vasopressin, and by local vascular tone modifications of nitric oxide (NO) and reactive oxygen species (ROS) (2). In chronic hypoxia, redistribution of the circulation is sustained, thus inducing physiological changes such as reduction in smooth-muscle and endothelial function and constantly increased flow in the cerebral and myocardial vasculature (62). Furthermore, a persistent increase in peripheral vascular resistance leads to remodeling of the cardiomyocytes and the walls of the heart and major vessels (2).

Hematologic changes. The major part of the intracellular adaptive responses in continuous hypoxia are regulated by transcription factor HIFα (hypoxia-inducible factor alpha) (54). Stabilization and activation of HIF-1 in hypoxia leads to activation of transcription of multiple genes, such as those producing EPO and VEGF (vascular endothelial growth factor), thus enhancing tissue oxygenation (54). Consequently, in tissue hypoxia, erythropoiesis, nucleated red blood cells, and hematocrit increase (6).

Metabolic and endocrine compensation. In hypoxia, swift hypothalamus-pituitary-adrenal (HPA) axis activation plays a key role in initiating fetal protective mechanisms. A significant increase in glucocorticoids influences plasma glucose, lipid, and protein concentrations, thus maintaining fetal homeostasis (1). The sympathetic nervous system is activated and catecholamines released regulate fetal cardiovascular and metabolic stress responses (65).

Such responses are crucial in acute stress circumstances, but in chronic conditions they have the potential to alter regulation mechanisms of the endocrine system and metabolism permanently. Indeed, in chronic hypoxia, the adrenal sensitivity to adrenocorticotropic hormone (ACTH) and the cortisol response are attenuated as compared to responses in normal pregnancy, and adrenal medullary responses appear to adapt to chronic hypoxia, as well (62). Thus, the adverse environment caused by intrauterine hypoxia predisposes the fetus to a variety of cardiovascular and metabolic pathologies later in life (1,66).

Fetal consequences of hypoxia

The effects of intrauterine hypoxia on the fetus vary by severity and duration of hypoxia, gestational age during hypoxia, and etiology of the insult (1). As a consequence of placental insufficiency, both nutrient and oxygen transfer are reduced (6). This leads to failure of the fetus to achieve its optimal growth potential (27). Fetal growth restriction, especially when not detected antenatally, is a major risk factor for stillbirth (67). Hypoxia, caused by placental insufficiency, is assumed to be an important contributor to most fetal deaths, also in high-income countries (68,69).

Antepartum intrauterine hypoxia may cause increased vulnerability to hypoxic ischemic adverse events at birth, especially in regard with fetal growth restriction (7,8). Recurrent undetected asphyctic events can occur during pregnancy, and this may lead to continuous and more severe injury during delivery (5,70). In a substantial portion of birth asphyxia cases the origin of asphyxia is antenatal (55).

Long-term consequences of intrauterine hypoxia

Neurologic implications. In hypoxia the metabolic rate of the brain tissue decreases, probably to reduce its oxygen demands (62). In the fetal brain, chronic hypoxia causes production of reactive oxygen species (ROS), potentially leading to cell damage and apoptosis (62). Fetal hypoxia, especially when related to growth restriction, contributes to long-term cognitive and neurologic abnormalities, (71). In term pregnancies, the etiology of CP is prenatal for approximately 70% to 80% of cases (72), one of the major contributors being fetal asphyxia (73). Birth asphyxia is associated with 10% of all cases of CP and 20% of CP among children born at term (5). In preterm births, however, antenatal causes explain roughly 17% of CP cases (73). Among preterm neonates, asphyxia is more frequently moderate to severe and leads to a greater risk for long-term morbidity and death, than among term neonates (55).

A recent study on fetal sheep demonstrated that chronic hypoxia leads to significant alterations in fetal neurodevelopment (74). The findings included reduced cortical folding, reduced neuronal density, hypervascularity, and impaired myelination. Such structural changes are associated with subsequent delay in cognitive performance, and resemble those abnormalities found among newborns with growth restriction and congenital heart diseases, being injured by intrauterine hypoxia (74).

A recent animal study on gene expression patterns has revealed inflammation patterns in the fetal brain after a hypoxic episode (62). Additionally, live bacteria identical to species in the placenta have been found in the fetal brain after transient hypoxia, possibly related to increased vascular permeability resulting from hypoxia (62). The significance of this recent discovery is not yet elucidated.

Fetal programming. Intrauterine hypoxia causes long-term health effects and increased risk for chronic diseases via fetal programing (75). Hypoxia causes an increase in reactive oxygen species (ROS), which can trigger long-term changes in gene expression patterns (3). Such epigenetic changes may affect multiple enzymes involved in steroidogenesis and stress hormones, potentially predisposing the fetus later in life to chronic cardiovascular and metabolic diseases. Intrauterine hypoxia is associated with depressed cardiac performance and with cardiomyopathies (3). In addition, hypoxia affects lung development by reducing the pulmonary circulation and breathing movements (4). Activation of hypoxia-induced factors lead to remodeling of the pulmonary vasculature and to increased vascular resistance, predisposing to development of pulmonary hypertension (76).

ANTENATAL AND INTRAPARTUM FETAL MONITORING

The purpose of fetal antenatal surveillance is detection of signs of evolving hypoxia and activation of adaptation mechanisms in its early stages. In most cases, fetal deterioration follows a certain pattern, beginning with changes in heart-rate tracings, followed by decrease in amniotic fluid volume and in fetal movements (77). Thus, intensive monitoring and treatment can focus on pregnancies with fetuses at increased risk for adverse outcomes, with timing and mode of delivery planned appropriately.

On the other hand, the main objective of fetal monitoring during labor is to identify those fetuses at immediate risk for perinatal asphyxia, and to prevent potential serious consequences such as HIE or perinatal death. Assurance of fetal wellbeing without unnecessary obstetric interventions is, as well, a priority (78).

Ultrasonography

Growth assessment. Evidence of improving pregnancy outcomes by routine late pregnancy ultrasound after 24 GW in a low-risk population is lacking (79). However, identifying small-for-gestational age (SGA) fetuses before delivery significantly reduces risk for adverse outcomes such as stillbirth, low Apgar score and umbilical pH, HIE, intracranial hemorrhage, neonatal seizures, CP, and infant death (30). However, identifying SGA during pregnancy is challenging, even by applying appropriate methods. A substantial proportion of fetuses that are SGA, up to 35%, remain undetected before birth (80).

Biophysical profile scoring (BPP) estimates fetal wellbeing by describing these variables: Fetal movements, tone, amniotic fluid volume, breathing, and heart rate activity (81). BPP reflects fetal metabolic state and oxygenation, along with the function and maturation profile of the nervous system (81). Evidence of BPP providing benefits for pregnancy outcome is controversial. A clear correlation between fetal acidosis and decreased fetal movements does exist (61), and studies have demonstrated an association of abnormal BPP with prenatal mortality and CP (82). Pregnancies with poor perinatal outcomes after women observing reduced fetal movements may reach 25%, and in one study more than half of the population reported decreased movements before fetal demise (83). One systematic review of high-risk pregnancies, however, found no improvement in perinatal outcome—no reduction in perinatal deaths nor low Apgar score at 5 min—but found an increased risk for cesarean section by means of fetal surveillance with BPP scoring (84).

Amniotic fluid volume assessment is a part of BPP scoring. Oligohydramnios is often associated with placental dysfunction and is considered to reflect decreased renal perfusion due to redistribution of the circulation (85). Reasonably, in one systematic review, an association of adverse delivery outcomes—such as Apgar score < 7 at 5 minutes and cesarean section for fetal distress—appeared with oligohydramnios (86). However, controversial studies do exist with no evidence of such increased risk, even among complicated high-risk pregnancies (85,87). Amniotic fluid volume can be estimated by measuring the deepest

vertical pocket or the amniotic fluid index, and these methods are equivalent in prevention of adverse outcomes (88).

Doppler flow ultrasound assessments are extensively utilized in uteroplacental hypoxia to detect fetal hemodynamic stress responses. Superficial trophoblast invasion during early stages of placentation and suboptimal remodeling of maternal vessels leads to high-resistance uteroplacental circulation and placental dysfunction (58). These abnormalities are detectable by Doppler flow changes such as increased resistance and persistent notching in uterine artery blood flow during early pregnancy (89).

Changes in fetal circulation are often progressive and follow certain patterns (15). Umbilical artery Doppler parameter alterations appear when more than half of the placental circulation is lost (90). The severity of a Doppler flow abnormality is proportional to the degree of hypoxia and acidemia. Thus, absent or reversed end diastolic flow patterns are late signs of a far advanced process (63,91). In high-risk pregnancies, umbilical artery Doppler ultrasound surveillance reduces risk of perinatal death (90). Following umbilical artery Doppler alterations, reduction in blood flow impedance in the middle cerebral artery (MCA) usually appears as a “brain sparing” effect (92). According to a recent systematic review and meta-analysis, MCA Doppler alone, however, show good predictive accuracy (93). Alterations in cerebroplacental ratio (CPR)—changes in relations between MCA and umbilical artery flow—are, however, associated with perinatal composite adverse outcome and emergency cesarean delivery for fetal distress (94,95).

As the cerebral and placental vascular impedances change, resistance in aortic blood flow increases (15,92). Abnormalities in aortic isthmus Doppler flow may help in early identification of fetuses at risk for intrauterine compromise and help in predicting perinatal and neurodevelopmental outcomes (96). As hypoxia progresses, an increase in venous flow resistance occurs, owing to hypoxemia of the myocardium (15,97). Alterations in venous Doppler flow profiles correlate well with fetal acidosis (91,97). Retrograde ductus venosus a-wave and pulsatile flow in the umbilical vein (UV) indicate fetal cardiac compromise (98).

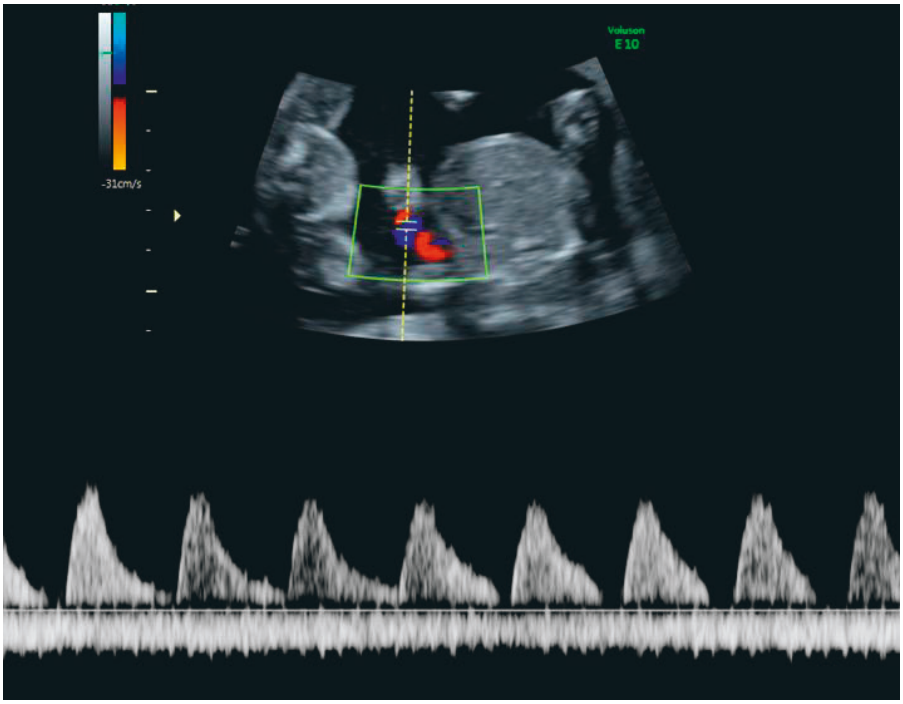

Figure 1. Intermittent absent end diastolic flow in monochorionic twin pregnancy complicated by selective intrauterine growth restriction. *By author*

Antenatal cardiotocography and computerized cardiotocography

Cardiotocography (CTG) is a method widely used in fetal surveillance. Basically, changes in heart rate patterns can predict ominous fetal hypoxia (60). During pregnancy, accompanying the developing autonomic nervous system and fetal maturation, heart rate patterns evolve (60). Heart rate baseline decreases, and the variability increases. The behavioral stages with this episodic activity and accelerations usually appear around 25 to 28 GW (6,99). Intrauterine hypoxia can delay maturation of the nervous system and reduce fetal responsiveness and also reduce the emergence of various behavioral stages (6,100).

Visual interpretation of the fetal heart rate patterns is subjective, and its consistency is affected by high intra- and interobserver variability (101). Evidence of improvement in reducing perinatal morbidity or mortality by utilizing traditional CTG monitoring is lacking (102). Computerized CTG (cCTG) is, however, a more objective method (99). The most useful cCTG indicator of fetal antepartum well-being is fetal heart rate variation, with the normal values of short-term variation by gestational age being well reported (99). In certain risk-pregnancies, reduction in short-term variability predicts metabolic acidosis and adverse neonatal outcomes (103). Some data exists on improvement in perinatal survival rates by use of cCTG, but the level of evidence is low and needs further study (102).

Intrapartum cardiotocography

Fetal heart rate monitoring is generally considered the gold standard of intrapartum fetal surveillance, with CTG tracings classified as normal, suspicious, or pathological (100). The basic normal intrapartum CTG features are described in the FIGO guidelines, including normal baseline between 110 and 160 bpm, normal variability with a bandwidth amplitude of 5 to 25 bpm, and accelerations from baseline being a sign of a neurologically responsive fetus (100). Decelerations are defined as decreases in the fetal heart rate below baseline of more than 15 bpm in amplitude and lasting more than 15 seconds. Decelerations are classified according to physiologic background as early, variable, late, or prolonged (100). Chemoreceptor-mediated heart rate responses, and late and prolonged decelerations are often related to fetal hypoxia or acidosis. Regular re-evaluation of the tracings during delivery is recommended, especially during the second stage of labor when fetal hypoxia may develop rapidly.

Numerous studies on CTG analysis have indicated considerable disagreement and variation (100,104). The early studies revealed that only a minority of suspicious and even pathological fetal heart rate recordings were associated with fetal acidemia (105). Specificity and the positive predictive value of suspicious and pathologic CTG tracings for acidemia and low Apgar score at birth are low (106). According to a recent Cochrane review, continuous CTG compared to intermittent auscultation had no effect on perinatal death, on CP, or on cord blood acidosis rates (107). Neonatal seizure rates, however, were lower in the continuous CTG group. Risks for cesarean section or instrumental vaginal delivery were higher in the group with continuous CTG tracing. The outcomes were comparable among low- and high-risk subgroups (107).

Figure 2. Pathologic CTG in the end of the second stage of labor. Umbilical artery pH at birth was 6.99 and BE -12.00, with Apgar score 4/6/6. *By author*

Adjunctive intrapartum surveillance technologies

Several technologies adjunctive to CTG allow fetal intrapartum surveillance, both to identify evolving hypoxia and to reduce unnecessary interventions (108).

Fetal scalp-blood sampling (FBS) for pH or lactate is an accessory, second-line method to CTG for assessing fetal wellbeing during labor. At birth, moderate neonatal acidosis can be defined as pH below the 10th percentile, meaning pH below 7.15 (109). Acidosis can be due to accumulation of pCO2, leading to increased production of H+ ions. Such a condition can occur and resolve rapidly and is infrequently associated with long-term adverse outcomes (109). Metabolic acidosis, however, is related to prolonged tissue hypoxia. Anaerobic metabolism produces lactate and H+ ions, thus depressing blood and tissue pH. This type of acidosis takes a longer time to resolve, with increased risk for impaired neurologic development and end-organ damage (109). Metabolic acidosis is defined as severe when the arterial base deficit is > 12 mmol/L, or lactate > 10 mmol/L.

Fetal scalp-blood analysis (FBS) was introduced almost 60 years ago to improve diagnosis of intrapartum fetal acidemia (105). The technique has some disadvantages; it is invasive and has several technical challenges, and each sample reflects only the current situation. Additionally, a blood sample from the fetal scalp probably does not represent the precise situation in the central circulation. However, when lactate measurement is normal during the last hour of labor, it strongly predicts normal oxygenation at birth (108). In detecting hypoxia during labor, FBS analyze for lactate and for pH are equally effective (110). Some evidence exists that FBS, as a method adjunct to CTG, reduces rates of operative interventions (109). Clear evidence as to the beneficial effect of fetal blood sampling on neonatal outcomes is lacking (107).

Fetal stimulation. Observational studies have shown that appearance of acceleration and normalization of CTG tracing in response to scalp stimulation is a consistent sign of fetal wellbeing, a negative predictive value comparable to a pH of 7.25 with FBS (108). A positive fetal scalp-stimulation test may reduce the need for FBS (108).

STAN. During hypoxia, fetal electrocardiogram ST-segment changes precede failure of cardiovascular function (111). Computer-assisted combined analysis of CTG and ST morphology changes, the STAN® concept, has been utilized since 2000 (108). The potential advantages of this technique are its less invasive approach and its continuous data on fetal status. The results of numerous randomized controlled trials (RCTs) and observational studies on ST segment analysis are controversial (108). Currently, the latest meta-analysis showed that monitoring of ST changes significantly reduces rates of operative deliveries and the need for FBS (112). The effect on neonatal acidosis has been debated, but in recent observational studies, a decrease in metabolic acidosis incidence has been detectable (113,114). Importantly, commencement of ST analysis technique is associated with a long-lasting learning curve, and the advantages of the method may emerge after a considerable delay following its introduction (114).

MANAGEMENT OF SELECTED RISK PREGNANCIES

The primary objective of fetal surveillance is to improve perinatal outcome by reducing mortality and morbidity. Since the options to influence fetal well-being during pregnancy are few, the most important opportunity for preventing adverse outcomes is timely delivery. This is, however, a major obstetric challenge. Unnecessary interventions can lead to excessive and potentially harmful obstetric procedures and to neonatal morbidity from prematurity. On the other hand, stillbirth or severe neonatal asphyctic injury can result from delayed actions on fetal distress.

Intrauterine growth restriction (IUGR)

Incidence, background, and definitions. Approximately 10% of neonates are born small for gestational age (27,28). In high-income countries, fetal growth restriction occurs in 3% to 9% of all births (115), a prevalence significantly—more than six-fold—higher in low- and middle-income countries (116).

Numerous maternal and fetal factors can lead to impaired fetal growth. These include environmental and maternal problems such as infection, malnutrition, reduced uteroplacental blood flow, and placental insufficiency, as well as fetal factors like chromosomal and structural abnormalities (70,117). Fetal growth restriction can be classified into three types according to fetal growth patterns (118). Such patterns generally reflect the etiology of the growth disorder, as well. In symmetric growth restriction, a proportional decrease in size of the head, abdomen, and long bones occurs. These fetuses can either be constitutionally small but normally developed fetuses with no risk for complications, or be fetuses with congenital chromosomal or genetic abnormalities leading to severe growth retardation. Fetuses with asymmetric FGR mainly have a smaller abdominal size, but the head and long bones are within normal limits. In such a growth pattern, the primary etiologic factor is placental insufficiency, putting these fetuses at increased risk for intrauterine hypoxia. In the third pattern, features of both the other two patterns are manifested, and etiologic factors consist of embryonic infections and toxic agents at early stages of pregnancy (118).

Diagnostic criteria for fetal growth restriction (FGR) or intrauterine growth restriction (IUGR) have, some extent of variation, but generally these terms refer to a fetus who fails to achieve its expected genetic growth potential (27). The WHO has defined FGR as fetal growth below the third percentile (-2 SD), but in clinical practice the most generally applied definitions include estimated weight or abdominal circumference less than the 10^{th} percentile (80,119,120). Such definitions include heterogenous groups of SGA fetuses comprising both the constitutionally small fetuses not at risk for adverse outcomes, and the fetuses not reaching their growth potential due to external factors; these are at increased risk for antenatal and postnatal complications (93,121-123). Nevertheless, in a major proportion of SGA pregnancies, fetuses are growth restricted, and most of the growth-restricted fetuses are SGA (30). Recently, an international expert panel introduced a consensus definition of

fetal growth restriction considering also the grade and distribution of growth restriction as well as Doppler flow parameters (123).

Controversy exists as to the advantages of individualized reference curves for fetal growth estimation. Proposals as to improvement of prediction of SGA neonates and adverse outcomes by customized growth charts are several (124-126). One recent study, however, has demonstrated similar fetal and newborn growth patterns among diverse geographical surroundings and ethnicities in which environmental characteristics are similar (127). Among fetuses identified as SGA, a change in abdominal circumference may be useful in detection of growth restriction (128). The time interval between the measurements should be long enough, up to three weeks, to avoid false positive results (129).

The two distinct phenotypes of IUGR are early and late-onset. They differ by timing of occurrence, patterns of evolution and of Doppler-flow parameters, and neonatal outcome (17,130). Considering perinatal outcomes, the best division between the two IUGR forms is at 32 GW (131). Early IUGR is more often related to pre-eclampsia, and the progression is more predictable (17,132). Late-onset IUGR is more difficult to detect, and more prevalent than is early IUGR (16,133).

Outcomes. The subject of fetal growth is crucial, since growth-restricted fetuses are at increased risk for mortality and morbidity (17,120,122). Among normally growing fetuses, risk for stillbirth is approximately 2/1000 births (134,135), a risk significantly higher among growth-restricted fetuses and proportional to the severity of growth restriction, three- to nine-fold that of normally growing fetuses, increasing up to 2.5% among fetuses weighing less than the 2.5th percentile (134,135). Consistent with these data, suboptimal fetal growth accounts for 25% to 43% of all stillbirths (31-33).

Incidence of neonatal death, of low Apgar score ≤ 3 at five minutes, and of umbilical artery pH ≤ 7.00 are significantly higher among term infants with birth weights at or below the 3rd percentile (30,136). Additionally, in this group, the need for intubation at birth, incidence of seizures, and sepsis are more common (136). In fetal growth restriction, the risk for perinatal asphyxia and moderate to severe HIE, intracranial hemorrhage, meconium aspiration, polycythemia, thrombocytopenia, immune dysfunction, hypoglycemia, and other metabolic abnormalities are increased (29,30). Those preterm neonates in particular with IUGR are more susceptible and have excessive prematurity-related morbidity: Hypothermia, hyperbilirubinemia, respiratory distress syndrome, bronchopulmonary dysplasia, pulmonary hemorrhage, necrotizing enterocolitis (NEC), infections, and intraventricular hemorrhage (17,29,137).

Furthermore, fetal growth restriction is associated with long-term adverse outcomes such as intellectual disability, CP, and cardiovascular and metabolic diseases (30,75,115). Brain-tissue volume alterations occur in association with IUGR (138). Distinct brain areas and neurological functions are affected at different stages of pregnancy, suggesting the evolving pathophysiology and vulnerability of the fetal brain with gestational age (139). A recent systematic review of neurodevelopmental outcomes after IUGR concluded that such

children are at risk for poorer neurodevelopmental outcomes from 6 months to 3 years of age (33). A wide variety of impairments of cognitive functions including delay in language and in adaptive behavioral development, and in learning and attention disabilities, as well as motor delay (33). Furthermore, children with IUGR had lower academic achievement, lower intelligence quotients (IQs), and more neurophysiological problems at age 9 to 10 years, than for normally growing gestational age-matched controls (33,140).

Management of IUGR pregnancy - timing of delivery. The purpose of antenatal surveillance in fetal growth restriction is to predict fetal asphyxia and enable delivery before irreversible injury or stillbirth occur. When fetuses at risk are identified, and monitoring is implemented efficiently, surveillance can reduce perinatal death risk significantly (90). However, timing of delivery in preterm pregnancy with severe IUGR constitutes a major obstetric challenge. Gestational age at birth strongly affects neonatal outcome, and the timing of the delivery thus includes critical balancing between the risks related to prematurity and inadequate tissue perfusion (141). In one large study on early growth restriction, gestational age was the primary determinant of survival until 27 GW, and of intact survival until 29 weeks (142). Indeed, before 28 weeks, prolongation of pregnancy by one day raised the chance of survival by 2% (142). Delaying delivery may lead to increased rate of stillbirths. However, a few days' delay does not affect overall perinatal mortality and appears to improve neurologic outcomes at age two (143,144). In a cohort study of near term SGA fetuses, delivery before 38 weeks was associated with higher risk of perinatal mortality than was expectant management (145). Beyond that point, continuing the pregnancy began to increase the perinatal mortality risk (145).

Several methods are available in surveillance of growth-restricted fetuses, ones such as CTG, biophysical profile assessment, and Doppler flow ultrasound. Some controversy as to the acuity and utility of the test combinations does exist. Among the majority of early IUGR fetuses, deterioration of parameters follows a classical pattern along with worsening hypoxia and acidemia (15,132,146). First appear alterations in Doppler parameters—in umbilical artery, MCA, and venosus indices—followed by reduced fetal breathing movement and amniotic fluid volume. Finally, loss of fetal movement and tone occurs, along with CTG signs of fetal distress (15,132,146). The time scale and sequence of alterations varies by gestational age and underlying pathology (15,16). In late onset IUGR, umbilical artery Doppler does not necessarily reveal fetal distress, and the classical sequence of Doppler alterations does not always appear (17,147,148). CTG changes reflecting brain hypoxia may occur at earlier phases of the sequence (17).

Computerized CTG tracings utilizing short-term variation analysis are better predictors of fetal distress in growth restriction than are traditional CTGs (103,149). Low short-term variation (STV) ≤ 3 ms is associated with high rates of metabolic acidemia (54%) and of neonatal death (8%) (103). Generally, STV tends to be lower among growth-restricted fetuses (150). According to current recommendations, computerized CTG analysis should be the preference in surveillance of SGA fetuses (119).

Biophysical profile score is a common tool for evaluation of hypoxia-related changes, with a reduced score associated with fetal acidosis, prenatal mortality, and CP (82,151). However, in chronic hypoxia, fetal movements can return to close to normal patterns, and ultimate loss of movements may appear as a late manifestation of fetal distress (60,146). Current evidence reveals high false-negative rates in detecting fetal acidemia among SGA fetuses; BPP is thus not considered an accurate surveillance method in such pregnancies (149,152). Reduced amniotic fluid volume can occur in an early phase of placental insufficiency (92), but as an isolated sign it fails to predict adverse pregnancy outcomes (85).

Doppler flow studies are in wide use in evaluation of growth restriction and of placental function among fetuses with growth below the 10th percentile (153). Vascular abnormalities in tertiary villi lead to high-resistance placental circulation and placental insufficiency (153,154), leading to progressive changes in Doppler flow profiles in maternal and fetal vessels (153,154).

Umbilical artery Doppler velocimetry is recommended as a primary surveillance method in fetal growth restriction (90,119). Especially associated with intrauterine hypoxia, and perinatal morbidity and mortality is absent or reverse end-diastolic flow in the umbilical artery (141,155,156). In addition, abnormal umbilical artery Doppler is related to long-term adverse neurologic outcomes (33). In high-risk pregnancies such as growth restriction, current evidence shows umbilical artery Doppler assessment significantly reducing risks of perinatal death and unnecessary obstetric interventions (90,154). Nevertheless, despite normal umbilical artery flow, small-for-gestational-age fetuses may still be at increased risk for neonatal morbidity and suboptimal neurodevelopmental outcome (157,158).

Redistribution of blood flow and cerebral vasodilatation both rise in response to chronic hypoxia. Brain-sparing as a neuroprotective reflex, nonetheless, cannot entirely compensate for the effects of hypoxia on neural tissues (159). Increased MCA end-diastolic flow leads to alterations in Doppler parameters such as reduced MCA PI or cerebroplacental ratio (CPR) (154,160,161). In late-onset growth restriction, these alterations in cerebral blood flow are associated with adverse perinatal outcomes such as neonatal metabolic acidosis, stillbirth, or perinatal mortality, and long-term neurological sequelae (33,162,163). In a recent meta-analysis, however, evidence did not support routine use of CPR or MCA Doppler in surveillance of late growth-restricted fetuses (95). In early-onset growth restriction, these flow parameters are not significant predictors of outcome (142).

Central venous circulation Doppler patterns reflect fetal right-ventricle status. Aberrations in ductus venosus flow—decreased, absent, or reversed flow during atrial contraction (a-wave)—may represent myocardial impairment and increase in right ventricular afterload (154). Such aberrations in IUGR are associated with fetal acidemia and increased mortality rates (141,149). Ductus venosus indices are predictive of neonatal outcomes (15,164), and can predict chances of intact survival after 29 pregnancy weeks (142). Systematic reviews have demonstrated the moderate predictive accuracy of DV Doppler for adverse perinatal outcome and perinatal mortality (98,165). A recent randomized study on timing delivery in preterm growth restriction indicate that delaying delivery until the DV a-wave has

disappeared causes a small excess in stillbirths, but also a significantly improved intact neurologic outcome at age of two years (166).

In placental insufficiency, fetal cerebral oxygenation is preserved if the net blood flow through the aortic isthmus remains antegrade (167). Retrospective and prospective studies have shown increased perinatal morbidity and mortality rates and suboptimal neurodevelopment among IUGR fetuses with abnormal aortic isthmus blood flow patterns (168,169). In one study of SGA fetuses with normal umbilical artery flow parameters, a considerable proportion had abnormal, even retrograde, blood flow in the aortic isthmus, demonstrating cardiovascular compromise (162). Thus, assessment of aortic isthmus Doppler blood flow may be of help in timing of delivery before appearance of hypoxic brain injury (96).

In early growth restriction, international guidelines recommend timing of delivery based on Doppler velocimetry findings. With absent end-diastolic flow in the umbilical artery before 32 GW, The Royal College of Obstetricians and Gynaecologists (RCOG) endorse delivery once venous Doppler findings become abnormal (119). The American College of Obstetricians and Gynecologists (ACOG), in turn, recommends delivery before 32 weeks, generally, in cases of most severe growth restriction with advanced Doppler findings such as reversed end-diastolic flow in the umbilical artery (120). However, with pathological umbilical artery Doppler findings, delivery no later than 37 GW is the recommendation(119,120). In late-onset growth restriction with normal umbilical artery Doppler, or among SGA fetuses, delivery is recommended between 37 and 40 GW, depending on other risk factors such as ACM Doppler velocimetry (119,120).

Screening and detection of fetal growth restriction. Adverse outcomes related to fetal growth restriction are reduced by accurate identification of growth restriction and structured fetal surveillance (30). However, a substantial proportion, as high as 15% to 35%, of SGA neonates, are not identified by ultrasonography assessments, regardless of method (80,153). A routine third trimester ultrasound for fetal biometry does not improve perinatal outcome in low-risk populations (79).

Placental-related fetal growth restriction arises primarily due to deficient remodeling of the uterine spiral arteries supplying the placenta during early pregnancy (59). Especially in selected risk-pregnancies, abnormal uterine artery Doppler indices predict pre-eclampsia and fetal growth restriction and may thus be beneficial in pregnancy management (119,170). Timely aspirin treatment in risk-pregnancies with abnormal uterine artery flow parameters may improve placental function and thus reduce risk for pre-eclampsia and fetal growth restriction (171). In a recent meta-analysis on first-trimester uterine artery Doppler, however, its specificity in predicting fetal growth restriction at any gestational age was high (93.3%), but its sensitivity low (15.4%) (89).

Several placenta-originating maternal serum biomarkers such as pregnancy-associated plasma protein A (PAPP-A), soluble fms-like tyrosine kinase 1 (sFlt-1), insulin-like growth

factor-1 (IGF-1), human chorionic gonadotropin (hCG), and alpha-fetoprotein (AFP) may be useful in risk-assessment of pregnancy (172).

Prevention of fetal growth restriction. Among selected high-risk pregnancies, timely began aspirin treatment can reduce IUGR incidence (173,174). Moreover, in such risk-pregnancies antithrombotic therapy with heparin has demonstrated promising results in preventing low neonatal birthweight (175). New treatments against pre-eclampsia, such as pravastatin, may prove useful in preventing associated fetal growth restriction (176). Similarly, treatments modulating placental circulation, like sildenafil, may improve fetal growth and maternal blood pressure during preeclampsia pregnancy (177).

Impact of gestational age on pregnancy outcome

Perinatal outcomes vary by gestational age. According to the WHO definition, preterm birth occurs at < 37^{+0} GW and post-term birth at ≥ 42^{+0} GW (178). The American College of Obstetricians and Gynecologists (ACOG) recommends a further division of term birth as follows: Early term (37^{+0} to 38^{+6} GW), full term (39^{+0} to 40^{+6} GW), and late term (41^{+0} to 41^{+6} GW) (179). Additionally, preterm births are further classified by gestational length as extremely preterm (< 28^{+0} weeks), very preterm (28^{+0} to 31^{+6} GW), and moderate (32^{+0} to 33^{+6} GW) to late preterm births (34^{+0} to 36^{+6} GW) (180). Regardless of these precise definitions, gestation-associated risk profiles are in fact a biological continuum. A U-shaped relationship between adverse pregnancy outcomes and gestational age occurs (34). The optimal gestational length appears to be reached between 39 and 41 completed weeks, and deviation from this may elevate risk for suboptimal outcomes (34).

Preterm birth rate approximates 11% of all births worldwide, ranging from 18% in sub-Saharan Africa to 5% in in northern Europe (181). During 2017, preterm birth occurred in 5.9% of pregnancies in Finland (41). Neonatal morbidity and mortality are significantly higher among neonates born before 37^{+0} GW, with risks inversely correlated with gestational age (34). Antepartum asphyxia frequently occurs in preterm births, with up to 50% of such asphyxia cases moderate to severe (55). Preterm births account, worldwide, for most neonatal deaths (181). Furthermore, preterm birth is associated with a considerable economic burden (181).

During the neonatal period and early childhood, preterm infants have elevated rates of temperature instability, respiratory distress, apnea, hypoglycemia, seizures, jaundice, kernicterus, NEC, feeding difficulties, and periventricular leukomalacia (PVL) (182). The frequencies of CP—as well as of intellectual and sensory disabilities—are inversely related to gestational age at birth, and neurodevelopmental disabilities and recurrent health problems require excessive rehospitalizations (182). Cognitive dysfunction such as school difficulties and behavioral problems may subsequently arise and continue into adolescence (182). Not unexpectedly, the risk for chronic health disorders appears to be higher among those born preterm (182).

Of the preterm births, those occurring after 32^{+0} GW—moderate and late preterm births—comprise up to 84% (181). As compared to full-term infants, these infants born close to term are also physiologically immature, with limited compensatory responses. Thus, such infants are at increased risk for acute infectious and respiratory disorders, early childhood mortality, neurodevelopmental and cognitive disabilities, and behavioral problems at school age (38).

Early-term birth, at 37^{+0} to 38^{+6} GW, occurs in high-income countries, in 17% to 27% of pregnancies, in Finland comprising 17% of births (183). Traditionally, studies have considered term births as a homogenous reference low-risk population. Recent evidence, however, demonstrates increased short- and long-term morbidity related to early term birth. Excessive neonatal admissions, prolonged hospitalizations, various health complications during early childhood, and developmental delay do occur among early-term infants, with considerable potential economic consequences (38). A significant amount of central nervous system growth and maturation—such as a 50% increase in cortical volume and a 25% increase in cerebellar development—occurs between 34 GW and 40 GW (34). One meta-analysis has demonstrated increased risk for CP as being related to birth at early term (34). Additionally, these children are at risk for a wide range of more subtle impairments such as difficulties in mathematics, behavioral and emotional problems necessitating medication, and social- and communication disadvantages (34,184). Lately, more attention has been on late preterm and early term birth rates, making avoidance of obstetric interventions and inductions at early term without medical indications the recommendation (183,185).

Late term and post-term pregnancies comprise those pregnancies continuing beyond 41 GW. The prevalence of post-term birth varies significantly among countries, occurring in European and North American countries in 0.4% to 8.1%, and its prevalence appears to be decreasing, along with a more active delivery induction policy (39,186). In Finland in 2016, the rate of post-term birth was 4% (41). During the same period, 19% of pregnancies were ongoing at 41^{+0} GW (41).

Perinatal and delivery outcomes in pregnancies beyond term. Pregnancies beyond 41 GW are at increased risk for perinatal morbidity and mortality (36,37). Post-term pregnancies ≥ 42^{+0} GW account for approximately 14% of stillbirths worldwide, contributing considerably to stillbirth rates in high-income countries, as well (12,35). Numerous studies show increases in perinatal mortality rates, including both stillbirths and neonatal deaths, among term pregnancies with advanced gestations (36,187-189). Several studies demonstrate a U-shaped relationship between gestational age and perinatal mortality (40,190).

Furthermore, risks for perinatal morbidity and complications are higher in post-term deliveries. Meconium aspiration syndrome (MAS), pneumonia, shoulder dystocia, and traumatic injuries occur more frequently in post-term deliveries (188). With advancing gestation, umbilical artery pH and oxygen partial pressure (pO2) tend to decrease, while acidosis, carbon dioxide (pCO2), and lactate increase (7,189,191). Among post-term deliveries, risks for perinatal asphyxia, seizures, and neonatal encephalopathy are preeminent (188,192). Long-term adverse outcomes are associated with advanced gestation. A Norwegian cohort study has demonstrated that intellectual performance

declines among individuals born beyond 41 GW (193). In a Swedish cohort study, risk for developmental delay was higher among children born post-term (194). Additionally, occurrence of long-term metabolic and cardiovascular risk factors is higher among children born post-term (195,196).

Maternal complications increase in deliveries beyond 40 GW as well. In post-term deliveries, cephalopelvic disproportion, labor dystocia, acute cesarean section, postpartum hemorrhage, and infections occur excessively (188).

Surveillance methods in post-term pregnancies. No clear cut-off gestational age exists for adverse outcome, and no antenatal surveillance method can entirely eliminate the risk for stillbirth or fetal compromise during delivery (191,197). Evidence from randomized controlled studies on advantages of surveillance in improving outcomes is lacking. Some studies suggest that fetal monitoring and routine ultrasound examination after 41^{+0} GW reduces perinatal mortality and severe neonatal morbidity, but no consensus exists as to test utilized or frequency of testing (198,199).

No evidence supports any benefits of maternal fetal movement counting (189). According to one systematic review, biophysical-profile assessment in high-risk pregnancies, including post-term pregnancies, fails to improve perinatal outcomes, and may even cause an increase in cesarean section rates (84). Low amniotic-fluid volume frequently occurs in late-term and post-term pregnancies and is generally considered a marker of chronic placental insufficiency and reduced kidney perfusion (200). However, in post-term pregnancies, oligohydramnios has not predicted adverse perinatal outcome, but such observation may lead to more obstetrical interventions (200,201).

Doppler assessments are unlikely to add considerable value in surveillance of late-term and post-term pregnancies (202). In such pregnancies, pulsatility indices of the umbilical artery, MCA, ductus venosus, or inferior vena cava do not differ between individuals with favorable and unfavorable outcomes (202-204). In uncomplicated pregnancies, beyond term gestation placental vascular resistance does not increase (205). Some controversy occurs as to the effectiveness of CPR in predicting adverse outcome in late and post-term pregnancies. Low CPR among pregnancies at term and beyond is associated with fetal distress and obstetric interventions during delivery, and with adverse perinatal outcome (206,207). The predictive value of CPR for adverse outcomes, however, is poor (202,207). One recent systematic review on term pregnancies suggests that CPR has some predictive utility and can serve as an adjunctive surveillance method in identifying fetuses at risk (208).

The characteristics of cCTG evolve along with advancing pregnancy (99). Certain changes appear in recordings during post-term gestation such as decreasing long- and short-term variation, increased proportion of traces without accelerations, higher proportion of large decelerations, more frequent and longer episodes of low variation, and increased basal fetal heart rate. Fewer tracings meet the criteria of normality, than in term pregnancies (99). In post-term pregnancies, signs of sympathetic activity such as increased fetal heart rate and small accelerations, may precede fetal distress during delivery (209). However, evidence on

utilization of computerized CTG as improving outcomes or predicting adverse events in post-term births is insufficient (203,210).

Controversies regarding induction of delivery and expectant management. Agreement on the optimal timing of delivery is lacking. European and American guidelines recommend beginning the surveillance and inducing delivery variably between the 41^{+0} and 42^{+0} GW (211). Severe adverse events such as HIE and perinatal mortality occur infrequently in low-risk populations, leading to considerable numbers of pregnancies needed to treat to improve outcomes, and to challenges in organizing studies sufficiently large to demonstrate beneficial effects (211). However, the number of deliveries needed to induce to avoid one perinatal death decreases continuously after the 41^{+0} GW (212).

Recently a large RCT comprising 1801 women demonstrated that induction of labor at 41 GW led to a lower rate of composite adverse outcomes including perinatal mortality, neonatal intensive care unit (NICU) admission, and Apgar score < 4 at five minutes, as compared to expectant management until 42 GW (213). The cesarean section rates and instrumental vaginal delivery rates between these groups were comparable (213). A recent meta-analysis on RCTs including low-risk term pregnancies with induction of delivery versus expectant management demonstrated a lower risk of perinatal death in the induction group, with the number needed to treat being 426 (95% CI 338 to 1337) (214). Furthermore, the rates of low Apgar scores < 7 at 5 minutes and NICU admissions, as well as cesarean-section rates, were lower in the induction group than in the expectant management group. Otherwise, maternal or neonatal complications between groups did not differ (214). An observational register study from Sweden showed that implementation of a more active induction policy post-term—from 43^{+0} GW to 42^{+0} GW—caused significant decreases in perinatal mortality, MAS, and low Apgar scores at birth (186). Controversial results have also been reported. A current register study on term deliveries utilizing propensity score method demonstrated increased risk for cesarean section until 41 GW, no effect on perinatal mortality and no low Apgar score, but increased risk for prolonged hospital stays related to induction of delivery (215).

The long-term health effects of delivery induction on offspring are somewhat controversial. One Danish cohort study suggests that introduction of a more active delivery-induction strategy to beyond-term pregnancies led to reduction in CP and in neonatal death rates (216). On the other hand, a Norwegian register study revealed an independent association between induction of labor and CP among term pregnancies (217). However, in a case-control study on HIE at term, induction of labor was unrelated to increased risk for encephalopathy (8). Induction or augmentation of delivery is associated with increased rates of autism spectrum disorders, especially among male children (218). The optimal management of pregnancies beyond 41^{+0} GW is still debated and further studied, since the absolute risk for adverse perinatal outcomes is low regardless of management or induction protocol (211,213).

Antenatal neuroprotection

Placental dysfunction leading to intrauterine hypoxia and growth restriction may necessitate preterm delivery. Prematurity is associated with increased risk for neurologic impairments, most frequently CP and cognitive dysfunction, such as intellectual impairment or developmental delay (219). While more delicate impairments like learning difficulties are included, preterm neonates are at 28% risk for developing at least one long-term complication and at 8% risk for multiple impairments (220). With earlier gestations, the risks for sequelae are greater. Fetal neuroprotection aims to prevent these adverse outcomes.

Magnesium sulphate (MgSO4) treatment for fetal neuroprotection, conferred to women at risk of preterm delivery before 30 to 32 GW, is associated with reduction in CP risk by 32%, along with a significant reduction in gross motor dysfunction rate (221). Antenatal magnesium treatment reduces all cases of CP, but also of moderate and severe CP alone (219). This effect is unassociated with any reduction in intraventricular hemorrhage- or cystic PVL rates (219). Magnesium neuroprotection does not result in increased perinatal mortality nor give rise to significant short- or long-term maternal or neonatal morbidities (219). Although details of the neuroprotective mechanism of magnesium are unknown, they appear to be complicated and related to treatment of timing (70,222). Its beneficial effect is independent on prematurity etiology (219). Recently, magnesium neuroprotection was included in several national guidelines and WHO recommendations (180).

Antenatal corticosteroid treatment is routine management in accelerating fetal lung maturation among women at risk for preterm delivery. In addition to improving lung development, treatment leads to a reduction in perinatal and neonatal death, NEC, need for mechanical ventilation, systemic infections, intraventricular hemorrhage, and PVL rates (220,223,224). Antenatal glucocorticoids probably enhance maturation of fetal cardiovascular responses to hypoxia, as well (2). Several randomized studies on antenatal corticosteroids among preterm births below 36 GW show a resultant decrease in CP rates (223), although a recent meta-analysis could not confirm this finding (72). Nevertheless, based on these data, in preterm neonates, corticosteroids appear to be neuroprotective. However, the role of antenatal corticosteroid treatment and subsequent hyperglycemia in context of asphyxia or HIE is unclear (70).

Emerging antenatal neuroprotective treatments against developing brain injury are under intensive study. Progesterone has shown potential neuroprotective benefits in animal models of birth asphyxia and in reducing inflammation and improving myelination (220). A recent meta-analysis failed to demonstrate a clear reduction in CP rates with progesterone treatment in prevention of preterm birth, however (72). Melatonin has multiple endogenous functions. It has demonstrated neuroprotective effects in animal models, probably due its antioxidative and cytokine-modulating features (220). Several other agents, such as creatine and allopurinol, may also have neuroprotective features when administered antenatally (72).

ADAPTATION AT BIRTH

Transition from fetus to newborn and adaptation to extrauterine life is a complex process, requiring rapid changes in most vital functions (4).

Physiological changes at normal birth

The first and most critical adaptations concern cardiovascular and pulmonary systems (225). Pulmonary vascular resistance decreases markedly by oxygen exposure at the first breaths (4). After fetal levels of 60%, neonatal oxygen saturation rapidly rises over 90% (4). After placental separation, on the other hand, systemic vascular resistance increases. These changes together lead to the closure of fetal circulatory shunts. Cardiac output and blood flow to the lungs, heart, kidneys, and intestines increase (4,225). Surfactant secretion into the fetal alveoli and fluid clearance from the lungs begin during the delivery process and are generally completed within two hours after birth (4).

Metabolic and endocrine changes occur at transition in order to maintain blood pressure, blood glucose, and free-fatty-acid levels (4). During normal birth, the HPA axis is activated, leading to substantial increases in production and in release of catecholamines, renin-angiotensin, and vasopressin. This is important in regulation of a wide variety of functions, such as in reducing insulin levels, raising liver glucose production, stimulating lung-fluid resorption, maintaining water homeostasis, and modifying the hemostatic system and nociception during the adaptation process (4,53,226). Hematologic changes include decreased production of fetal hemoglobin and decreased secretion of EPO, resulting in reduced erythropoiesis in neonates (4).

Effect of delivery mode on adaptation

During normal birth, the endocrine stress response is activated, with the mode of delivery strongly affecting these responses. Several hormones such as ACTH, corticotropin-releasing factor, cortisol, adrenalin, noradrenalin, β-endorphin, and vasopressin are significantly lower among neonates born by primary cesarean section than by vaginal delivery (53,226,227). This can inevitably affect neonatal adaptation processes and may even have a long-term impact on neurologic development and health (228). Fetal distress and assisted vaginal delivery can further enhance the stress response.

Pulmonary adaptations, for instance, are triggered during delivery, and liquid absorption is enhanced by β-receptor-agonist stimulation throughout normal delivery process (4). Primary cesarean section is associated with increased risk for respiratory morbidity. Among term infants born via primary section, the incidence of transient tachypnea and RDS are double the incidence of those via vaginal birth (225). Furthermore, physiologically increased levels of AVP support water homeostasis after birth. Water loss, and consequently weight loss, is more pronounced after primary cesarean than after vaginal delivery (53).

Challenges in neonatal transition

A considerable proportion of neonates, up to 10%, require some clinical intervention at birth for facilitating the transition (225). Approximately 1% need extensive resuscitation (225).

Several circumstances, such as gestational age at birth, maternal health conditions, and pregnancy and delivery complications, affect the neonatal transition stage. Chronic intrauterine hypoxia changes catecholamine regulation and fetal sensitivity to glucocorticoids and can interrupt adaptive mechanisms at birth, as well as affect long-term metabolism (1). Perinatal hypoxia and asphyxia suppress initiation of breathing in term and preterm births (4). Persistent pulmonary hypertension of the newborn is a severe neonatal condition with continuously elevated pulmonary vascular resistance and extrapulmonary shunting, leading to neonatal hypoxia (225). Increased risk for this disorder is associated with both post-term and late preterm births (225). Preterm infants are more prone to pulmonary complications due to this relatively lower lung volumes and immature lung-fluid clearance mechanisms (225). Furthermore, the antioxidant defense system is immature until late gestation and predisposes preterm infants to damage caused by relative oxidative stress at birth (229).

Most neonates cope well with adaptations required by the transition to extrauterine life, but clinicians caring for neonates should be aware of the risks and prepared to provide timely treatment if necessary (225).

PERINATAL ASPHYXIA

In asphyxia, impairment of respiratory gas exchange leads to tissue hypoxia and accumulation of lactic acid, resulting in metabolic fetal acidosis. Such condition further deteriorates accompanied by reduced clearance of carbon dioxide (5,60).

Mechanisms of asphyxia

Short-term accumulation of carbon dioxide— isolated respiratory acidemia—is quickly reversible and cause no fetal injury (78). Prolonged hypoxia, however, shifts the cellular energy metabolism towards anaerobic processes. The increased amount of intracellular hydrogen ions eventually leads to depletion of buffering mediators. Consequently, base deficit increase and metabolic acidosis arise, predisposing the fetus to tissue injury (78).

Intrapartum aberrations and complications may affect placental function and fetal oxygenation. The most common cause of fetal hypoxia and acidosis is excessive uterine contractile activity during delivery (100). Maternal complications such as respiratory distress, cardiorespiratory arrest, sudden hypotension due to aortocaval compression or after epidural or spinal analgesia, placental abruption and uterine rupture, can interrupt fetal oxygenation. Other less frequent complications leading to hypoxia and acidosis are umbilical-

cord complications, fetal hemorrhage, shoulder dystocia, or retention of the after-coming head in a breech delivery (78).

Fetuses suffering from intrauterine hypoxia are more susceptible to adverse intrapartum events. Perinatal asphyxia originates during the antepartum period from underlying chronic maternal or fetal conditions in approximately 50%, whereas during delivery occur 40%, and in the early postnatal period 10% (10,230). However, in term pregnancies, the majority of fetal asphyxia cases occur in pregnancies without known antepartum risk factors (5). Consequently, pregnancies at zero risk for intrapartum fetal asphyxia do not exist (231).

Incidence and consequences of asphyxia

Perinatal asphyxia requiring significant resuscitation at birth occurs in high-income countries in approximately 20/1000 to 25/1000 live births (5,232). In preterm births, asphyxia occurs even more frequently, in 73/1000 births, and it is moderate or severe in half of the cases (55). Globally, neonates suffering from birth asphyxia annually amount up to four million (9). Asphyxia is the second most frequent cause of death within the first 28 days, contributing up to 10.5% in children younger than 5 years (233). Furthermore, it is estimated to contribute to millions of antepartum and intrapartum stillbirths worldwide, and an equal number of children develop permanent neurologic sequelae such as CP, epilepsy, intellectual disability, and sensorineural impairments (9,12). In the literature, some inconsistency exists among definitions of asphyxia, studies reporting highly variable rates of adverse outcomes among asphyxiated neonates, severe deficits occurring in 5% to 100% (10).

HYPOXIC ISCHEMIC ENCEPHALOPATHY (HIE)

Owing to efficient compensation mechanisms, the majority of the neonates exposed to perinatal asphyxia will recover without developing long-term sequelae. Constant severe metabolic acidosis eventually results in, however, a vital-organ and cerebral-injury cascade, leading to neonatal HIE (10,11). Globally, HIE incidence varies between countries parallel with neonatal mortality rates, occurring in low-mortality countries in 1-3/1000 live births and in countries with a high neonatal mortality rate in 12/1000 (70,116). Among term neonates, HIE incidence approximates one to three cases per 1000 births (234). However, a recent cohort study suggested that the HIE rate among preterm neonates is considerably higher, up to 37 cases of moderate to severe HIE per 1000 (235).

Definition and diagnosis

The clinical definition of encephalopathy consists of disturbed neurologic function in a neonate born beyond 35^{+6} GW (22). Manifestations of this condition are subnormal level of consciousness or seizures, difficulty with initiating and maintaining respiration, hypotonia and depressed reflexes, abnormal oculomotor or pupillary movements, and weak or absent

sucking (22). Several mechanisms, ones such as birth asphyxia and intrapartum events, metabolic and genetic disorders, congenital neurologic conditions, infections, and medications, can contribute to neonatal encephalopathy and consequent neurologic impairment. Brain injury can occur during pregnancy or in the neonatal period at multiple points. Neonatal hypoxic ischemic encephalopathy (HIE) specifically refers to the neurologic implications of perinatal asphyxia (22).

Approximately 50% to 80% of neonatal encephalopathy is of hypoxic ischemic origin (11). A careful assessment to determine etiology and identify these neonates is essential, since these neonates can significantly benefit from timely action and intensive care treatment (11). Certain findings, such as low Apgar score of < 7 at 5 and 10 minutes, arterial cord blood pH < 7.0 and BE < -12, and early imaging evidence of cerebral edema, indicate the probability of an intrapartum hypoxic event as the origin of neonatal encephalopathy (22,78). Evidence of peripartum hypoxic events and severe delivery complications such as placental abruption or abnormal fetal heart tracings generally help in identifying affected neonates, but the false-positive rate in CTG is high, and predictive patterns are usually evident only during the final hours before birth (8,231). HIE diagnosis is based on clinical manifestations, imaging, and electrophysiological monitoring (20). The most sensitive method in neonatal encephalopathy is magnetic resonance imaging (MRI); nevertheless, the full extent of the defect is not evident until a week after the injury (22).

Brain injury patterns in HIE

Following hypoxic ischemic insult, neuronal injury proceeds in phases (70,236-238). The acute neuronal injury occurs due to disruption of the oxygen and energy supply to the brain. Loss of integrity of the neuronal-cell membrane leads to widespread depolarization, cerebral edema, and primary neuronal death (238). A latent period of approximately 6 hours follows the acute injury, during which, some neuronal cells recover. Subsequently, a secondary phase of delayed cell death occurs. Due to failure of mitochondrial function, energy and oxidative metabolism deteriorate. Oxidative stress and inflammation, along with reperfusion injury by toxic neurotransmitters from damaged brain areas, result in additional neural cell death (70,238). A considerable portion of the brain injury and neuronal loss occurs during this secondary phase. Additionally, seizures frequently appear at this phase of injury (237). After initial injury in severe HIE, progressive evolution of cell death occurs over hours and days (70). Subsequently, 3 to 4 days after the initial event, the tertiary phase of continuing injury arises. This phase can last from weeks to years and is characterized by chronic inflammation and by epigenetic changes which affect neuronal repair and reorganization (70,238). Associative infections and inflammation may exacerbate the extent of the injury and may impair treatment effectiveness (11).

Staging of HIE

Severity of consequences in HIE depends mostly on the degree of the encephalopathy (10,239). The widely applied original Sarnat system divides the grade of encephalopathy into three stages (116,239). Stage I is the mildest. In this stage, neonates characteristically demonstrate generalized sympathetic tone and hyper-alertness with excessive deep-tendon reflexes. In Stage II, generalized parasympathetic tone occurs, along with strong distal flexion and mild hypotonia. Additionally, seizures frequently appear. Stage III is the most severe form of encephalopathy with clinical signs of remarkably decreased level of consciousness or coma, soft tone, decreased deep-tendon reflexes, and clearly abnormal electroencephalography (EEG) (78,116). Other ways of staging proposed include the widely implemented modified scoring system by Thompson; this is simple and accurate, a clinically applicable method for evaluating and monitoring neurologic signs and predicting outcomes in asphyctic neonates (24).

Clinical consequences

HIE is associated with high risk of neonatal death or neurodevelopmental impairment. Approximately 15% to 20% of affected neonates die during their first 28 days, and up to 25% of those who survive suffer from permanent neurological sequelae (240,241). HIE can lead to wide range of neurologic impairments from subtle cognitive and behavioral problems to severe sensorineural and intellectual disability, epilepsy, and CP (11,116). The severity and location of damage is influenced by degree and duration of the insult, and by developmental maturity of the brain (242). Preterm fetuses tolerate longer periods of hypoxia, hypoperfusion, and hypotension without permanent brain and organ injuries (70). On the other hand, late preterm neonates between 34 and 36 GW are especially vulnerable to ischemic events due to the extensive growth phase of their central nervous system during this period (19). Severity of multisystem organ dysfunction does not necessarily correlate with grade of brain injury, and can affect cardiovascular, pulmonary, gastrointestinal, renal, metabolic, and hematological systems (22,78).

The diagnosis and prognosis of neonatal HIE is presently based on clinical signs and symptoms, radiologic imaging, and electrophysiological monitoring (20). The traditional Sarnat Score is considered valuable in estimating outcomes at mild and severe stages. Most neonates with stage I mild encephalopathy develop no major long-term neurologic impairments. Outcomes among neonates with stage II moderate encephalopathy are more challenging to estimate, although risk of long-term sequelae or death is increased, affecting up to 20% to 30% of these neonates (240). In severe stage III encephalopathy, most of the affected neonates either die or develop major long-term neurological sequelae such as CP and intellectual disabilities (19).

Perinatal events are associated with approximately half the cases with CP (243). Most children with CP—from 50% to 60%— are born at term (72). In 10% to 20% of these children, the etiology is related to acute HIE (78,243). Rates of CP subsequent to HIE range from 10%

to 13% among survivors of moderate to severe encephalopathy. In particular, CP of the spastic quadriplegic or dyskinetic type is associated with intrapartum asphyxia and HIE at term (78). Sensorineural defects such as hearing loss and visual function abnormalities are increased in neonates with HIE (11). Furthermore, long-term impairments associated with HIE involve memory function, learning, and behavioral and neuropsychiatric development (11). Studies have reported negative effects of Stage I HIE on behavioral functions at the age of 9 to 10 years (18). Even mild stages of neonatal encephalopathy may thus be associated with long-term adverse outcomes affecting daily life (18). Importantly, a substantial proportion of neurologic impairments are hidden until later in childhood (244).

NEONATAL OUTCOME MEASURES

An unequivocal marker for predicting neonatal outcome after perinatal asphyxia and subsequent HIE is lacking. Only a small proportion of the neonates with evidence of intrapartum asphyxia will develop severe neurological impairments (245). All methods presently used, as acid-base balance, clinical examination including Apgar score, radiologic imaging, and electrophysiology have certain limitations. Nevertheless, along with the introduction of potent neuroprotective treatments such as therapeutic hypothermia, the importance has risen to swiftly and surely identify those neonates who could benefit from interventions. Novel biological and physiological prognostic markers are emerging, but the current "traditional" indicators remain valuable tools in evaluation of neonates during the first postnatal hours.

Current asphyxia markers

Acid-base balance quantified from the umbilical artery at birth presents the oxygenation status of neonate and may reveal fetal hypoxia and metabolic acidosis during birth (78). Acidosis is an early sign of fetal distress during delivery. Uterine contractions, even during normal vaginal birth, cause mild intermittent hypoxia and consequent respiratory acidemia, leading to a slight decrease in pH and an increase in pCO2 (246,247).

Metabolic acidosis is defined as umbilical artery pH below 7.00 and base deficit (BD) above 12 mmol/L (21,78,248). However, a continuum exists that shows increasing risk for encephalopathy along with deepening acidemia (21,22). Serious adverse outcomes such as neonatal encephalopathy, seizures, low 5-minute Apgar scores, neonatal unit admission, and death begin to increase with pH values below 7.10 and BE values below -10 mmol/L (21,78). Umbilical artery pH less than 7.00 raises the risk for abnormal outcome in up to half the affected neonates (245). Maintenance of cardiovascular adaptations and defense mechanisms deteriorates in severe acidemia with arterial pH < 7.05, predisposing the fetus to brain injury (2). One meta-analysis demonstrated an association of low umbilical artery pH with increased neonatal mortality, HIE, intraventricular hemorrhage, PVL, and CP (249). Metabolic acidosis can be identified by umbilical artery lactate concentration (78). Umbilical artery lactate values beyond 10 mmol/L are associated with short-term neonatal adverse

outcomes, and children with elevated lactate values in their scalp samples tend to be at increased risk for fine motoric and cognitive dysfunction at age four years (247,250). The positive predictive value, sensitivity, and specificity of acidosis at birth in predicting brain injury is, however, low (11,245). Even in severe metabolic acidosis at birth, the majority of neonates will be free of neurologic complications (78).

Apgar score is a standardized tool for assessment of neonatal condition immediately after birth and is an important indicator of need for resuscitation (251). This scoring system evaluates vital cardiovascular, pulmonary, and neurologic functions by five distinct components: color, heart rate, reflexes, muscle tone, and respiration (78,251). Numerous factors affect Apgar score assessment. It is a subjective estimation and therefore predisposed to interobserver variability, and moreover is dependent on the neonate's physiologic maturity and congenital malformations, as well as on maternal medications (251). Thus, low Apgar score alone is not a specific marker of intrapartum distress. Furthermore, in cases of hypoxia, Apgar score is depressed only when hypoxia is severe and prolonged (78).

Apgar score cannot serve in predicting individual adverse neurologic outcome (251). As a prognostic marker of asphyxia and HIE outcomes, Apgar score has low sensitivity and specificity (245). Up to 80% of neonates with Apgar score ≤ 7 at 5 min will have a normal outcome (11,245). However, population-based studies demonstrate an increased risk for CP and mortality among neonates with a low Apgar score of < 5 at 5 and 10 minutes of age (22,78). Especially the change in score between 1 and 5 minutes represents the response of the neonate to resuscitation, and the degree of prolonged Apgar abnormality correlates with risk for neurological disability (22). In one Norwegian cohort study, low Apgar score at 5 minutes strongly associated with CP, with 10% to 17% of the children with an Apgar score less than 4 developing such a condition (252). A recent cohort study from Sweden demonstrated an inverse association of Apgar score at 5 and 10 minutes with risk for epilepsy and CP among term pregnancies (253). Low Apgar score < 7 at 5 minutes is associated with increased risk for respiratory distress, need for assisted ventilation or intubation, NICU admission, and HIE (254). Among neonates suffering from asphyxia, Apgar score at 5 minutes predicts HIE and amplitude-integrated electroencephalography (aEEG) abnormalities (255). A large register-based study on more than a million live births demonstrated an association of low Apgar score at 5 minutes with increased risk for neonatal and infant death, the strongest association appearing at term (256).

Clinical examination and neurologic estimation of the newborn are affected by medication, treatment, and resuscitation. Modified Apgar systems, ones involving also neonatal maturity and interventions, may be more sensitive and specific in predicting birth asphyxia and severe adverse neurologic outcomes, than is the conventional Apgar score system (23). The Sarnat Score system provides a summary score for describing neonatal outcome within 24 hours after birth (239). This system is useful in predicting outcome at mild and severe grades of encephalopathy (240). Outcomes of neonates with moderate encephalopathy are, however, more challenging to predict (240). Furthermore, neurological status may evolve—either improve or deteriorate—during the first days after birth, and again, treatment and

interventions affect assessment (240). Up to 15% to 20% of neonates with an initial grading of mild HIE later turn out to show more severe encephalopathy (18). Improved standardized methods reduce interobserver differences, and modified methods may better take the evolving nature of HIE into account (257,258). Nevertheless, a significant correlation exists between neurologic outcome at 2 years of age and Sarnat score at 24 hours, as well as with clinical examination at discharge from hospital exists (240). Similarly, the predictive values of the Thompson score system for neurologic outcome at one year are remarkable, particularly when the highest score and prolongation of abnormal signs are combined (24).

Electrophysical monitoring by continuous multichannel electroencephalography (EEG) is nowadays the gold standard in assessing brain function and grading neonatal encephalopathy (11). Amplitude-integrated EEG (aEEG) is a feasible and user-friendly technique for assessing high-risk neonates (259). Its interpretation is based on pattern recognition and is in good accordance with standard EEG (259). Among neonates with HIE, aEEG assessment 3 to 6 hours after birth consistently predicts neurodevelopmental outcome at the age of one year (259). The outcomes are strongly related to the severity of EEG abnormalities (11). A normal or slightly abnormal EEG at 6 hours of age predicts normal neurodevelopment at the age of 2 years (260). Because EEG findings tend to subsequently improve, poor outcomes are predictable among individuals with continuously abnormal recordings (260). Interventions such as therapeutic hypothermia affect evolution of EEG patterns in HIE and consequently influence its prognostic ability (11).

Severe fetal acidemia predicts early onset of the neonatal seizures frequently occurring in moderate and severe HIE (11,261). In detecting such seizures, multichannel EEG is essential, since aEEG alone fails to detect low-amplitude seizures (11). Occurrence of seizures after perinatal asphyxia considerably affects neonatal outcome. Persistent electrographic seizures are associated with brain injury and correlate with poor long-term neurodevelopmental outcomes (262-264).

Neuroradiology – brain imaging – has advanced significantly during recent decades. Still the first-line method in imaging neonatal brain is, however, cranial ultrasonography (265). It is valuable in screening those neonates admitted to the NICU during the first days of life and can be useful in differential diagnosis of encephalopathy and congenital brain abnormalities (265,266). Furthermore, certain neonates cannot be transferred from the NICU for further imaging. Currently, MRI, including diffusion weighted MRI, is considered the most sensitive modality and the method of choice in imaging HIE (266). A combination of imaging modalities such as cranial ultrasonography and MRI during the first days, prove of use to differentiate between perinatal injury and antenatal injury (266). Brain-imaging techniques may also prove useful in predicting the outcome after HIE. Alterations in Doppler parameters such as increase in cerebral blood-flow peak velocities, have demonstrated predictive value for one-year neurologic outcomes among asphyxiated newborns (267). Injuries may, however, not be visible until 24 hours or more after the insult; the injury's full extent may be evident only 7 to 21 days afterward (22,259).

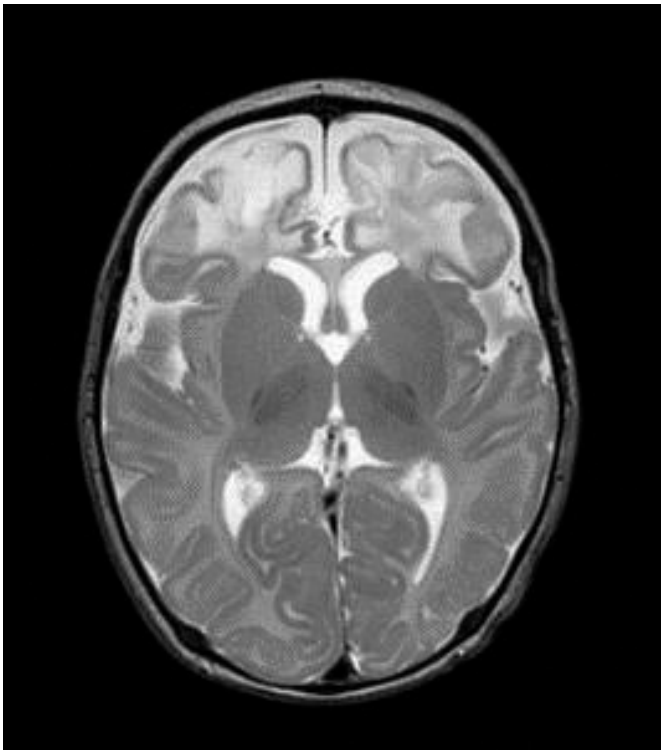

Figure 3. Widespread hypoxic ischemic encephalopathy injury in neonatal MRI: Bilateral restricted diffusion in frontal cortical and subcortical areas, along with watershed areas. Signal changes in corpus callosum, thalami, and basal ganglia. *By author*

Novel markers in the prediction of the outcome

More precise and earlier markers for diagnosing and predicting the outcome of HIE are crucial. The best method may be a combination of several consistent markers such as physiological, neuroradiological-, and biomarker-assessment composed together (11).

Advances in neuroradiology have enhanced understanding of the mechanisms and timing of brain injury (266). Improvements in MRI have confirmed that certain injury patterns in HIE are of prognostic and diagnostic value and have aided in development of treatments such as hypothermia timing (266). In HIE, novel MRI techniques may serve as biomarkers of neurodevelopmental outcome (266). Proton MR spectroscopy (^{1}H-MRS) is specific and sensitive in identifying the lesions, and a potent predictor of outcome after HIE (268). After HIE, novel quantitative MRI techniques such as diffusion tensor imaging tractography and arterial spin labeling predict cognitive and motor outcome (269,270). Some limitations, such as correct timing of imaging and influence of treatment on findings, affect the prognostic value of neuroradiologic modalities, as well (266).

Heart rate variability (HRV) describes the parasympathetic and sympathetic control of heart rate and is easily and non-invasively measured by routinely recorded electrocardiogram (ECG) (271). Abnormalities in heart rate variability are associated with pathological EEG and MRI findings, and reduced HRV may show potential in assessment of HIE grading and prediction of long-term outcomes (271,272).

Biomarkers are one focus of this active research field, especially biomarkers with the potential to predict neurodevelopmental outcome. Currently, no definitive blood biomarker of HIE is in clinical use, but several novel biomarkers have demonstrated promising diagnostic and prognostic features in pilot studies (11,19,273). Some of these biomarkers, ones such as interleukins IL-6 and IL-6, and activin-A, are, in HIE, significantly elevated in the umbilical cord blood already at birth (11), while other markers like neuron-specific enolase (NSE) and protein S100B rise after a slight delay following the initial insult (11,273). The basis of utilization of these biomarkers is diverse. Some biomarkers are related to inflammation, because cytokine and chemokine levels vary according to the phase of the injury and of the recovery processes (20). Specific neurobiomarkers such as glial fibrillary acidic protein (GFAP), NSE, protein S100B, and Tau, are principally brain constituents which are measurable in biological fluids in relation to HIE (19). Additionally, vasoactive agents like adrenomedullin, hematopoiesis-regulating hormone EPO, and markers of tissue damage evaluated in other situations (such as troponin and copeptin in cardiac distress in emergency departments) are potential prognostic markers for HIE (19,20,48,49,274). Studies have demonstrated significant alterations in metabolomics and transcriptomics among neonates with perinatal asphyxia and HIE, and these may have diagnostic value (11). In addition to blood, other biological fluids such as cerebrospinal fluid, urine, and saliva are potential targets for biomarker evaluation. Even maternal blood samples can be of use to detect increased risk for HIE, since quantification of circulating microRNAs can reveal fetal hypoxia during pregnancy and delivery (275).

The objective is that a biomarker, or a combination of markers, could be assessed quickly and easily at certain time-intervals to screen for brain injury, to identify neonates suitable for neuroprotective intervention, to monitor the progression of disease, and to provide reliable short- and long-term prognoses (11,19,20).

NEONATAL NEUROPROTECTION

HIE is a major problem globally, causing a vast humane and economic burden for patients, families, and society. It is associated with high mortality, with death rates among affected neonates from 10% to 60%. Furthermore, at least a quarter of those who survive develop severe neurodevelopmental sequelae (237). HIE, being the single most common contributor to disability worldwide, accounts for 10% of all disability-adjusted life-years (116). Thus, effective neuroprotective prophylactic actions and treatments are urgently needed.

Therapeutic hypothermia

Nowadays, is therapeutic hypothermia the treatment of choice for HIE in term and near-term neonates (13,14). The time-window for therapeutic interventions occurs during the latent phase following the primary hypoxic ischemic insult, before the secondary injury begins. Introducing therapeutic hypothermia as a neuroprotective treatment has improved the prognosis of moderate and severe encephalopathy significantly (244). A meta-analysis of RCTs has clearly demonstrated that therapeutic hypothermia in term- and late-preterm neonates with moderate to severe HIE reduces death and major neurodevelopmental disability among survivors at 18 or 24 months of age, with an absolute risk reduction of 15%, when initiated within 6 hours after birth (237). Especially important, therapeutic hypothermia significantly reduces CP. Neonates with moderate encephalopathy seem to benefit more from hypothermia than those with a severe condition. Notably, therapeutic hypothermia improves survival without increasing severe impairments (237). Studies on long-term outcomes after therapeutic hypothermia are still ongoing, but, some follow-up studies, such as the Total Body Hypothermia for Neonatal Encephalopathy Trial (TOBY), have demonstrated promising neurodevelopmental outcomes at age six to seven years: a significantly larger proportion of children in the hypothermia group than in the control group survived without neurologic impairments (45% vs. 28%, RR 1.60; 95% CI 1.15 to 2.22) (244). Furthermore, hypothermia-treated children had less CP (21% vs. 36%, p=0.03) or moderate or severe disability (22% vs. 37%, p=0.03) than did their control group. However, despite improvements in therapeutic intervention, of treated neonates with moderate or severe encephalopathy, up to 25% still die, and of survivors, 20% suffer from sensorimotor and cognitive impairments (244).

Patient selection and indications for therapeutic hypothermia. Therapeutic hypothermia is recommended for neonates at or beyond 36 GW with moderate to severe HIE, especially in areas and countries with abundant resources (276). The criteria for hypothermia should be in accordance with protocols published in large trials including evidence of acute hypoxic ischemic event, such as Apgar score ≤ 5 at 10 minutes, resuscitation ≥ 10 minutes, or severe acidosis - pH < 7.0 or BE ≤ 16mmol/L - within one hour after birth (276,277). Clinical signs of moderate to severe encephalopathy should include at least three abnormal neurologic signs concerning either level of consciousness, spontaneous activity, posture, tone, primitive reflexes, or the autonomic nervous system, and aEEG may assist in diagnosing the severity of encephalopathy (24,277). Therapeutic hypothermia should be initiated within the first 6 hours of birth and last for 72 hours (277). The therapeutic-hypothermia time window is narrow, and currently the enhancement of neurodevelopmental outcomes seems to require diagnosis and initiation of hypothermia after birth to occur earlier (14). However, considering the evolving nature of HIE, the existing indicators are limited, and the grading is not consistent during the first 6 h of life (14,18).

Neonates with mild HIE comprise 50% of the affected cases and are generally considered a low-risk group (278). However, in a recent meta-analysis, up to 25% of neonates initially diagnosed with mild HIE have had an abnormal outcome defined as CP, death, or later poor performance on standardized neurodevelopmental tests age 18 months or older (278).

Consistently, adverse cognitive and neuromotor findings occurred in a study on long-term outcomes at age 5 years, with survivors of mild HIE demonstrating cognitive outcomes similar to those of children with moderate encephalopathy without hypothermia treatment (279). Unfortunately, among neonates with mild or no encephalopathy, the predictive value of any abnormal neurologic sign is extremely poor, with positive predictive values for adverse outcomes ranging from 0% to 54% (18).

The balance of risks and benefits of therapeutic hypothermia for mild HIE is unclear. Treatment of all the cases would promote possible adverse effects such as cardiac dysfunction, arrhythmias, coagulopathy, thrombocytopenia, leukocyte dysfunction, and pulmonary hypertension, as well as invasive treatment, delayed oral feeding, and separation from families (14,237). New strategies, perhaps biomarkers, are essential for optimal assessment of neonates with HIE, and for selection of patients suitable for such therapeutic interventions.

Novel emerging therapies

Although therapeutic hypothermia has improved outcomes after moderate and severe HIE, in clinical trials, despite the despite the hypothermia treatment, neonates who still die or suffer from disabilities such as CP, intellectual impairments, and epilepsy, may reach 40% to 45% (241,280,281). Additionally, some neonates in need of neuroprotection have no access to therapeutic hypothermia. Preterm neonates and neonates with mild HIE are not currently eligible for hypothermia, and the safety and efficacy of therapeutic hypothermia for HIE in low- and middle-income countries is still to be established (241). Thus, adjunctive neuroprotective therapies are necessary.

Ongoing studies aim at developing low-cost and easily available supplementary and alternative neuroprotective therapies to improve neonatal outcomes worldwide. Several promising options, both exogenous agents and agents based on endogenous induction of protective mechanisms, are currently in focus (14,282).

Recombinant erythropoietin (rEPO) is one of the most promising neuroprotective agents. In addition to regulating erythropoiesis, EPO has potential neuro-, cardio-, and nephroprotective effects, as well as beneficial effects on wound healing (283). EPO modulates injury response by receptor-mediated cell-specific action and by non-specific effects. EPO's anti-apoptotic, anti-inflammatory, antioxidative, anti-excitotoxic, and cytoprotective features contribute to neuroprotection. Furthermore, EPO promotes the proliferation, maturation, and differentiation of oligodendrocytes and neurons, and activates the neurogenesis and angiogenesis essential for injury repair processes (14,284). Thus, EPO may improve neurologic outcomes by acutely reducing brain injury, and by promoting long-term beneficial effects for neuronal repair and regeneration (281). Exogenously administered recombinant EPO has demonstrated neuroprotective effects plus the ability to reduce the amount of damage in the brain after hypoxia (285-287).

In preterm neonates, exogenous EPO is currently utilized to reduce the need for red cell transfusions (288). A recent meta-analysis on erythropoiesis-stimulating agents in preterm neonates suggests that in addition to reducing rates of red blood cell transfusions, early administration of erythropoiesis-stimulating agents significantly reduces rates of IVH grade III and IV, PVL, and NEC (288). Mortality rates were unaffected. Early EPO treatment appeared to reduce brain damage by reducing neurodevelopmental impairment at age 18 to 22 months, but the results in individual studies were conflicting (288). In a randomized study of 800 preterm neonates under the age of 32 GW, among neonates in the rEPO treatment group, incidences of IVH, PVL, NEC, and sepsis fell (289). Additionally, the rate of moderate to severe neurological disability at 18 months of corrected age was significantly lower in the EPO group than in the placebo group (7% vs. 19%, $p < 0.001$) (289). No difference in adverse events, such as thrombosis, hypertension, polycythemia, or retinopathy of prematurity occurred (289). However, another study comprising 450 preterm neonates failed to demonstrate any improvement in short-term outcomes including retinopathy of prematurity, IVH, sepsis, NEC, enterocolitis, bronchopulmonary dysplasia, and mortality after rEPO treatment as compared to the placebo-treated group (290). Confirmation of the advantages of EPO treatment in preterm neuroprotection requires further studies.

EPO treatment as monotherapy has improved neurologic outcome also in several studies on term neonates with HIE. A small case-control study on neonates with mild to moderate HIE demonstrated reduced blood NO concentrations and improved electroencephalographic findings at 2 weeks of age, and fewer neurodevelopmental abnormalities at 6 months among neonates treated with EPO as compared to findings in neonates receiving conventional care (291). Two randomized studies on moderate to severe HIE found no effect on mortality from EPO therapy (292,293). Nevertheless, the first study indicated an improvement in long-term neurologic and developmental outcomes at 18 months among infants with moderate HIE in the EPO treatment group (292). The more recent study demonstrated, at 19 months of age in the EPO monotherapy group in both moderate and severe HIE, compared to children in placebo group, decreased risks for CP and need for anticonvulsants, and fewer brain abnormalities in MRI (293).

Because therapeutic hypothermia is the standard HIE treatment, adjuvant neuroprotective treatments during hypothermia may lead to further improvements in outcomes. A phase-II randomized trial for neonates with moderate-to-severe HIE examined rEPO as an adjunctive treatment during therapeutic hypothermia (281). Brain MRI revealed substantially less moderate to severe brain injury, subcortical, and cerebellar injuries in the rEPO group as compared to the placebo group. Furthermore, after rEPO therapy motor outcomes at one year were improved (281).

Melatonin is an endogenously synthesized neurotransmitter serotonin-derivative with anti-apoptotic, anti-inflammatory, and effective antioxidant- and free-radical scavenger functions (220,282). It inhibits the production of proinflammatory cytokines and easily crosses physiologic barriers (220,282). Melatonin is a promising neuroprotective agent: In a recent small randomized study as an adjunctive therapy with hypothermia, the melatonin group

had a lower mortality rate, fewer seizures, less white matter injury on MRI, and improved intact survival at 6 months as compared with hypothermia only (294).

Magnesium sulphate, which reduces glutamate-mediated excitotoxic damage after a hypoxic ischemic insult, is widely used and recommended for neuroprotection in preterm birth (282). In small randomized studies on term neonates with moderate to severe asphyxia or encephalopathy, postnatal magnesium sulphate treatment has significantly improved short-term neonatal outcomes (295,296). In magnesium-treated groups, rates of clinical neurologic and EEG aberrations, abnormalities in computerized tomography and MRI, and parenteral feeding were significantly lower. Long-term effects on neurodevelopment and the safety of usage remain to be confirmed before adoption of magnesium therapy to wider use (282).

Stem cells derived from umbilical cord blood may be beneficial in HIE cases for neuroprotection (220,241). Endothelial progenitor cells which maintain and regulate vascular integrity and responses and multipotent mesenchymal stem cells enhancing tissue repair are especially promising possibilities (220). In animal studies on neonatal hypoxia-ischemia, stem cells have reduced white matter injury and motor impairments (297).

Allopurinol—a xanthine oxidase inhibitor—is a potential treatment in HIE, acting against oxidant injury caused by free radicals and superoxides (282). A small randomized follow-up study on neonatal allopurinol treatment demonstrated significantly less severe adverse neurodevelopmental outcomes among the neonates in the allopurinol-treated group with moderate HIE than suffered by control neonates (298). Ongoing studies are evaluating allopurinol as an adjunctive therapy with hypothermia (ALBINO Trial). Furthermore, some evidence exists on the neuroprotective effectiveness of antenatal allopurinol treatment. In a study on allopurinol treatment for fetal hypoxia, lower cord blood levels of protein S100B, a brain injury biomarker, occurred in neonates treated with allopurinol than in neonates with no maternal medication (299).

Xenon—a noble gas used as inhalational anesthetic in adults—has displayed its neuroprotective ability in animal models (14). It inhibits N-methyl-D-aspartate (NMDA) glutamate receptors, thus reducing excessive glutamate concentrations and subsequent seizures after hypoxic ischemic injury (14,220). In a recent small randomized study on Xenon as an adjunct therapy with hypothermia, but initiated within 12 hours after birth, no difference between the xenon-treated group and control group appeared as measured by advanced MRI imaging techniques on neuronal tissue metabolism and integrity (300). Trials with different timeframe are ongoing (CoolXenon3 Trial).

Additional therapeutic agents being studied as adjunctive therapies with hypothermia include the anticonvulsants topiramate and levetiracetam, N-acetylcysteine—owing to its antioxidant and anti-inflammatory properties, and monosialoganglioside to support cell-membrane integrity (14,220,282).

SELECTED BIOMARKERS OF ASPHYXIA IN PERINATAL MEDICINE

Rationale for searching biomarkers

In certain obstetric circumstances, accurate timing and prediction of outcome of the delivery is challenging despite advanced monitoring methods (202,204,210,301). In diabetic pregnancies, for instance, normal CTG tracings, BPP scoring, and Doppler flow measurements are not always sufficient to assure fetal well-being (55). In IUGR pregnancies, Doppler flow parameters can progress in an unexpected manner (107,108); in post-term pregnancies, the value of various surveillance methods in preventing adverse outcome is not evident (109). Abnormal findings may appear as late manifestations of fetal distress and even by standardized surveillance methods, among risk-pregnancies unexpected fetal deaths occur (54,302). Thus, additional information on fetal wellbeing is desirable. Several new fetal biomarkers can detect fetal intrauterine hypoxia and to guide management of risk-pregnancies.

Currently, the only gold standard treatment for moderate and severe HIE is therapeutic hypothermia (20,241). The challenge is the narrow time-window in selecting patients and initiating treatment. Evaluation of an asphyxiated neonate and diagnosis of HIE utilizes clinical and biochemical signs, radiologic imaging, and electrophysiological monitoring (20). Such methods have certain limitations. MRI biomarkers appear after some delay following the insult (303), and the sensitivity and specificity as predictive markers in HIE of arterial cord blood pH values and Apgar scores are low (11).

Up to 20% of newborns with an initial estimation of mild HIE later develop abnormal short-term outcomes related to encephalopathy, and 40% have a Bayley score ≤ -1 SD—for either cognition, or motor, or language skills—at age 19 months (14,18). Infants with mild HIE have shown adverse cognitive and neuromotor outcomes at 5 years (14). Furthermore, among preterm neonates, more accurate prognostic markers are urgently needed. Correlations of diagnoses with defined status at initial discharge from hospital with severe neurodevelopmental outcome are poor. Of neonates classified initially as healthy, adverse neurologic outcomes such as CP or intellectual disability can appear in up to 20% by age of two years (235). Thus, biomarkers could serve as adjunctive methods in screening neonates with subclinical or progressive lesions at early stages, and in monitoring progression of the injury and effectiveness of treatments (19,20).

Erythropoietin (EPO)

EPO—a pleiotropic glycoprotein hormone—is the main regulator of erythropoiesis in adults, newborns, and fetuses (42). The primary stimulus for EPO production is hypoxia, with its the production rate related to the blood partial oxygen pressure (pO2) (304). Both EPO- and EPO-receptor synthesis increase in response to proinflammatory cytokines and reactive oxygen species, as well (305). Hypoxia-inducible transcription factors (HIF-1, HIF-2) and hepatic

nuclear factor-4 (HNF-4) modulate EPO synthesis in oxygen-sensitive fibroblasts (286,306). Acute hypoxia in adult human leads to activation of EPO synthesis within 90 minutes (304). In animal studies, plasma concentrations of EPO begin to increase two to three hours after hypoxia (307).

During the first and second trimesters, fetal EPO is produced in the yolk sac and liver. During the third trimester, the synthesis shifts gradually to peritubular fibroblasts in the kidneys (308). In addition to the kidneys, some synthesis of EPO and its receptors occurs in other tissues such as the lungs, spleen, blood capillaries, brain, uterus, and Leydig cells (305,308). It has been hypothesized that, during severe fetal hypoxia, EPO is produced also in the placenta, as in ovine studies (309). EPO and its receptors occur in human fetal membranes (310,311), and placental EPO gene expression is increased in fetal growth restriction (312). Furthermore, differences between umbilical venous and arterial EPO concentrations may indicate placental EPO synthesis (313).

Functions of EPO. EPO-receptor activation leads to signal transmission cascades affecting various cellular functions such as apoptosis, proliferation, and differentiation (305). Within the bone marrow, EPO prevents apoptosis of erythroid progenitor cells, consequently resulting in erythrocyte synthesis (286). In addition to regulating red cell production, EPO plays a significant role in tissue-damage response (284). Tissue-protective effects of EPO arise in the central nervous system, peripheral nerves, heart, and retina (314-317). These two differing actions are mediated by different receptor isoforms each with a different affinity to EPO (318). Low concentrations of EPO are sufficient to cause increasing erythropoiesis in anemia; however, non-hematological actions require higher concentrations (318,319). EPO has short- and long-term tissue effects. Those in the short term include anti-apoptotic, anti-inflammatory, and antioxidant properties, as well as bleeding-limiting actions which locally reduce blood flow and contribute to thrombus formation (284,305,320). Long-term effects comprise erythropoietic, angiogenesis promoting, and neurotrophic actions (284,287,305,318,321).

EPO in pregnancy and delivery. In the course of normal pregnancy, maternal EPO concentrations rise two- to four-fold; the timing and magnitude of changes are highly variable (322). Normal physical activity and exercise during pregnancy shows no effect on fetal EPO concentrations (323). Maternal anemia, however, raises fetal EPO, possibly reflecting fetoplacental hypoxemia (324). In complicated pregnancies such as ones with pre-eclampsia, placental hypoxia may induce local EPO production, leading to increased maternal EPO concentrations (325). Maternal EPO can participate in humoral regulation of fetoplacental circulation by suppressing vasoconstrictive factors and may consequently provide a defensive mechanism to improve oxygenation of hypoxic fetus (326).

Since EPO does not cross the placenta, EPO in the fetal circulation is of fetal origin (327,328). The half-life of fetal EPO is unknown, but in newborns it is 2 to 4 hours (25,329). In the majority of studies of normal pregnancies, EPO plasma levels remains stable after the second trimester (246,330,331). Some studies on healthy and complicated pregnancies have, however, found an increasing trend in EPO levels towards term, and found a correlation of

EPO plasma levels with gestational age (332-334). In pregnancies beyond 40 weeks, cord plasma EPO levels seem to increase progressively, most probably reflecting weakening placental function (335,336).

EPO is transferred to amniotic fluid via some unknown mechanism, and its secretion into urine is considered an insufficient mechanism to explain its highly increased levels in hypoxia (25). Possible sources of EPO in amniotic fluid are the fetal membranes and the placenta (310-312). EPO concentrations in fetal plasma are higher than in amniotic fluid, but the concentrations correlate well in uncomplicated as well as in complicated pregnancies (246,337,338). Increased EPO production can be measured in amniotic fluid, but in hypoxia, am-EPO increases more slowly than fetal plasma EPO does, probably due to a delay in transfer (25). A moderate increase in am-EPO level may thus implicate early fetal hypoxia (25). In animal studies, EPO levels in amniotic fluid start to rise 6 hours later than the fetal plasma levels, approximately 9 hours after the beginning of hypoxia (307).

Uterine contractions during normal vaginal delivery raise fetal EPO concentrations, and after uncomplicated vaginal birth, EPO concentrations in umbilical cord serum are higher than after planned cesarean sections (339). EPO levels in fetal plasma start to rise 6 hours after rupture of the membranes (323). These phenomena are most probably related to normal physiological processes and adaptation mechanisms at birth.

Signs of fetal distress and EPO. In fetal hypoxia, EPO levels rise. Amniotic fluid EPO (am-EPO) is considered a biomarker of chronic hypoxia (25). In cord plasma, however, high EPO values may indicate acute hypoxia, as well (340). Elevated EPO levels have been detectable in cord plasma and amniotic fluid in various complicated pregnancies (25). It is assumed that in pathologic pregnancies when hypoxia has induced EPO synthesis, its secretion accelerates, and EPO levels increase exponentially (338,341,342). Such an increase occurs irrespective of the cause of fetal hypoxia (25).

In several studies, cord plasma and am-EPO levels correlated inversely with umbilical artery pH, BE, and pO2, and directly with lactate and pCO2 values (246,337,342-344). Neonates with elevated EPO concentrations in amniotic fluid have decreased umbilical artery pH, BE, and pO2 at birth (338,342). Among risk-pregnancies, neonates with low Apgar score < 7 at one and five minutes have higher am-EPO concentrations than do vigorous newborns (338,345). Additionally, elevated am-EPO concentrations are associated with increased risk for neonatal intensive care admission (338,342).

Various signs of fetal distress have shown an association with elevated EPO levels. Abnormal fetal heart-rate tracings during delivery are related to increased cord-plasma and amniotic-fluid EPO concentrations (340,346,347). On the other hand, in high-risk pregnancies, am-EPO level can rise well before non-reassuring characteristics appear in heart tracings (338,342). Meconium staining of amniotic fluid is common, occurring at term deliveries in 7% - 22% (348,349). Its role as a hypoxia marker is controversial; nevertheless, it has been associated with elevated cord plasma EPO levels at birth (336,349,350). In addition, such elevated cord plasma EPO concentrations are associated with MAS (351). Among otherwise uncomplicated

deliveries, neonates with a nuchal cord had higher amniotic EPO concentrations without any effect on cord plasma EPO (352), probably reflecting transient hypoxic events before delivery resulting from cord entanglement.

EPO in complicated pregnancies. Pregnancies with non-hypoxic complications such as cholestasis in pregnancy and prolonged rupture of the membranes are unrelated to increased fetal EPO concentrations (305,353). On the other hand, in complicated pregnancies involving various mechanisms leading to hypoxia, elevated EPO levels occur (313). Such an elevation in am-EPO can be considered as a sign of ominous fetal compromise (25).

Elevated levels of EPO in cord plasma and amniotic fluid occur in relation to fetal growth restriction, especially in hypertensive pregnancies (338,347,354). Low birthweight < 2500 g is associated with elevated am-EPO levels (345). In addition, serum EPO levels may correlate with gestational-age-related fetal size in IUGR (355). One study evaluating am-EPO values in normally grown and SGA infants suggests that higher EPO values could differentiate growth-restricted neonates from constitutionally small ones (356). In monochorionic twins showing discordant growth, the smaller fetus tends to have higher serum EPO values (357), but in some studies, this association has occurred only in relation to abnormal umbilical artery Doppler flow parameters (331). One study on IUGR pregnancies and Doppler flow parameters demonstrated increasing cord serum EPO concentrations in parallel with advancing fetal cardiovascular distress and deteriorating Doppler flow parameters (358).

In cases of stillbirth, what may prove useful in investigating the cause of fetal death is am-EPO evaluation. For days after fetal demise, EPO levels in amniotic fluid remain stable, with elevated levels detectable in deaths from chronic hypoxic events (359). A recent study demonstrated that most of its stillbirths, up to 88%, among structurally normal fetuses, involved placental dysfunction and hypoxia-based mechanisms of death. Am-EPO levels were significantly higher in stillbirths than in control pregnancies, especially in cases with risk factors for adverse pregnancy outcome, such as maternal hypertension or diabetes (69).

Hypoxic complications and stillbirths occur relatively often in diabetic pregnancies (342,360). Both hyperinsulinemia and hyperglycemia may lead to fetal hypoxemia. In type-1 diabetes, maternal glycated hemoglobin (HbA1c) levels reflecting long-term glucose balance may be directly correlated with fetal plasma and am-EPO levels (25,361).

Maternal obesity is becoming a more common obstetric challenge, also being a significant risk factor for intrauterine fetal death. In a recent retrospective cohort study, obesity was associated among term pregnancies with approximately 25% of stillbirths, a risk related to obesity severity (362). One possible mechanism might be increasing fetal hypoxia related to increasing maternal body mass index (BMI). With regarding to such hypoxia, cord-blood EPO concentrations are correlated with maternal BMI (363,364), as well as with poor pregnancy outcomes (364). In addition, maternal obesity promotes production of reactive oxygen species and hampers fetal iron supply, factors modulating fetal EPO levels (363,365).

In chronic fetal anemia, EPO values remain relatively low, unless hypoxia acutely worsens (341). This may be explainable by effective cardiovascular adaptations during this slowly progressing condition, or by accelerated EPO metabolism in anemia (319,341).

Maternal smoking is associated with elevated cord plasma EPO levels independent of fetal growth restriction (366,367). Furthermore, these EPO levels are correlated with number of cigarettes smoked (368). Maternal opioid- or other illicit-substance abuse, however, is not significantly associated with cord blood EPO levels or with other hypoxia biomarkers such as S100B (334). Maternal alcohol consumption during pregnancy is related to elevated fetal cord serum EPO levels, but any connection with hypoxia remains unclear (369).

EPO and adverse outcomes. High am-EPO levels predict adverse neonatal outcomes such as increased risk for prolonged intensive care unit admission, cardiomyopathy, metabolic disturbances, NEC, and neurologic morbidity (338,342,345). Furthermore, among asphyxiated neonates, high cord blood EPO is related to neonatal death or abnormal neurologic outcomes such as CP or delayed mental development at age two (274,340). In preterm births, elevated umbilical cord plasma EPO predict grade 3 to 4 IVH, independent of gestational age (370).

High endogenous serum EPO levels during the first days of life predict increased risk for respiratory morbidity and severe NEC in extremely preterm infants. These levels, however, are unrelated to signs of postnatal hypoxia (371). High blood EPO values among preterm newborns are also associated with higher concentrations of inflammation-related proteins (372). On the other hand, EPO has tissue-protective functions and has potential in neonatal neuroprotection (281,284,285,287). Therefore, effects of endogenous and exogenous EPO are somewhat controversial (371). The mechanisms of EPO action are complex, and relationship of EPO with perinatal outcomes, especially with long-term outcomes, is yet not fully elucidated.

S100B

S100B is a calcium-binding protein expressed mainly in the central nervous system in glial cells, Schwann cells, and neurons, but also in extra-neuronal tissues (19,43). Its level in biological fluids; cerebrospinal fluid, peripheral and cord plasma, serum, urine, saliva, and amniotic fluid, is regarded as a biomarker of brain distress and of cell injury in the nervous system (43,373,374). It is associated with variable conditions including traumatic brain injury, stroke, neurodegenerative, congenital, and psychiatric diseases, as well as with extra-cranial injuries, which reduces its specificity (43,44).

The exact function of intracellular S100B is still unknown. It participates in multiple intracellular activities such as cell proliferation, survival, and differentiation, as well as in regulation of calcium homeostasis and enzyme activation (43). Initially, S100B was assumed to leak from damaged neuronal cells, but recent evidence on active secretion of the protein, especially in association with metabolic stress, has emerged (373,375). S100B released into

the extracellular space interacts with adjacent cells in a manner dependent on its concentration and on environmental features of the tissue (19,376). At physiological low concentrations, S100B has paracrine trophic effects such as enhancing neuronal survival and promoting neurite extension and muscle regeneration (377). At high concentrations in a neural-injury response, however, S100B may trigger pathological processes leading to toxic and proinflammatory effects (43).

In perinatal medicine, S100B is detectable in fluid compartments such as in amniotic fluid, cord plasma and serum, cerebrospinal fluid, serum, urine, and saliva. S100B, a relatively small protein with a half-life of only one hour, is capable of passing the brain-blood barrier into the peripheral blood stream (19). In high-risk pregnancies, fetal S100B appears also in maternal bloodstream (378). To some extent, S100B protein may be of placental origin (379). Cord plasma S100B concentrations correlate with gestational age (380) and are related to mode of delivery, with higher values in vaginal births (381).

High amniotic fluid S100B concentrations can predict intrauterine death (45). In pregnancies with chronic fetal hypoxia, amniotic fluid S100B and EPO concentrations are correlative (382). Elevated S100B concentrations in cord plasma are also related to acidemia and asphyxia at birth (46,47). In addition, in asphyxiated newborns, elevated umbilical cord serum S100B concentrations predict development of moderate to severe HIE, and concentrations correlate with risk for neurological damage and death until six years of age (47). Increased S100B values in serum may predict abnormal cerebral hemodynamics and hemorrhage at an early phase, before occurrence of any clinical or radiological signs (19). Among neonates with perinatal asphyxia and HIE, high levels of serum S100B predict neonatal death and development of CP (383). Serum and urine levels of S100B within one week after delivery correlate with grade of HIE, risk for white-matter lesions and long-term neurologic morbidity, and risk of death (373,383,384). After perinatal asphyxia, neonates with hypothermia treatment show serum S100B levels that are lower (385). In addition, serum S100B levels in hypothermia-treated HIE neonates are predictive of long-term neurodevelopmental outcome at 15 months and at age 2 (385,386). Saliva S100B measurement is a non-invasive and easily achievable method and proving sensitive and specific as a marker of abnormal outcome in asphyxiated neonates with HIE (19).

S100B is an extensively studied biomarker with reference values available for various biological fluids (19). It can serve as a prognostic marker and serve in monitoring the progression of certain disorders, but further data on correlations with radiologic findings and long-term outcomes are necessary (19,43).

Arginine-vasopressin and copeptin

Arginine-vasopressin (AVP), also known as antidiuretic hormone (ADH), regulates homeostasis of the cardiovascular and renal systems and is an important physiological stress hormone (387). AVP is synthesized in the hypothalamus and released from the pituitary in response to various stimuli such as decreased arterial pressure, reduced cardiac output

volume, and hypoxia (387,388). AVP is involved in regulation of numerous crucial body functions such as activation of the HPA axis, water absorption and homeostasis, behavioral and cognitive functions in the central nervous system, and hemostatic activity of platelets and endothelium (53,389). Direct measurement of AVP is, however, challenging because of its short half-life, pulsatile secretion, and platelet binding (389). Copeptin, however, a stable part of the vasopressin precursor molecule, is produced in equimolar ratio with AVP (389). Copeptin can thus serve for indirect demonstration of AVP production. As a novel biomarker, copeptin may be useful in diagnosis and prognosis of various conditions such as myocardial infarction, stroke, traumatic brain injury, and seizures in adult emergency departments (48-50,390,391).

During normal birth, release of fetal stress hormones occurs, ones including catecholamines, cortisol, and vasopressin (52,53,226). This endocrine activation supports the newborn's transition phase and enhances breathing, adaptation of its cardiovascular system, thermogenesis, and glucose and water homeostasis (53,392). After vaginal birth, AVP and copeptin concentrations in cord plasma are significantly higher than after planned cesarean section (53). Uterine contractions cause transient episodes of fetal hypoxemia without inevitably affecting heart rate patterns or reducing arterial pH (26,53). Acute arterial hypoxia strongly stimulates AVP and copeptin excretion (393), and these brief hypoxemic events during contractions are sufficient to trigger AVP- and copeptin release (26). One hypothesis is that this phenomenon prepares the fetus for transition by activating the fetal HPA axis before birth (26,53).

During the perinatal period, variable stressors enhance AVP and copeptin release (53). As compared to concentrations in planned cesarean section, AVP and copeptin plasma concentrations exceed those following acute cesarean section. Correspondingly, after assisted vaginal birth, the concentrations are higher than after spontaneous vaginal birth (51,53,392,394). Chronic stress resulting from IUGR is related to elevated cord-plasma copeptin concentrations (394). High concentrations of copeptin occur also in association with chorioamnionitis, non-reassuring CTG tracings, and perinatal asphyxia, both in pre-term and in term deliveries (51,52,395). Cord plasma AVP is of fetal origin (396). Concentrations of AVP and copeptin correlate with other cord-blood stress markers like pH, BE, and lactate (51-53,395). The highest cord plasma and serum values for AVP and for copeptin occur in association with perinatal hypoxia-ischemia (26,52,274,397).

Cord-serum copeptin concentrations of asphyxiated neonates are only two-fold higher than are those of vigorous healthy newborns (52). The effects of AVP may differ between tissue hypoxia and normoxia (53). In a recent study on asphyxiated neonates, serum copeptin levels in the early neonatal period were higher among neonates with poor outcome (273). In addition, pre-term infants developing IVH have significantly elevated cord plasma copeptin concentrations at birth (395). Copeptin correlates with neuronal-damage biomarkers such as NSE, and high serum concentrations occur in the early phase after delivery (273). Thus, copeptin appears to be a promising biomarker of long-term neurodevelopment (273).

Current feasibility of the biomarkers EPO, S100B, and copeptin

Technologies for swift laboratory assessments of these biomarkers do exist. For copeptin, rapid point-of-care testing is newly available (398). However, round-the-clock availability of assessments means increased financial costs. Thus, their current availability is limited (Table 1).

Table 1. *Feasibility of the biomarkers EPO, S100B, and copeptin*

	EPO	S100B	Copeptin
Sample sources			
Amniotic fluid	+	+	-
Neonatal samples			
Umbilical serum/plasma	+	+	-
Plasma/Serum	+	+	+
Cerebrospinal fluid	-	+	-
Urine	-	+	-
Saliva	-	+	-
[a]Current availability			
Amniotic fluid	3 times/week	Round the clock	-
Umbilical serum/plasma	Weekdays	Round the clock	-
Plasma/Serum	3 times/week	Round the clock	3 times/week
[a]Current cost/sample			
Amniotic fluid	18.70 e	54 e	-
Umbilical serum/plasma	21 e	52 e	-
Plasma/Serum	18.70 e	50 e	35.10 e

[a]HUSLAB: Helsinki University Hospital laboratory

AIMS OF THE STUDY

The aims of this study were to evaluate the role of biomarkers in identifying fetuses at risk for hypoxia-caused complications and in predicting neonatal outcomes after birth asphyxia. Additionally, we aimed to evaluate the association of gestational age with incidence of perinatal asphyxia and long-term neurologic morbidity in term and post-term pregnancies.

The specific aims were the following:

I To evaluate the association of amniotic fluid EPO levels with neonatal outcomes in preterm pregnancies < 34 GW complicated by IUGR.

II To investigate whether, among relatively low-risk pregnancies, amniotic fluid EPO levels prior to labor can identify the individual fetuses at risk for intrapartum distress.

III To examine the value of cord-serum copeptin as a biomarker for birth asphyxia, as compared with the value of EPO and of S100B.

IV To evaluate the association of gestational age at birth – early-term, full-term, or postterm – with asphyxia, neurologic morbidity, and perinatal mortality.

SUBJECTS AND METHODS

STUDY POPULATIONS AND OUTCOME MEASURES IN BIOMARKER STUDIES (I-III)

All biomarker studies were conducted at the University Hospital of Helsinki, Finland, Department of Obstetrics and Gynecology. Gestational ages were defined by fetal crown–rump length measurement at the first trimester ultrasound screening. Birth weight z-score in all the studies we defined according to the Finnish population standardized for gestational age (399). Inclusion and exclusion criteria (I-III) appear in Table 2.

Table 2. *Inclusion (+) and exclusion (-) criteria in Studies I-III*

Inclusion (+) and Exclusion (-) Criteria	Study I	Study II	Study III
Intrauterine growth restriction	+	-	-
Preterm pregnancy	+	-	-
Postterm pregnancy	NA	+	+
Planned cesarean section	+	-	-
Planned vaginal delivery	-	+	+
Singleton pregnancy	+	+	+
Rhesus alloimmunization	-	-	-
Medicated gestational diabetes or DM 1	-	-	-
Pre-eclampsia or severe hypertension	+	-	-
Fetal or chromosomal anomaly	-	-	-
Fetal distress before delivery	+	-	-
Maternal infection	-	-	-
Amniocentesis > 7 days before delivery	-	NA	NA
Lacking umbilical cord samples	-	-	-

NA: Not applicable

Study I

The study population comprised 98 women with singleton pregnancies complicated by IUGR, in which am-EPO determinations were performed during pregnancy. The study design was a retrospective case series, one covering pregnancies with preterm deliveries between 24 and 34 GW during the years 2004 to 2012. Here, IUGR was defined as estimated fetal weight ≤ -2 SD of the mean of the Finnish standard population, corresponding to growth as ≤ 3th percentile (399). After the exclusion of the 32 women shown in Table 2, the final study group comprised 66 women with delivery by cesarean section. These pregnancies were divided into two groups according to pathologic am-EPO and normal/intermediate am-EPO levels for data analysis. The main outcome measure was the association of am-EPO level with short-term and composite adverse neonatal outcomes.

Study II

This prospective observational study comprised 121 women with induction of labor by amniotomy. Women with pregnancies beyond 37^{+0} GW we recruited between September 2012 and December 2014. Indications for induction included prolonged pregnancy (beyond 41^{+0} GW), fear of childbirth, maternal exhaustion, mild pregnancy-induced hypertension, complications in a previous pregnancy, high-pool rupture of membranes in the absence of delivery contractions, gestational diabetes (non-medicated), fetus large-for-gestational age in a non-diabetic mother, unstable presentation, polyhydramnios, or intrahepatic cholestasis of pregnancy. According to our study protocol, no women with severe complications potentially causing fetal hypoxia were recruited (Table 2). Two women were excluded due to neonatal conditions, one with VACTERL syndrome and the other with nonketotic hyperglycinemia. After further exclusion of the 26 pregnancies lacking umbilical serum EPO (us-EPO) measurements, the final study group comprised 93 pregnancies. These 26 excluded pregnancies did not differ from the study population's in terms of am-EPO levels, cesarean section rate, umbilical artery pH or BE, or Apgar score < 7 at one or five minutes. For data analysis, the 78 vaginal deliveries were divided into two groups according to gestational age: term (37^{+0}- 40^{+6} GW) and prolonged pregnancies (41^{+0} GW). The outcome measures were association of EPO levels with short-term neonatal and delivery outcomes—umbilical pH, BE, Apgar score and intrapartum fetal distress—and with gestational age at birth.

Study III

The study population comprised 151 singleton births at or beyond 37^{+0} GW. Women were recruited for this prospective observational study between May 2012 and April 2013. For patient recruitment, low one-minute Apgar score < 4 allowed the swift identification of 72 neonates with suspected birth asphyxia. Additionally, controls recruited were 79 neonates with one-minute Apgar score ≥ 4. Nine complicated pregnancies were excluded (Table 2). Additionally, one neonate was excluded due to a chromosomal anomaly and one for mitochondrial disease. Thus, the final study group comprised 140 neonates. They were divided into asphyxia and control groups for data analysis. Birth asphyxia we defined as the neonate's meeting two of these three criteria: umbilical artery pH < 7.1, umbilical artery BE ≤ -12 mmol/L, and five-minute Apgar score < 7 (21,248). All deliveries were planned as vaginal. The main outcome measure was association of biomarker levels with birth asphyxia.

STUDY POPULATION AND OUTCOME MEASURES IN THE STUDY ON BIRTH ASPHYXIA AND LONG-TERM NEUROLOGIC MORBIDITY (IV)

The population of this cohort study comprised 1 138 109 women with singleton term- and postterm deliveries and their infants born between 1989 and 2008. During the study period, gestational age was determined either by mother's last menstrual period, or by first-trimester ultrasonography. Deliveries of unknown gestational age numbered 7 230 (0.6%). Such deliveries were excluded from our study. In addition, we excluded stillbirths from all

analyses, except from perinatal mortality analysis. Live births included thus numbered 1 129 481. Here, deliveries were divided into the following categories: early-term (37^{+0}– 38^{+6} GW), full-term (39^{+0}– 41^{+6} GW), and postterm (≥ 42^{+0} GW). Five-year periods included 1989–1993, 1994–1998, 1999–2003, and 2004–2008. Perinatal deaths comprised both stillbirths and early neonatal deaths during the first seven days of life. Newborns with major congenital anomalies were excluded from mortality analyses. The main neonatal outcomes were associations of gestational age with birth asphyxia, perinatal mortality, and with long-term neurologic morbidity at age four years – including CP, epilepsy, intellectual disability, and sensorineural defects.

CLINICAL DATA SOURCES

In the biomarker studies (I-III), hospital charts provided the data on maternal and delivery characteristics including BMI in early pregnancy, in vitro fertilization, parity, gestational diabetes, smoking, chronic hypertension, medications used before and during the pregnancy, ultrasonography data recordings, gestation at delivery, and the primary indications for cesarean sections. Hospital charts provided the data on neonatal outcomes, such as one- and five-minute Apgar score, umbilical artery pH and BE, birthweight, and neonatal diagnoses and complications, as well.

The Finnish Medical Birth Register (MBR) maintained by the National Institute for Health and Welfare (THL), provided data for our cohort study (IV). The MBR gathers baseline data concerning pregnancies, deliveries, and the newborn's outcome during the first days of life on all live births and stillbirths beyond 22^{+0} GW and/or with birthweight ≥ 500g. Data collected for analyses covered the following variables: in vitro fertilization, smoking, parity, maternal age (<20, 20–34, and ≥35 years), delivery induction, mode of delivery, gestational age at birth, gender, birth weight < 2 500 g or ≥ 4 000 g, gestational age adjusted birth weight, SGA (< -2 SD) and large for gestational age (> +2 SD), diagnosis of MAS, low umbilical artery pH (< 7.00 and 7.00 – 7.10), and Apgar score < 4 at one and five minutes. Data on one-minute Apgar score were available for all newborns during the whole study period, whereas data on five-minute Apgar score were reported in the MBR since 2004, comprising 230 408 births (83.8%). Data on umbilical artery pH were included in the MBR in 1990 and were available for 519 210 births (45.9%). These data are collected at the time of birth from the mother's prenatal charts. To complete the MBR data, basic information on the missing newborns (0.1%) are obtained from the Central Population Register and the Cause of Death Register.

Data on diagnoses related to pregnancies and deliveries, plus children's long-term neurologic diagnoses at age of four years, came from the Hospital Discharge Register (HDR) maintained by THL, containing nationwide linkable data on all inpatient hospital discharges. This register includes all inpatient and outpatient visits registered as due to CP, epilepsy, intellectual disability, and sensorineural defects (involving visual impairment and deafness) in public hospitals. Only occasionally are children treated in private hospitals and children who emigrated before established diagnoses missing from the HDR. A neurologic disorder at four years was recorded in the study if the child was detected in the HDR with ICD-9 (1989–1995)

or ICD-10 (1996–2008) codes for neurologic diagnoses (Figure 4). THL retains information on major congenital anomalies in the Register on Congenital Malformations. All the data linkages were performed by use of unique personal identity codes anonymized by relevant authorities. CP is typically evident within the first two years of life, and practically always by the age of three to four years (400). The Finnish public health care system invites all children to undergo annual physical examinations; thus, all neurologic diagnoses are consistently recognized by the age of four. Furthermore, multidisciplinary evaluations in secondary or tertiary pediatric neurology units are requirements for diagnosis of CP, epilepsy, intellectual disability, or sensorineural defects. These diagnoses are based on medical history, ultrasonography, and on MRI data, as required.

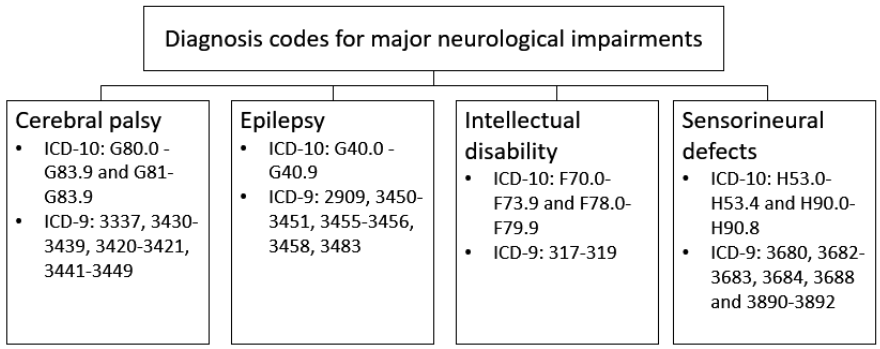

Figure 4. Diagnosis codes for major neurological impairments from the international classification of diseases (ICD) 9 (years 1989- 1996) and ICD 10 (years 1997-2008). Seikku et al. 2016 (Study IV), modified with permission by American Academy of Pediatrics

DEFINITIONS

In all the studies, we defined obesity as BMI > 30 kg/m^2. The diagnosis of gestational diabetes was based on the oral glucose tolerance test plasma glucose levels with values of ≥ 5.3 mmol/L (fasting), ≥ 10.0 mmol/L (1 h) or ≥ 8.6 mmol/l (2 h) according to the American Diabetes Association (401). The American College of Obstetricians and Gynecologists (ACOG) has defined chronic hypertension in pregnancy as blood pressure ≥ 140/90 mmHg before 20 weeks of gestation (402). Gestational hypertension was defined as a blood pressure level of ≥ 140/90 mmHg in a previously normotensive woman after 20 weeks of gestation, without proteinuria (402). Pre-eclampsia was defined as a blood pressure level of ≥ 140/90 mmHg after 20 weeks of gestation with proteinuria more than 0.3 g per day, or with severe symptoms in a woman with previously normal blood pressure (402). MAS was defined (403) as the presence of meconium in both amniotic fluid and neonatal trachea, massive bilateral patchy infiltrates of the lung in thoracic radiograph, and profuse pleural fluid effusions. NEC was defined according to Bell's staging system (404). Neonates fulfilling radiological and clinical criteria of stages II and III—"definite" and operatively treated stages—were included.

Ultrasound examinations and Doppler flow characteristics of the umbilical artery were carried out by perinatologists. Findings of absent or reverse end-diastolic flow were recorded if noted constantly. Oligohydramnios was defined as an amniotic fluid index less than four. Non-reassuring fetal heart rate was defined as the presence of decelerations or of a prolonged decrease in short- and long-time variability. Abnormal biophysical profile was defined as decreased or absent fetal movements and tone in ultrasound examination for 30 minutes, oligohydramnios, or non-reassuring fetal heart rate patterns.

Analysis of total perinatal mortality and early neonatal death was related to total number of births in the same gestational week. Data on stillbirths extracted from the MBR by use of the ICD codes were correlated with the numbers of ongoing pregnancies in the beginning of the gestational week.

In Study I, composite adverse neonatal outcome was defined as the occurrence of at least one of the following diagnoses: necrotizing enterocolitis (NEC), grade III–IV intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), or cerebral infarction. Study II defined composite adverse outcomes if at least one of the following criteria applied: umbilical artery pH less than 7.15, umbilical artery BE less than -12 meq/L, one or five-minute Apgar less than 7, or emergency cesarean section for fetal distress (203).

SAMPLES AND ASSAYS

Amniotic fluid samples

Amniotic fluid samples for EPO measurements came either from amniocentesis (I), at delivery by cesarean section (I), or vaginally at induction of labor by amniotomy (II). Perinatologists performed the amniocentesis under ultrasound guidance by an aseptic technique within 7 days before delivery. In cesarean section, the amniotic fluid samples were obtained after hysterotomy and before amniotomy by syringe. Collection of vaginal samples was at the time of amniotomy from the vaginal pool with a plastic cup and syringe. The EPO-level analysis occurred only after birth, and they had no influence on clinical management.

We compared am-EPO levels in vaginally collected samples with those obtained by amniocentesis in five patients who had an amniocentesis for fetal lung maturation assessment followed by induction of labor by amniotomy. The correlation between these sampling methods was strong ($r = 0.9$, $p = 0.037$).

Umbilical cord samples

Collection of umbilical cord blood samples was immediately after birth (I, II, III). Umbilical artery pH, BE, pO2, and pCO2 measurement occurred routinely with the Radiometer ABL800 Flex blood gas analyzer (Radiometer, Copenhagen, Denmark).

Biomarker assays

EPO levels in amniotic fluid (I, II) and umbilical cord serum at birth (II, III) we measured with a solid-phase immunochemiluminometric assay (Immulite 2000XPI, Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The intra-assay coefficient of variation (CV) of the assay was 3.6% to 6.8%, and the total CV 6.4% to 10.3% for the concentration range 4–615 IU/L. The detection limit was 1.0 IU/L, with functional sensitivity (CV of 20%) of 1.5 IU/L. The Immulite standard of measuring EPO in amniotic fluid changed during Study I. EPO levels analyzed by the old standard were correlated with the new Immulite 2000 EPO standard by use of a regression equation. We classified am-EPO levels into three categories (normal/intermediate/abnormal) as previously described (338). The normal level of EPO in umbilical cord serum we defined as < 40 IU/L (25).

We measured copeptin levels in umbilical serum with a sandwich enzyme-linked immunosorbent assay (ELISA). The inter-assay variability was 5.8%. Fifteen serum samples were analyzed with both the ELISA and the BRAHMS copeptin Kryptor assay used earlier (51,52). A highly significant correlation occurred (Pearson r = 0.9793, p < 0.0001), covering the full range of values obtained (6.0 to 4 637 pmol/L, median 381.2 pmol/L). Thus, copeptin concentrations obtained with the ELISA we converted to Kryptor concentrations to enable direct comparisons of our data with the earlier copeptin results.

We measured serum S100B levels with an electro chemiluminometric immunoassay by Modular e170 analyzer (Roche Diagnostics). The detection range was 0.005 µg/L, and functional sensitivity < 0.02 µg/L. The intra-assay CV was < 2.1%, and the inter-assay variation 6.4%.

STATISTICAL ANALYSES

Characteristics of the newborns and their mothers appear as means with standard deviations (SD) in normally distributed continuous variables, by medians with interquartile range in skewed distributed variables, and in categorial variables by number of values and percentages. We compared groups by using the Mann-Whitney U-test or the $\chi 2$ test, when appropriate. Comparisons of continuous variables in more than two groups were by the Kruskal-Wallis test. We calculated correlations by Spearman´s rank correlation coefficient test or Pearson's test, as appropriate. Statistical outliers ($p < 0.01$) for copeptin and S100B ($n = 1$ for each) were excluded from the study population prior to performance of calculations. The receiver operating characteristics (ROC) curves were constructed and areas under the curves (AUC) determined to evaluate diagnostic accuracy of am-EPO in predicting adverse neonatal outcome (I) and accuracy of copeptin in diagnosing birth asphyxia (III). Risk factors for neonatal asphyxia and neurologic adverse outcome we evaluated by logistic regression analyses using multivariate models, described as odds ratios (ORs) with 95% confidence intervals (CIs).

Statistical analyses were with the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) versions 18.0 (I), 20.0 (IV), or 22.0 (II, III). Additionally, GraphPad Prism 6 (III) and SAS version 9.3 (SAS Institute, Inc, Cary, NC, USA) were utilized. All the tests were two-sided, with probability (p) values of < 0.05 considered statistically significant.

ETHICS

The local Ethics Committee of the Department of Obstetrics and Gynecology, Helsinki University Hospital, Finland (105/13/03/03/2012, 105/13/13/03/2012), approved the biomarker studies (I, II, and III). All participants provided informed written consent to participate in the study. THL, as the register keeper, provided the necessary authorization for Study IV, as required by national data protection legislation (THL/1200/5.05.00/2012).

RESULTS

BIOMARKER STUDIES (I-III)

Table 3 presents selected demographic and clinical characteristics of the populations in Studies I, II, and III.

Table 3. *Characteristics of the study populations*

	STUDY I (n=66) n (%)		STUDY II (n=93) n (%)		STUDY III (n=140) n (%)	
Gestational weeks, median (range)	30.3	(25.6- 34.0)	41.9	(37.0-42.4)	40.9	(37.4-42.2)
Maternal age, mean (SD)	32.3	(6.4)	30.6	(5.4)	31.1	(5.6)
BMI, mean (SD)	26.1	(5.9)	24.1	(5.3)	24.1	(5.2)
Primiparity	41	(62.1)	35	(37.6)	63	(45.0)
Gestational diabetes	5	(7.6)	11	(11.8)	18	(12.9)
In vitro fertilization	6	(9.1)	3	(3.2)	7	(5.0)
Smoking	10	(15.2)	9	(9.7)	13	(9.3)
Delivery mode						
Spontaneous vaginal	NA		74	(79.6)	56	(40)
Vacuum extraction	NA		4	(4.3)	33	(23.6)
Cesarean section	66	(100)	15	(16.1)	51	(36.4)

SD: standard deviation

BMI: body mass index

GW: gestational weeks

NA: not applicable

Study I on 66 IUGR pregnancies compared 56 (84.8%) pregnancies with normal or intermediate am-EPO levels with 10 (15.2%) pregnancies with abnormal EPO levels. Among the whole population in this study, hypertensive disorders were excessive, with up to 31.8% of the women (n = 21) having chronic pre-pregnancy hypertension, and with 18.2% (n = 12) developing pregnancy-induced hypertension, and 40.9% (n = 27) with pre-eclampsia. Study II compared am-EPO and us-EPO levels in 30 (32.3%) term pregnancies to 63 (67.7%) prolonged pregnancies ≥ 41^{+0} GW. Study III comprised 140 term and postterm pregnancies, which were divided into two groups for data analysis: 27 (19.3%) neonates with birth asphyxia and 113 (80.7%) healthy controls.

No statistically significant differences emerged in maternal or delivery characteristics between any of these study groups. Maternal BMI correlated with am-EPO (r = 0.323, p = 0.008) in Study I. Other baseline characteristics of the study populations, including the hypertensive disorders in Study I, failed to correlate with biomarker levels.

AMNIOTIC FLUID AND UMBILICAL SERUM EPO (AM-EPO AND US-EPO) (I-III)

Am-EPO and us-EPO levels in Studies I, II, and III are shown in Table 4. In Study II, am-EPO levels in samples collected before delivery contractions correlated with us-EPO levels at birth

(r = 0.480, p < 0.000), and this correlation was sustained after vaginal delivery (n = 78, r = 0.513, p < 0.000).

Table 4. *Amniotic fluid and umbilical serum levels (I-III)*

	Study I (n=66)	Study II (n=93)	Study III (n=140)
Amniotic fluid EPO IU/L (*median, IR*)	9.0 (4.9 – 16.0)	5.9 (3.2-10.7)	NA
Abnormal amniotic fluid EPO (*n, %*)	10 (15.2)	3 (3.2)	NA
Umbilical serum EPO (*median, IR*)	NA	32.9 (19.5-57.5)	48.6 (22.2-121.5)
Abnormal umbilical serum EPO (*n, %*)	NA	28 (36)	83 (65)
IR: Interquartile range, NA: Not applicable			

Correlation of EPO with gestational age (I-III)

In Study I on preterm IUGR pregnancies, am-EPO did not correlate with gestational age (p = 0.674). Among term and prolonged pregnancies (II), a correlation between am-EPO level and gestational age occurred (r = 0.261, p = 0.012) (Figure 5). Am-EPO levels were higher in prolonged pregnancies ≥ 41^{+0} GW (median 7.1 IU/L), than in term pregnancies (median 3.9 IU/L) (p = 0.005).

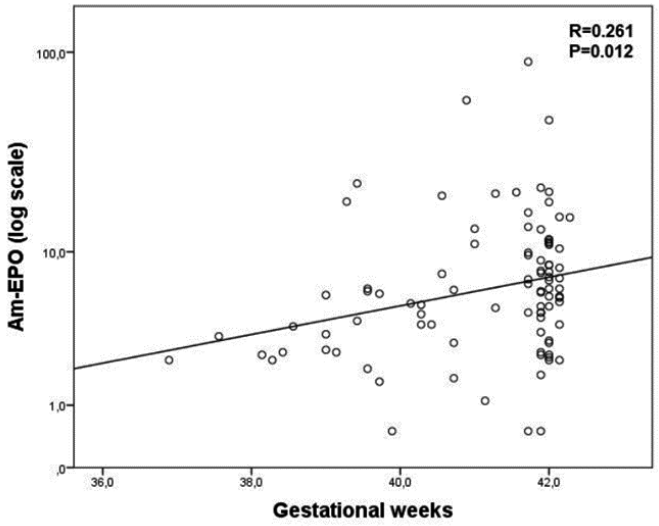

Figure 5. Amniotic fluid EPO levels related to gestational weeks. Seikku 2019 et al. (Study II), modified with permission by Elsevier

In Study II, us-EPO levels in umbilical serum correlated with gestational age among the 78 (83.9%) vaginal deliveries (r = 0.250, p = 0.027). The us-EPO levels did not differ between

prolonged and term pregnancies (p = 0.057) (Table 5). Correspondingly, in Study III, EPO levels in cord blood correlated with gestational age at birth (r = 0.451, p < 0.0001).

Table 5. *A comparison of term and prolonged pregnancies in the vaginal delivery group (n=78) (II)*

	Term pregnancies 37^{+0}- 40^{+6} GW n= 23 (29.5%)		Prolonged pregnancies ≥ 41^{+0} GW n=55 (70.5%)		
	Median	(IR)	Median	(IR)	P-value[a]
Amniotic fluid EPO	3.9	(2.7-6.2)	7.1	(4.6-11.3)	0.026
Umbilical artery pH	7.32	(7.24-7.36)	7.26	(7.16-7.32)	0.017
Umbilical artery BE	-4.5	(-6.1 - -2.2)	-5.5	(-7.0 - -3.1)	0.145
Umbilical serum EPO	20.3	(11.5-51.5)	34.0	(23.6-55.7)	0.057
Abnormal umbilical serum EPO (n,%)	8	(34.8)	20	(36.4)	1.000

[a]*Mann Whitney U-test*

GW: gestational weeks, IR: interquartile range, BE: Base Excess, EPO: Erythropoietin

Am-EPO and fetal parameters (I)

Absent end-diastolic flow in the umbilical artery did not associate with abnormal EPO in amniotic fluid (Table 6). However, along with further deterioration in umbilical artery flow—in reversed end diastolic flow—an association with abnormal EPO arose (p = 0.042). Abnormal biophysical profile was associated with abnormal am-EPO (p < 0.001), whereas oligohydramnios was not.

Table 6. *Umbilical artery Doppler flow and biophysical profile characteristics according to am-EPO values. Seikku et al. 2015 (Study I), modified with permission by John Wiley and sons*

	am-EPO < 27 IU/L n=56 (%)		am-EPO ≥ 27 IU/L n=10 (%)		p-value[a]
Absent end-diastolic flow	33	58.9	6	60.0	0.404
Reverse end-diastolic flow	4	7.1	3	30.0	0.042
Abnormal biophysical profile[b]	7	12.5	8	80.0	<0.001
Oligohydramnios	13	23.2	5	50.0	0.080

[a]*Chi-squared or Fisher's Exact Test, p < 0.05 is considered significant*

[b]*Defined as reduced fetal movements and tone, and/or non-reassuring FHR*

am-EPO: amniotic fluid EPO

EPO and neonatal outcomes (I-III)

Study I. Growth restriction (≤ 2.0 SD) was observable in 62 (94%) of the neonates at birth, severe growth restriction (birthweight ≤ 3.0 SD) comprising 53% of the population (n = 35). Degree of growth restriction did not correlate with am-EPO levels (p = 0.852) (Table 7).

Table 7. *Selected neonatal characteristics and outcomes by amniotic fluid EPO levels. Seikku et al. 2015 (Study I), modified with permission by John Wiley and sons*

	am-EPO < 27 IU/L n=56 %/(SD)		am-EPO ≥ 27 IU/L n=10 %/(SD)		p-value
Gestational weeks at birth (mean)	30.5	(2.1)	29.5	(3.1)	0.203
Male	31	55.4	6	60.0	0.498
Birthweight (g, mean)	1064	(303)	890	(424)	0.240
SD of birthweight					0.288
SD ≤ -3.0	28	50.0	7	70.0	
SD > -3.0	28	50.0	3	30.0	
Apgar score 1 min < 7	24	42.8	6	60.0	0.492
Apgar score 5 min < 7	10[a]	20.9	4	40.0	0.202
Composite adverse neonatal outcome[b]	3	5.4	8	80.0	< 0.001
NICU admission	53	94.6	9	90.0	1.000
Length of NICU admission (days, mean)	24.0	(25.4)	42.4	(35.4)	0.167
Total mortality	2	3.6	2	18.2	0.106
Early neonatal mortality (≤ 28days)	1	1.8	0		
Infant mortality (>28 days to 6 months)	1	1.8	2	18.2	

[a] *Missing data of six newborns*

[b] *Defined as intraventricular hemorrhage, periventricular leukomalacia, cerebral infarction and/or necrotizing enterocolitis*

NICU: Neonatal intensive care unit, am-EPO: amniotic fluid erythropoietin

p < 0.05 is considered significant

T-test with continuous variables and χ^2-or Fisher's Exact Test with categorical variables

Among preterm IUGR pregnancies, an inverse correlation occurred between levels of am-EPO and umbilical artery pH (p = 0.001, r = -0.433) and BE (p = 0.001, r = -0.418) (Figure 6). Furthermore, pH and BE at birth were lower among the neonates with abnormal am-EPO (p = 0.027 and p = 0.007).

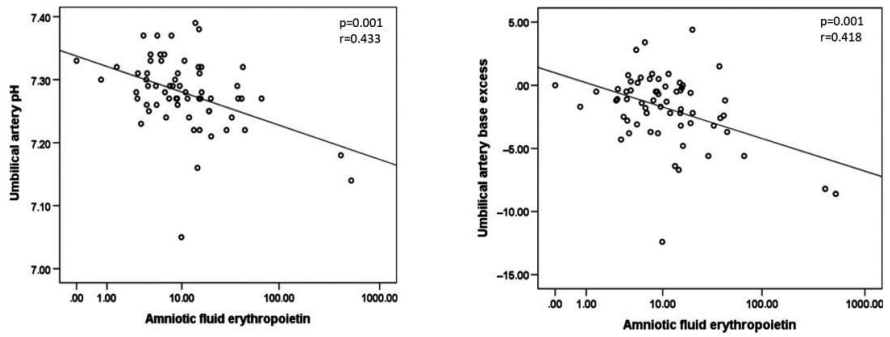

Figure 6. Correlation of amniotic fluid EPO with umbilical artery pH and base excess (log scale)

Composite adverse neonatal outcome, defined as intraventricular hemorrhage, periventricular leukomalacia, cerebral infarction, or NEC, was significantly more common among those neonates with abnormal am-EPO ($p < 0.001$). In predicting composite adverse neonatal outcome by the receiver operating characteristic (ROC) curve, the best cut-off value of am-EPO was 25.0 IU/L with sensitivity of 63.6% and specificity of 96.4%, and with AUC 0.803 (95% CI 0.633 – 0.947, $p = 0.002$). Neither umbilical artery flow parameters nor gestational age at birth associated with composite adverse neonatal outcome.

No stillbirths occurred in the study population. The overall mortality of the population at age 6 months was 6.1%, with one perinatal death at ≤ 7 days and three infant deaths, all due to prematurity-related complications. Mortality was not higher in the group with abnormal am-EPO levels (Table 7).

Study II. In term- and prolonged low-risk pregnancies (II), abnormal am-EPO level occurred in only three (3.2%) (Table 4), with no correlation of am-EPO levels with umbilical artery pH or BE ($r = 0.092$, $p = 0.381$, and $r = 0.051$, $p = 0.626$, respectively). Neither was there a correlation of umbilical cord serum EPO level with umbilical artery pH nor BE ($p = 0.897$, $p = 0.390$).

Composite adverse outcomes—defined as umbilical artery pH ≤ 7.15, umbilical artery BE ≤ -12 meq/L, one or five-minute Apgar < 7, and/or emergency cesarean section for fetal distress—occurred in 18 of the deliveries (19.4%). Am-EPO or us-EPO levels were no higher among those pregnancies ending in adverse outcomes, than among the uncomplicated deliveries ($p = 0.903$, and $p = 0.059$, respectively). Furthermore, no difference in adverse outcomes appeared between the term (10%) and prolonged pregnancies (28.2%) ($p = 0.162$).

Vaginal delivery succeeded in 78 (83.9%) pregnancies. Indications for acute cesarean sections included three with fetal distress during delivery, seven with labor dystocia, four chorioamnionitis, and one fetal malpresentation. Am-EPO levels were comparable among women with vaginal delivery (median 6.0 IU/L, range 0.5–58.2 IU/L) and cesarean section (median 5.8 IU/L, range 1.6–89.8 IU/L) ($p = 0.350$). No difference emerged in cesarean section rates between the term and prolonged pregnancies ($p = 0.232$).

Study III. In this study, the birth asphyxia group comprised neonates with two of these three criteria: umbilical artery pH < 7.10, umbilical artery BE ≤ -12 mmol/L, or a 5-min Apgar score < 7 (Table 8).

Us-EPO-level values did not correlate with umbilical artery pH ($r = -0.145$, $p = 0.144$). However, correlations appeared between us-EPO levels and umbilical artery BE ($r = -0.174$, $p = 0.04$), and five-minute Apgar score ($r = -0.291$, $p < 0.001$), but no difference in us-EPO between asphyxia and control groups ($p = 0.683$).

Table 8. *Comparison of delivery and neonatal characteristics of the study populations (III). Summanen et al. 2017 (Study III), modified with permission by Karger*

	Asphyxia group (n=27)		Control group (n=113)		p-value
Gestational weeks at birth	41.1	(40.0 - 41.6)	40.7	(39.7 - 41.7)	0.372
Spontaneous vaginal delivery	7	(25.9)	49	(43.4)	0.257
Vacuum extraction	9	(33.3)	24	(21.2)	0.309
Emergency cesarean section	11	(40.7)	40	(35.4)	0.727
Male	17	(63.0)	66	(58.4)	0.828
Birthweight	3520	(3260-3945)	3656	(3282-3991)	0.499
SD of birthweight	-0.13	(-1.02 - 0.62)	0.02	(-0.67 - 0.81)	0.385
Apgar score at 5 min	6	(4-7)	8	(6-9)	**<0.0001**
Apgar score at 10 min	8	(6-9)	9	(8-9.5)	**0.001**
Blood gas analysis from umbilical artery					
pH	7.03	(6.97-7.08)	7.21	(7.14-7.30)	**<0.0001**
base excess	-12.7	(-14.4 to -11.1)	-5.80	(-7.85 to -2.85)	**<0.0001**
pO2 (kPa)	1.9	(1.1 - 2.8)	2.4	(1.7-3-3)	0.029
pCO2 (kPa)	10.1	(9.2 - 12.7)	7.8	(6.7-9.2)	**<0.0001**
Umbilical serum erythropoietin, IU/L	71.7	(22.2-116.0)	46.1	(22.2-124.5)	0.683
Umbilical serum S100B ug/L	0.33	(0.19-0.63)	0.31	(0.24-0.45)	0.712
Umbilical serum copeptin, pmol/L	2279	(1476-3144)	973.7	(321-1961)	**<0.0001**

Data are presented as medians (interquartile ranges) or n (%)

Mann Whitney U-test with continuous variables and χ^2-or Fisher's Exact Test with categorical variables

$p < 0.05$ is considered significant

Neonates with two of the following criteria: pH <7.10, base excess ≤-12 mmol/L, 5-min Apgar score <7

Delivery characteristics and EPO

In Study II, us-EPO levels among the vaginal deliveries (median 32.7 IU/L) and the acute cesarean sections (median 32.9 IU/L) were comparable (p = 0.222).

Study III comprised 89 (63.6%) vaginal births and 51 (36.4%) acute cesarean sections. Vacuum extractions occurred in 37.1% of the 33 vaginal births, with no difference in delivery mode between asphyxia and control groups (Table 8). EPO levels were higher among neonates born by cesarean section than born vaginally—both spontaneously and by vacuum extractions (medians 116.0 IU/L, 38.3 IU/L, and 32.3 IU/L, respectively, p = 0.0002 and p = 0.001), with no difference in EPO levels between spontaneous vaginal deliveries and vacuum extractions occurred (p = 0.983).

Umbilical cord serum EPO levels did not correlate with total duration of labor or duration of the second stage of labor in either of the studies II (p = 0.287 and p = 0.783) or III (p = 0.276 and p = 0.763).

UMBILICAL SERUM S100B (III)

The median umbilical serum S100B level in the study population was 0.31 ug/L (range 0.04 – 4.03 ug/L). Umbilical serum S100B levels did not correlate with gestational age (r = -0.002, p

= 0.984). Among male neonates, S100B levels were higher than among those who were female (medians 0.33 ug/L and 0.28 ug/L, p=0.038).

Umbilical serum S100B level did not correlate with umbilical artery pH (r = -0.125, p = 0.144). Nevertheless, a correlation did appear between S100B level and umbilical artery BE (r = -0.218, p=0.01). Umbilical serum S100B correlated with Apgar score at 5 and 10 minutes (r = -0.204, p = 0.016 and r = -0.205, p = 0.016), but no difference appeared in S100B levels between the birth asphyxia group and unaffected controls (p = 0.712) (Table 8).

We found no differences in S100B levels between spontaneous vaginal, vacuum-extraction, or cesarean-section deliveries (S100B medians 0.30 ug/L, 0.33 ug/L, and 0.33 ug/L, respectively; p = 0.476). S100B was not correlated with total duration of delivery nor with duration of second stage of delivery (r = 0.125, p = 0.244, and r = 0.104, p = 0.333).

UMBILICAL SERUM COPEPTIN (III)

Median umbilical serum copeptin level was 1217.5 pmol/L (range 0.0 - 7107.6 pmol/L), with no correlation of copeptin levels with gestational age at birth, or with the sex of the neonate, occurred (r = 0.064, p = 0.452, and p = 0.337). Copeptin levels correlated significantly with birth weight (r = -0.171, p = 0.044).

Umbilical serum copeptin levels correlated with arterial pH (r = -0.622, p < 0.001) and with umbilical artery BE (r= -0.37, p <0.001). Additionally, copeptin correlated with Apgar score at 5 and 10 minutes (r= -0.230, p <0.001, and r = -0.168, p = 0.048). Copeptin levels were higher among those neonates with birth asphyxia than among the unaffected controls (medians 2 279 and 974 pmol/L, p < 0.001). By receiver operating characteristic (ROC) curve, the best cut-off value of copeptin was 1 522 pmol/L in predicting asphyxia (sensitivity of 77% and a specificity of 70%), with the AUC of 0.76 (95% CI 0.69 - 0.86) (Figure 7).

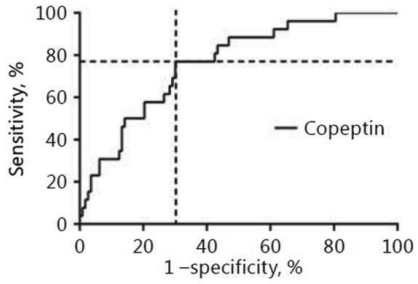

Figure 7. ROC curve for cord serum copeptin levels in relation to birth asphyxia. The dashed lines demonstrates the optimal discriminative cutoff of 1522 pmol/L, with sensitivity 77% and specificity 70%

Copeptin levels and delivery characteristics

Umbilical serum copeptin medians according to delivery mode were as follows: spontaneous vaginal birth 1052.7 pmol/L, vacuum-assisted birth 1611.7 pmol/L, and acute cesarean section 793.8 pmol/L (p=0.004). Copeptin levels were higher among neonates born vaginally than among those born by cesarean section (p = 0.020). A difference occurred especially between vacuum-assisted vaginal births and cesarean sections (p = 0.001), but also in vacuum-assisted births as compared with spontaneous vaginal births (p = 0.019). Among vaginally born neonates, copeptin levels increased as a function of total labor duration (r = 0.327, p = 0.002), as well as of duration of the second stage of labor (r = 0.279, p = 0.009).

COMPARISON OF UMBILICAL SERUM BIOMARKERS COPEPTIN, EPO, AND S100B (III)

Copeptin was the only biomarker correlating with umbilical artery pH (Table 9). The correlation of umbilical artery BE with copeptin was stronger than was its correlation with EPO or S100B. Additionally, only their copeptin levels were significantly higher among neonates with birth asphyxia. Furthermore, copeptin levels increased as a function of labor duration among vaginally born neonates. Copeptin levels correlated with S100B levels (r = 0.295, p < 0.001). Us-EPO correlated with neither copeptin nor S100B (r = 0.106, p = 0.218 and r = 0.24, p = 0.780, respectively).

Table 9. *Correlations of the biomarkers with neonatal variables*

	Copeptin (n=139)		**S100B (n=139)**		**Erythropoietin (n=140)**	
	r	**p**	**r**	**p**	**r**	**p**
Gestational age	0.064	0.451	-0.002	0.984	**0.451**	**<0.0001**
Birth weight	**-0.171**	**0.044**	-0.024	0.784	-0.037	0.667
Apgar score at 5 min	**-0.300**	**<0.001**	**-0.204**	**0.016**	**-0.291**	**0.001**
Apgar score at 10 min	**-0.168**	**0.048**	**-0.205**	**0.016**	-0.138	0.104
Umbilical artery pH	**-0.622**	**<0.0001**	-0.125	0.144	-0.145	0.144
Umbilical artery BE	**-0.637**	**<0.0001**	**-0.218**	**0.010**	**-0.174**	**0.039**

p < 0.05 considered as significant

ASPHYXIA AND NEUROLOGIC MORBIDITY (IV)

Table 10 demonstrates maternal and delivery characteristics of this study covering 1 129 481 live births, including 214 465 (19.0%) early-term and 55 189 (4.9%) postterm births.

Table 10. *Maternal and delivery characteristics of the study population. Seikku et al. 2016 (IV), modified with permission by American Academy of Pediatrics*

	Total		Early-term 37^{+0} - 38^{+6} GW		Full-term 39^{+0} - 41^{+6} GW		Postterm ≥ 42^{+0} GW		Early-term vs Full-term	Postterm vs Full-term
	n	(%)	N	(%)	n	(%)	n	(%)	p-value[a]	p-value[a]
	1 129 481		214 465	(19.0)	859827	(76.1)	55 189	(4.9)		
Study period (5 years)										
1989-1993	304 929	(27.0)	56 201	(18.4)	233 560	(76.6)	15168	(5.0)		
1994-1998	285 729	(25.3)	55 138	(19.3)	217 471	(76.1)	13120	(4.6)		
1999-2003	263 857	(23.4)	51 573	(19.5)	199 990	(75.8)	12294	(4.7)		
2004-2008	274 966	(24.3)	51 553	(18.7)	208 806	(76.0)	14607	(5.3)		
Maternal characteristics										
Maternal age, mean (±SD)	29.7	(±5.3)	30.1	(±5.5)	29.5	(±5.3)	43584	(±5.3)	<0.001	<0.001
in vitro fertilization	15 759	(1.4)	5 252	(2.5)	9 962	(1.2)	545	(1.0)	<0.001	<0.001
Smoking	168 671	(14.9)	33 432	(15.6)	126 201	(14.7)	9038	(16.4)	<0.001	<0.001
Nulliparity[b]	453 889	(40.2)	83 331	(38.9)	341 561	(39.7)	28997	(52.5)	<0.001	<0.001
Mode of the delivery										
Spontaneous vaginal delivery	887 081	(78.5)	152 803	(71.2)	694 462	(80.8)	39816	(72.1)	<0.001	<0.001
Planned section	75 679	(6.7)	28 401	(13.2)	46 108	(5.4)	1170	(2.1)	<0.001	<0.001
Emergency section	92 424	(8.2)	21 808	(10.2)	62 040	(7.2)	8576	(15.5)	<0.001	<0.001
Vacuum extraction	67 365	(6.0)	9 369	(4.4)	52 548	(6.1)	5448	(9.9)	<0.001	<0.001
Forecps	1 604	(0.1)	243	(0.1)	1 273	(0.1)	88	(0.2)	<0.001	0.498
Breech vaginal delivery	4 437	(0.4)	1 669	(0.8)	2 714	(0.3)	54	(0.1)	<0.001	<0.001
Induced delivery	166 794	(14.8)	33 780	(15.8)	108 175	(12.6)	24839	(45.0)	<0.001	<0.001

GW, gestational weeks

[a]t-test, chi square-test, and test for relative proportions, p < 0.05 considered significant

[b]Data missing on 1 331 births

Delivery induction was more common among both early-term (15.8%) and postterm births (45.0%) than among full-term births (12.6%) (OR 1.12, 95% CI 1.11–1.14, and OR 6.30, 95% CI 6.19–6.42, respectively). Additionally, the rate of emergency cesarean delivery was higher for early-term (10.2%) and postterm births (15.5%) than for full-term births (7.2%) (OR 1.40, 95% CI 1.37–1.42, and OR 2.31, 95% CI 2.25–2.36, respectively).

Table 11 includes the neonatal characteristics, and Figure 8 presents the incidences of low umbilical artery pH ≤ 7.10, and low Apgar scores at 1 and 5 minutes.

Table 11. *Neonatal characteristics of the study population. Seikku et al. 2016 (IV), modified with permission by American Academy of Pediatrics*

	Total		Early-term 37+0 - 38+6 GW		Full-term 39+0 - 41+6 GW		Postterm ≥ 42+0 GW		Early-term vs Full-term	Postterm vs Full-term
	n	(%)	n	(%)	n	(%)	n	(%)	p-value[a]	p-value[a]
	1 129 481		214 465	(19.0)	859827	(76.1)	55 189	(4.9)		
Male	574 820	(50.9)	113 479	(52.9)	433 022	(50.4)	28 319	(51.3)	<0.001	<0.001
Apgar at 1 min < 4	9 056	(0.8)	2 031	(1.0)	6 284	(0.7)	737	(1.3)	<0.001	<0.001
Apgar at 5 min (n[b])	230 408		43 947		174 749		11 712			
Apgar at 5 min < 4	634	(0.3)	148	(0.3)	417	(0.2)	69	(0.59)	<0.001	<0.001
Umbilical artery pH (n[c])	519 210		99 579		392 309		27 322			
Umbilical artery pH < 7.00	2 613	(0.5)	513	(0.5)	1 886	(0.5)	214	(0.8)	0.081	<0.001
Umbilical artery pH 7.00-7.10	15 384	(3.0)	2 202	(2.2)	11 902	(3.0)	1 280	(4.7)	<0.001	<0.001
Birthweight[d]										
< 2500g	13 622	(1.2)	9 858	(4.6)	3 710	(0.4)	54	(0.1)	<0.001	0.002
> 4000g	227 735	(20.2)	15 794	(7.4)	191 436	(22.3)	20 505	(37.2)	<0.001	<0.001
Birthweight, mean (±SD)	3 598	(±486)	3 290	(±487)	3 658	(±448)	3 859	(±446)	<0.001	<0.001
< -2SD	21 343	(1.9)	4 242	(2.0)	16 070	(1.9)	1 031	(1.9)	<0.001	0.998
> +2SD	32 497	(2.9)	6 270	(2.9)	24 691	(2.9)	1 536	(2.8)	0.198	0.233
Meconium aspiration	1 891	(0.2)	143	(0.1)	1 481	(0.2)	267	(0.5)	<0.001	<0.001

GW: gestational weeks

[a]t test, chi square test, and test for relative proportions, p < 0.05 considered significant

[b]Data available on 230 408 births (years 2004-2008)

[c]Data available on 519 210 births

[d]Data missing on 295 births

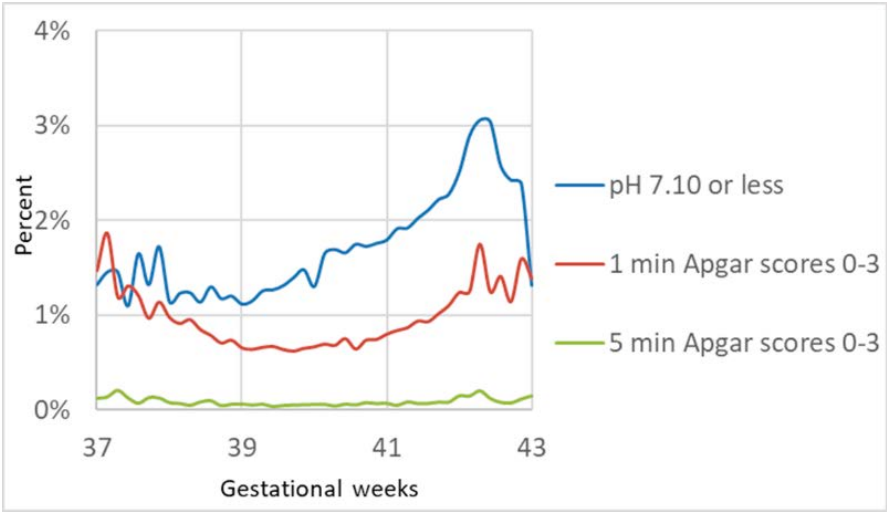

Figure 8. Incidences of low umbilical artery pH and low Apgar score at 1 and 5 minutes according to gestational weeks. Seikku et al. 2016 (IV), modified with permission by American Academy of Pediatrics

Early-term birth and early morbidity

Early-term birth was an independent risk factor for low Apgar score at 1 and 5 minutes, as analyzed by logistic regression (OR 1.03, 95% CI 1.03–1.04, and OR 1.24, 95% CI 1.04–1.49, respectively). In contrast, early-term birth was not associated with low umbilical artery pH ≤7.10 (OR 0.83, 95% CI 0.79–0.87) or pH <7.00 (OR 1.06, 95% CI 0.96–1.18).

Postterm birth and early morbidity

By logistic regression analysis, postterm birth was an independent risk factor for low Apgar score at 1 and 5 minutes (OR 1.26, 95% CI 1.26–1.26, and OR 1.80, 95% CI 1.43–2.34, respectively). Furthermore, postterm birth was an independent risk factor for low umbilical artery pH ≤7.10 (OR 1.26, 95% CI 1.19–1.34) and pH <7.00 (OR 1.18, 95% CI 1.02–1.37]. MAS was more common in postterm than in full-term pregnancies (OR 3.20, 95% CI 3.20–4.16).

The risk factors for long-term morbidity

The results of our risk factor analysis for neurologic morbidity are in Table 12. Induction of delivery was not associated with increased risk for CP, but an association with risk for epilepsy emerged. Emergency cesarean section was associated with neurologic morbidity: CP, epilepsy, intellectual disability, and sensorineural defects.

Low Apgar score at 1 and 5 minutes were independent risk factors for these sequelae, as was low umbilical artery pH—especially pH < 7.00, but even at pH values from 7.00 to 7.10 (Table 12).

Table 12. *Risk factor analysis for cerebral palsy, epilepsy, mental retardation, and sensory neural defects. Seikku et al. 2016 (Study IV), modified with permission by American Academy of Pediatrics*

		Cerebral Palsy		Epilepsy		Intellectual Disability		Sensorineural Defects[a]	
	n = 1 129 481	Unadjusted OR (CI)	**Adjusted OR (CI)**	Unadjusted OR (CI)	**Adjusted OR (CI)**	Unadjusted OR (CI)	**Adjusted OR (CI)**	Unadjusted OR (CI)	**Adjusted OR (CI)**
Maternal risk factors									
In vitro fertilization	**15 759**	1.02 (0.72-1.47)	**0.82 (0.57-1.18)**	1.21 (0.96-1.52)	**1.09 (0.86-1.38)**	0.92 (0.64-1.32)	**0.79 (0.55-1.13)**	1.18 (0.98-1.42)	**0.79 (0.55-1.13)**
Smoking	**168 671**	1.32 (1.19-1.48)	**1.24 (1.11-1.39)**	1.07 (0.98-1.16)	**1.03 (0.95-1.12)**	1.15 (1.03-1.28)	**1.07 (0.96-1.19)**	1.27 (1.20-1.35)	**1.07 (0.96-1.19)**
Nulliparity[b]	**453 889**	1.02 (0.94-1.11)	**0.88 (0.80-0.97)**	0.97 (0.91-1.03)	**0.89 (0.83-0.95)**	0.92 (0.85-1.00)	**0.83 (0.75-0.90)**	1.00 (0.96-1.05)	**0.83 (0.75-0.91)**
Maternal age <20 years	**30 276**	1.41 (1.12-1.77)	**1.46 (1.15-1.84)**	1.23 (1.04-1.45)	**1.30 (1.10-1.55)**	1.30 (1.04-1.63)	**1.43 (1.13-1.79)**	1.35 (1.19-1.53)	**1.43 (1.13-1.79)**
Maternal age >35 years	**186 213**	1.24 (1.11-1.38)	**1.16 (1.03-1.29)**	1.09 (1.01-1.18)	**1.03 (0.96-1.12)**	1.22 (1.10-1.35)	**1.14 (1.02-1.26)**	1.05 (0.98-1.11)	**1.14 (1.02-1.26)**
Induced labour	**166 794**	1.14 (1.02-1.28)	**1.05 (0.94-1.18)**	1.14 (1.06-1.24)	**1.13 (1.04-1.22)**	1.29 (1.17-1.43)	**1.21 (1.09-1.35)**	1.21 (1.14-1.29)	**1.21 (1.09-1.35)**
Emergency section	**92 424**	2.50 (2.24-2.79)	**2.03 (1.80-2.29)**	1.44 (1.31-1.58)	**1.37 (1.25-1.52)**	1.67 (1.48-1.89)	**1.47 (1.30-1.68)**	1.17 (1.08-1.26)	**1.48 (1.30-1.68)**
Planned section	**75 679**	1.31 (1.12-1.52)	**1.43 (1.22-1.68)**	1.35 (1.22-1.50)	**1.43 (1.29-1.60)**	1.31 (1.13-1.51)	**1.37 (1.18-1.59)**	1.16 (1.06-1.27)	**1.37 (1.18-1.59)**
Breech vaginal delivery	**4 437**	5.98 (2.47-14.44)	**5.64 (2.30-13.81)**	0.56 (0.08-3.98)	**0.55 (0.08-3.92)**	1.06 (0.15-7.53)	**0.94 (0.13-6.78)**	NA	**NA**
Vacum extraction	**67 365**	1.05 (0.88-1.26)	**1.08 (0.89-1.30)**	1.13 (1.00-1.27)	**1.17 (1.03-1.32)**	1.08 (0.92-1.28)	**1.15 (0.97-1.36)**	1.09 (0.99-1.20)	**1.15 (0.97-1.36)**
Neonatal risk factors									
Postterm birth ≥ 42^{+0} GW	**55 189**	1.06 (0.87-1.29)	**1.03 (0.84-1.26)**	1.03 (0.90-1.18)	**1.00 (0.87-1.15)**	1.20 (1.01-1.43)	**1.19 (1.00-1.43)**	0.99 (0.88-1.10)	**0.96 (0.86-1.07)**
Early term birth 37^{+0}- 38^{+6}	**214 465**	1.52 (1.38-1.68)	**1.40 (1.27-1.55)**	1.20 (1.11-1.29)	**1.14 (1.06-1.23)**	1.46 (1.33-1.60)	**1.39 (1.27-1.53)**	1.27 (1.20-1.34)	**1.24 (1.17-1.31)**
Male	**574 820**	1.32 (1.21-1.44)	**1.30 (1.19-1.42)**	1.09 (1.03-1.15)	**1.09 (1.03-1.16)**	1.49 (1.37-1.62)	**1.51 (1.39-1.64)**	1.09 (1.04-1.14)	**1.09 (1.04-1.14)**
Apgar < 4 at 1 min	**9 056**	11.09 (9.51-12.95)	**6.20 (5.13-7.49)**	3.94 (3.32-4.66)	**2.89 (2.39-3.51)**	4.88 (3.94-6.04)	**3.19 (1.39-1.64)**	2.01 (1.66-2.42)	**3.21 (2.52-4.10)**
Apgar < 4 at 5 min	**634**	16.77 (11.19-25.13)	**2.20 (1.40-3.47)**	6.52 (4.18-10.17)	**2.30 (1.42-3.74)**	9.91 (5.94-16.56)	**2.82 (1.62-4.92)**	3.26 (1.95-5.43)	**2.88 (1.66-5.01)**
Umbilical artery pH < 7.00	**2 613**	12.11 (9.26-15.84)	**3.66 (2.70-4.96)**	3.89 (2.83-5.35)	**2.15 (1.53-3.01)**	3.55 (2.26-5.59)	**1.66 (1.02-2.68)**	2.86 (2.14-3.84)	**1.64 (1.02-2.65)**
Umbilical artery pH 7.00-7.10	**15 384**	1.96 (1.50-2.56)	**1.49 (1.13-1.96)**	1.48 (1.20-1.82)	**1.32 (1.06-1.64)**	1.42 (1.06-1.91)	**1.23 (0.91-1.66)**	1.64 (1.40-1.93)	**1.23 (0.91-1.66)**
Birth weight[c]									
< -2SD	**21 343**	3.54 ('2.97-4.23)	**2.76 (2.30-3.31)**	2.23 (1.92-2.59)	**2.04 (1.75-2.38)**	3.92 (3.34-4.59)	**3.67 (3.11-4.32)**	1.81 (1.59-2.07)	**3.66 (3.11-4.32)**
> +2SD	**32 497**	1.10 (0.86-1.41)	**1.02 (0.79-1.30)**	0.84 (0.69-1.02)	**0.79 (0.65-0.96)**	0.84 (0.65-1.10)	**0.76 (0.58-0.99)**	0.92 (0.80-1.07)	**0.76 (0.58-0.99)**
Meconium aspiration	**1 891**	5.34 (0.75-38.31)	**1.54 (0.20-11.63)**	2.53 (0.35-18.11)	**1.54 (0.21-11.18)**	9.65 (2.38-39.16)	**5.19 (1.24-21.68)**	NA	**NA**

Logistic regression multivariate models, results given as OR (95% CI)

[a]Sensorineural defects including severe impaiments of vision and hearing

[b]Data missing on 1331 births

[c]Data missing on 295 births

NA: Not applicable

In logistic regression analysis, early-term birth was another independent risk factor for CP, epilepsy, intellectual disability, and sensorineural defects (Table 12). Among children born early term, 14.0% of newborns with pH < 7.10 had one-minute Apgar < 4, and 37.7% of newborns with pH < 7.00 had one-minute Apgar < 4.

Postterm birth was an independent risk for intellectual disability. However, no associations with CP, epilepsy, or sensorineural defects appeared (Table 12). Among post-term births, 8.8% newborns with pH < 7.10 had one-minute Apgar < 4, and 33.3% of newborns with pH < 7.00 had one-minute Apgar < 4. MAS was associated with intellectual disability, but not with CP or epilepsy (Table 12).

Data on long-term neurologic impairment rates according to gestational age are in Table 13. The incidence of CP decreased over the study period from 0.26% during the years 1989 to 1993 to 0.15% during 2004 to 2008 (p < 0.001). During the same time periods, the incidences of epilepsy (from 0.19% to 0.48%), intellectual disability (from 0.16% to 0.25%), and sensorineural defects (from 0.09% to 1.04%) increased (p < 0.001 for all). No changes occurred in rates of early-term or postterm birth rates during the study period.

Table 13. *Neurological impairments at age 4. Seikku et al. 2016 (Study IV), modified with permission by American Academy of Pediatrics*

	Deliveries		Cerebral Palsy		Epilepsy		Intellectual disability		[a]Sensorineural defects	
Gestational weeks	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
	1129481		2298	(0.20)	4410	(0.39)	2337	(0.21)	7117	(0.63)
37^{+0}- 38^{+6}	214465	(19.0)	607	(0.28)	966	(0.45)	596	(0.28)	1638	(0.76)
39^{+0}- 40^{+6}	638931	(56.6)	1205	(0.19)	2429	(0.38)	1200	(0.19)	3862	(0.60)
41^{+0}- 41^{+6}	220896	(19.6)	373	(0.17)	793	(0.36)	405	(0.18)	1273	(0.58)
≥ 42^{+0}	55189	(4.9)	113	(0.21)	222	(0.40)	136	(0.24)	344	(0.62)

[a]*Sensorineural defects include severe impairments of vision and hearing*

PERINATAL MORTALITY (IV)

Total perinatal mortality of the study population, excluding neonates with major congenital anomalies, was 1.3 per 1000 births. Neonates born at early term had the highest incidence, with 2.5 deaths per 1000 births. Among full-term neonates, the incidence was 1.0 per 1000 and postterm neonates 0.9 per 1000 births. The risk for early neonatal death within the first seven days was higher after early-term and postterm births, than after full-term births (Table 14). Stillbirth risk, as related to ongoing pregnancies, was lower in both early-term and postterm births (Table 14). Total perinatal mortality rates decreased during the study period mostly due to a decreased stillbirth rate (p < 0.001). Early neonatal death rate, however, did not fall significantly (p = 0.071) (Figure 9).

Table 14. *Risk for perinatal mortality in early- and postterm birth as compared to full term birth[a]. Seikku et al. 2016 (Study IV), modified with permission by American Academy of Pediatrics*

	Early term 37^{+0}- 38^{+6} GW		Postterm ≥ 42^{+0} GW	
	OR	(95% CI)	OR	(95% CI)
Stillbirth[b]	0.49	(0.43-0.55)	0.67	(0.46-0.98)
Early neonatal death at ≤ 7 days	1.73	(1.25-2.40)	2.06	(1.31-3.25)
Total perinatal mortality[c]	2.40	(2.14-2.69)	0.91	(0.69-1.22)

[a]Children with major congenital anomalies excluded

[b]Related to ongoing pregnancies

[c]Including stillbirth and early neonatal death related to births on the specific gestational week (GW)

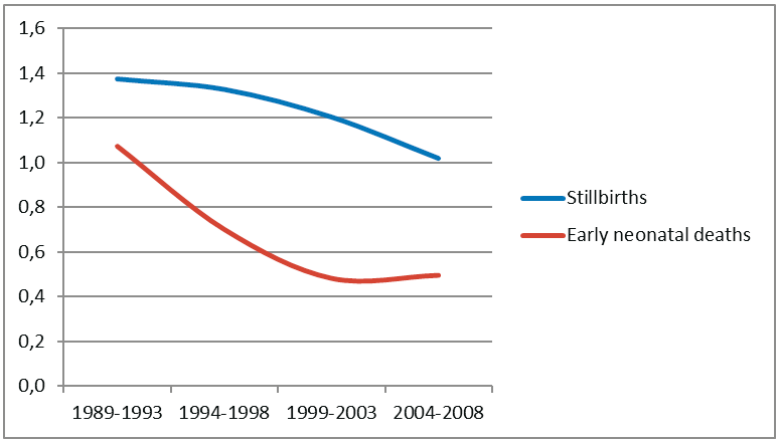

Figure 9. Perinatal mortality rates during years 1989 - 2008 (IV)

DISCUSSION

BIOMARKER STUDIES (I-III)

Alterations in biomarker levels during delivery are at least in part due to physiological fetal adaptations in the transition phase. “Ischemic preconditioning” refers to a phenomenon in which an ischemic insult below the damage-causing threshold activates endogenous protective mechanisms. Such priming may reduce the effect of subsequent, possibly more severe, insults (405). The extent of any advantage from these protective responses depends on circumstances. Some reactions, such as increased AVP secretion during birth, may be necessary and useful under normal conditions, but be harmful in association with asphyxia (53). Interpretation of the biomarker evaluations is thus complex. The same phenomenon may be related to the physiological decrease in umbilical artery pH during normal birth (406), a phenomenon possibly involved in triggering adaptation processes.

AMNIOTIC FLUID AND UMBILICAL SERUM EPO (AM-EPO AND US-EPO) (I-III)

EPO in amniotic fluid is a biomarker of chronic fetal asphyxia (25). Increased EPO levels in fetal plasma or in umbilical cord serum at birth may also reflect subacute hypoxia (340). Elevated EPO levels have been associated with decreased umbilical artery pH, BE, and Apgar score, as well as with increased risk for neonatal intensive care admission in various pathologic pregnancies (25,344,345). On the other hand, EPO has tissue- and neuroprotective features (281,284,313). Thus, increased EPO levels may reflect preconditioning and an effort toward tissue protection, even though endogenous EPO levels may not always prove sufficient (371).

Am-EPO sampling methods

Both methods of am-EPO sampling—amniocentesis and vaginal sample at amniotomy—have been used, sometimes as equivalent methods in the same study (340,345). The presence of blood in the amniotic fluid tends to elevate EPO values, whereas meconium has no influence on EPO levels (345). We compared amniotic fluid samples collected first by amniocentesis, followed by samples collected at amniotomy in the same patients, and found a substantial correlation between these methods. Amniocentesis is a relatively safe invasive method, with complications requiring immediate delivery occurring in only 0.7% (407). More common complications such as preterm rupture of the membranes and bleeding, occur in an estimated 3.6% (408). In our study, no complications due to amniocentesis occurred. However, the advantages of vaginally obtained—noninvasive sampling—needs further evaluation.

Am-EPO and maternal characteristics (I, II)

Maternal obesity has been associated with increased risk for IUGR, especially when utilizing customized centiles for fetal-growth estimation (409). Additionally, perinatal mortality risk increases in correlation with maternal BMI (409). In Study I, we found a correlation of maternal BMI with am-EPO. This may reflect increased risk for hypoxia with higher BMI among IUGR pregnancies. However, among relatively low-risk pregnancies (II), no such association was evident.

Earlier studies have demonstrated higher am-EPO levels in pregnancies with hypertensive complications than in uncomplicated pregnancies (338). We found no association of am-EPO levels with hypertensive disorders (I), which may be explained by our study population's comprising only pregnancies complicated by fetal growth restriction.

Correlation of EPO with gestational age (I-III)

In uncomplicated pregnancies—during normoxia—fetal EPO levels remain relatively stable throughout the second and third trimesters (330). However, studies have demonstrated increasing EPO levels beyond 41 GW (335). Consistent with this, among the preterm pregnancies (I), am-EPO did not correlate with gestational age, whereas among the term and prolonged pregnancies (II) a correlation of am-EPO level with gestational age appeared, with significantly higher EPO levels among prolonged pregnancies. Furthermore, EPO levels in the umbilical serum in the term and prolonged pregnancies (II, III) correlated with gestational age. In us-EPO, the difference between term and prolonged pregnancies was non-significant (II), which is understandable, as the risk for adverse perinatal outcomes in term pregnancies increases gradually after 40 pregnancy weeks (191). Increasing levels of EPO along with advancing gestation are in line with the hypothesis that gradual weakening of placental function and incipient relative hypoxia explains the increased risk for fetal compromise in prolonged pregnancies.

Am-EPO and parameters of fetal wellbeing (I)

Widely established in management of pregnancies with fetal growth restriction is Doppler flow assessment. Abnormalities in umbilical-artery flow parameters such as absent or reversed end-diastolic flow, are associated with intrauterine hypoxia and increased risk of perinatal mortality (155,156). Our study is, to the best of our knowledge, the first to report am-EPO in relation to umbilical artery Doppler flow changes. We found an association between reversed—but not absent—end-diastolic flow in the umbilical artery and abnormal am-EPO levels. This finding is consistent with the increasing risk for fetal hypoxia along with deteriorating umbilical artery flow, as are earlier findings on IUGR pregnancies demonstrating a positive correlation between umbilical artery pulsatility (PI) index and cord serum EPO at birth (358). In our study, an association of umbilical artery Doppler flow changes with adverse neonatal outcome was, however, lacking. This is in line with some

earlier findings of increased morbidity in IUGR pregnancies even in the absence of umbilical artery flow abnormalities (301) and suggests that prediction of chronic fetal hypoxia and adverse neonatal outcomes cannot be based on umbilical Doppler flow parameters alone.

Biophysical profile assessments serve in detecting hypoxia-related changes in fetal status (151). Abnormal biophysical-profile findings are associated with fetal acidosis, stillbirth, and CP (82). We demonstrated a strong association of abnormal biophysical profile with abnormal am-EPO levels. Biophysical profile scoring is, however, a subjective method, and its clinical utility is limited. Furthermore, an abnormal biophysical profile may appear at late stages of fetal hypoxia and indicate irreversible fetal compromise (60). Placental insufficiency generally leads to decreased amniotic volume, which may occur at earlier stages than do the other changes in biophysical profile (92). In our study, oligohydramnios was not significantly related to abnormal EPO levels, indicating that subtle weakening of placental function does not cause significant changes in EPO levels. When the indication is for immediate delivery, EPO assessment by amniocentesis is no longer useful.

Correlation of am-EPO with us-EPO (II)

A correlation exists between EPO levels in amniotic fluid and in fetal plasma, as well as with umbilical cord serum after delivery by planned cesarean section (25,338). EPO levels in fetal plasma rise in response to hypoxic events more quickly than do the levels in amniotic fluid (25). Consequently, umbilical cord serum EPO levels may be elevated in subacute hypoxia during delivery. We confirmed that EPO levels in amniotic fluid before the delivery contractions remain correlated with us-EPO levels at birth, even after the stress caused by vaginal delivery.

Associations of EPO with umbilical artery pH and BE (I-III)

Increased levels of EPO in amniotic fluid and fetal plasma indicate chronic hypoxia and can be detectable in various pathological pregnancies such as Rhesus alloimmunization, fetal growth restriction, pre-eclampsia, and maternal type 1 diabetes (25). High fetal and am-EPO levels have been associated with acute adverse neonatal outcomes such as decreased umbilical cord pH, BE, and pO2, and increased risk for intensive-care-unit admissions (25). Moreover, increased umbilical plasma EPO levels at birth are associated with increased mortality rates and risk for neurodevelopmental abnormalities at age two (274).

In our study on IUGR pregnancies (I), am-EPO showed an inverse correlation with umbilical artery pH and BE, with abnormal am-EPO levels being associated with significantly lower umbilical artery pH and BE values, consistent with findings in several studies (342,344). However, in a study by Girsen et al. on IUGR pregnancies, only cord blood BE correlated with EPO levels at birth (358). In their study, one-third of the population had a vaginal delivery, and EPO levels were determined in cord blood samples. Similarly, in our study on term asphyxiated neonates with various delivery modes (III), no correlation of umbilical cord

serum EPO with umbilical artery pH occurred, but EPO levels correlated with umbilical artery BE. The variation in delivery modes and in sampling methods among these studies may explain the difference in findings, since uterine contractions during even normal vaginal birth can affect umbilical cord pH and EPO concentrations at birth (246,339). The increased am-EPO levels and decreased pH and BE values in Study I most probably reflected actual intrauterine circumstances caused by chronic fetal hypoxia.

In our study on apparently low-risk pregnancies (II), as expected, few abnormal am-EPO samples were detectable. Furthermore, low umbilical artery and BE values were uncommon. These features of the study population probably explain the lack of correlations of amniotic fluid and umbilical cord serum EPO with umbilical artery pH and BE levels. Possibly, however, increased EPO levels reflect fetal stress, even if they do not correlate with pH (333). Elevated EPO levels may occur without decreases in umbilical cord pH and BE, since these parameters can return to normal levels soon after the hypoxic event (336).

EPO and neonatal outcomes (I-III)

Study I. Composite adverse neonatal outcome was strongly associated with abnormal am-EPO values. Adverse outcomes included cerebral complications—intraventricular hemorrhage, periventricular leukomalacia, and cerebral infarction—as well as NEC. Our findings are, therefore, in line with those of a study demonstrating that asphyxiated infants with subsequent neurodevelopmental sequelae such as CP or developmental delay may show at birth increased cord plasma EPO levels (274). Furthermore, am-EPO associates with other biomarkers reflecting oxidative stress and neuronal injury, ones such as oxidative and nitrosative stress biomarkers, as well as protein S100B (382,410).

Among our study population, all neonates developing NEC had abnormal EPO levels in their amniotic fluid, consistent with the fact that asphyxia elevates risk for NEC among neonates with growth restriction (411). Generally, growth restriction causes increased neonatal prematurity-related morbidity (137). In our study, gestational age at birth was not associated with composite adverse neonatal outcome, however.

The best cut-off value of am-EPO for predicting adverse neonatal outcome was 25.0 IU/L, in substantial agreement with the earlier definition of pathologic EPO level (338).

Study II. In late-term and prolonged pregnancies, adverse perinatal outcomes such as decreased umbilical artery pH and BE values, low Apgar score, intrapartum fetal distress, and meconium aspiration occur more frequently than in term pregnancies (36,412). We saw only a few cases of intrapartum fetal distress, or of neonates with low Apgar score or low umbilical artery pH and BE; and our definition of composite adverse outcome was relatively mild. Thus, we were unable to demonstrate any association between antenatal am-EPO levels and adverse outcomes among these apparently low-risk pregnancies.

Study III. Relatively mild asphyxia criteria included neonates with at least two of the following criteria: umbilical artery pH < 7.10, umbilical artery BE ≤ 12.0 mmol/L, and/or five-minute Apgar score < 7. Us-EPO correlated with Apgar score at five minutes; nonetheless, it was not higher among the neonates with asphyxia than among the control neonates. This may reflect the nature of EPO as less a biomarker of acute asphyxia than of chronic hypoxia.

Delivery characteristics and EPO (II, III)

Umbilical cord serum EPO levels differ by mode of delivery, with higher levels following vaginal birth than in birth via planned cesarean section (25,339). In Study II, am-EPO levels between women who succeeded in vaginal delivery and those who ended with acute cesarean section were comparable. Thus, in this low-risk population, am-EPO did not appear to predict intrapartum fetal distress. Nor did any difference appear between delivery modes in umbilical cord serum EPO levels (II). The explanation is most likely the exposure to delivery contractions, as all the deliveries in this study were primarily planned as vaginal, and all the neonates experienced varying stress from uterine contractions.

In Study III, us-EPO levels were higher among neonates born by acute cesarean section than by spontaneous or assisted vaginal birth. This may reflect antenatal conditions leading to acute cesarean section. However, no difference appeared in cesarean section rates between asphyxia and control groups. Furthermore, the total duration of labor or duration of second-stage labor showed no correlation with EPO levels in umbilical serum in either of the studies, suggesting that normal delivery contractions activate EPO synthesis, but do not typically lead to an exponential increase in EPO levels.

Clinical implications and future directions of EPO determination

Significance of am-EPO assessment in management of IUGR pregnancy (I). Timing the delivery in preterm IUGR pregnancies—ergo, finding a balance between risk for stillbirth and risk for prematurity—is a major obstetric challenge. Risk for stillbirth is significantly increased in IUGR pregnancies (134). In the GRIT study, delay of delivery by a few days led to increased stillbirth rates, but the total numbers of perinatal deaths in the immediate-delivery and in the delayed-delivery groups were equal (143). Moreover, a trend towards increased neurological disability among the immediate-delivery group occurred at 2 years of follow-up (144). In our study on IUGR pregnancies, no stillbirths occurred, and total mortality was well comparable with rates from other studies on IUGR (143,302).

Am-EPO assessment in IUGR pregnancies may be an additional method in fetal surveillance and in optimizing delivery timing. When noninvasive surveillance methods cannot confirm the wellbeing of the fetus, or if uncertainty remains, amniocentesis may be justified to obtain additional information. Elevated am-EPO levels can be a warning of emerging problems several days before alterations in other parameters, ones such as CTG (338).

Delivery may be safely delayed if am-EPO level is not abnormal, but in cases of moderately elevated levels, careful monitoring is necessary. In cases of abnormal EPO levels, especially when other surveillance methods are non-reassuring, immediate delivery requires consideration. Further studies on the association of abnormal EPO levels with long-term neurologic outcomes in IUGR are essential.

Am-EPO and prediction of adverse outcome in prolonged and low-risk pregnancies (II). We hypothesized that am-EPO assessment before labor contractions predicts adverse delivery outcomes. Among the low-risk study population, however, abnormal EPO levels and adverse outcomes were rare, and it was impossible to demonstrate such a predictive value for am-EPO. Demonstrating such an association would probably have required considerably larger populations. We thus concluded that, in predicting delivery outcomes among low-risk populations am-EPO assessment may not prove clinically useful.

Nevertheless, our study demonstrated that EPO levels in amniotic fluid collected vaginally at amniotomy are consistent with the levels in samples collected by amniocentesis. This observation could be useful in selected high-risk pregnancies, where either am-EPO assessment at amniotomy or at spontaneous rupture of membranes could prove predictive of the outcomes. In one earlier study, higher EPO levels in amniotic fluid and umbilical cord serum have been detectable among those deliveries which ended in an acute cesarean section than among spontaneous vaginal births (340). The am-EPO values can prove of value in determining delivery mode, since fetuses with abnormal EPO values may not tolerate the stress of delivery contractions.

Furthermore, the correlation between amniotic fluid and umbilical serum EPO levels was persistent after vaginal delivery. This information may prove useful in estimating the timing of possible hypoxic events among asphyctic neonates and in differentiating chronic from acute hypoxia. Further studies on these issues are warranted.

Umbilical cord serum EPO, and delivery and neonatal outcomes (II, III). In Study II, a considerable portion of the population had, at birth, abnormal umbilical cord serum levels, and median cord serum EPO level was close to our definition of abnormal EPO level. In most of the cases, however, neonatal adverse outcomes were absent. Instead, among the neonates born by cesarean section for fetal distress, us-EPO levels were abnormal in all cases. This may reflect either fetal response to stressful events during delivery, or antenatal conditions contributing to decreased resilience to stress caused by delivery contractions. That us-EPO levels were not significantly higher among asphyxiated neonates (III), may be explained by the nature of EPO as a marker of chronic hypoxia more than as a marker of acute hypoxia (25). Additionally, the relatively mild asphyxia criteria we used may have influenced study findings.

The mechanisms of EPO effects are complex. Association of EPO with neonatal outcomes, especially with long-term outcomes, is not fully elucidated as yet. Studies are warranted on the long-term neurologic health of children who, during pregnancy and at birth, show increased EPO values.

UMBILICAL SERUM S100B (III)

At early stages of acute asphyxia, S100B plasma levels increase (413,414). Among asphyxiated neonates, elevated S100B levels are associated with IVH or HIE (379). In a recent report on a small case series, elevated cord serum S100B levels were related to cord blood acidosis, severity of aEEG pattern abnormalities, HIE stages II and III, and 6-year neurodevelopmental health (47). S100B levels have been correlated with gestational age (380), but contradictory findings have emerged (47).

Our study found no correlation of umbilical serum S100B with arterial pH at birth. More importantly, a correlation with umbilical artery BE and with Apgar score at 5 and 10 minutes occurred. No difference in S100B levels between asphyxiated and unaffected controls appeared, however, most probably explained by our relatively mild definition of asphyxia and our selection method for our control population. We found no correlation of gestational age with S100B levels, as has been reported earlier (19). Furthermore, we failed to confirm others' findings of higher S100B levels among neonates born vaginally (19). What may explain this disparity is that our study population included no planned cesarean sections. The significance of S100B assessment in umbilical cord serum in predicting asphyxia and HIE thus requires further study.

UMBILICAL SERUM COPEPTIN (III)

One of the essential adaptation mechanisms at birth is activation of the HPA axis, which results in release of AVP from the pituitary. This release is further enhanced by various stressors such as infections and hypoxia (51,52,395). AVP is highly unstable, and its secretion is measurable by its surrogate peptide copeptin, derived from the same hypothalamic precursor molecule and released in concentrations equal to those of AVP (389). Among normal births, copeptin levels show a negative correlation with umbilical cord blood pH and BE (51). These levels are further increased in birth asphyxia (52). Furthermore, high levels of copeptin among preterm neonates at birth are associated with adverse neonatal outcomes such as IVH (395).

Copeptin levels and neonatal outcomes

Consistent with earlier data, we demonstrated a highly significant correlation of copeptin with umbilical artery pH and BE. Its strong correlation with BE—the most important single criterion for metabolic acidosis and birth asphyxia (248)—is of special interest. In line with this finding, copeptin levels among the asphyxiated neonates were significantly higher than levels in unaffected controls. The moderate sensitivity and specificity parameters in the ROC analysis in our study, as compared to earlier numbers (52), are related to our inclusion criteria of the control group with Apgar score ≥ 4, and rather mild asphyxia criteria.

Copeptin levels and delivery characteristics

Umbilical serum copeptin levels are higher after vaginal delivery than after delivery by planned cesarean section (51). Consistently, our study's copeptin levels were higher among neonates born vaginally—spontaneous and assisted vaginal birth numbers combined—than among cesarean-section neonates. Copeptin levels, as previously reported, were highest among assisted vaginal births (51). The difference between spontaneous vaginal births alone and acute cesarean sections was non-significant. This is attributable to the study population comprising only neonates who had experienced variable periods of labor contractions before being born, either vaginally or by cesarean section.

Additionally, we demonstrated a correlation of copeptin with duration of labor and with duration of the second stage of labor. This finding is in line with recent observations that even a few contractions are enough to activate the release of AVP and copeptin, and that its half-life of 30 minutes allows copeptin to accumulate during delivery (26,415). The accumulating copeptin may also reflect stress of delivery, which is advantageous for adaptation at transition within normal limits, but may turn out to be overwhelming if continuing for excessively long, as in cases of labor dystocia.

Clinical implications of copeptin assessment at birth

According to our findings, copeptin is a highly promising biomarker of neonatal distress and asphyxia, having the potential to become part of a routine examination. Serum copeptin serves as a diagnostic and prognostic biomarker in certain adult emergency departments (49,50). Thus, this assessment could be easily implemented for neonatal intensive care units. Recently, a rapid immunoassay for copeptin point-of-care testing became available (398), to improve the suitability of copeptin assessment in clinical practice. Future studies are warranted on the correlation of copeptin with HIE stage and long-term neurologic outcomes. Sequential copeptin evaluations may enhance the prognostic value of this biomarker and thus call for further study. Wider clinical utilization of copeptin requires introduction of quick assessments and the easy availability of these tests in daily practice.

COMPARISON OF UMBILICAL SERUM BIOMARKERS (III)

Our distinct biomarkers have different bases and profiles in perinatal asphyxia (25,51,414). AVP—measured by copeptin—plays a central role in neonatal adaptation at birth, protein S100B reflects neuronal injury, and EPO is primarily a biomarker of chronic hypoxia. Consequently, these variable features mostly explain the differences that appear in our study.

Copeptin turned out to be the only biomarker associating significantly with birth asphyxia. We were unable to demonstrate the potency of either S100B or EPO as biomarkers of acute birth asphyxia. This may reflect the acuteness of the asphyxia among the study population

or may be related to the inclusion criteria and the asphyxia definition that we applied. Our definition is, however, supported by the evidence that falling in the tenth centile of umbilical artery pH at birth of approximately 7.15, and having a five-minute Apgar score less than seven both associate with neonatal morbidity (254,255,406).

Furthermore, copeptin was the only biomarker which increased as a function of labor duration. Thus, our study suggests that, as a predictor of acute birth asphyxia, copeptin is the most potential of these three biomarkers.

ASPHYXIA AND NEUROLOGIC MORBIDITY IN TERM AND POST-TERM PREGNANCIES (IV)

In many countries, the elective delivery induction rate at early term and after the 41^{+0} GW has increased substantially, leading to a decrease in number of post-term births, and an increasing number of early-term births (416). In Finland, such routine inductions have been less common, even though induction of delivery is more frequent among early-term and postterm pregnancies. During the 20-year study period, the overall delivery-induction rate increased (41), but the early-term birth rate remained unchanged, and post-term birth rate slightly increased. Furthermore, our perinatal mortality and long-term morbidity numbers were comparable with those reported earlier (187,417-419). The controversial association of delivery induction with CP (216,217) did not arise here.

Early morbidity in early-term and postterm pregnancies

Low Apgar score at 5 and 10 minutes in term deliveries is frequently related to acidosis and perinatal asphyxia and predicts increased risk for neonatal morbidity and mortality (252,254,420). In earlier studies, long-term neurologic sequelae such as decreased cognitive function (421), epilepsy (253,422), and CP (252,253) have been associated with low Apgar score. Our strongest risk factor for neurologic morbidity was low Apgar score at one minute. This may in part be due to limitations in the MBR data on five-minute Apgar score. Nevertheless, this finding is in line with recent cohort-study findings of a strong association of one-minute Apgar score with long-term neurologic sequelae (423). Furthermore, low one-minute Apgar score < 7 predicts CP in moderately and late preterm births, as well (424). Consistent with earlier studies, both postterm and early-term birth were associated with increased risk for low Apgar score (190,418).

Low umbilical artery pH at birth associates with increased risk of neurologic morbidity and neonatal mortality (249,425). Risk for neonatal encephalopathy increases along with deepening acidemia (21). The predictive value of birth acidosis for neurologic sequelae is poor, however (245,425). We demonstrated an association of low umbilical artery pH—at pH values between 7.00 and 7.10—with long-term neurologic sequelae. Even in low-risk pregnancies, along with advancing gestation, umbilical artery pH tends to decrease (189,191). Consistent with this, postterm birth was confirmed to rise as an independent risk

factor for low umbilical artery pH. In contrast, and in line with earlier reports, early term birth was not associated with low umbilical artery pH (426).

Long-term neurologic morbidity risk factors

Neurologic complications and developmental abnormalities are assumed to occur excessively among children born postterm (189,427). Among term pregnancies, the risk for CP appears in a U-shaped pattern, with the highest risk occurring at 37 GW and beyond 42 weeks (400). However, contradictory reports exist on the association of CP with postterm births, with early-term births frequently included in reference groups (400,428). We demonstrated the highest risk for CP among births at early term, whereas not confirming elevated risk among births at postterm. Similarly, we could not confirm the earlier findings of an association of postterm birth with epilepsy (429), whereas early-term birth did associate with epilepsy and sensorineural defects. The highest IQ values occur among individuals born at term, with lower IQs in early-term and postterm births (193,430). This is consistent with our demonstrating elevated risk for intellectual disability among both early-term and postterm births.

The fact that emergency cesarean section associates with neurologic morbidity most probably reflects the circumstances leading to cesarean section, such as fetal distress during delivery. Interestingly, emergency cesarean deliveries were more frequent among early-term and postterm births than among full-term births.

Despite its associations with low pH and with low Apgar score, postterm birth did not elevate risk for long-term neurologic sequelae, as did early-term birth. This may be explained by early-term neonates' relative physiologic immaturity and increased vulnerability (426).

Time periods

The five-year periods in the study revealed some changes in adverse outcomes possibly related to changes in clinical management. The incidences of MAS and low one-minute Apgar score increased, while incidence of low umbilical artery pH remained stable. The rates of early-term and postterm pregnancies remained substantially unchanged during the entire study period, and thus cannot explain this. Furthermore, we demonstrated increasing incidences of epilepsy, intellectual disability, and sensorineural defects, all of which may reflect improved diagnostics and advanced data-collection systems. Overall, our neurologic morbidity rates were well comparable to internationally published numbers (253). In contrary to earlier reports of an unchanging prevalence of CP worldwide (419), we found a tendency of CP numbers to have decreased during the study period in Finland. A similar decrease in CP rates was earlier reported in Finland among children born moderately and late preterm (424).

Clinical implications and future considerations

Gestational age has an influence on neonatal outcomes, even after completion of gestational weeks defined as term pregnancy. Risks for various neonatal complications are increased at both extremes of term and postterm birth.

We observed an association of early-term birth with short-term morbidity and long-term neurologic sequelae. These findings are well in line with earlier findings of a relationship of neurologic impairments with early-term birth and support the recent recommendations on avoiding unnecessary deliveries in early-term gestation (34,183,184). Such recommendations do not apply to medical indications for deliveries. Future studies may further elucidate the effect of pregnancy complications on neonatal outcomes at early term.

We confirmed the increased risk for birth asphyxia and for short-term morbidity among neonates born postterm, but the impact on long-term neurologic health was less important than earlier reports led us to expect (189,427). Intellectual disability was the only neurologic outcome associated in our study population with postterm birth. Conceivably, postterm birth seems to encompass fewer risks than previously assumed, especially among pregnancies receiving inclusive health care. Furthermore, management of pregnancies beyond 41 GW affects a high proportion of pregnant women. This emphasizes the need for further studies on the optimal management of pregnancies beyond 41 GW (211).

PERINATAL MORTALITY IN TERM AND POSTTERM PREGNANCIES (IV)

In the literature, perinatal mortality rates—including both early neonatal deaths at ≤ 7 days and stillbirths—rise along with advancing gestations in term pregnancies, from 0.7 cases per 1000 deliveries at gestational week 37 up to 5.8 at 43 GW (36,187,189,412). Several studies, however, describe a U-shaped relationship of gestational age with mortality rates among term births (40,190). Some studies have observed the highest risk for perinatal mortality in early-term births (187,431), consistent with our findings. In one earlier study, perinatal mortality rates were elevated in those countries with a substantial proportion—more than 4%—of postterm deliveries (39). Our results were in contrast to this, possibly reflecting the high quality and availability of the basic health-care system and pregnancy-management protocols in Finland.

In high-resource countries, stillbirth risk estimates range from 1.6 to 5.3 cases per 1000 deliveries (189,432). The most realistic way to put stillbirth risk into perspective is to compare its rate to numbers of pregnancies ongoing at the same gestation (187,431). Calculated by such a method, the risk for stillbirth in our study population was lower in early-term and postterm pregnancies than in full-term pregnancies. This is consistent with some findings of no increase in stillbirth numbers beyond postterm (188).

Total perinatal mortality decreased as expected during the study period, owing mostly to our decrease in stillbirth numbers. We observed an increased risk of perinatal mortality among

early-term births, but no such risk associated with postterm birth. Considering postterm births, our results were in line with those of a recent Finnish study (215). In weighing these findings, the low absolute number of perinatal deaths must be considered, as well. Additionally, studies on the influence of pregnancy complications on perinatal mortality numbers are essential.

STRENGTHS AND LIMITATIONS OF THE STUDIES

In our study on IUGR (I), all of the women delivered by cesarean section; thus, delivery mode had no effect on umbilical-cord blood parameters. Women with conditions potentially altering fetal EPO concentration we excluded. Therefore, we were able to evaluate changes in EPO levels related specifically to IUGR. We had limited data on MCA and ductus venosus Doppler, owing to our retrospective study design with its long study period. The seven-day cut-off from sampling to delivery may underestimate actual EPO levels, since rapid elevations in EPO concentrations occur in pathological pregnancies. Our study population comprised patients with amniocentesis performed for non-reassuring findings in Doppler parameters or in their biophysical profile and may hence not be totally representative of unselected IUGR pregnancies.

The study on term- and postterm pregnancies (II), included only apparently low-risk pregnancies. We were thus able to evaluate the impact of gestational age on EPO levels without any possible influence of fetal hypoxia on these levels. However, we cannot totally exclude the possible effect of indication for delivery induction on EPO values. The number of umbilical cord blood samples that were lacking was considerable, and some selection of patients may have occurred. However, the outcomes of the study population and those lacking samples were comparable.

The study on birth asphyxia biomarkers (III) used relatively mild asphyxia criteria, which may explain some mitigation of the findings. Data on clinically estimated HIE grade of the neonates and the need for resuscitation procedures would have provided valuable additional information. Furthermore, the design of the patient recruitment and final study group allocation led probably to a less vigorous control group as compared to the standard population.

The small sample size in all the biomarker studies (I, II, and III) caused some limitations. Multivariant analyses were not performed in these studies partly because in small populations such analyses are not relevant. Power analyses in these studies were also lacking. It is challenging to define a sufficient difference with clinical relevance for biomarker levels in these study compositions.

The study on early and postterm births (IV) comprised a large population-based cohort, and this allowed evaluation of rare adverse events. The homogenous population in Finland during our study period is an additional strength. We recognized the limitations common in observational register studies. The registers provided limited data on several factors such as

indication of delivery induction, umbilical artery BE values, and five-minute Apgar score. The study population also included high-risk pregnancies and infants with congenital anomalies, which may have influenced timing of delivery and exaggerated morbidity among early-term birth. We included all children at age four years with any diagnosis of palsy; thus, on rare occasions children may not have had CP, but other kinds of palsies. During the long study period, pregnancy-dating practice changed. This may have led to underestimation of the effect of gestational age on CP during the earliest five-year periods, before routine ultrasonography dating (400).

CONCLUSIONS

In pregnancies complicated by IUGR before 34 GW, high EPO levels in amniotic fluid were associated with decreased umbilical artery pH and BE, and with increased risk for neonatal morbidity. We suggest that in selected pregnancies with severe preterm IUGR, am-EPO assessment may prove a useful additional tool in fetal monitoring and in optimizing timing of delivery.

Am-EPO levels correlated with gestational age in term- and prolonged pregnancies, and with us-EPO, even after delivery contractions. EPO levels were higher in prolonged pregnancies than in term pregnancies. In this study, however, am-EPO levels were not related to adverse outcomes. Therefore, determining EPO levels in amniotic fluid at induction of labor turned out not to be useful in routine practice for predicting delivery outcomes among low-risk pregnancies. However, EPO levels in amniotic fluid can be easily and safely determined from samples obtained by amniotomy at induction of labor. Further studies on am-EPO assessment, either at amniotomy or in spontaneous rupture of membranes, are justifiable in selected high-risk pregnancies. In such circumstances, abnormal EPO level could guide delivery management with more intensive fetal monitoring, or even influence decisions concerning eventual delivery mode.

Our study on umbilical serum biomarkers indicates that copeptin proved to be a high-potential biomarker of acute birth asphyxia and neonatal distress and may thus become a routinely used assessment among neonates in poor condition at birth. Serum copeptin serves as a diagnostic biomarker of suspected myocardial infarction in adult emergency departments. Recently, a sensitive and rapid assay for point-of-care testing has emerged, that could easily be introduced to neonatal intensive care units, as well. Correlation of copeptin levels with severity of HIE and long-term neurologic outcomes calls for further study. In this study population, S100B and EPO proved ineffective as biomarkers of acute asphyxia. Combined data on fetal Doppler flow parameters and biomarkers also need further examination in a prospective study on high-risk pregnancies such as IUGR.

Neonatal outcomes vary by gestational age at birth. Our study demonstrated that numerous complications were increased at both extremes of term and postterm birth. Early-term birth between 37^{+0} and 38^{+6} GW was associated with low Apgar score, increased long-term neurologic morbidity, and perinatal mortality. Among postterm births ≥ 42 GW, asphyxia and intellectual disability were more frequent than after full-term birth, but general neurologic morbidity and perinatal mortality were not increased. Accordingly, birth in the early-term period appears to elevate risks involving long-term neurologic health, whereas birth at postterm seems to confer fewer risks than previously assumed.

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