Informative Fetal Blood: Umbilical Cord Blood Analytes to Predict Neonatal Problems and Diseases Occurring Later in Life

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Mehreen Zaigham

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Abstract

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It is an undisputed fact that the assessment of umbilical cord blood gases gives the most objective evaluation of the condition of the newborn at birth. The fetus develops in an intrauterine environment that has a lower partial pressure of oxygen than the maternal circulation and it has several defense mechanisms by which it can protect itself from an acute hypoxic event, at least for a short while. Sustained hypoxia, however, can lead to perinatal asphyxia and the risk of hypoxic brain injury. The analysis of cord blood gases may help to identify asphyxiated infants at risk for permanent neurological damage/death already at birth when most clinical and radiological signs are still absent.

With the revelation that cord blood gases vary with gestational age in term pregnancies, *Study I* used a large, prospectively collected database of cord arterial and venous blood gases that were meticulously sorted to ensure the highest data quality. We examined changes in pH across a wide range of preterm, term and postterm pregnancies. It was shown that both umbilical cord arterial and venous pH decreased linearly with increasing gestational age and that the median 5-minute Apgar score was less than 10 before 31 weeks of gestation.

Large-for-gestational-age (LGA) infants have increased insulin resistance and an increased risk of neonatal morbidity and mortality. Normal cellular metabolism is reliant on sufficient glucose and oxygen supplies with lactate being the end-product of anaerobic metabolism. In *Study II*, the cord blood glucose and lactate concentrations of LGA infants were compared to appropriate-for-gestational-age (AGA) infants in normal as well as acidotic conditions at birth. Using low pH and base excess (BE) as a proxy for sustained intrapartum hypoxia, we found that LGA infants matched AGA infants in their metabolic responses to intrapartum hypoxia and the diabetic status of the mother did not hamper this ability.

On the verge of survival is another vulnerable group, extremely preterm infants (i.e. born <27 gestational weeks). They have immature body systems and are at high-risk for neurodevelopmental handicap as well as death. Using the national, prospectively collected database of the “Extremely Preterm Infants in Sweden Study” (EXPRESS), *Study III* explored a relatively unknown area i.e. the value of cord blood gases in the prediction of death or disability in extremely premature infants. We discovered that cord arterial pH was significantly higher in extremely preterm infants as compared to term infants, however, low cord arterial pH and BE did not seem to be a crucial factor in the risk for neurodevelopmental outcome or death by 6.5 years of age.

The last two studies, *Study IV* and *V* focused on the potential value of specific neurological proteins [Ubiquitin carboxy-terminal hydrolase 1 (UCH-L1), Glial fibrillary acidic protein (GFAP) and S100B] sampled from cord blood at birth, as potential biomarkers of hypoxic brain injury. Using codes from the International Classification of Diseases (ICD), we identified infants with moderate and severe hypoxic ischemic encephalopathy (HIE). The stored cord serum of these infants was tested for neurological biomarkers and their respective concentrations were compared to biomarker concentrations in matched healthy controls. We found that UCH-L1 and GFAP were not significantly increased in the cord blood of affected infants. However, S100B concentrations were elevated in infants with moderate/severe HIE and positively correlated with the severity of disease including risk for permanent handicap and death.

Key words: Umbilical cord blood, hypoxia, gestational age, Apgar score, large-for-gestational-age, base excess, pH, neurodevelopmental outcome, extremely preterm, biomarker, mortality.

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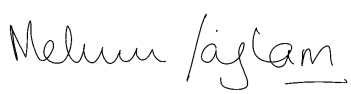
Signature  Date 2019-03-15

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MADE IN SWEDEN

To my children, Ismail and Alisha

*"**Imagine** with all your mind. **Believe** with all your heart.*
***Achieve** with all your might."*

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In summary, this thesis sheds light on the informative value of umbilical cord blood.

Original Papers

This doctoral thesis is based on the following original papers which are referred to in the text by using their Roman numerals:

I. **Zaigham M**, Källén K, Olofsson P. Gestational age-related reference values for Apgar score and umbilical cord arterial and venous pH in preterm and term newborns. *Submitted manuscript.*

II. **Zaigham M**, Källén K, Olofsson P. Assessment of lactate production as a response to sustained intrapartum hypoxia in large-for-gestational-age newborns. Acta Obstet Gynecol Scand. 2018;97(10):1267-73.

III. **Zaigham M**, Källén K, Maršál K, Olofsson P. Lack of associations between umbilical cord blood gases in extremely preterm newborns and neurodevelopmental outcomes at 6.5 years of age. *Submitted manuscript.*

IV. **Zaigham M**, Lundberg F, Hayes R, Undén J, Olofsson P. Umbilical cord blood concentrations of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) in neonates developing hypoxic-ischemic encephalopathy. J Matern Fetal Neonatal Med. 2016;29(11):1822-8.

V. **Zaigham M**, Lundberg F, Olofsson P. Protein S100B in umbilical cord blood as a potential biomarker of hypoxic-ischemic encephalopathy in asphyxiated newborns. Early Hum Dev. 2017;112:48-53.

List of Abbreviations

aEEG	Amplitude-integrated electroencephalography
ANCOVA	Analysis of covariance
AGA	Appropriate-for-gestational-age
ApH	Umbilical cord arterial pH
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BD	Base deficit
BD_{blood}	Base deficit in blood
BD_{ecf}	Base deficit in extracellular fluid
BE	Base excess
BMI	Body mass index
CI	Confidence Interval
CNS	Central nervous system
CS	Cesarean section
CSF	Cerebrospinal fluid
ECLIA	Electro chemiluminescent immunoassays
EDD	Estimated date of delivery
EEG	Electroencephalography
EXPRESS	Extremely Preterm Infants in Sweden Study
FPGS	Fractional placental glucose supply
GDM	Gestational diabetes mellitus
GFAP	Glial fibrillary acidic protein
Hb	Hemoglobin
HbF	Fetal hemoglobin
HIE	Hypoxic-ischemic encephalopathy
IGF	Insulin-like growth factor
IVF	In vivo fertilization
IVH	Intraventricular hemorrhage
ICD	International Classification of Diseases
IUGR	Intra-uterine growth restriction
kPa	Kilopascal
K-W test	Kruskal-Wallis test

LDH	Lactate dehydrogenase
LMP	Last menstrual period
LGA	Large-for-gestational-age
M-W test	Mann-Whitney *U* test
MRI	Magnetic resonance imaging
NICU	Neonatal intensive care unit
N-HLR	Neonatal heart-lung-resuscitation
nM	Unit of measurement, nanomolar in reference to hydrogen ion concentration
OR	Odds ratio
P	Probability
pCO_2	Partial pressure of carbon dioxide
pO_2	Partial pressure of oxygen
pH	Negative log of the hydrogen ion concentration
r^2	Coefficient of determination
RR	Relative risk
S100B	S100 protein including the subunits αβ and ββ
SD	Standard deviation
SGA	Small-for-gestational-age
SVD	Spontaneous vaginal delivery
UCH-L1	Ubiquitin carboxy-terminal hydrolase L1
VpH	Umbilical cord venous pH
WD%	Weight deviation in percent from the gestational age-adjusted mean weight

Preface

He makes you, in the wombs of your mothers, in stages, one after another in three veils of darkness. Such is Allah your Lord and Cherisher: to Him belongs (all) dominion. There is no god but He: then how are ye turned away (from your true Center)?

The Quran 39:6

The umbilical vein carries oxygen as well as nutrients to the fetus whilst umbilical artery transports the waste products of fetal metabolism for exchange to the placenta. Scientists are just beginning to understand the true potential of cord blood sampling. From giving an objective assessment of the condition of the newborn at birth, to the early detection and diagnosis of devastating perinatal conditions; cord blood is truly the "*elixir of life"*. The overall purpose of this thesis was to further understand some of the utilizations of fetal cord blood in addition to elucidating the associations of specific analytes within cord blood to the risk of death or disability later on in life.

To relate abnormal analyte values in cord blood to events occurring later in life, reference intervals for these analytes are necessary. Our research group has previously determined reference curves for arterial pH for gestational weeks 37 to 43 and for arterial and venous lactate for weeks 24 to 43. In ***Study I*** we wanted to complete the project by determining cord arterial and venous pH from preterm to postterm pregnancies as well as the normal distribution of the Apgar score at birth.

Metabolism is dependent on the availability of oxygen and energy. In the fetus, the main energy source is glucose. A well-nourished fetus builds up glycogen stores from which glucose is easily released by glycogenolysis during aerobic metabolism. Glucose enters the citric acid cycle releasing energy-rich adenosine triphosphate (ATP). As a measure of the metabolic rate, fetal plasma lactic acid (lactate) increases with advancing gestational age. During episodes of sustained oxygen depletion, where the oxygen consumption is higher than the oxygen delivery, aerobic metabolism switches to anaerobic metabolism with the production of lactate in substantial amounts. Each glucose molecule then creates just 2 ATP.

With the start of the second stage of labor, the increased intensity and strength of uterine contractions can temporarily compromise oxygen transfer to the fetus via the maternal circulation. The fetus has different mechanisms to be able to withstand short episodes of hypoxia but with sustained hypoxia and the concomitant anaerobic metabolism, hydrogen ions and lactate accumulate in increasing concentrations. A low pH and base excess (BE) can then lead to the development of metabolic acidosis and

perinatal asphyxia, which can lead to permanent neurological damage and death in extreme cases.

Elevated lactate concentrations in fetal scalp blood and in umbilical cord blood have previously been shown to be measures of perinatal asphyxia and metabolic acidosis at birth. Researchers have debated on whether the growth restricted fetus, i.e. small-for-gestational-age (SGA) infant can have a restricted ability to produce lactate secondary to immature glucose metabolic systems. Lactate would then be falsely low relative to the degree of hypoxia and anaerobic metabolism. However, in a recent study we found that growth restricted fetuses had an intact capacity to produce lactate when exposed to severe hypoxia. Little is known about the lactate production capabilities of over-nourished fetuses, i.e. large-for-gestational-age (LGA) infants during hypoxic conditions intrapartum. In ***Study II*** we wanted to find answers by investigating changes in cord blood glucose and lactate concentrations during normal and hypoxic conditions at birth in diabetic and non-diabetic pregnancies.

Fetal life conditions in-utero have a significant impact on the individual's development and health later on in life. For example, growth restriction in utero increases the risk of developing heart disease and atherosclerosis during adulthood. Advances in perinatal medicine, with modern neonatal intensive care units (NICUs), have led to an increase in the survival of extremely premature infants i.e. born before 28 completed weeks of gestation. The EXPRESS (Extremely Preterm Infants in Sweden Study) concluded that the 1-year survival of liveborn infants from 22+0 to 26+6 weeks of gestation was around 70%. This was an outstanding figure by any international standards, however, these infants are often in need of prolonged care in NICUs and their recovery is marred by numerous short-term and long-term complications. ***Study III*** was designed to investigate the potential predictive value of cord blood acidosis to mortality and neurodevelopmental impairment later on in life for extremely preterm infants.

It is well known that fetuses exposed to severe hypoxia at birth have a greater risk of developing hypoxic-ischemic encephalopathy (HIE) during the early neonatal period. Moderate and severe HIE may cripple the affected neonate with neurodevelopmental handicaps for life. The pathogenesis of HIE involves two main stages: an initial ischemic phase characterized by cell death within areas of poor blood perfusion, and a reperfusion phase after 2–6 hours with apoptotic cell death and extension of the affected areas. Hypothermia treatment has been shown to be neuroprotective and therapeutic in the prevention of the second stage of neuronal cell injury. However, this treatment must be started within a critical time window of 6 hours after birth to have any affect. Complicating the situation even further, clinical signs and symptoms are often few with absent radiological findings during this critical time window. Biochemical markers for

hypoxic brain injury have been discussed as adjunctive tools in the diagnosis of perinatal brain injury especially if they can be detected in cord blood at birth.

Our research group found that the unspecific cell injury marker, lactate dehydrogenase (LDH) in cord blood at birth, has a fair predictive value on the development of HIE stages II and III and the risk of subsequent sequalae and death. With ***Study IV*** **and** ***V***, we hoped to further explore neuronal biomarkers in cord blood with similar studies on proteins: Ubiquitin carboxy-terminal hydrolase 1 (UCH-L1), Glial fibrillary acidic protein (GFAP) and S100B.

1. Introduction

Embryonic development

The umbilical cord develops during the 4th to 8th week of gestation. The amnion, which is a membrane that closely covers the embryo when it is first formed, keeps expanding with the growth of the embryo. The amnion expands until it covers the entire embryo except for the umbilical area where the connecting stalk and the yolk sac emerge [1] (Figure 1).

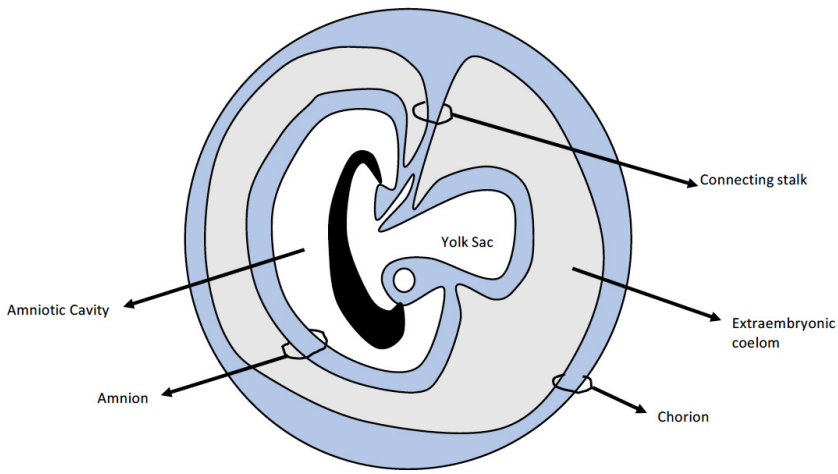

Figure 1:
Embryonic development of the umbilical cord week 4

Increase in the volume of the amniotic fluid causes the amnion to swell until it completely takes over the chorionic space. When the amnion is in contact with the chorion, it causes the two extra-embryonic mesoderm layers that cover both membranes, to loosely fuse together. With embryonic folding, the amnion encircles the following to constitute the "umbilical cord".

By the end of the 5th gestational week, blood flow has been established [2]. Initially, the umbilical cord is very thick and short in length but later lengthens to allow for fetal movements and even coils in the amniotic cavity. A number of elements degenerate by the 12th week of embryonic development, leaving only the body stalk with the umbilical vessels (2 arteries, 1 vein).

The umbilical vessels are surrounded by an amniotic epithelial layer. The connective tissue of the body stalk and the amnion convert into the umbilical cord connective tissue or "*Wharton's jelly*". This is an elastic and resistant tissue that possibly protects the umbilical vessels.

Anatomy of the umbilical cord

The umbilical cord is a tubular structure that connects the fetal circulation to the placental circulation. Mention of the umbilical cord can be dated back to 384-322 BC where Aristotle identified it as a connection between the mother and unborn child [3].

A fully developed umbilical cord consists of (Figure 2):

- two umbilical arteries which have a slightly thicker, double layered smooth muscle wall, without an elastic layer
- one umbilical vein, which has a thinner muscular wall containing a subintimal elastic layer
- remnant of the allantois
- Wharton's jelly
- surrounding single layer of amnion which is continuous with the placental surface and fetal skin

The average diameter of the umbilical cord is about 2 cm and it decreases with the progress of the pregnancy secondary to a reduction in the water content of the Wharton's jelly [4].

The cord has an average length of 50-60 cm at term. It can, however, range from 30 cm to 100 cm (or more) in length with both excessively long and short cord lengths associated with adverse outcomes. An extremely long cord may encircle the neck of the fetus and cause problems at the time of delivery; or a very short cord may lead to problems during delivery by pulling at its attachment to the placenta [5, 6]. It approximately 1% of cases, "true-knots" can be formed in the cord, mostly due to the

fetus passing through a loop during labor. The majority of true knots are loose and thus clinically irrelevant. However, if a true knot forms early in pregnancy, active fetal movements can interfere with fetal circulation and effectively strangulate the fetus.

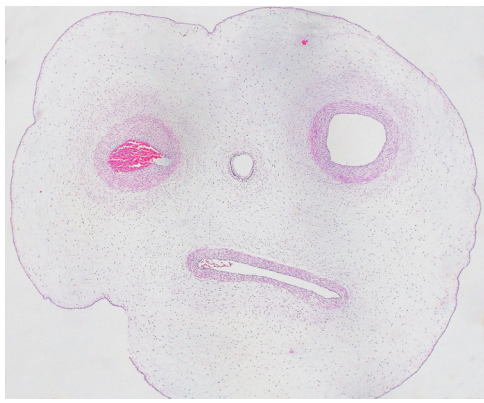

Figure 2:
A cross-sectional image of the umbilical cord. Courtesy Ed Uthman. Accessed January 8, 2019. (Available from: https://www.flickr.com/photos/euthman/3276268079/)

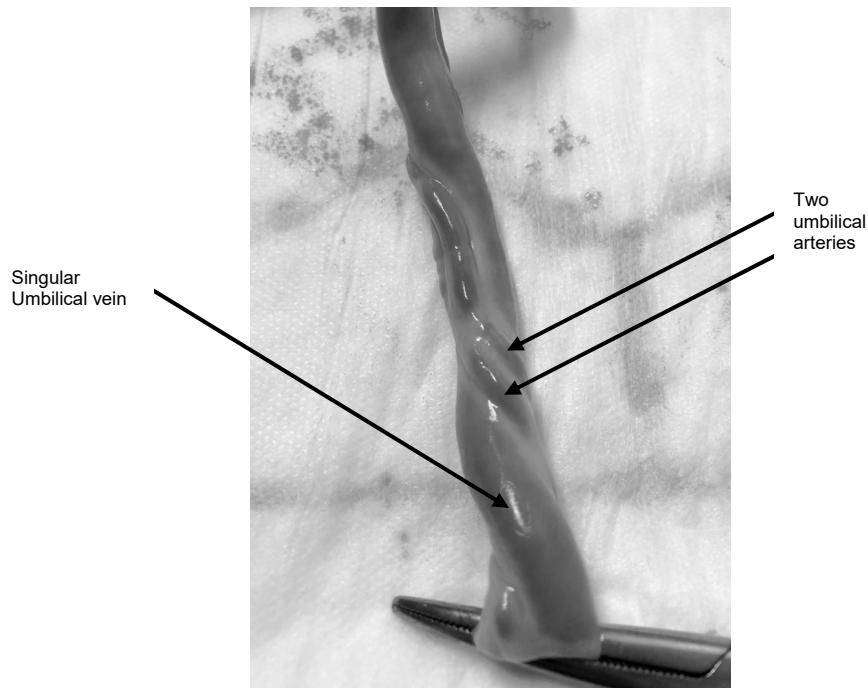

Figure 3:
Image of the umbilical cord, double clamped at a planned Cesarean Section at the Department of Obstetrics and Gynecology, Malmö. The prominent umbilical vein is seen which is crossed by two tubular umbilical arteries.

The umbilical cord vein, unlike other veins of the body, transports *oxygenated* blood to the fetal heart whilst the two umbilical arteries return *deoxygenated* blood to the placenta. In certain cases, (1/200 newborns) due to atresia or agenesis, one may come across a singular umbilical artery where the left artery is more frequently absent [7].

Cord blood sampling technique

The true acid-base status of the fetus is reflected from sampling the umbilical arteries which, as mentioned earlier, carry deoxygenated blood back to the placenta for replenishment. Cord arterial pH and base deficit (BD) have been found to strongly correlate with neonatal morbidity and mortality [8, 9].

Sampling from the umbilical vein provides information primarily on placental function but recent studies have demonstrated the value of venous cord blood variables as powerful markers of fetal acidemia [10, 11]. It is therefore important for obstetric staff to know the correct sampling technique for both the umbilical artery and vein.

Method

They are two accepted approaches. The *unclamped method* involves using an assistant nurse or additional midwife (obstetrician with cesarean section) to puncture the cord directly after birth without the use of any clamps (Figure 4). The *clamped method*, on the other hand, involves the identification of a 10 to 20 cm segment of the umbilical cord which is double clamped as soon as possible after the delivery of the baby [8] (Figure 5). Delayed umbilical cord clamping has effects on arterial and venous blood gases and lactate concentrations [12, 13, 14].

The umbilical vein is easily identified as the prominent dark blue coil in the cord. Adjacent to the umbilical vein, one can identify 2 thinly tubular, translucent coils, the umbilical arteries. With a pre-heparinized syringe, pierce the umbilical artery first and draw approximately 0.5-1 ml of blood into the syringe. Remove the needle from the cord and push out all air bubbles from the syringe. Air bubbles can lead to inaccurate results from the blood gas analyzer. Repeat the procedure with a separate pre-heparinized syringe for the umbilical vein

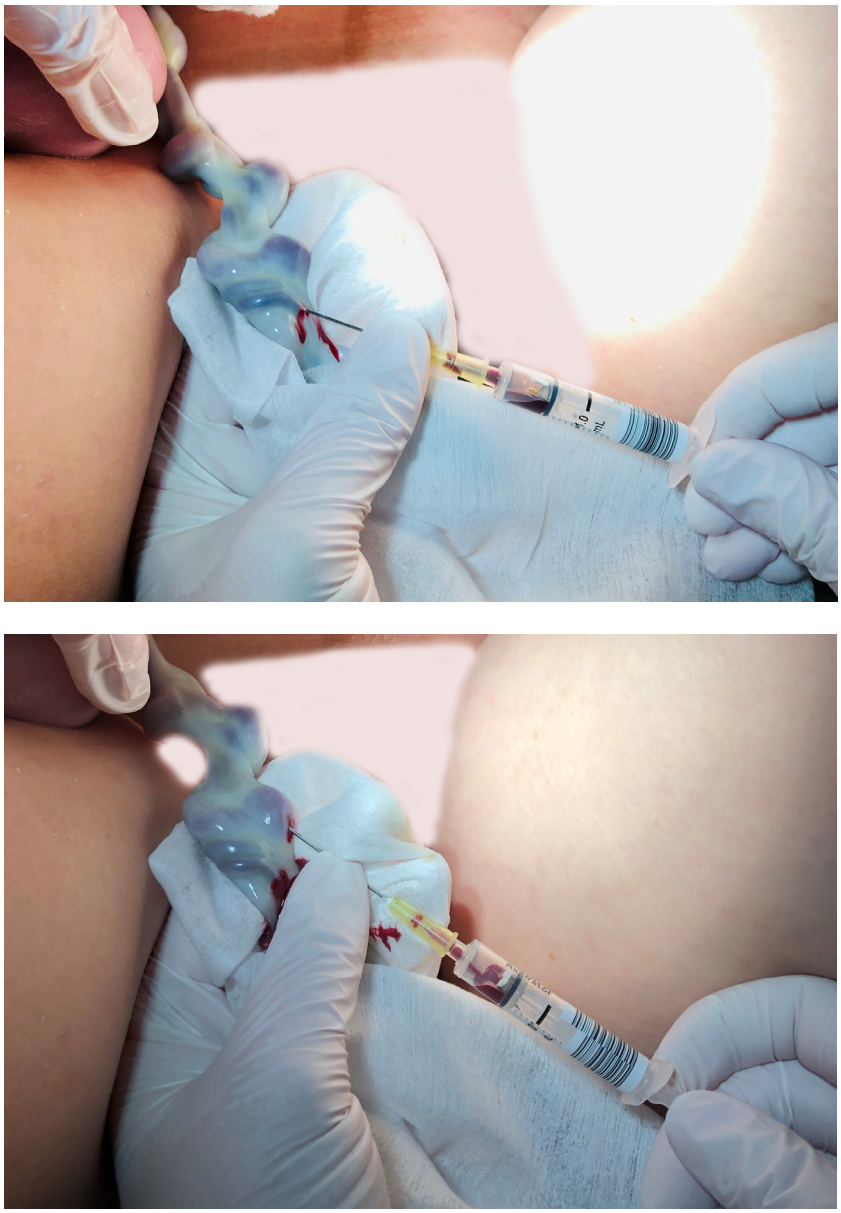

Figure 4:
Unclamped method with a vaginal delivery: First image shows the umbilical artery being sampled immediately after the birth of the baby, using a pre-heparinized syringe. The second image shows cord blood being drawn from the umbilical vein in a separate pre-heparinized syringe.

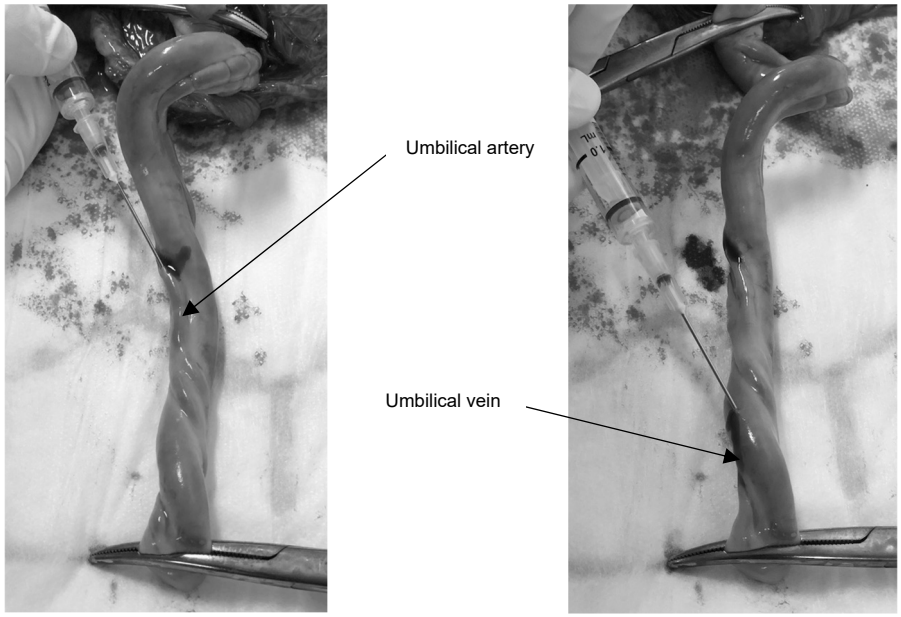

Figure 5:
Double clamped method with a planned cesarean section: A 10-20 cm section of the umbilical cord is double clamped after birth. First image shows the umbilical artery being sampled. The second image shows a cord sample being drawn from the more prominent umbilical vein.

It should be noted that commercial syringes containing lyophilized heparin are available for use (Figure 6) and these are preferable to the older method of "flushing" syringes with heparin since Kirshon and Moise [15] reported that the addition of even 0.2 mL of 10 000 U/mL of heparin with 0.2 mL of blood can significantly decrease the pH, pCO_2 and bicarbonate of the sample.

It is easy to draw a sample from the umbilical artery on the surface of the placenta, in case one has difficulty obtaining a sample from the umbilical cord [16]. In this regard, it is useful to remember that the arteries always cross over the vein on the surface of the placenta.

Once both samples are procured, strive to analyse the samples within 30-60 minutes for accurate results. In practise, the umbilical artery is usually sampled before the vein due to its smaller size and ability to collapse completely if the blood of the larger umbilical vein is drawn out first. One can confirm correct identification of the vessels by analysing the results since the umbilical artery has a lower pH and higher pCO_2 than the umbilical vein.

Figure 6:
Two pre-heparinized syringes (in this case dried heparin already packed within the syringes) are prepared before delivery of the newborn.

Fetal acid-base balance

Physiology

Fetal metabolism results in the production of acids including both volatile (carbonic acid) and non-volatile acids (organic acids). These acids are neutralized by buffers to keep the extracellular pH within a critical range. This function is vital for the optimal functioning of different fetal organ systems including the central nervous system and the cardiovascular system which would be compromised by even minute changes in pH [17]. Extreme changes in pH can be fatal for the fetus and thus the difference between maternal and fetal pH is limited to 0.05 to 0.10 units in normal pregnancies.

pH is the negative logarithm of the hydrogen ion concentration [H^+] and a measure of the degree to which a solution is acidic or alkaline. It is directly related to concentration of bicarbonate (base) and inversely related to the concentration of carbonic acid (acid). In 1916, the **Henderson-Hasselbalch equation** was first described [18] highlighting the relationship between blood pH and the components of the H_2CO_3 (carbonic acid) buffering system:

$$\mathbf{pH = pK + \log (base / acid)}$$

$$\mathbf{pH = pK + \log (HCO_3^- / H_2CO_3)}$$

$$\mathbf{pH = 6.1 + \log [HCO_3^- (mEq/L)] / [pCO_2 (mmHg)]}$$

pK is the negative logarithmic scale of any rate constant (in this case the scale of relative acid strength). The HCO_3^- represents the "metabolic" component, while the H_2CO_3 (or pCO_2) represents the "respiratory" component.

Normal adults have a pH range which is slightly alkaline from 7.35 to 7.45.

Table 1:
Important acid-base terminology

Term	Definition
Acidemia	Increased concentration of hydrogen ions [H+] in blood
Acidosis	Increased concentration of hydrogen ions [H+] in tissue
Asphyxia	Hypoxia with metabolic acidosis
Base deficit	HCO_3^- concentration below normal
Base excess	HCO_3^- concentration above normal
Hypoxemia	Decreased oxygen content of blood
Hypoxia	Decreased level of oxygen in tissue
Acid	It is a substance that can give up a hydrogen ion [H+]
Base	It is a substance that can accept a hydrogen ion [H^+]

Buffers tend to resist changes in their pH when a small amount of acid [H^+] or base (OH^-) is added. A *buffer system* comprises of a weak acid (donates the proton) and its conjugate base (acceptor of the proton).

Progesterone induced hyperventilation leads to a substantial reduction in the partial pressure of CO_2 (pCO_2) in the maternal circulation during pregnancy which in turn facilitates the diffusion of CO_2 and O_2 across the placenta. Efficient buffering ensures that there are only marginal changes in maternal arterial pH since the chronic reduction in pCO_2 with enhanced renal clearance and decreased bicarbonate concentrations can affect maternal pH. The maternal acid-base system is in a state of *compensatory respiratory alkalosis* [19, 20].

Fetal acids

Carbonic acid

Normal cellular metabolism in the fetus results in the production CO_2 and H_2O (aerobic glycolysis). Carbonic acid (H_2CO_3) is produced in erythrocytes using the enzyme *carbonic anhydrase* by the hydration of CO_2.

$$\mathbf{CO_2 + H_2O \overset{\textbf{Carbonic anhydrase}}{\Leftrightarrow} H_2CO_3 \Leftrightarrow HCO_3^- + H^+}$$

The greater the rate of metabolism, the greater will be the oxygen consumption of the fetus and consequent CO_2 production and H_2CO_3 generation. Carbonic acid in turn, readily disassociates to CO_2 and H_2O and these can easily diffuse across the placenta to the maternal circulation. As mentioned earlier, the transfer of CO_2 from the fetus to the mother is facilitated by the lower pCO_2 in the maternal circulation with a pressure gradient for CO_2 in the intervillous space of approximately 1.8 kPa.

The **Bohr** and **Haldane effects** facilitate the exchange of O_2 and CO_2 across the placenta. The "*Bohr effect*" describes the shift of the oxyhemoglobin dissociation curve to the right by $[H^+]$ which in return reduces the affinity of Hb for O_2. The "*Haldane effect*" refers to the increased capacity of deoxygenated blood to carry carbon dioxide as compared to oxygenated blood.

As CO_2 from the fetal side diffuses into the maternal circulation, there is a resulting increase in $[H^+]$ in the maternal intervillous space, which in turn reduces the affinity of maternal hemoglobin for O_2, facilitating O_2 transfer to the fetus. Simultaneously, the relative reduction of CO_2 on the fetal side causes the fetal blood to become slightly alkaline which results in the increased ability of fetal hemoglobin to pick up O_2. As the Bohr effect occurs on both maternal and fetal sides of the circulation, it has been called the "*Double Bohr effect*" (Figure 7).

Likewise, a "*Double Haldane effect*" is also seen on maternal and fetal sides of the placenta when fetal hemoglobin becomes oxygenated to release CO_2 which diffuses over to the maternal circulation and has an increased affinity to bind to maternal hemoglobin which just became deoxygenated [21]. In addition to its action at the maternal-fetal barrier, the Haldane effect has great importance out in peripheral tissue.

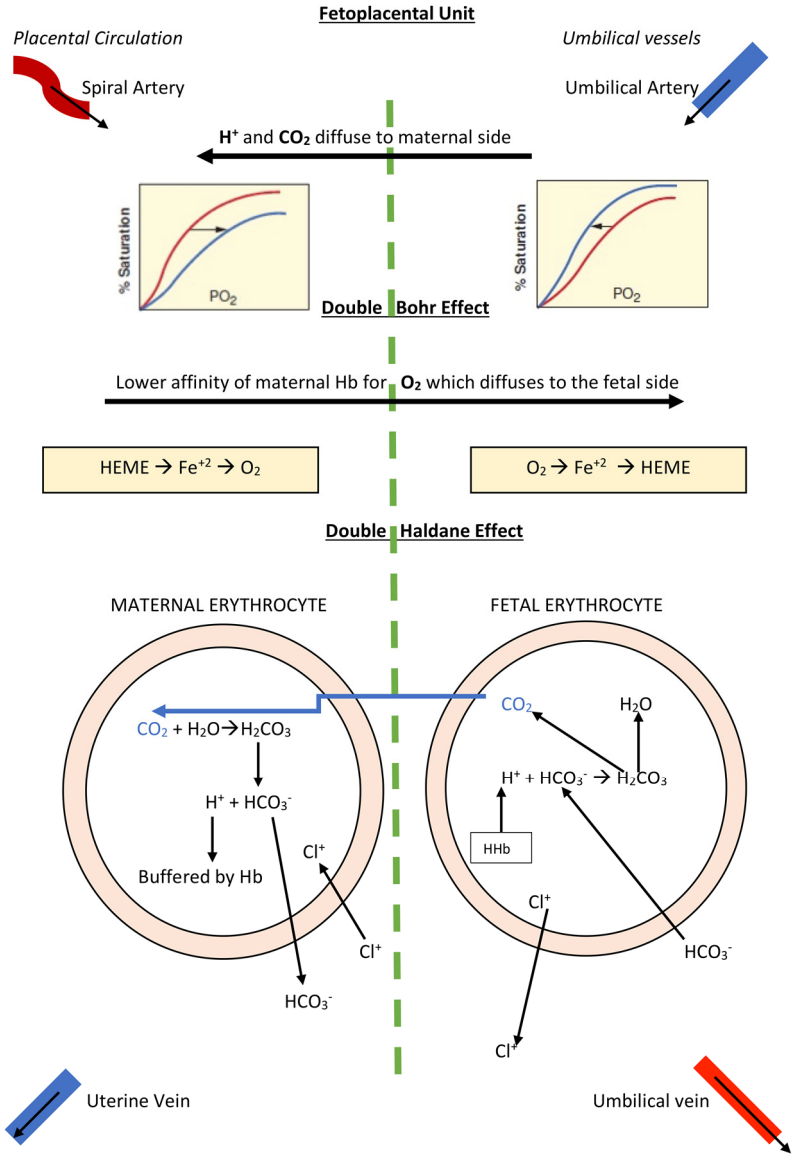

Figure 7:
The Double Bohr and Haldane effects. Picture modified from Shnider and Levinson's Anesthesia for Obstetrics, Fifth Edition [22].

The fetus also has a higher hemoglobin concentration than the mother with *fetal hemoglobin* (HbF) having a greater affinity for O_2. All these factors optimize O_2 uptake from the maternal circulation.

Organic Acids

These are produced when there is a lack of oxygenation i.e. hypoxia. Fetal hypoxia can be the result of:

- Compromised maternal oxygenation e.g. maternal respiratory disease
- Decreased placental perfusion e.g. preeclampsia, chronic hypertension, hypotension/hypovolemia
- Compromised transfer of blood from the placenta to the fetus e.g. placental abruption, cord compression

When oxygenation is compromised, the oxidative metabolism of glucose to CO_2 and H_2O is impaired leading to anaerobic metabolism that results in the production of organic acids like lactate and ketoacids [19]. Organic acids are not readily cleared via the placenta and tend to accumulate in the fetus. This accumulation of organic acids can deplete the fetal buffering systems and lead to *mixed acidemia* (respiratory and metabolic acidemia) with low pH, HCO_3^- and high pCO_2 or *metabolic acidemia* with low pH and HCO_3^- but normal pCO_2 (Table 2).

Table 2:
Types of Acidemia

Term	Definition
Metabolic	Normal pCO_2 and decreased HCO_3^-
Respiratory	Increased pCO_2 and normal HCO_3^-
Mixed	Increased pCO_2 and decreased HCO_3^-

Fetal buffering systems

The two buffers that are of greatest significance in fetal acid-base balance are *plasma bicarbonate* and *hemoglobin*. Quantitatively less important buffers include erythrocyte bicarbonate and inorganic phosphates [20].

Of all the CO_2 produced in the fetus, 5% is carried freely in plasma as carbonic acid, 20% is bound to proteins and 75% is transported as bicarbonate.

Bicarbonate is produced from CO_2 within erythrocytes using the enzyme carbonic anhydrase. The bicarbonate produced enters the plasma in exchange for chloride. The abundance of carbonic anhydrase gives the erythrocytes a high affinity for CO_2 and bicarbonate resulting in the rapid conversion of CO_2 and H_2O to bicarbonate and $[H^+]$. There is a shift of the carbonic acid equation to the right (blue arrow):

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+$$

The major component of this vital buffering system is intracellular, although extracellular carbonic acid and bicarbonate also play a significant role due to the involvement of the volatile element of CO_2. In addition to this buffering system, inorganic phosphates and various proteins such as albumin have buffering capacities as well.

Base excess

Introduced by Siggaard-Andersen and Engel [18] in the 1950s, base excess (BE) is a measure of the non-respiratory, metabolic component of acid-base balance. In 1977, Siggaard-Andersen later went on to present the Van Slyke equation for the calculation of BE [23]:

$$\mathbf{BE = [(HCO_3^-) - 24.4 + (2.3 \times Hb + 7.7) \times (pH - 7.4) \times (1 - 0.023 \times Hb)]}$$

BE is the amount of base or acid needed to restore the pH to a physiological level of 7.4 at a pCO_2 of 5.33 kPa (40 mm Hg) at 37° C (or 98.6° F). BE is thus an artificial measure that is calculated and *not* analyzed directly from the blood gas analyzer.

It can be calculated either in the blood as actual BE, or in the extracellular fluid compartment as standard BE. If BE is negative then the term *base deficit* (BD) is used instead.

Clinically, BD helps to distinguish between respiratory and metabolic acidemia. In the initial stages of impaired feto-placental circulation, hypoxemia and hypercapnia result in decreased pH but BD remains normal (respiratory acidemia). However, with sustained hypoxia, anaerobic metabolism kicks in to effect and the BD rises secondary

to lactic acidosis and buffer consumption. BD corresponds linearly to lactic acid concentrations and correlates to neonatal neurological morbidity [24].

Metabolic acidosis in obstetrics is defined as a low umbilical cord pH (usually pH <7.1, <7.05, or <7.0) with high BD (usually >12.0 or >16.0 mmol/L). A cord arterial base deficit ≥12 mmol/L (corresponding to >2 standard deviations above the mean) [25] is commonly accepted as the cut-off value for predicting an increased risk of moderate or severe newborn complications [24, 26, 27]. Values between 12 to 16 mmol/L have been found to be associated with increased infant mortality, moderate to severe neonatal encephalopathy, multiorgan failure, and long-term neurologic dysfunction [9, 26, 28, 29].

Different to adults, the hypoxic fetus could have significantly large differences in BD value when calculated in blood vs extracellular fluid. It should be noted that BD in blood (BD_{blood}) and BD in extracellular fluid (BD_{ecf}) are calculated differently using algorithms which vary from one blood gas analyzer to the next [27, 30].

The fetus has a higher hemoglobin and lower plasma protein concentration than the adult. The fetus even has a relatively larger extravascular fluid compartment where [H^+] ions accumulate after production via metabolism. Hydrogen ions generally exist outside the blood compartment and thus BD_{ecf} has been speculated to best represent fetal exposure and response to hypoxia [31].

The most frequently used BD_{ecf} algorithm is derived from the Siggaard- Andersen [23] acid-base chart:

$$\mathbf{BD_{ecf} = - 0.9149 \times [0.23 \times pCO_2 \times 10^{(pH - 6.1)} - 24.1 + 16.21 \times (pH - 7.4)]}$$

Labor

The progressive fall of maternal pCO_2 during pregnancy reaches a nadir at mean 31.3 mmHg. BD increases to -3.5 mEq/L and standard bicarbonate decreases to 21.3 mEq/L by the 3rd trimester. The pH remains unchanged [20].

When uterine contractions begin with the onset of labor, maternal blood flow to the placenta becomes intermittently strangulated once the intrauterine pressure exceeds 30 mmHg [32]. However, under normal circumstances, studies have shown that the umbilical artery blood flow is not adversely affected by uterine contractions [33, 34, 35] but all fetuses born vaginally show a fall in pH including increase in pCO_2 and BD, which is more pronounced during the second stage of labor and during delivery [36]. On the

other hand, infants born by elective cesarean section have been shown to have higher pH values reflecting the lack of a strenuous vaginal delivery [37].

Compensatory mechanisms with sustained hypoxia

A healthy fetus can withstand mild to moderate hypoxia with the help of sufficient glycogen reserves. However, sustained hypoxia leads to the accumulation of CO_2 in the blood i.e. hypercapnia. The fetal heart rate increases to speed up blood circulation to the peripheral tissues and eliminate CO_2 in addition to enhancing O_2 uptake from the maternal circulation via the Bohr effect. Fetal movements decrease followed by a redistribution of the blood circulation to vital organs such as the brain, heart and adrenal glands. The fetus valiantly tries to compensate for hypoxia and a "brain-sparing" effect comes in to force [38] but if the sustained lack of O_2 is not lifted, anaerobic metabolism predominates, leading to lactate accumulation and acidosis.

Acidosis

Definition

There is no universal agreement on the definition of acidosis with different countries reporting cut-off values from between pH 7.10 to 7.00. At Skåne University Hospital, Sweden, obstetricians and neonatologists usually use a cord arterial pH equivalent to <7.10.

Yeh et al. [39] published an observational cohort study including over 50 000 non-anomalous term infants and reported an increased risk for adverse neurological outcome at pH <7.10. Malin et al. [9] in a meta-analysis of 51 articles in which cord pH at birth was compared to neonatal or long-term outcome (over 480 000 infants), showed that the strongest association between neonatal morbidity and pH was at a pH equivalent or lesser than 7.0 [Odds Ratio (OR) 12.5; 95% Confidence Interval (CI) 6.1-25.6]. It is important to keep in mind that previous studies by our research group have clearly shown that pH decreases with gestational age and thus fixed cut-off values may not predict all cases of fetal acidosis [40].

Gestational age

It is defined as a measure of the age of a pregnancy which can be taken from the woman's last menstrual period (LMP), a known date of fertilization e.g. in vitro fertilization (IVF) or by obstetric ultrasonography. The gestational age is expressed in completed days or completed weeks (e.g. events occurring 280 days *to less than 287 days* after the onset of the last normal menstrual period are considered to have occurred at 40 weeks of gestation) [41]. The gestational age can be used to calculate the estimated date of delivery (EDD). The EDD is generally taken as 280 days from the onset of the LMP.

Naegele's rule is still used in certain parts of the world to date pregnancies. The EDD is calculated by adding a year and subtracting 3 months from the LMP and adding seven days [42]. For example: if the LMP is 2018-08-18, then the EDD will be 2019-05-25. This method, however, assumes that the patient has a 28-day menstrual cycle with fertilization occurring on day 14 and since this is not the case in many women [43], there was a need for a more accurate dating system.

The most accurate measurement of gestational age (and subsequently EDD) is obstetric ultrasonography performed before 22+0 weeks of gestation. The American College of Obstetricians and Gynecologists goes so far as to declare pregnancies "*suboptimally dated*" if they lack ultrasonographic confirmation/revision before 22+0 weeks of gestation [44].

In Sweden, free antenatal care is provided to all pregnant women. The first visit, which is also called the "**booking visit**", takes place around the 9th to 12th week of gestation. All relevant data is recorded in individual pregnancy charts during this visit including the first day of the LMP. The vast majority of pregnant women then undergo a routine ultrasound examination around 16-19 weeks of gestation by trained midwives to detect anomalies and register a gestational age based on measurement of the *fetal biparietal diameter* and *femur length* according to Persson and Weldner [45, 46].

Birth classification

A **fullterm** pregnancy in humans is 280 days or 40 weeks. **Postterm** refers to pregnancies from gestational week 42+0 onwards. According to the World Health Organization (WHO), a **preterm birth** is defined as "babies born alive before 37 weeks of pregnancy are completed". Based on gestational age, preterm births are further divided into 3 subgroups: extremely preterm (from 22+0 to less that 28 weeks), very preterm (28 to 32 weeks), moderate to late preterm (32 to 37 weeks) with late preterm birth (34 to less than 37 completed weeks) [41] (Table 3).

Table 3:
Birth classification terminology

Classification	Gestational age in weeks
Preterm	< 37 weeks
-Late preterm	34+0 to 36+6
-Moderate preterm	32+0 to 33+6
-Very preterm	28+0 to 31+6
-Extremely preterm	22+0 to 27+6
Term	37+0 to 41+6
-Early term	37+0 to 38+6
-Full term	39+0 to 40+6
-Late term	41+0 to 41+6
Postterm	≥ 42+0 weeks

Apgar score

Initially published in 1953, the Apgar score was a simple and quick method to assess the health of the newborn infant immediately after birth. Virginia Apgar (Figure 8), an American anesthesiologist, originally developed the score to quantify the effects of obstetric anesthesia on newborns [47, 48].

The scoring system comprises of five simple parameters (Table 4) summarized by the backronym: **a**ctivity, **p**ulse, **g**rimace, **a**ppearance and **r**espiration. Each parameter in allocated points from 0-2, with a minimal score of 0 and maximum score of 10 points. The Apgar score is calculated in most maternity centers at 1, 5 and 10 minutes after birth of the infant. In certain situations, for example neonatal heart-lung-resuscitation (N-HLR), the scoring system can be performed at 15, 20 and 30 minutes after birth. In such cases, the Apgar score can be used to assess the neonate's response to resuscitation.

It is famously said that "every baby born in a modern hospital anywhere in the world is looked at first through the eyes of Virginia Apgar" [49].

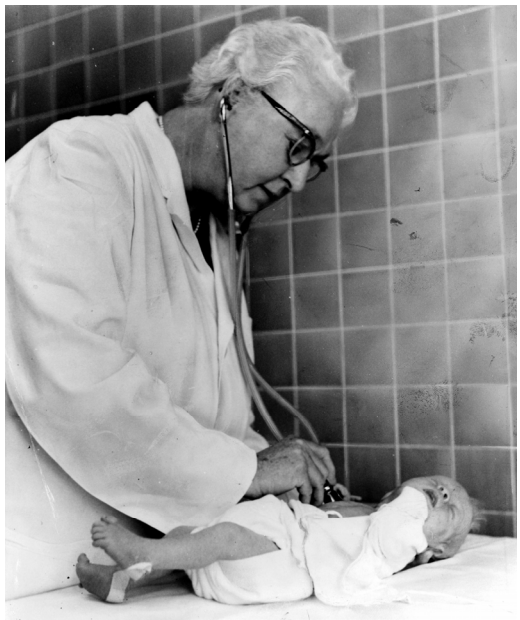

Figure 8:
Virginia Apgar, physician who founded the Apgar Score. Picture from the United States Library of Congress's Prints and Photographs division, New York World-Telegram and the Sun Newspaper Photograph Collection. Digital ID cph.3c31540.

Tabel 4:
Apgar Scoring System

Parameter		2	1	0
A	**Activity (Muscle tone)**	Active	Flexed limbs	Absent
P	**Pulse**	>100 beats per minute	<100 beats per minute	Absent
G	**Grimace (Reflex irritability)**	Prompt response to stimuli	Minimal response to stimuli	Floppy
A	**Appearence/Skin color**	Pink	Pink body, blue extremities	Cyanotic/blue
R	**Respiration**	Vigorous cry	Slow and irregular	Absent

Interpretation of the Apgar score

The Apgar score has been shown to have clinical significance in assessing the condition of the newborn immediately after birth and as a useful tool in measuring the response of the neonate to N-HLR. The 5-minute score has been found to correlate to survival in infancy [50].

The scoring system is divided into 3 ordinal categories: Apgar 0-3 *low*, Apgar 4-6 *intermediate* and Apgar 7-10 *normal* [51]. In general, neonates with normal scores are deemed to be in good health whilst neonates with low Apgar scores are in poor health and need on-going medical attention.

It is incorrect to use Apgar scores to predict long-term mortality and morbidity in neonates since, by itself, the Apgar score has little predictive value [52]. A low Apgar score cannot be used as a proxy for birth asphyxia since birth asphyxia can also result from different maternal-fetal conditions like the administration of maternal drugs, congenital anomalies, preterm birth etc. which by themselves can result in low Apgar scores at birth [53].

In an effort to highlight the limitations of the Apgar score, the American Academy of Pediatrics in conjunction with the Committee on Obstetric Practice of the American College of Obstetricians and Gynecologists, published an article titled: "*Use and Abuse of the Apgar Score*" to limit the use of the scoring system in predicting birth asphyxia and subsequent neurological outcome [54]. However, well published studies continue to underline the importance of the Apgar score by showing strong correlations of the 5-minute score with neonatal death and morbidity [55, 56].

Fetal metabolism

Metabolism relies on sufficient sources of oxygen and energy. Glucose is the primary substrate for fetal metabolism, accounting for approximately 80% of fetal energy requirements. Alternative fuels like lactate, ketoacids, amino acids and fatty acids account for the remaining 20% of fetal energy requirements [57].

The growing fetus is completely reliant on the maternal glucose pool for its own glucose supplies. Maternal glucose readily and continuously diffuses across the placenta aided by the process of "*facilitated diffusion*". The fetal glucose concentration is 0.56 - 1.11 mmol/L lower than the maternal glucose concentration which leads to a glucose gradient that aides placental transfer of glucose to the fetus [58]. Fluctuations in maternal glucose levels can be rapidly reflected in fetal glucose levels.

Maternal and fetal hormones like insulin, insulin-like growth factors (IGF), glucagon and catecholamines also regulate glucose transfer. With progressing gestational age and increasing fetal mass, the energy requirements of the fetus intensify and the placenta successfully meets these added demands by increasing placental glucose transport. There is an increase in the number of fetal glucose transporters along with a greater maternal-fetal glucose gradient via lower fetal glucose levels secondary to fetal insulin

activity. The family of proteins in charge of maternal-fetal glucose diffusion are known as glucose transporters (GLUT) of which GLUT-1 is the most abundant. GLUT-1 activity and expression is regulated by insulin, IGF and other hormones. With increasing gestational age, enhanced expression of GLUT-1 and GLUT-3 accelerate placental glucose transport. GLUT-3 has a strong affinity for glucose and thus is efficient even at low maternal glucose levels [57].

During the third trimester, extra substrate is converted to **glycogen** and stored in the fetal liver, heart and skeletal muscle. The enzymes *glycogen synthase* and *glycogen phosphorylase* regulate the storage and breakdown of this glycogen. Fetal hyperglycemia stimulates fetal glycogen deposition via glycogen synthase. Glycogen synthase is also activated by increasing insulin levels. On the other hand, fetal hypoglycemia can activate glycogen phosphorylase which in return results in the break-down of glycogen to release glucose [59].

Aerobic and Anaerobic glycolysis

Energy-rich ATP is produced by the breakdown of glucose (glycolysis). Glucose is converted to glucose-6-phosphate which is converted to pyruvate with the release of 2 ATP (Figure 9). The oxidation of pyruvate to acetyl-CoA initiates the aerobic *Citric Acid Cycle* (also known as the *Krebs cycle*).

Oxygen fuels the conversion of oxidized nicotinamide adenine dinucleotide (NAD^+) to reduced nicotinamide adenine dinucleotide (NADH) via the acceptance of a hydrogen ion [H^+]. Since the citric acid cycle is a closed loop, the last step of the pathway regenerates the starting molecule meaning that NAD^+ is regenerated when NADH transfers its electron to O_2 via the electron transport chain with the production of ATP. Each molecule of glucose yields two acetyl-CoA molecules and the net product of the citric acid cycle is 36 ATP, 6 CO_2 and H_2O.

Pyruvate can also be converted to lactate by the enzyme *lactate dehydrogenase* (anaerobic glycolysis).

$$\textbf{Pyruvate + NADH + } H^+ \xleftrightarrow{\textit{Lactate dehydrogenase}} \textbf{Lactate + } NAD^+$$

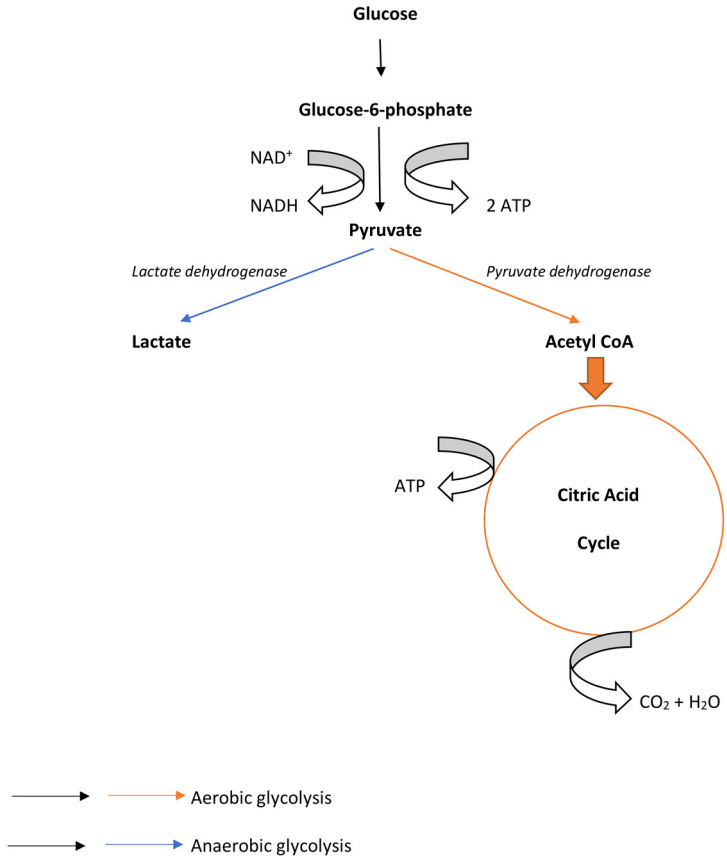

Figure 9:
Systematic illustration of Aerobic and Anaerobic glycolysis modified from Despopoulos & Silbernagl [60]

Lactate production is the only means for oxygen depleted tissues to utilize glucose for the production of ATP. Anaerobic glycolysis yields just 2 ATP molecules whilst aerobic glycolysis yields 2 ATP from glycolysis and an additional 36 ATP from the Citric Acid cycle.

Glucose changes with birth

The stress of vaginal delivery stimulates the release of fetal catecholamines which induce *glycogenolysis* in the fetal liver and the release of glucose. Increased pyruvate concentrations lead to lactate production [61]. With birth, infant glucose concentrations fall rapidly and reach minimum levels 1 hour after delivery. The neonate's regulatory mechanisms bounce into effect with a surge of catecholamines and glucagon levels accompanied by decreased insulin secretion. In addition, the fetal liver intensifies *gluconeogenesis* and *glycogenolysis* resulting in rising glucose concentrations which stabilize approximately 3 hours postpartum. Additional energy is provided via lipolysis and the production of ketone bodies and lactate which serve as alternative sources of energy [57, 62]. There is also increased expression of GLUT-3 in the brain and GLUT-4 in the muscle tissue [63, 64].

However, fetuses with small energy stores of glycogen (e.g. IUGR), hyperinsulinemia (e.g. large-for-gestational-age) or increased utilization of glucose (e.g. strenuous labor) may have an impaired metabolic transition postpartum leading to an increased risk for hypoglycemia. Such infants need close postpartum monitoring to help prevent the potential hazardous neurodevelopmental effects of severe hypoglycemia. Regulation of glucose levels in extremely preterm infants is also challenging considering their immature glucose regulatory systems and lack of sufficient glycogen reserves. These infants are highly susceptible to hypoglycemia after delivery and often need continuous glucose infusions at the neonatal intensive care units (NICUs).

Pedersen's Hypothesis

Pedersen launched his hypothesis in the 1960s and postulated that maternal hyperglycemia (in diabetic mothers) increased placental glucose supply to the fetus resulting in increased fetal insulin secretion and higher concentrations of fetal glucose and lactate. This *fetal hyperinsulinism* causes lipogenesis and macrosomia (birthweight > 4000 g). Contrary to previous belief, it was not the chronic hyperglycemia but rather the increased fluctuations in glucose levels that resulted in "glucose stimulated insulin secretion". This hypothesis explained why diabetic mothers with normal HbA_{1c} had babies with macrosomia, underscoring poorly controlled postprandial sugar levels [65].

The correlation between maternal hyperglycemia, fetal hyperglycemia and ensuing fetal hyperinsulinism is now well established [66] but is not limited to just diabetic mothers [67].

Lactic acid and lactate

Lactic acid was discovered by the Swedish chemist Carl Wilhelm Scheele in 1780 from sour milk (*lact-* Latin for milk). Lactate is the anion that results from the disassociation of lactic acid (Figure 10) and is produced in oxygen deprived tissues as a result of anaerobic glycolysis.

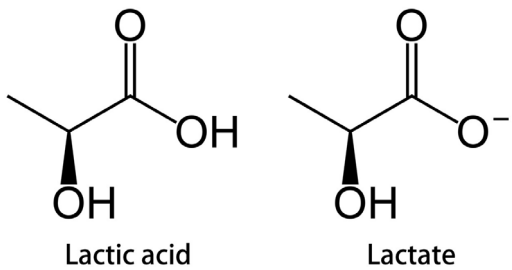

Figure 10:
Molecular structure of lactic acid and lactate

The highest concentration of lactate is in plasma and the lowest is in whole blood [68]. Lactate can be reconverted to pyruvate using the enzyme lactate dehydrogenase and the vast majority of lactate is eliminated by this reconversion. Small amounts of lactate can even be eliminated in the urine (Figure 11).

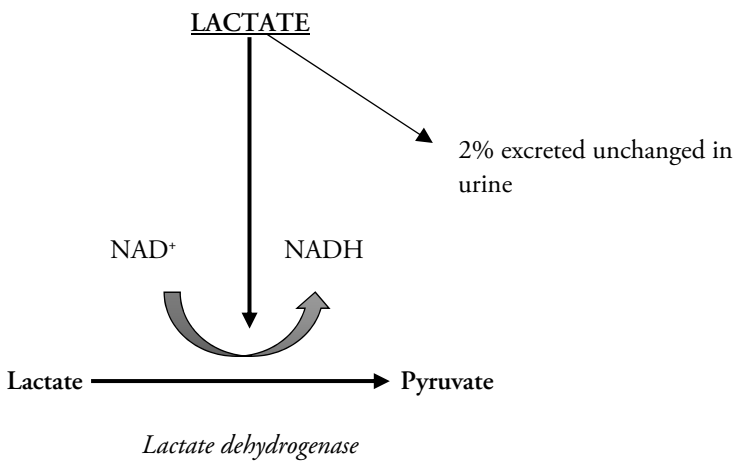

Figure 11:
Conversion of lactate to pyruvate utilizing the enzyme lactate dehydrogenase

Lactate measurement in cord blood was a welcome new alternative to assess the "oxygen-debt" of the fetus in hand with pH and BD values. Wiberg et al. [69] published gestational age-related cord blood values for arterial and venous lactate. Mean cord arterial lactate was significantly higher that cord venous lactate, suggesting the gradual development of an intrauterine *physiological lacticemia*. This was further highlighted by a significant increase in lactate levels with progressing gestational age.

During the second stage of labor, bearing down efforts together with powerful uterine contractions, exponentially increase the intra-abdominal pressure which can surpass the placental perfusion pressure leading to diminished blood perfusion to the fetus. This physiological intermittent hypoxia leads to the build-up of lactate within the fetus. Studies have shown that fetal pH is lower towards the end of the second stage of labor as compared to the first stage [70, 71]. Nordström et al. [72] in a prospective observational study measured serial fetal and maternal lactate concentrations during the second stage of labor. It was shown that there was a positive maternal-fetal lactate gradient during the first hour of the second stage of labor with lactate levels increasing more rapidly in the mother than the fetus. On average, fetal lactate increased about 1 mmol/L for every 30 minutes of bearing down. At delivery, the study even showed a positive umbilical arterial-venous lactate difference which led the researchers to speculate that fetal anaerobic metabolism was the main reason behind the fetal lactate increase.

Holzmann et al. [73] found lower scalp blood lactate levels in large-for-gestational-age (LGA) fetuses as compared to appropriate-for-gestational-age (AGA) fetuses in a series of non-reassuring cardiotocograms. This, in turn, raised the question of whether LGA fetuses have an impaired ability to produce lactate when exposed to hypoxia. The study concluded that hypoxic LGA fetuses had no impaired ability to produce lactate but since they used lacticemia as a proxy for hypoxia in the comparison of fetuses with scalp lactate >4.8 mmol/L, selection bias was introduced, and thus a difference could not be expected.

Birth weight

It is the body weight of the neonate with birth.

The natural distribution of birth weight varies according to geographical region with an average of 3553 g (grams) for infants with Swedish background and 2910 g with Pakistani origin [74]. In general, the normal range for birthweight is between 2500g to 4000g and infants with birthweight within this range are referred to as AGA. Infants

with a birthweight greater than normal are called LGA whilst infants with lower than normal birthweight are called *small-for-gestational-age* (SGA).

Large-for-gestational-age (LGA)

Definition

There is no fixed consensus but in general, an infant with a birthweight greater than the 90th percentile for gestational age is considered LGA. Certain experts recommend that this definition should be restricted only to infants + 2 standard deviations above the mean birth weight (i.e. 97th percentile) since these infants are at greater risk for neonatal morbidity and mortality [75, 76]. To illustrate the differences between these two alternative definitions, Alexander et al. [77] published national reference values for fetal growth in the United States in which infants born at 40 gestational weeks had a 90th percentile cut-off > 4000 g and those at the 97th percentile > 4400 g.

Macrosomia on the other hand, refers to excessive intrauterine growth regardless of gestational age. The prevalence of infants ≥ 4000 g is approximately 9 percent with wide variations among countries [78].

Table 5:
Grading system and incidence for macrosomia

Grade	Birthweight	Incidence in USA in percent births [79]
I	4000 to 4499 g	6.6
II	4500 to 4999 g	0.9
III	> 5000 g	0.1

For Sweden, Surkan et al. [80] reported an increase in the mean birthweight and percentage of LGA births during the period 1992 to 2001 with an estimated risk of LGA birth >23% over the 10-year study period. This was speculated to be as a result of increased maternal obesity and decreased maternal smoking.

Risk factors and Maternal diabetes

A number of genetic, intrauterine and environmental factors play a significant role in influencing in-utero *growth acceleration*. Of the maternal factors, pre-pregnancy body mass index (BMI), gestational weight gain and maternal diabetes are all prominent

causes of LGA. The Lifestyle in Pregnancy study by Vinter and colleagues [81] showed limitation in gestational weight gain in women in the lifestyle intervention group vs no intervention group but no differences in obstetric outcomes could be acknowledged.

Hyperglycemia secondary to maternal diabetes results in fluctuating glucose levels which in turn lead to fetal hyperglycemia and fetal hyperinsulinemia. As mentioned earlier, fetal hyperinsulinemia stimulates liver glycogen production and lipid synthesis with accumulation of fat in adipose tissue. This growth acceleration is particularly prominent in insulin-sensitive tissues like the liver, skeletal muscle, cardiac muscle and subcutaneous fat.

LGA associated neonatal complications

As compared to AGA, the LGA neonate has a considerably increased risk for neonatal morbidity and even mortality as compared to AGA. Among the major neonatal complications, the following are most relevant:

Birth injury

LGA predisposes infants for shoulder dystocia and birth injury including clavicular fracture and brachial plexus injury. The greater the grade of macrosomia, the greater risk for birth injury [76]. Spellacy et al. [82] demonstrated that LGA infants with a birthweight from 4500 to 5000 g had a three-fold higher risk of birth injury if they were born vaginally as compared to cesarean delivery (9.3 versus 2.6%).

Respiratory distress

It is increased due to several reasons but especially because of an increased risk of respiratory distress syndrome (RDS), particularly in cases of maternal diabetes where infants are more likely to be born prematurely. According to Boulet et al. [76] the risk of RDS was 1.15 (95% CI 1.1-1.22), 1.84 (95% CI 1.68-2.01), and 3.7 (95% CI 3.11-4.4) times greater for infants with grades 1, 2, and 3 macrosomia, respectively. The higher risk for cesarean delivery in the LGA group also results in an increased risk of transient tachypnea in the newborn. LGA fetuses have an increased risk of meconium aspiration and according to Boulet et al. [76] the risk was 1.28 (95% CI 1.23-1.34), 1.65 (95% CI 1.52-1.79), and 2.61 (95% CI 2.15-3.16) times greater for infants with grades 1, 2, and 3 macrosomia, respectively.

Hypoglycemia

After delivery, the neonate's continuing supply of glucose from the mother stops leaving glucose generation up to the neonate's own body systems. It is well known that LGA infants have an increased risk for hypoglycemia after birth, with hyperinsulinism labelled as the main culprit [67]. A study using the Perinatal Registry from the Netherlands [83] showed that the incidence of hypoglycemia was 19 and 15 percent in all LGA infants and in LGA infants of nondiabetic mothers, respectively.

Perinatal Asphyxia

LGA infants in general and LGA infants of diabetic mothers in particular, are at increased risk for perinatal asphyxia [75, 82, 84]. According to Boulet et al. [76] the risk of a 5-minute Apgar score < 3 was 1.3 (95% CI 1.21-1.39), 2.0 (95% CI 1.76-2.29), and 5.2 (95% CI 4.09-6.62) times greater for infants with grades 1, 2, and 3 macrosomia respectively. It has been speculated that the reason behind this might be due to hypoglycemia secondary to fetal hyperinsulinism as well as birth complications like shoulder dystocia.

Neonatal mortality

LGA neonates have an increased risk for death only when the birthweight >5000 g, relative risk 2.69 (95% CI 1.91-3.8) times greater than the mortality rate in AGA infants [76].

The Preterm infant

Definition

It is an infant born before 37 completed weeks of gestation. Preterm infants are also defined according to birthweight since the certainty of gestational age estimation is not reliable in all countries of the world. In such cases, all infants with birthweight less than 2500 g are considered preterm. Birthweight can, however, lead to an overestimation in the preterm rate especially in certain regions of the world such as South East Asia, where a substantial number of neonates are born SGA [85].

The global burden

The WHO estimated that 14.9 million newborns were premature as of 2010, a figure representing a preterm birth rate of 11.1% worldwide [86]. Sub-Saharan Africa and South Asia accounted for over 60% of these preterm births with an estimated 9.1 million premature births (12.8%) annually. On the other side of the spectrum, a recently published report by the National Board of Health and Welfare, Sweden [87] noted that of the 119 794 infants born in Sweden during 2016, only 5.6 % were born preterm.

With the rate of preterm birth increasing worldwide, the associated neonatal morbidity and mortality is also increasing. Complications due to preterm birth continue to be the leading cause of death among children under 5 years of age, leading to 1 million deaths during 2015 [88].

Advances in perinatal medicine, with modern NICUs, has led to increased survival even in **extremely premature infants** i.e. born before 28 completed weeks of gestation. The Extremely Preterm Infants in Sweden Study (EXPRESS) (Figure 12) concluded that in Sweden, the incidence of extreme prematurity (infants born < 27 completed weeks of gestation) was approximately 3.3 per 1000 infants and the overall perinatal mortality 45% (from 93% at 22 weeks to 24% at 26 weeks) [89]. All-in-all, 1-year survival in liveborn infants from 22+0 to 26+6 weeks of gestation was 70%. This is an outstanding figure by any international standards, however, these infants often need prolonged care in NICUs and their recovery is marred by numerous short-term and long-term complications as listed in Table 6.

Tabel 6:
Prematurity and some of its complications [90-94]

Parameter	Outcome
Respiratory problems	Respiratory Distress Syndrome (RDS) Apnea Chronic Lung disease of prematurity
Cardiovascular problems	Persistant ductus arteriosus (PDA) Pulmonary Hypertension Low blood pressure short-term Increased blood pressure later on in life
Body temperature regulation	Lack of subcutaneous fat deposits and increased body surface area as compared to body volume lead to problems in temperature regulation
Central Nervous System	Intraventricular bleeding Periventricular leucomalacia (PVL) Long-term hearing impairment Blindness and myopia
Neurodevelopmental delay	Mild to severe disorders including cognitive, motor issues Cerebral palsy
Gastrointestinal system	Necrotizing enterocolitis (NEC) General problems in absorption of nutrition

Umbilical cord blood gases in extremely premature infants

The EXPRESS noted that of all surviving children born extremely preterm with active perinatal care, approximately 73% had mild or no disability with neurodevelopmental assessment at 2.5 years of age [95]. Other studies indicate that up to one third of survivors will suffer from neurodevelopmental sequelae [96, 97]. Physicians are unsure how to best counsel parents about the neurodevelopmental future of these very tiny babies.

Whilst cord blood gases and the diagnosis of perinatal asphyxia are strong predictors of death and/or neurodevelopmental outcome in term infants, few studies address this issue in extremely premature infants. Randolph et al. [98] in a retrospective study of extremely low birthweight infants, defining cord blood acidosis as pH < 7.00 or BE < -12, found that both pH and BE associated significantly with death/neurodevelopmental sequelae [OR=2.5 (CI: 1.6, 4.2); and OR=1.5 (CI: 1.1, 2.0) respectively]. However, the study was based on birthweight and not on gestational age. In addition, very little is known about the maturation of the extremely premature infants' acid-base systems and thus it remains to be seen if cord blood gases can help predict long-term neonatal outcome in this high-risk group.

Figure 12:
Courtesy EXPRESS study. Accessed 10 January 2019. Available at: http://express-study.se/wp-content/uploads/2013/09/Håller-tumme.jpg

Perinatal Asphyxia

For the parents involved as well as the practicing obstetrician, perinatal asphyxia is a catastrophe of epic proportions. Delivering a neonate that has undergone severe and prolonged lack of oxygen during birth is an alarming situation which can have potentially life-threatening consequences. Thankfully, Scandinavia enjoys some of the lowest rates of perinatal asphyxia 0.5-0.6% (defined as pH < 7.00 and Apgar < 7 at 5 minutes [99] alongside some of the best documentation in its birth registers in the world [100].

Asphyxia is broadly defined as a condition of impaired blood exchange which leads to progressive hypoxemia and hypercapnia [101]. A number of risk factors have been identified (Table 7) of which IUGR seems to play the strongest role in antepartum factors [102] [Relative risk (RR) 38.2, 95% CI 9.4-154.8] whilst intrapartum emergencies, such as placental abruption or uterus rupture, can led to a fourfold increase [103].

Tabel 7:
Risk factors for for perinatal asphyxia [102, 103, 104]

Risk factors		Conditions
Antepartum	Maternal	Maternal age and unemployment Family history of neurological disorder Thyroid disease Moderate or severe bleeding during pregnancy
	Placental	Severe preeclampsia Postterm pregnancy (>41 gestational weeks) Placental lesions: trombosis, inflammation etc.
	Fetal	Intra-uterine growth restriction (IUGR) LGA
Intrapartum	Persistent occipitoposterior position	
	Shoulder dystocia	
	Emergency cesarean section preceded with or without failed vacuum extraction	
	Operative vaginal delivery	
	Intrapartum emergencies: uterine rupture, placental abruption, cord prolapse, maternal shock or death	
	Inflammatiory conditions: maternal feber, chorioamnionitis, prolonged rupture of membranes	

The fetal brain is especially vulnerable to hypoxia-ischemia which results in energy loss of mitochondrial function. This subsequently leads to membrane depolarization, brain edema, increased intracellular calcium and other pathological cascade reactions including the production of reactive oxygen and nitrogen species [105, 106]. When perinatal

brain injury is suspected to stem from a hypoxic-ischemic event, clinicians use the term "hypoxic ischemic encephalopathy" (HIE) to describe the condition.

Hypoxic ischemic encephalopathy (HIE)

With an incidence between 2-9 per 1000 term births [107-110], the neuronal injury associated with HIE develops in two phases. There is an initial *ischemic phase* that is characterized by cell death in areas of poor blood perfusion. This is followed after 2-6 hours by a more damaging *reperfusion phase* which is characterized by apoptotic cell death and extension of the affected areas [111-113].

According to Sarnat and Sarnat [114] HIE can be classified into three stages according to the degree of clinical signs and symptoms:

Stage I (Mild) lasts <24 hours after birth and is characterized by hyper-alertness, uninhibited Moro and stretch reflexes, sympathetic effects but the electroencephalogram is normal.

Stage II (Moderate) is characterized by obtundation, hypotonia, strong distal flexion and multifocal seizures. Electroencephalography (EEG) shows a periodic pattern that can sometimes be preceded by continuous delta activity.

Stage III (Severe) in which infants are stuporous, flaccid with suppressed brain stem and autonomic functions. The EEG is isopotential or has infrequent periodic discharges.

It is important to realize that the above-mentioned signs and symptoms are observed only once the neurological injury has already been established. The permanent neurological handicap caused by moderate to severe HIE requires substantial monetary resources from the affected family and the society in general to finance long-term rehabilitation, physiotherapy etc. [115]. Whilst stage moderate to severe HIE are strongly associated with long-term neurological morbidity and mortality [109, 116-120] treatments like *therapeutic hypothermia* have been shown to reduce death and disability if initiated within a narrow therapeutic window of 0-6 hours after delivery [121, 122]. In fact, hypothermia is the *only* existing therapy available for infants with HIE which underlines the critical importance of early diagnosis and subsequent treatment of all infants suspected to have suffered from a hypoxic-ischemic event during birth.

Fetuses exposed to prolonged hypoxia at birth may show one or more combinations of the following: a 5- and 10-minute Apgar score <5, seizures, pathological EEG, cord blood acidemia with arterial pH <7.10 or 7.00, base deficit ≥12 mmol/L or high initial

lactate levels. Unfortunately, the mentioned biochemical and clinical measurements have been shown to have poor predictive value [123-126] which has led researchers to explore potential neurological biomarkers of hypoxic brain injury.

Biomarkers of hypoxic brain injury

Latest developments in the field of hypoxic brain injury include the hunt for potential *biomarkers* of neuronal damage. These biomarkers can be detected in a variety of neonatal fluids including cord blood, urine, cerebrospinal fluid (CSF), saliva etc. Of these, cord blood has been hailed as the ultimate "holy grail" of such sources since it can be sampled directly at the time of birth, giving obstetricians and neonatologists the opportunity to diagnose HIE as well as initiate therapy well within the 6-hour therapeutic window (personal communication, Dr. Ronald Hayes: 7th International conference of Biomarkers for Brain Injury, Lund Sweden 2015).

A number of different biomarkers have been studied including S-100B, glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase-L1 (UCH-L1), neuron specific enolase (NSE), tau protein, miRNA, lactate dehydrogenase (LDH) and different cytokines among many others. With regard to the current thesis, the following proteins were of particular interest:

S100B

Discovered by Moore in 1965, S100B is a member of the S100 protein family. The protein derived its name from its solubility in 100% ammonium sulphate at neutral pH [127]and is localized intracellularly across a wide range of cells. It has alpha (α) and beta (β) subunits and it was the β-subunit which was thought to be brain-specific for several years but was later shown to be present even in non-neuronal tissues [128].

S100B is a calcium binding protein and is concentrated in the astrocyte cells of the central nervous system (CNS). Studies found increased levels of S100B in a variety of biological fluids in association with severe CNS damage and normal S100B concentrations were found to reliably exclude major CNS trauma. The excellent negative predictive value of S100B as well as the relative ease and reproducibility of its measurement including a short half-life of 1-hour which made longitudinal monitoring possible; all made S100B an ideal biomarker for brain injury [129]. This in turn, inspired researchers to investigate the potential role of S100B in the diagnosis of *perinatal* brain injury.

As mentioned earlier, the diagnosis of perinatal asphyxia relies heavily on a combination of documented obstetrical and medical factors in combination with laboratory and radiological evidence in addition to clinical assessment. A biomarker that directly indicated neuronal cell damage, such as S100B, would be critical in the correct identification and management of such cases at a time when radiological evidence was still silent.

S100B is detectable in cerebrospinal fluid, amniotic fluid, blood, saliva and urine of fetuses/neonates at risk for perinatal asphyxia [130]. Initial adult-studies showed the usefulness of the protein to detect the occurrence and the extent of traumatic brain damage [131, 132]. Later perinatal studies confirmed that S100B concentrations were increased in blood 48-72 hours before any clinical, laboratory or radiological signs of intraventricular hemorrhage (IVH) became apparent in preterm infants [133] or HIE in full-term infants [134, 135].

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1)

UCH-L1 is a cytoplasmic enzyme that is highly specific to neurons and neuroendocrine cells [136, 137]. It is found abundantly in neuronal tissue and averages 1-5% of the total soluble brain protein. UCH-L1 was used previously as a histological marker of neurons [138]. During normal and pathological conditions, UCH-L1 has been found to play an important role in cellular protein degradation and is involved in the removal of excessive, oxidized or misfolded proteins [137, 139]. In a pilot study, Douglas-Escobar et al. [140] explored the relationship of serum UCH-L1 taken from HIE neonates against the risk for neurodevelopmental outcome later in life. The study demonstrated an early increase in UCH-L1 levels already at 0-6 hours postpartum in HIE cases which was associated with affected motor development later on in life.

Glial fibrillary acidic protein (GFAP)

GFAP is a cytoskeleton intermediate filament protein specific to astrocyte cells and released in to the blood stream after ischemic cell injury and the eventual disruption of the blood-brain barrier (BBB) [141, 142]. It is functionally involved in regeneration and gliosis, thus increasing concentrations are released in to the blood steam with brain injury [143]. Several studies have shown elevated levels of GFAP in the serum samples taken from neonates suffering from HIE [144, 145]. Chalak et al. [146] investigated the role of several proteins in a cohort of neonates suffering from encephalopathy with hypothermia treatment. They identified GFAP as the only serum biomarker at birth that correlated significantly with other indicators of birth depression and multiple

organ dysfunctions. The study reported that GFAP concentrations remained significantly higher in neonates with HIE II-III vs HIE I at different time points after birth.

However, very few studies have explored GFAP in cord blood. Ennen et al. [145] compared cord blood GFAP concentrations in HIE stage II-III neonates with healthy controls and found no differences. However, samples obtained 6 hours after birth showed significantly higher GFAP concentrations in HIE cases than controls signalling the possible later onset of GFAP release as compared to other biomarkers.

2. Aims and Objectives

The overall aim of this thesis was to establish the value of umbilical cord blood sampling at birth as well as to investigate the possible connection between different analytes within cord blood, to the risk of death or neurodevelopmental outcome both in the neonatal period as well as later on in life.

Specific objectives:

I. To define umbilical cord arterial and venous pH reference values in preterm, term and postterm deliveries. To define reference values for Apgar score in preterm, term and postterm deliveries.

II. To study changes in umbilical cord glucose concentrations in AGA and LGA newborns of mothers with and without diabetes in non-acidemic and acidemic conditions. To study changes in umbilical cord lactate concentrations in AGA and LGA newborns of mothers with and without diabetes in non-acidemic and acidemic labor.

III. To investigate neurological outcomes at 6.5 years of age of birth hypoxia in infants born between 22+0 to 26+6 weeks of gestation.

IV. To investigate the relationship of GFAP and UCH-L1 concentrations in the umbilical cord blood of asphyxiated newborns to the risk of hypoxic ischemic encephalopathy, severity of disease and death/neurodevelopmental outcome as compared to healthy controls.

V. To investigate the relationship of S100B concentration in the umbilical cord blood of asphyxiated newborns to the risk of hypoxic ischemic encephalopathy, severity of disease and death/neurodevelopmental outcome as compared to healthy controls.

3. Materials and Methods

If there was no individual variability, medicine would be a science not an art.

Sir William Osler 1849-1919

Umbilical cord blood samples

The maternity centre at Skåne University Hospital in Malmö (Kvinnokliniken), initiated the routine sampling of umbilical cord blood at birth in Sweden back in August 1981. Skåne University Hospital in Lund joined in soon afterwards and thus cord blood gas data from both Malmö and Lund can be considered epidemiological and representative of the population for this region.

Midwives and nursing staff were trained in the proper technique to obtain cord blood gases. They were instructed to puncture, first the umbilical artery, and then the umbilical vein immediately after the birth of the newborn. Blood was to be collected in 2 mL pre-heparinized syringes and this blood was to be analyzed within 15 minutes for optimal results. During the study period, as per local tradition, midwives in Lund extracted samples without clamping the umbilical cord whilst in Malmö, midwives would double-clamp the cord prior to sampling. In cesarean deliveries, the umbilical cord was double-clamped at both centres. The data obtained from these cord blood samples was used in *Study I* and *II*.

Radiometer ABL

New blood gas analyzers were installed in Malmö and Lund Maternity Centres in the year 2000. The Radiometer ABL 735 (Radiometer A/S, Copenhagen, Denmark), replaced the older ABL 520 model. This machine was a comprehensive analyzer with optional wet chemistry in addition to blood gas determinations. For example, the

analyzer measured lactate, glucose, fetal hemoglobin (HbF), total Hb, pH, pO_2 and pCO_2 among other analytes.

In the analyzer, the term "electrode" referred to a sensory unit with both electrode and electrode jacket which held the electrode solution and membrane [147]. The analyzer employed two different measuring principles for electrodes:

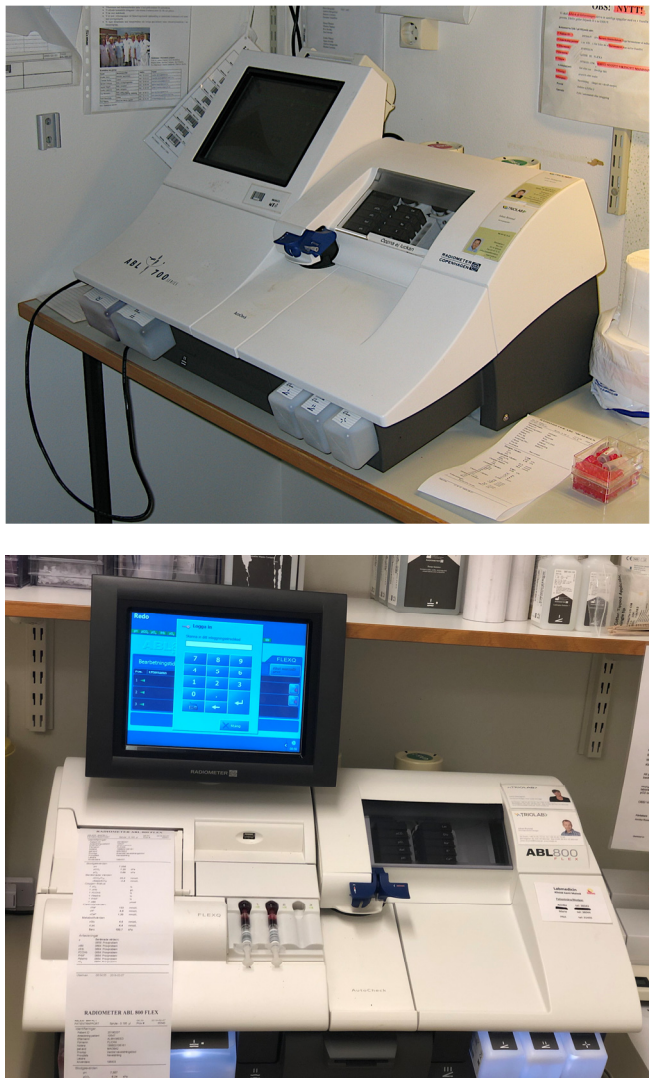

Figure 13:
Radiometer ABL. First image: ABL Model 700. Second image: ABL Model 800 in use currently at the Maternity Center, Skåne University Hospital, Malmö.

Potentiometry

This was based on the principle that the potential of an electrode chain was recorded using a voltmeter and related to the concentration of the sample. This measuring principle was applied for calculating pH, pCO_2 and electrolyte electrodes for potassium, sodium, calcium and chloride.

Amperometry

Used the principle that the magnitude of an electric current flowing through an electrode chain was in turn proportional to the concentration of the substance being oxidized or reduced at an electrode in the chain. This measuring principle was applied for calculating pO_2, glucose and lactate electrodes.

All analyzer results could be printed out but they were also automatically stored in each individual analyzer hard disk. These results were exported to an external database for statistical analyses. During the period 2000-2010 we compiled a database comprising of approximately 80 770 samples. Of these, 32 287 women had paired arterial and venous cord blood samples. This database was used in *Studies I* and *II.*

It is also pertinent to mention that the Radiometer reported pH and pCO_2 values using 3 decimal places (e.g. pH=7.324). Two decimal places were used for pO_2 values (e.g. pO_2=9.20) and only one decimal place for BD, lactate and glucose (e.g. lactate=1.7) [147]. In addition, the origin of the sample, labelled "cord artery" or "cord vein" needed to be entered manually by the person running the sample. The analyzers were routinely calibrated by trained biomedical analysts.

Clinical obstetric and neonatal data

Data on obstetric and neonatal outcome was collected from a regional computerized database, the Perinatal Revision South Register (PRSR) [148]. Participating hospitals included the eleven southern-most maternity units in Sweden. Specific protocols were designed for obstetric care, neonatal care and autopsy findings. Data collection was started on September 1, 1994. It was estimated that the PRSR received data on about 20 000 deliveries annually. Unfortunately, the PRSR has been dismantled as of December 31, 2015. Data obtained from the PRSR was used in *Study I* and *Study II*.

The Extremely Preterm Infants in Sweden Study (EXPRESS)

With recent advances in perinatal and neonatal medicine, the limit of survival is successively decreasing leading to an increasing number of extremely preterm infants being born today [149]. With this background, the EXPRESS was founded with the overall aim to study mortality as well as short-term and long-term morbidity in extremely preterm infants (Figure 14).

Figure 14:
Official logo of the Express. Accessed 5 January 2019. Available from: www.express-study.se

Participants

This national population-based, prospective study comprised of all infants born at 22+0 to 26+6 weeks of gestation between April 1, 2004 and March 31, 2007 from all the seven Swedish healthcare regions. Perinatal and neonatal data was collected prospectively by designated research coordinators (one obstetric and one pediatric coordinator) at each healthcare region. Gestational age was based on ultrasound dating in 95% of pregnancies. Live-born infants and stillbirths were defined according to the recommendations of the World Health Organization [150]. During the three-year study period, 1011 extremely preterm infants were born in Sweden before 27 completed gestational weeks. Of these, 707 were live-born (70%) and 304 stillborn (30%) [89]. *Study III* derived its cases from the participants of the EXPRESS.

Umbilical cord serum bank

Between 1969 and 2001, the maternity unit at Skåne University Hospital in Malmö had routinely collected maternal venous and umbilical cord blood. Mixed arterial and venous cord blood was collected by passive drainage into test tubes. The test tubes were refrigerated overnight at + 8 °C for sedimentation. The supernatant serum was collected the following morning and frozen in polypropylene plastic test tubes at – 20 °C until analysis. Several thousand serum samples lie stored away in this serum bank and have been used for several previous studies. All samples were labelled with the mother's personal number and put in boxes sorted according to the date of delivery. This serum bank was used for *Study IV* and *V*.

Descriptive Statistical Methods

Simple linear regression analysis

The association between a numerical outcome and a numerical exposure can be illustrated using simple linear regression analysis. It is used to estimate the best-fitting straight line to describe this association and only one exposure variable is considered. It also provides an estimate of the correlation coefficient, which measures the closeness (strength) of the linear association.

If y is the outcome and x is the exposure variable; linear regression gives the straight line that best describes how y increases or decreases with an increase in x. The regression line equation is:

$$\boldsymbol{y = \beta 0 + \beta 1\, x}$$

In the equation, β is the Greek letter beta and $\beta 0$ and $\beta 1$ are the parameters or regression coefficients. $\beta 0$ is the intercept (the value of y when x=0) and $\beta 1$ the slope of the line (the increase in y for every unit increase in x).

Two assumptions are made when performing linear regression. Firstly, for any value of x, y is normally distributed. Secondly, the magnitude of the scatter of the points about the line is the same throughout the length of the linear regression line.

The strength of the linear association between the outcome and the exposure variable is measured by the ***correlation coefficient, r***. This is always a number between -1 and

+1. If *r* equals to zero, it shows that the variables are not associated. Positive *r* values show that *x* and *y* tend to be high and low together and a value close to the maximum value of 1, shows a very strong association. When illustrated on a scatter plot, all the points would then lie exactly on the straight line in this case. The *P* value is used to describe the probability that there is a linear relationship in regression analysis.

Multiple regression analysis

It is used to examine the dependency of a numerical outcome variable on several exposure variables. Multiple regression analysis can be carried out using any number of exposure variables.

Considering the effect of two exposure variables (*x*1 and *x*2) on an outcome variable (*y*), the multiple regression model will be:

$$\boldsymbol{y = \beta 0 + \beta 1\, x1 + \beta 2\, x2}$$

Intercept β0 is the value of the outcome *y* when both exposure variables *x*1 and *x*2 are zero.

Polynomial regression analysis

Polynomial regression is a form of regression analysis in which the relationship between the independent variable *x* and the dependent variable *y* is demonstrated as an *n*th degree polynomial in *x*.

It fits a nonlinear relationship between the value of *x* and the corresponding conditional mean of *y*, denoted E (*y* |*x*), and has been used to describe nonlinear phenomena such as the progression of disease epidemics. It is considered to be a special case of multiple linear regression.

Kendall rank correlation coefficient (tau)

It is used to establish whether two variables may be regarded as statistically dependent. The Kendall rank coefficient is non-parametric because it does not rely on any assumptions on the distributions of *x* or *y* or the distribution of (*x*, *y*).

Values between -1 to 1 are reported using the tau (τ), where 0 refers to no relationship between the variables and 1 is a perfect relationship.

Kendall's tau has a number of advantages over other non-parametric rank tests [e.g. Spearman's rank correlation coefficient (rho)] including the following:

- The distribution of Kendall's tau has better statistical properties.
- The interpretation of Kendall's tau in terms of the probabilities of observing the agreeable (concordant) and non-agreeable (discordant) pairs is very direct.
- In most of the situations, the interpretations of Kendall's tau and Spearman's rank correlation coefficient are very similar and thus invariably lead to the same inferences.

Mann-Whitney *U* test (M-W test)

It is also known as the "Wilcoxon rank sum test" and is a non-parametric test used to assess whether an outcome variable differs between two exposure groups i.e. if the median difference between pairs of observation from the two exposure groups is equivalent to zero. No assumptions are made regarding the normal distribution of the population and thus the M-W test is regarded as the non-parametric counterpart of the *t*-test.

The M-W test makes the following assumptions:

- Sample is randomly drawn from the population.
- Each observation within a sample is independent and cannot be in the other group.
- Ordinal measurement scale is presumed.

The *P* value describes the difference between the mean rank of the two exposure groups and the sample sizes. The test is robust for outliers and is suitable for small sample sizes. According to Mundry and Fisher [151], the permitted threshold for sample sizes (N) is N_1= 3 or 4 and N_2 >12; N_1=>4 and N_2 >10.

Kruskal-Wallis test (K-W test)

The Kruskal-Wallis test, or "one-way ANOVA on ranks", is another non-parametric test for checking whether samples originate from the sample distribution. It is similar to the M-W test but is used for comparisons between three or more groups and is considered to be the non-parametric alternative to the one-way ANOVA.

When the K-W test is significant, the only information obtained is that at least two groups are different and it cannot specify which groups of the independent variable are statistically significant from each other and the Mann-Whitney *U* test can help in distinguish the groups from one another.

The assumptions made include:

- Samples are drawn randomly from the population.
- Observations are independent of each other.
- Measurement scale for the dependent variable is ordinal.

Chi-squared test (χ^2 test)

This test is used to determine whether there is a significant difference between the expected and observed frequencies in one or more categories. The greater the differences between the observed and expected values, the larger will be the value of χ^2.

$$\chi^2 = (\text{observed-expected})^2/\text{expected}$$

It should be noted that the χ^2 test is valid when the total number is more than 40, regardless of the individual expected values. It is also valid when the total number is between 20 and 40, provided all the individual expected values are greater or equivalent to 5.

Fisher's exact test

It is a test of significance and compares two proportions when the numbers in the contingency tables are very small. However, the test is valid for all sample sizes.

McNemar test

The McNemar's test is a chi-squared test based on the number of discordant pairs which should be at least 10. In other words, the method is used to determine differences on a dichotomous dependent variable between two related groups. This test is commonly used to analyse matched pairs and case-control studies.

Transformations

All data sets do not satisfy the assumption of normality, for example, a distribution can be positively skewed referring to the different standard deviations in different groups within the data set. Similarly, the relationship between an outcome and an exposure variable may not be linear thus negating the assumptions of linear and multiple regression analyses. Such issues can be overcome by using transformations to change (or transform) the data to a different scale of measurement.

Logarithmic transformation is the commonest form of transformation in which each individual value within a variable is replaced by its logarithm.

$$u = log\ x$$

x= original value, u= transformed value

Different types of transformations can be used in different situations. For example, with a positively skewed distribution one can use logarithmic transformation, but in the case of negatively skewed distributions, square ($u = x^2$) or cubic ($u = x^3$) versions are used.

Analysis of Covariance (ANCOVA)

ANCOVA is used in examining the differences in the mean values of the dependent variables that are related to the effect of the controlled independent variables while considering the influence of the uncontrolled independent variables.

t test

This test compares the means of two unmatched groups and assumes that the values follow a normal (Gaussian) distribution. The *t* test is sometimes also known as the "unpaired *t* test" or the "student's t-test" to distinguish it from the "paired *t* test" which is utilized for paired measurements.

Receiver Operating Characteristic (ROC) curve

When binary classifications come from a numerical variable, utilizing a cut-off value, the performance of different cut-off values can be judged using a ROC curve. The ROC curve is a plot of the sensitivity against 1 – specificity, for different cut-off values. The

area under the ROC curve would yield an area of 1 if the cut-off value had 100 % sensitivity and 100 % specificity.

Logistic regression

Logistic regression was developed by statistician David Cox in 1958 [153] and is most commonly used for the analysis of binary outcome variables. It can be used in a number of settings including: to examine the effect of a single exposure variable, to compare a binary outcome variable between two exposure (or treatment) groups, to compare more than two exposure groups and to examine the effect of an ordered or continuous exposure variable.

Logistic regression provides a flexible means of analysing the association between a binary outcome and a number of exposure variables. Unlike linear regression, logistic regression does not look at the relationship between the two variables as a straight line. Instead, it uses the natural logarithm function to elucidate the relationship between the variables and utilizes test data to find the coefficients. Using the logistic equation, the function can then predict the future results using these coefficients.

[Descriptive statistical analyses explained with the help of **Essentials of Medical Statistics by BR Kirkwood** [152]]

Ethical Approval

Study I, II, IV and *V* were approved by the Regional Research Ethics Committee in Lund, Sweden (Dnrs 2009/222 and 2012/5) and *Study III* (Dnr 2004/42 and 2009/524).

Paper-specific Materials and Methods

Study I

Data was collected from the routine sampling of umbilical cord blood gases at Malmö and Lund Maternity Centres. These samples were collected from March 2001 to July 2010 in line with established routines which were outlined earlier in this chapter.

All analyses were transferred electronically from the analyzers hard drives to a computerized statistical program (STATVIEW® version 5.0.1, SAS Institute, Cary, NC, USA). Prerequisites for inclusion in the study comprised of the following: all blood samples could be identified without doubt in the analyzers' hard drive by their unique maternal personal identification number, site of sampling (arterial, venous), place of origin (labor and delivery ward), and time and date of analysis. In addition, all samples where the analyzer quality check system reported an error for poor calibration, temperature error, electrode instability, air bubbles at the electrode etc, were excluded from the study. These strict criteria ensured good data quality in our database.

We discarded all samples that could not be identified as originating from umbilical cord blood, unpaired arterial-venous samples, or paired samples not fulfilling the validation requirement that cord venous pH (VpH) should be at least 0.02 units higher than cord arterial pH (ApH) [154]. Lastly, all included samples needed matching clinical data which was retrieved using maternal personal identification numbers and cross-referencing data from the regional Perinatal Revision South Register [148].

In total, we could identify 27 175 cases. Figure 15 describe the exclusion of cases to the final series of 18 584 newborns included in the statistics. The study population represented 35.9% of the total of 75 793 deliveries during the period.

Statistical analyses

Associations between continuous variables were investigated with simple linear and polynomial regression analyses. To minimize the influence of outliers, we also used weighted linear regression analysis when appropriate. The possible influence of more than one independent variable was investigated with multiple regression analysis. Median values and percentiles were calculated with the weighted average method for robustness against outliers. When skewed distributions appeared, logarithmic and exponential transformations were performed to gain the best of fit.

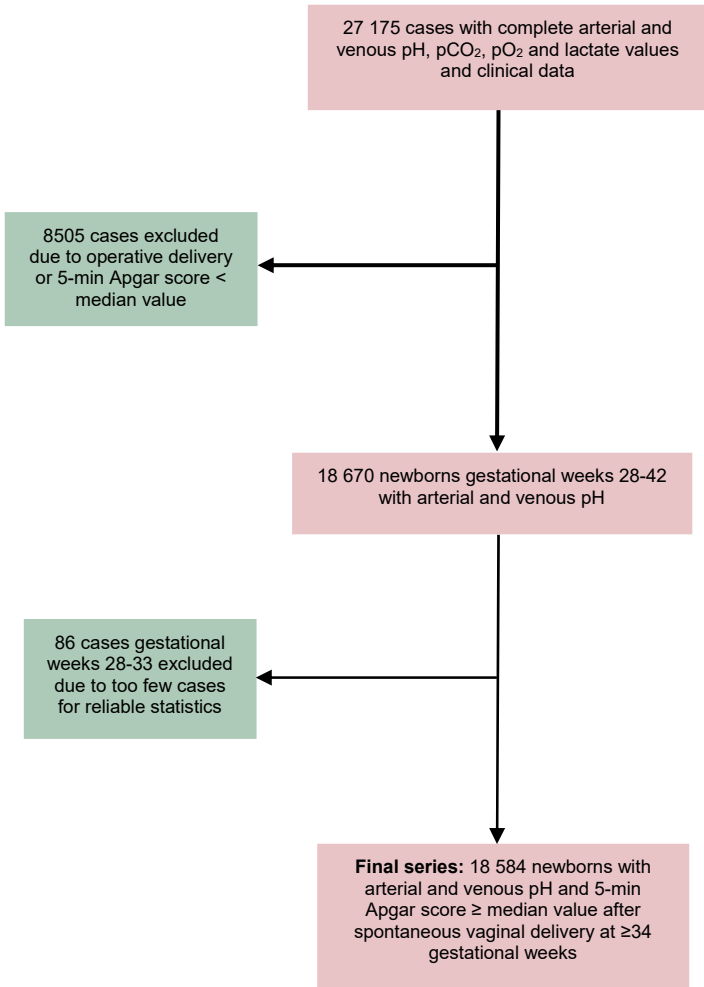

Figure 15:
Flowchart describing exclusion of cases to final series of 18 584 newborns included in calculations of arterial and venous umbilical cord blood pH reference values.

Analysis of covariance (ANCOVA) was used to elucidate the effect of mode of delivery on arterial and venous pH at birth. Statistics were performed with aid of the StatView® computer software (SAS Institute, version 5.0.1, Cary, NC, USA) and IBM SPSS Statistics for Windows, version 23.0 (IBM Svenska AB, Stockholm, Sweden). Values are reported as mean ± standard deviation and median with 2.5th and 97.5th percentiles. A two-tailed P <0.05 was considered statistically significant.

Study II

Originating from the same database of cord blood samples as *Study I*, we found that 28 727 newborns had a complete panel of results with pH, lactate and glucose from both vessels. Additional exclusion criteria in this study included: cases not having a venous-to-arterial pH gradient of ≥0.02, no match in our electronic obstetric database, delivery before 37 weeks or by elective cesarean section, multiple gestations, small-for-gestational-age newborns and cases without information on birthweight. The final cohort comprised of a total of 17 358 cases with complete and validated data.

A normal pH was defined as a value equal or greater than the gestational age-adjusted mean value minus 2 SD and neonatal acidemia as pH less than the mean minus 2 SD [40].

BD_{ecf} was calculated post hoc with the recommended algorithm (Introduction: Base excess). There was no established reference curve for BD relative to gestational age and the gestational age-dependency was adjusted for by the regression equation [27]:

$$\mathbf{BD = 4.288 + 0.0319 \times (GA - 280.0905)}$$

*GA denotes gestational age in days

Using the regression equation, all individual values were adjusted to a fictive gestational age of 280 completed days (40+0 weeks). Metabolic acidosis was defined as an arterial pH less than the mean minus 2 SD combined with BD_{ecf} less than 12.0 mmol/L when adjusted to 280 days. Lacticemia was defined as an arterial lactate value above the gestational age-adjusted mean value +2 SD according to Wiberg et al. [14].

To estimate the transplacental supply of glucose to the fetus, the *fractional placental glucose supply (FPGS)* to venous cord blood was calculated as:

FPGS = ([glucose vein] – [glucose artery])/ (glucose artery) concentration per kg placental weight.

AGA was defined as a birthweight within the 10th to 90th percentile interval, LGA as above the 90th percentile, and SGA as below the 10th percentile relative to our fetal weight reference curve [155].

The study was carried out in the South of Sweden where a 75-g oral glucose tolerance test is routinely performed at all maternal healthcare service centres around the 28th week of gestation. Gestational diabetes mellitus (GDM) was defined as a 2-hour capillary blood glucose value ≥9.0 mmol/L tested with a HemoCue blood glucose analyzer (HemoCue AB, Ängelholm, Sweden) in 2 consecutive blood samples [156]. Women with a 2-hour value between 7.8 and 8.9 mmol/L were retested after 1 week. Women diagnosed with GDM were followed at a specialist antenatal care clinic until delivery, as were women with type 1 or type 2 pre-gestational diabetes mellitus. All diabetic mothers were pooled in to one group.

Statistical analyses

The Mann-Whitney *U* test was used for group comparison of continuous variables, simple and multiple linear regression analysis and Kendall's rank correlation coefficient (tau) for correlation between variables, and the Chi-square test and Fisher's exact test for comparisons of categorical variables. Multiple logistic regression analysis was used to estimate influences of dependent variables. A two-tailed $P < 0.05$ was considered statistically significant. Statistics were performed with aid of the StatView® computer software (SAS Institute, version 5.0.1, Cary, NC, USA). Values are reported as mean ± SD and median with range.

Study III

The Study Population

During the recruitment period of the EXPRESS from 2004 to 2007, a total of 1011 extremely premature infants were included in the study, all born before 27 completed weeks of gestation.

Perinatal and neonatal data were prospectively collected for all infants. Survivors included 707 infants of which 494 (70.0%) survived to 1 year of age. Of the infants that survived the 1st year of life, 456 were assessed at 2.5 years' corrected age and compared with a control group of children 2.5 years of chronological age who had been born at term [95]. At the chronological age of 6.5 years, the cohort was asked to undergo a comprehensive neurodevelopmental assessment. Eight children died between 1 and 6.5 years of age leaving 486 children (68.7% of all live births) that were eligible for participation in this neurodevelopmental assessment.

Neurodevelopmental assessment

Comprehensive neurodevelopmental assessment had been performed in the surviving infants at 2.5 and 6.5 years of corrected age by certified psychologists for cognition, language and motor development using the Swedish version of the Wechsler Intelligence Scale for Children, fourth edition (WISC-IV) [157]. The neurodevelopmental index (NDI) was calculated by summing up values for motor developmental index-Bailey assessment charts (MDI) and neuro-motor assessment. NDI was subdivided into four classes of severity [90]. If values for NDI were missing at the 6.5 years assessment, we used values from the 2.5 years assessment instead to sustain reasonable statistical power in our evaluations.

Umbilical cord arterial blood gases

Infants with available umbilical cord arterial blood gas values for pH and BE were included in calculations of median and percentile values relative to gestational age, as well as in comparisons of neurodevelopmental outcomes. Since BE is a variable that is calculated and not measured by the blood gas analyzer, BE was calculated post hoc as BE in extracellular fluid (BE_{ecf}), using the same equation as in *Study II*:

$$\mathbf{BE_{ecf} = -0.9149 \times (0.23 \times pCO_2 \times 10^{[pH\ -6.1]} - 24.1 + 16.21 \times [pH - 7.4])\ mmol/L,}$$

where pCO_2 values are in kPa.

Statistical analyses

Simple linear regression analysis was used to estimate the association between cord pH and gestational age, and analysis of covariance (ANCOVA) was used to estimate the possible influence of mode of delivery (cesarean section versus no cesarean section). Median and percentile values for pH and BE_{ecf} relative to gestational age were calculated. For pH, mean and SD were calculated in the total study population. Comparison of pH values between groups were performed with the unpaired *t*-test. ROC curves were constructed with calculations of AUC to identify the optimal discriminatory binary cut-off values of pH and BE_{ecf} (normal/abnormal blood gases) to predict death and survival with and without neurodevelopmental sequelae. OR with 95% CI and multiple logistic regression analysis were used in such calculations, with adjustments for the possible influences of gestational age and mode of delivery. Statistics were performed with aid of the StatView ® computer software (SAS Institute,

version 5.0.1, Cary, NC, USA), IBM SPSS Statistics for Windows, version 23.0 (IBM Svenska AB, Stockholm, Sweden) and MedCalc Software (Mariakerke, Belgium).

Study IV and V

Retrieval of HIE cases

Five regional and local obstetric, neonatal and rehabilitation medicine databases were searched for neonates with HIE born between 1990 and 2001 at the maternity unit of Skåne University Hospital in Malmö, Sweden. Using the International Classification of Diseases (ICD) 9th revision (ICD-9, Swedish version ICD-9-SE) diagnose codes for infants suffering from HIE were identified.

All infants that matched the ICD codes had their medical charts scrutinized by a senior neonatologist, Dr Fredrik Lundberg, to determine if they truly met the diagnostic criteria for HIE stage II or III according to Sarnat & Sarnat [114]. HIE stage II cases were identified in the medical records as lethargic with muscle hypotonia, decreased suck and Moro reflexes, overactive stretch reflexes, seizures and miotic pupils. HIE stage III cases were stuporous and flaccid, absent stretch, suck or Moro reflexes, mydriatic pupils with no reaction to light stimulus, no breathing and seizures absent or to some lesser extent.

HIE infants that survived had regular follow-up care up to 6 years of age at pediatric and/or rehabilitation outpatient clinics.

Selection of controls

Using the maternity unit logbooks, each HIE case was matched to four controls with similar gestational age and neonate gender. We selected two controls born close before the matching HIE case and two born soon after. The first two controls that were found with the appropriate amount of umbilical cord sera stored frozen in the serum bank were selected as the final controls. This meant that each HIE case was matched with a total of two controls. We did not match for mode of delivery.

Retrieval of clinical data

We retrieved clinical data from the pediatric and rehabilitation medical journals in HIE cases. For both cases and controls, local obstetric databases provided data about pregnancy progress, birth and perinatal outcome.

Umbilical cord serum bank samples

As mentioned earlier, serum from cord blood samples was frozen and stored at the Malmö Maternity Unit Serum Bank. All samples for *Study IV* and *Study V* were retrieved from this serum bank.

Assessment of severity of adverse outcome

The following comparisons were made between cases and controls:

- umbilical cord arterial pH at birth
- amplitude-integrated electroencephalography (aEEG) classification
- stage of HIE
- death or permanent sequelae up to an age of at least 6 years

The definition and diagnostic criteria for cerebral palsy were used according to international guidelines [158] and according to the national Swedish quality register for cerebral palsy follow-up [159].

Umbilical cord artery pH

As mentioned earlier, umbilical cord arterial and venous blood sampling has been routinely performed at the Skåne University Hospital in Malmö. Cord blood acidemia was defined as a cord artery pH below a gestational age-adjusted mean value minus 2 standard deviations (SD) [40]. A pH < 7.00 [27] was also noted.

aEEG classification

The aEEG was classified according to Toet et al. [160]:

1. Continuous normal voltage (no periods of low amplitude)
2. Discontinuous normal voltage (low amplitude periods lasting < 10 s)
3. Burst-suppression
4. Continuous extremely low voltage
5. Flat tracing (isoelectric)

Continuous or discontinuous normal voltage traces with no abnormal changes were classified as normal; burst-suppression, continuous extremely low voltage and flat tracing were classified as abnormal [160]. The classification took into consideration epileptic activity (single or repeated seizures, saw-tooth pattern, status epilepticus). A single seizure, not confirmed by aEEG and not requiring anti-epileptic treatment, was regarded as benign. We thus classified neonates with normal background activity aEEG as having normal aEEG patterns.

Laboratory methods

Study IV

UCH-L1 and GFAP in the serum samples were analysed in one batch using sandwich electro chemiluminescent immunoassays (ECLIA) according to the standard operating protocol of Banyan Biomarkers, Inc., Alachua, FL, USA. All samples were analysed concomitantly with serial dilution of a calibrator protein that was prepared and assayed under the same conditions as the serum samples. The calibrator dose-response curve was used to calculate the serum sample concentration. This method has been authorized for research use in accordance with the US Food and Drug Administration (FDA) guidance for bioanalytical method validation as applicable for biomarker assays. The level of interference for UCH-L1 was ≤5% and for GFAP ≤20%. The coefficient of variation for intra- and inter-assay precisions is <10% [146, 161]. The quantification range of UCH-L1 was 0.1-9 ng/mL with a lower limit of detection of 0.045 ng/mL. The quantification range for GFAP was 0.03-50 ng/mL with a lower detection limit of 0.008 ng/mL.

Study V

After extraction from the cord serum blood bank, the samples were vortexed and centrifuged at 2000g for 10 minutes according to standardized protocol of the

Department of Laboratory Medicine, Skåne University Hospital in Malmö. A volume of 130 µL of cord serum was transferred to standardized tubes for analysis. All cord serum samples were analyzed in the same batch.

The samples were analysed using one-step immunometric sandwich-method with electrochemiluminescence immunoassay (ECLI) (Elecsys S100, ref. 03175243190, Mannheim, Germany) using Cobas analyzer 6000 system with e601/e602 modules (Roche Diagnostics International, Rotkreuz, Switzerland). The principle method behind this process is based on using an antigen-antibody reaction that results in the emission of light. The light intensity is directly proportional to the concentration of S100B [162]. S100B concentrations from 0.005 to 39 µg/L can be detected by this method.

However, since S100B is an intracellular protein, hemolysis in the samples could influence the analysis [162]. To exclude the effect of hemolysis, free Hb in samples was determined by mixing 10 µL serum with 100 µL 0.01% Na_2CO_3 in a half well microplate reader (UV-star, Greiner Bio-One, Frickenhausen, Germany). After centrifugation for 1 minute at 900g, the absorbance was noted at 405, 450 and 380 nm. The Hb concentration was then calculated according to the Harboe equation [163] and corrected for shorter light path-length in the microplate.

Statistical Analyses

Associations between continuous variables were determined by simple regression analysis and Kendall rank correlation coefficient (tau), as appropriate. Multiple regression analysis included independent variables showing a *P* value of <0.20 at simple linear regression analysis. Group comparisons of continuous variables were performed with the Mann-Whitney *U* test (M-W test) for two groups and the Kruskal-Wallis test (K-W test) for three or more groups. The recommended threshold sizes for using non-parametric statistical tests were followed [151]. Dichotomous categorical variables were compared between groups with the Chi-square test or Fisher's exact test, as appropriate, and homogeneity with the McNemar test. Statistics were performed with aid of StatView ® computer software (SAS Institute, Cary, NC, USA) and VassarStats' website for statistical computation (http://vassarstat.net/) [164]. A two-tailed *P* value < 0.05 after correction for ties was considered statistically significant.

4. Results and Comments

Paper I: Gestational age-related reference values for Apgar score and umbilical cord arterial and venous pH in preterm and term newborns

Despite much literature on reference values of acid-base status in umbilical cord blood at birth, there are yet no studies performed to determine gestational age- dependent references in cord venous blood and no studies on preterm acid-base standards. Similarly, the normal reference range of the 5-minute Apgar score for term and preterm infants has not yet been determined.

Results

Table 8 illustrates the demographic characteristics of the study population. The distribution of 5-minute Apgar score across different gestational weeks in the series of 27 175 validated cases is displayed in Table 9. There were too few newborns before 28 weeks for robust calculation of percentiles in individual weeks and we thus merged those into one group.

Simple linear regression analysis showed a negative association between umbilical cord arterial pH (ApH) and gestational age (P <0.001, r^2=0.022); quadratic and cubic linear regression analyses showed a better r^2 value (both P <0.001, r^2=0.025).

Since the aim of the study was to estimate "normal" pH reference values, all newborns with Apgar scores less than the median score were excluded from statistics on pH. This step ensured inclusion of only "vigorous" newborns.

The effect of mode of delivery was evaluated using ANCOVA (Table 10), showing significantly higher ApH in newborns delivered by elective (N=2069) than by emergency cesarean section (N=2197), and significantly lower ApH in newborns delivered by vacuum extraction or forceps (N=1559) compared with spontaneous vaginal delivery (N=21 350) (P <0.001 for all estimates). Since mode of delivery significantly affected ApH, we restricted our statistical analyses to include only

spontaneous, non-instrumental vaginal deliveries. As a result of this sorting, the number of newborns in gestational weeks 28-33 became too few (N=86) for robust estimations of reference values in these weeks.

Tabel 8:
Demographic characteristics of the study population and the total obstetric population in Lund and Malmö during the study period 2001-2010

Characteristics	Study Population N= 27 175	Total Population[a] N= 75 793
	Number (%) or median (range)	Number (%) or median (range)
Maternal age (years)	31 (14-48)	31 (13-57)
Singleton pregnancy[b]	26 623 (98.0)	73 384 (96.8)
Preeclampsia	183 (0.7)	579 (0.8)
Pre-pregnancy diabetes	257 (0.9)	657 (0.9)
Mode of delivery		
Spontaneous vaginal delivery	21 350 (78.6)	58 831 (78.4)
Instrumental delivery	1559 (5.7)	3961 (5.3)
Cesarean section	4266 (15.7)	12 272 (16.3)
Gestational age		
Preterm delivery (<37+0 w)	1656 (6.1)	5682 (7.5)
Term delivery (37+0 to 41+6 w)	24 087 (88.6)	66 115 (87.3)
Postterm delivery (≥42+0 w)	1432 (5.3)	3934 (5.2)
Birthweight		
SGA	853 (3.1)	2124 (2.8)
AGA	24 874 (91.5)	70 652 (93.5)
LGA	1448 (5.3)	2773 (3.7)
5-minute Apgar score		
0-3	48 (0.2)	539 (0.7)
4-6	209 (0.8)	863 (1.1)
≥7	26 918 (99.0)	74 352 (98.2[c])
Umbilical cord arterial blood gases		
pH	7.269 (6.646-7.551)	7.26 (6.51-7.70)
pCO_2 (kPa)	7.08 (2.25-18.3)	7.23 (0.21-17.9)
pO_2 (kPa)	2.71 (1.00-35.2)	3.03 (0.004-13.8)
Lactate (mmol/L)	4.4 (0-25.0)	-

SGA, small-for-gestational-age (birthweight less than mean – 2SD); AGA, appropriate-for-gestational-age (within mean ± 2SD); LGA, large-for-gestational age (more than mean + 2SD).
[a] Data retrieved from the regional Perinatal Revision South Register [148]. Data were incomplete in 0.1-1.0% of cases and calculations were then performed on available data.
[b] For twin pregnancies, data from only the first twin was included in the study.
[c] 98.1% adjusted to 98.2% according to the largest remainder method.

Tabel 9:
Percentile distribution of 5-minute Apgar score relative to gestational age in a series of 27 175 newborns with validated umbilical cord blood gas values

Gestational age (weeks)	Number of cases	Percentiles						
		5	10	25	50	75	90	95
< 28	74	2.8	3.5	6	7	9	10	10
28	23	1	5.4	7	8	10	10	10
29	28	5	6.9	8	9	10	10	10
30	39	5	5	8	9	10	10	10
31	66	6	6.7	8.8	10	10	10	10
32	69	6	7	9	10	10	10	10
33	119	7	7	9	10	10	10	10
34	212	7	8	9	10	10	10	10
35	375	7	8	9	10	10	10	10
36	651	8	9	10	10	10	10	10
37	1607	9	9	10	10	10	10	10
38	4553	9	9	10	10	10	10	10
39	6530	9	9	10	10	10	10	10
40	7360	9	9	10	10	10	10	10
41	4037	9	9	10	10	10	10	10
42	1381	8	9	10	10	10	10	10
> 42	51	8.6	9.2	10	10	10	10	10
TOTAL:	**27 175**							

Tabel 10:
Covariance analysis illustrating the effect of mode of delivery on mean arterial pH at birth in 27 175 newborns. Non-instrumental vaginal deliveries were used as reference for estimations. Umbilical arterial pH was significantly higher in cases with cesarean section and lower for intrumental vaginal deliveries.

Mode of delivery	ApH	Standard error	t	*P*	95% Confidence Interval	
					Lower bound	Upper Bound
Intercept	7.474	0.012	605.1	0.000	7.450	7.498
Gestational age	-0.005	0.000	-16.95	0.000	-0.006	-0.005
Non-instrumental vaginal delivery (N=21 350)	0	-	-	-	-	-
Instrumental delivery (ventouse/ forceps) (N=1559)	-0.057	0.002	-23.85	0.000	-0.061	-0.052
Emergency cesarean section (N=2197)	0.022	0.002	10.98	0.000	0.018	0.026
Elective cesarean section (N=2069)	0.024	0.002	12.29	0.000	0.020	0.028

The final series for calculation of pH reference values then comprised of 18 584 newborns with a complete panel of ApH, cord venous pH (VpH) and Apgar score ≥ median value at 5 minutes after spontaneous vaginal delivery at ≥34 gestational weeks (Figure 15).

Weighted quadratic regression analyses were performed on percentile values of ApH and VpH according to increasing gestational age (P<0.001 for both estimates) (Table 11 and Figures 16 and 17).

A linear decline in both ApH and VpH was observed with advancing gestational age. Median pH at 34+0 weeks of gestation was 7.284 in the umbilical artery and 7.333 in the vein. At 42+3 weeks, median arterial pH was 7.241 and in the vein 7.316.

Table 11:
Percentile values for pH in the umbilical cord artery and vein relative to gestational age at birth (N= 18 584 cases)

Gestational age (weeks + days)	Umbilical artery pH			Umbilical vein pH		
	2.5th percentile	Median	97.5th percentile	2.5th percentile	Median	97.5th percentile
34+0	7.112	7.284	7.424	7.163	7.333	7.466
34+3	7.113	7.285	7.423	7.164	7.336	7.467
35+0	7.113	7.285	7.422	7.165	7.339	7.468
35+3	7.114	7.285	7.421	7.166	7.341	7.468
36+0	7.114	7.285	7.419	7.166	7.344	7.468
36+3	7.113	7.284	7.418	7.165	7.345	7.468
37+0	7.113	7.282	7.416	7.165	7.346	7.468
37+3	7.112	7.281	7.415	7.164	7.346	7.467
38+0	7.111	7.278	7.412	7.162	7.346	7.467
38+3	7.110	7.276	7.411	7.161	7.345	7.466
39+0	7.108	7.273	7.408	7.158	7.343	7.465
39+3	7.107	7.270	7.406	7.156	7.341	7.463
40+0	7.105	7.266	7.404	7.153	7.338	7.462
40+3	7.103	7.262	7.401	7.150	7.335	7.460
41+0	7.100	7.257	7.398	7.146	7.331	7.458
41+3	7.098	7.253	7.396	7.143	7.327	7.456
42+0	7.094	7.246	7.393	7.138	7.321	7.453
42+3	7.091	7.241	7.390	7.134	7.316	7.451

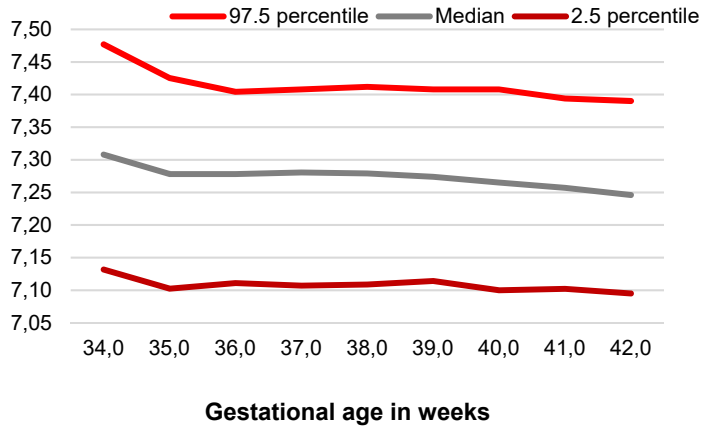

Figure 16:
Weighted quadratic regression analysis based on 18 584 vigorous newborns with median and percentile values of umbilical cord arterial pH relative to gestational age (*P*<0.001). For detailed pH values, see Table 11.

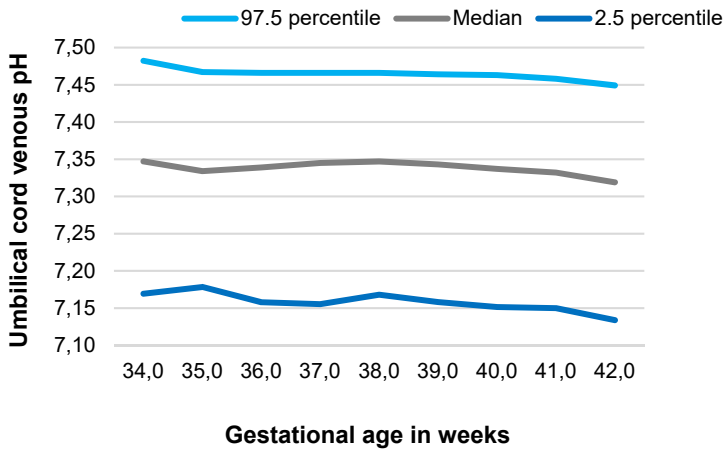

Figure 17:
Weighted quadratic regression analysis based on 18 584 vigorous newborns with median and percentile values of umbilical cord venous pH relative to gestational age (*P*<0.001). For detailed pH values, see Table 11.

Comments

The main findings of this study included the determination of the normal distribution of the 5-minute Apgar score in preterm, term and postterm newborns in an unselected population of 27 175 newborns. We know of no other similar large study. When not stratified for mode of delivery, the median 5-minute Apgar score was 7 before 28 weeks,

8 at 28 gestational weeks, 9 at 29-30 weeks and 10 from 31 weeks onwards. As the study population represented the total population, the lower median scores in moderately and extremely preterm newborns may reflect differences in maturity rather than depressed vitality. Inevitably, Apgar scoring is subjective and the generalizability of our results is thereby limited by inter-observer [165] and regional differences [166].

This study also defined gestational age-related reference values for pH in arterial and venous umbilical cord blood in a series of 18 584 vigorous preterm, term and postterm newborns after spontaneous vaginal delivery. By progress of gestation, we found that both arterial and venous median pH fitted a slightly downward quadratic linear regression curve. By meticulous purging from the database of non-optimal blood gas analyses, we believe these reference values are well suited for use in clinical practice and research.

We excluded all operative deliveries and newborns not showing a normal vitality, since we were not interested in describing pH values for adverse outcomes. Therefore, despite the large series, we could not create reference values for premature newborns before 34 gestational weeks due to too few cases to perform robust statistics. This can be explained not only by our strict inclusion criteria, but also by the fact that preterm newborns are often growth restricted and often delivered by cesarean section [89].

There is no global consensus on the definition of umbilical cord blood acidemia. It has been defined as fixed umbilical artery pH cut-offs from <7.00 to <7.20 [9] irrespective of gestational age, but it is well known that pH decreases with progression of pregnancy [27, 40, 167]. Maternity units in Sweden define a cord artery pH of less than 7.10 as acidemic since this value corresponds to the mean value minus 2 SD in the population [168, 169]. The American College of Obstetricians and Gynecologists and the National Institute for Health and Clinical Excellence recommend the use of an arterial pH less than 7.00 as a clinically useful fixed cut-off to identify neonates at high risk for serious neonatal morbidity and mortality [170, 171]. However, our research group has previously established that gestational age-adjusted values of pH, base deficit and lactate in cord blood at birth are better associated with the newborn's vitality than fixed cut-off values [172].

Malin et al. [9] found a strong association between low cord arterial pH and short-term and long-term adverse outcomes, with a clear dose-response relation. It is, however, important to be aware that statistical definitions of low pH values in cord blood based on population studies are not necessarily reflecting biological adverse effects of tissue acidosis in the individual. It seems that only in combination with decreased vitality is a low cord pH predictive [173]. Furthermore, at moderate degrees of acidemia, where pH is below 7.10 but above 7.00, a neuroprotective mechanism may be triggered: at this ApH level Dennis et al. [174] found no neurodevelopmental delay at 4.5 years of age and

Svirko et al. [175] even found that lower arterial pH from stressful vaginal deliveries correlated to higher literacy and non-verbal intelligence score at ages 6-8 years.

A clinical implication of our findings is that gestational age-related reference values, rather than fixed reference values, better reflect the normal maturity processes and physiological changes occurring with advancing gestational age. Additionally, when settling what is normal and what is abnormal, gestational age-related reference values for cord pH might make a difference in neonatal management and in the assessment of insurance and litigation cases.

Summary

The study determined the distribution of Apgar scores at 5 minutes across different gestational ages in a series of 27 175 newborns, well representing the total obstetric population. The median Apgar score increased with progress of gestation and was 10 from 31 weeks onwards. Based on a robust clinical material with validated paired arterial and venous umbilical cord blood samples from 18 584 births, gestational age-related reference values for pH from vigorous newborns were calculated. Both arterial and venous pH were found to decrease linearly with increasing gestational age, from a median of 7.284 at 34+0 weeks to 7.241 at 42+3 weeks in the cord artery. One might regard such a decrease of only 0.043 pH units as unimportant, but in terms of hydrogen ion concentration, it represents an increase by 10% from 52.000 nM to 57.412 nM [176] over the time period.

Paper II: Assessment of lactate production as a response to sustained intrapartum hypoxia in large-for-gestational-age (LGA) newborns

Using cord blood acidemia as a proxy for intrapartum hypoxia, we wanted to compare the umbilical cord blood glucose and lactate concentrations relative to AGA and LGA birthweight cohorts in diabetic and non-diabetic pregnancies during aerobic and anaerobic conditions. We hypothesized that LGA fetuses would have higher glucose and lactate levels than AGA fetuses during non-acidemic conditions, but that during acidemia they would show lower lactate values. In addition, since insulin inhibits the glycogenolysis of glycogen to glucose, endogenous glucose might be abated and we therefore also wanted to estimate the maternal glucose supply across the placenta.

Results

Demographic data of the study population is shown in Table 12. The umbilical cord arterial plasma glucose concentration was positively associated with gestational age (r^2=.015, P <0.0001) and negatively associated with weight deviation (WD%) (r^2 = .006, P < .0001), but not with birthweight (P = .89). Using multiple linear regression analysis, glucose concentration was found to be independently associated with both gestational age (P < .0001) and WD% (P < .0001).

Similarly, the umbilical cord arterial plasma lactate concentration was positively associated with gestational age (r^2 = .020, P < .0001) and negatively associated with WD% (r^2 = .007, P < .0001), but not with birthweight (P = .97). Multiple linear regression analysis showed that lactate concentration was independently associated with both gestational age (P < .0001) and WD% (P < .0001).

Cord artery concentrations of glucose and lactate were linearly correlated (r^2 = .21, P < .0001) and both variables were negatively correlated with umbilical cord arterial pH (r^2 = .094 and .62, respectively, P < .0001). Positive linear correlations were found between glucose and lactate concentrations within both the AGA and LGA birthweight groups (r^2 = .21 and .20, respectively, P < .0001).

Acidemia occurred as often in the AGA group (438/14 569, 3.0%) as in the LGA group (80/2789, 2.9%) (P = .69). When calculated for acidemic newborns, the positive significant associations between glucose and lactate remained in both the AGA group (n = 438; r^2 = .053, P < .0001) and the LGA group (n = 80, simple linear regression analysis, r^2 = .048, P = .050; Kendall's tau 0.23, P = .002) (Figure 18).

In all, 141 women had pre-gestational diabetes and 433 had gestational diabetes mellitus (GDM), resulting in a total of 574 women with diabetes. Diabetes was significantly more common among LGA cases (5.4%) than AGA cases (2.9%) (Table 12). Comparing pre-gestational diabetes and GDM for demographic characteristics presented in Table 12, significant differences were found for spontaneous vaginal delivery (SVD) (94/141 vs 363/433, P < .0001), gestational age (275 ± 8 days vs 277 ± 8 days, P = .009), and birthweight (3876 ± 498 g vs 3646 ± 437 g, P < .0001), but not for other variables (P ≥ .08). There were no significant differences in cord arterial glucose (P = .32) and lactate (P = .49) concentrations, or rates of acidemia (P=.21), BD_{ecf} (P=.30), metabolic acidosis (P=1.0) or lacticemia (P = .18). Significant differences were found for LGA (66/141 vs 86/433, P < .0001), WD% (13.4 ± 16.4 [median 12.0] vs 4.4 ± 12.2 [median 2.0], P < .0001), and cord artery pH (7.22 ± 0.08 vs 7.24 ± 0.08, P = .018) between the types of diabetes.

Table 12:
Demographic characteristics of the study population

Characteristics	AGA n=14 569				LGA n= 789				Significance of difference (*P*)
	n	%	Mean ± SD	Median; range	n	%	Mean ± SD	Median; range	
Maternal age (years)			30.1 ± 5.1	30; 13 - 48			31.1 ± 4.8	31; 15 - 46	<0.0001
Nulliparity	7345	50.4			994	35.6			<0.0001
Severe preeclampsia	30	0.2			4	0.1			0.66
Diabetes	422	2.9			152	5.4			<0.0001
SVD	12 610	86.6			2353	84.4			0.003
Gestational age (days)			280 ± 8	281; 259 - 303			279 ± 9	279; 259 - 302	<0.0001
Birthweight (g)			3586 ± 347	3580; 2600 - 4755			4298 ± 354	4285; 3415 - 6360	N/A
WD (%)			0.1 ± 7.3	0.1; -14.0 - 14.0			21.2 ± 6.9	19.2; 14.1 - 66.2	<0.0001
5 min Apgar score <7	93	0.6			29	1.0			0.029

AGA, appropriate-for-gestational age, birthweight 10th to 90th percentile; LGA, large-for-gestational age, birthweight > 90th percentile; SVD, spontaneous vaginal delivery; N/A, not applicable.
Statistics performed with the Mann-Whitney *U* test, Chi-square test, or Fisher's exact test.

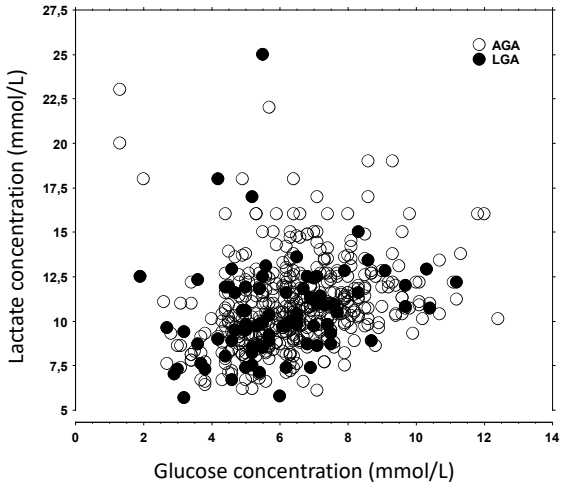

Figure 18:
Plot of umbilical cord blood arterial glucose-to- lactate values in 80 large-for-gestational-age (LGA) and 438 appropriate-for-gestational-age (AGA) acidemic newborns. Acidemia was defined as a cord artery pH below the gestational age-adjusted mean value minus 2 standard deviations.

Glucose concentration relative to birthweight groups, diabetes and acidemia

Table 13 shows the umbilical artery glucose concentration relative to birthweight cohorts, diabetes, and acidemia. At acidemia, glucose was significantly higher in all groups. Glucose was significantly lower in non-diabetes LGA than non- diabetes AGA, but not in diabetes LGA vs diabetes AGA. In addition, significantly higher glucose levels were observed in diabetes than non-diabetes in LGA cases, but not in AGA cases.

Lactate concentration relative to birthweight groups, diabetes and acidemia

Table 14 shows the umbilical artery lactate concentration relative to birthweight cohorts, diabetes, and acidemia. At normal pH, lactate was lower in LGA cases than in AGA cases in non-diabetes, but in cases of diabetes there was no difference. No significant differences were observed between non-diabetes and diabetes, either for LGA or for AGA cases (Figure 19). In the presence of acidemia, lactate was significantly higher in all groups. However, there were no significant differences between LGA and AGA, either for non-diabetes (P = .084) or for diabetes.

Table 13:
Umbilical cord arterial plasma glucose concentration (mmol/L) relative to birthweight cohorts, diabetes and arterial pH in 17 358 singleton deliveries at ≥37 gestational weeks. Values are mean ± SD and median (range).

	AGA n=14 569			LGA n= 789			AGA vs. LGA	
	Non-diabetes n=14 147	Diabetes n=422	Diabetes vs. non-diabetes (*P*)	Non-diabetes n=2637	Diabetes n=152	Diabetes vs. non-diabetes (*P*)	Non-diabetes (*P*)	Diabetes (*P*)
Normal pH[a] n=16 840	n=13 723 4.8 ± 1.3 4.6 (1.7-13.6)	n=408 4.9 ± 1.4 4.8 (1.8–10.7)	0.18	n=2565 4.5 ± 1.3 4.3 (1.9-12.3)	n=144 4.7 ± 1.5 4.3 (2.2-10.6)	0.70	<0.0001	0.067
Acidemia[a] n=518	n=424 6.4 ± 1.8 6.3 (1.3-12.4)	n=14 6.6 ± 1.8 6.5 (3.1-9.6)	0.67	n=72 5.9 ± 1.9 5.5 (1.9-11.2)	n=8 7.0 ± 1.2 7.0 (5.5-9.1)	0.039	0.005	0.58
Normal pH vs. Acidemia (*P*)	<0.0001	0.0005		<0.0001	0.0007			

[a] Normal pH was defined as cord artery pH ≥ the gestational age-adjusted mean value minus 2 SD, and acidemia as pH < mean minus 2 SD.
Statistics were performed with the Mann-Whitney *U* test.
For comparisons of AGA vs. LGA and diabetes vs. non-diabetes the table should be read horizontally, and between normal pH and acidemia vertically.

Table 14:
Umbilical cord arterial plasma lactate concentration (mmol/L) relative to birthweight cohorts, diabetes and arterial pH in 17 358 singleton deliveries at ≥37 gestational weeks. Values are mean ± SD and median (range).

	AGA n=14 569			**LGA n=2789**			**AGA vs. LGA**	
	Non-diabetes n=14 147	**Diabetes n=422**	**Diabetes vs. non-diabetes (*P*)**	**Non-diabetes n=2637**	**Diabetes n=152**	**Diabetes vs. non-diabetes (*P*)**	**Non-diabetes (*P*)**	**Diabetes (*P*)**
Normal pH[a] n=16 840	n=13 723 5.0 ± 2.0 4.7 (1.2-16.0)	n=408 4.8 ± 2.0 4.5 (1.5-11.7)	0.069	n=2565 4.6 ± 1.9 4.4 (1.2-15.0)	n=144 4.8 ± 2.1 4.6 (1.6-13.5)	0.38	<0.0001	0.98
Acidemia[a] n=518	n=424 10.9 ± 2.4 10.6 (6.1-23.0)	n=14 10.1 ± 2.2 9.6 (7.1-15.0)	0.17	n=72 10.4 ± 2.9 10.0 (5.7-25.0)	n=8 10.9 ± 1.7 10.4 (8.6-13.1)	0.28	0.084	0.29
Normal pH vs. Acidemia (*P*)	<0.0001	<0.0001	-	<0.0001	<0.0001	-	-	-

[a] Normal pH was defined as cord artery pH ≥ the gestational age-adjusted mean value minus 2 SD, and acidemia as pH < mean minus 2 SD.
Statistics were performed with the Mann-Whitney *U* test. For comparisons of AGA vs. LGA and diabetes vs. non-diabetes the table should be read horizontally, and between normal pH and acidemia vertically.

Fractional placental glucose supply (FPGS)

Data for FPGS calculation were available in 16 647 cases (placental weight missing in 711 cases). Among acidemic cases, non-diabetes LGA cases had a higher FPGS than non-diabetes AGA cases (P = .027), but there was no difference for diabetes cases (P = .61). In comparisons between diabetes and non- diabetes cases, there were no significant differences within the categories AGA and LGA, respectively, with or without acidemia ($P \geq .11$).

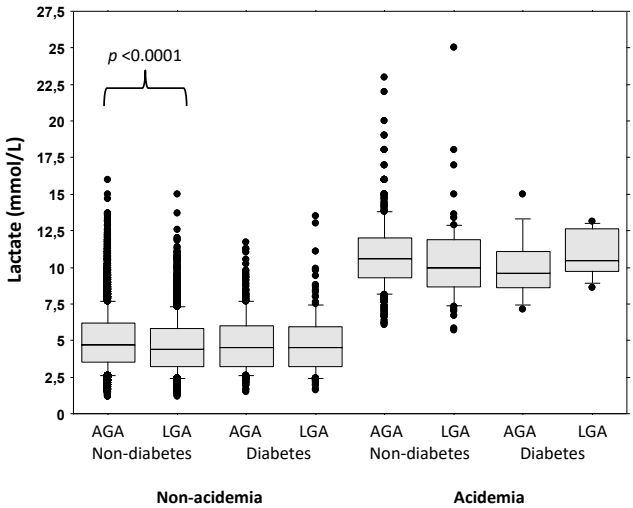

Figure 19:
Umbilical cord blood lactate concentration at birth in non-acidemic and acidemic appropriate-for-gestational-age (AGA) and large-for-gestational-age (LGA) birthweight groups of newborns compared between mothers with and without diabetes. At paired comparisons, a significant difference was found only between non-acidemic non-diabetes AGA and LGA. With acidemia, lactate increased in all birthweight groups compared to normal pH (P < .0001). For details, see Table 14.

Comments

This study showed that despite having lower cord arterial glucose levels relative to AGA fetuses during aerobic conditions, LGA fetuses had similar lactate production capabilities when exposed to sustained intrapartum hypoxia leading to acidemia. This was found in LGA fetuses of both non-diabetic and diabetic mothers. In all birthweight groups, during anaerobic metabolism, glucose was mobilized concomitantly with increased production of lactate. Furthermore, when estimating the "fractional glucose supply" by the placenta, we found that the supply in general decreased during anaerobic metabolism, but it was not impaired in LGA compared with AGA cases.

Considering the association between LGA and hyperinsulinism, a simultaneous occurrence of fetal hyperinsulinemia and hyperglycemia was not supported by this study. During both aerobic and anaerobic conditions, the cord glucose concentration was lower in non-diabetes LGA cases than non-diabetes AGA cases. This trend was similar but not significant during aerobic conditions in diabetes LGA. Furthermore, there was a weak but significant negative correlation between the cord glucose concentration and WD%, though the 0.6% impact of WD% on glucose values is negligible.

It thus appears that fetal hyperglycemia is much more a "fetal glucose hypermetabolism" rather than chronic hyperglycemia, with postprandial pulsatile hyperglycemia stimulating fetal insulin secretion [57]. Chronic hyperinsulinemia in LGA fetuses could explain the lower glucose concentration observed during aerobic conditions, enhancing glucose uptake by peripheral tissues and accelerating fetal growth; however, this did not prevent a sufficient mobilization of glucose reserves and lactate production during hypoxic stress.

Summary

Considering cord blood acidemia at birth a proxy for intrapartum hypoxia, this population-based comparative study showed that, compared with AGA fetuses, LGA fetuses had an intact ability to produce lactate in response to sustained intrapartum hypoxia. Maternal diabetes did not hamper the ability of LGA fetuses to mobilize glycogen stores for the production of glucose and lactate in strenuous situations during labor.

Paper III: Lack of associations between umbilical cord blood gases in extremely preterm newborns and neurodevelopmental outcomes at 6.5 years of age

Umbilical cord blood gas analysis is most objective method to assess the condition of the term infant at birth. Studies on umbilical cord blood gases in term infants have shown strong correlations with low pH and BE to increased risk for death or neurodevelopmental outcome later on in life. However, very little is known about the normal distribution of cord blood gases in extremely preterm infants nor the possible predictive value of umbilical blood gases in this high-risk infant group.

Results

Among the 705 live-births, 492 infants (69.8%) survived until 1 year of age (Table 15) and eight children died between 1 and 6.5 years of age. Among infants who survived their first year of life, 456 (92.7%) were assessed for neurodevelopmental development at 2.5 years of corrected age, and 486 (98.8%) at 6.5 years.

Table 15 shows the availability of cord blood gas data relative to survival and mortality. Data availability for cord arterial pH, pCO_2 and BE_{ecf} was 44.0-45.7%.

Table 16 shows the clinical characteristics of the infants with cord blood gas data. In total, 322 of 705 live-born infants had cord arterial pH determined, with the highest proportions at 25 and 26 gestational weeks (54%).

Table 15:
Analyses of umbilical cord blood gases at birth in relation to mortality and survival in all live-born neonates at 22-26 weeks of gestation in Sweden from April 2004 to March 2007

	Number of cases (N)	Cases with umbilical cord blood gases					
		pH		pCO_2		BE_{ecf}	
		n	%	n	%	n	%
Death <24 h after birth	106	18	17.0	17	16.0	17	16.0
Death 1-6 days	45	20	44.4	19	42.2	19	42.2
Death 7-27 days	35	15	42.9	13	37.1	13	37.1
Death 28-364 days	27	15	55.6	15	55.6	15	55.6
Alive at 365 days	492	254	51.6	246	50.0	246	50.0
Total	705	322	45.7	310	44.0	310	44.0

BE_{ecf}, base excess in extracellular fluid

Changes in cord arterial blood gases with advancing gestational age

Table 17 shows percentile values of cord arterial pH and BE_{ecf} at various gestational weeks, with Figure 20 illustrating variations in pH with advancing gestational age. Linear regression analysis showed no significant change in cord arterial pH with advancing gestational age (P=0.61, r^2=0.001); when adjusted for mode of delivery (cesarean section in 132/322=41%), the association was still not significant (ANCOVA, P=0.63).

The mean pH ± SD value over strata to be 7.279 ± 0.131, i.e. the ± 2SD range was 7.017 to 7.541. Figure 21 shows pH values of the 322 extremely preterm newborns plotted and the mean ± 2SD values indicated, together with the corresponding mean ± 2SD of term newborns, where values in the extremely preterm group were significantly higher than in the term group (unpaired t-test, P <0.0001).

Table 16:
Characteristics of extremely preterm livebirths with details of umbilical cord blood gas analyses

	Total N=705	pH n=322		pCO_2 n=310		BE_{ecf} n=310	
	N	n	%	n	%	n	%
Tertiary care center							
Stockholm	106	45	42.5	44	41.5	44	41.5
Uppsala	82	38	46.3	37	45.1	37	45.1
Linköping	34	11	32.4	9	26.5	9	26.5
Lund	80	40	50.0	39	48.8	39	48.8
Göteborg	78	44	56.4	44	56.4	44	56.4
Örebro	19	8	42.1	8	42.1	8	42.1
Umeå	56	47	83.9	47	83.9	47	83.9
Maternal age (years)							
<20	26	13	50.0	13	50.0	13	50.0
20–34	475	229	48.2	219	46.1	219	46.1
≥35	204	80	39.2	78	38.2	78	38.2
Parity							
Nullipara	433	198	45.7	192	44.3	192	44.3
Multipara	272	124	45.6	118	43.4	118	43.4
Mode of delivery							
Vaginal	350	131	37.4	124	35.4	124	35.4
Cesarean section	355	191	53.8	186	52.4	186	52.4
Body mass index (kg/m^2)							
<18.5	13	9	69.2	8	61.5	8	61.5
18.5–29.9	504	229	45.4	220	43.7	220	43.7
≥30	116	45	38.8	45	38.8	45	38.8
Comorbidity							
Preeclampsia	73	40	54.8	39	53.4	39	53.4
Chorioamnionitis	114	58	50.9	58	50.9	58	50.9
Placental abruption	76	44	57.9	40	52.6	40	52.6
Gestational age at birth							
22 wks	49	6	12.2	6	12.2	6	12.2
23 wks	101	31	30.7	30	29.7	30	29.7
24 wks	144	63	43.8	61	42.4	61	42.4
25 wks	202	110	54.5	105	52.0	105	52.0
26 wks	207	112	54.1	108	52.2	108	52.2
SGA	113	57	50.4	55	48.7	55	48.7

SGA, small-for-gestational-age

Table 17:
Percentiles of umbilical cord arterial pH and BE_{ecf} relative to gestational age at birth

	Percentiles						
	5	**10**	**25**	**50**	**75**	**90**	**95**
pH by gestational weeks							
22-23	6.89	7.04	7.27	7.33	7.41	7.42	7.46
24	6.97	7.07	7.21	7.31	7.36	7.42	7.46
25	6.99	7.07	7.22	7.31	7.35	7.40	7.43
26	7.03	7.16	7.27	7.32	7.35	7.39	7.46
BE_{ecf} (mmol/L) by gestational weeks							
22-23	-16.44	-12.18	-7.20	-4.42	-2.64	-1.47	0.87
24	-14.52	-10.69	-7.26	-4.15	-1.71	0.12	1.72
25	-15.68	-12.13	-6.96	-4.38	-2.27	0.31	1.56
26	-10.24	-8.78	-5.46	-3.30	-0.26	1.58	2.51

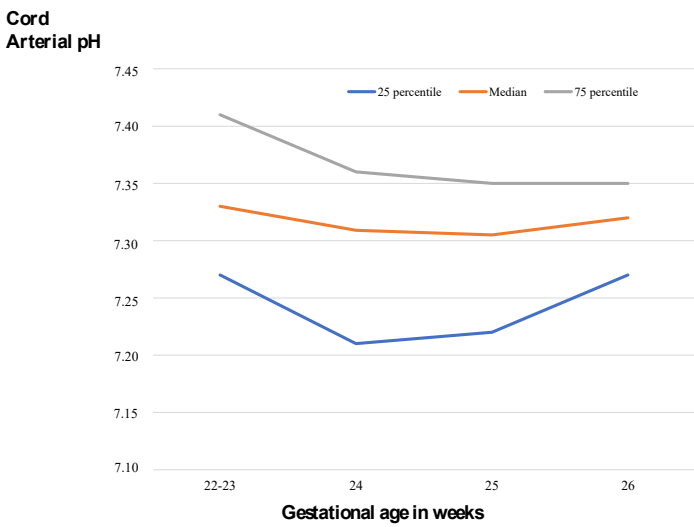

Figure 20:
Umbilical cord arterial pH relative to gestational age in 322 extremely preterm newborns

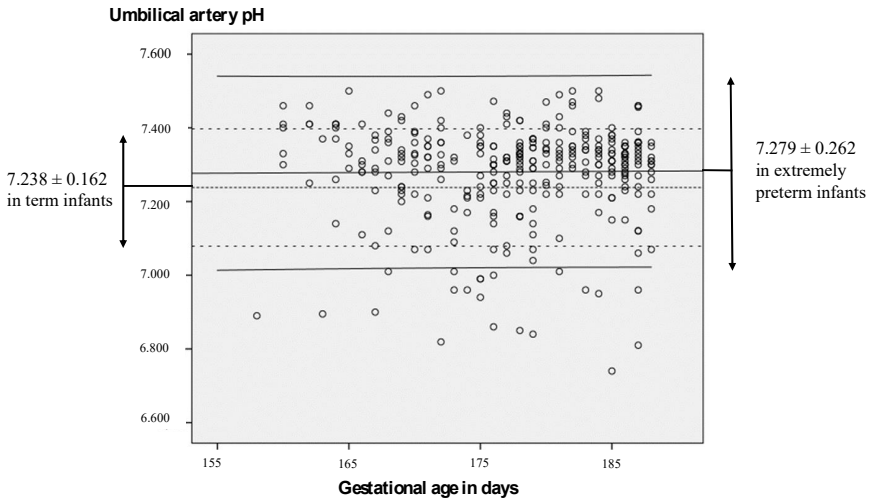

Figure 21:
Scatterplot and range (mean ± 2 standard deviations) of umbilical cord arterial pH values obtained in the current study of 322 extremely preterm newborns relative to the normal reference interval for 24 390 term newborns reported by Kitlinski et al. [40] .The difference between groups was statistically significant (unpaired *t*-test, *P*<0.0001).

ROC curves to identify optimal discriminatory cut-offs of blood gas values

ROC curves were calculated for pH and BE_{ecf} relative to three outcome variables (i.e. death up to 6.5 years of age, moderate or severe neurodevelopmental outcome at 6.5 years, and intact survival) which showed AUC values of 0.49-0.54, indicating a poor separation capacity and thus no optimal cut-off values could be identified.

Mortality and neurodevelopmental outcome at 6.5 years of age

Since no optimal cut-off values for prediction of adverse outcomes in relation to cord blood gas values could be identified by the ROC curves, we used the 10th percentile values in the subsequent multiple logistic regression analyses: 7.08 for pH and -9.76 mmol/L for BE_{ecf}. These cut-off values were used binary (normal/abnormal) in relation to the outcome variables mentioned in Table 18. When adjusted for differences in gestational age and delivery mode, low pH or BE_{ecf} values were not significantly related to an increased risk of death or neurodevelopmental outcome up to 6.5 years of age.

Table 18:
Odds ratio of umbilical cord arterial cord blood gas values < 10th percentile to predict risk of mortality and impaired neurodevelopmental outcome at 6.5 years of age

Outcome	Number of cases with complete data	pH <7.08	pH ≥7.08	Odds ratio		Adjusted odds ratio		Multiple logistic regression: *P* value after adjustments[a]
		n/N	n/N	OR	95% CI	AOR[a]	95% CI	
Death <6.5 years	299	10/28	61/271	1.9	0.8–4.4	1.7	0.7–4.1	0.20
Moderate or severe neurodevelopmental impairment at 6.5 years	228	7/18	74/210	1.2	0.4–3.1	1.0	0.4–2.9	0.96
Intact survival	299	11/28	136/271	0.6	0.3–1.4	0.7	0.3–1.7	0.45
		BE_{ecf} < -9.76	BE_{ecf} ≥ -9.76					
		n/N	n/N					
Death <6.5 years	287	10/29	55/258	1.9	0.9–4.4	1.8	0.8–4.2	0.18
Moderate or severe neurodevelopmental impairment at 6.5 years	222	7/79	12/143	1.1	0.4-2.8	1.0	0.4-2.8	1.0
Intact survival	287	12/29	131/258	0.7	0.3-1.5	0.8	0.3-1.7	0.53

OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval
BE_{ecf} values are in mmol/L
[a] Statistical analyses adjusted for gestational age at birth (in days) and cesarean section (yes/no)

Comments

The study could not establish that extremely preterm infants with abnormally low pH or BE_{ecf} at birth, would run a higher risk of mortality or neurodevelopmental outcome later in life. Since abnormally low pH and BE values are proxies of severe intrapartum hypoxia, it appears that mortality and morbidity in extremely preterm infants is reliant on factors other than intrapartum hypoxia and the development of acidosis. Similarly, normal blood gases did not predict an intact survival.

Based on previous studies with cord blood obtained from cordocentesis [167] or at birth [27, 40], we expected that the pH values would decline by progression of gestation, but no significant changes were found over the interval 22-26 weeks. However, the pH values were significantly higher in our cohort of extremely preterm infants than in a series of term newborns[40] used for comparison. Thus, previously established evidence that cord pH decreases with progression of pregnancy, was not challenged.

Although the cord pH values in extremely preterm newborns were significantly higher than in term newborns, the mean minus 2SD value was lower than in term newborns, 7.017 vs 7.076. This finding can be explained by the population sizes, being 322 newborns in the present study and 24 390 in the term group. With a larger cohort, the variance range would be narrower [177]. Extremely preterm birth (< 27 weeks of gestation) is thankfully an uncommon phenomenon with an incidence of only 3.3 per 1000 infants [89]. Therefore, the prospect of performing a larger study than ours on extremely preterm infants is challenging, since our series was collected nation-wide during a 3-year period in Sweden with 10 million inhabitants.

Summary

In clinical practice, obstetricians and neonatologists often struggle when counselling parents about the neurodevelopmental future of their extremely preterm infant. A low cord pH is strongly associated with risk for death and poor neurological outcome in term infants [9], but the results of this study indicate no such correlation for infants born <27 gestational weeks. Hence, it seems that intrapartum hypoxia with acidosis is not a risk factor that is critical in the assessment of the long-term morbidity and mortality of these infants. Little is known about acid-base regulation in extremely preterm infants and the specific relevance of acidosis in this high-risk group is therefore still uncertain.

Paper IV: Umbilical cord blood concentrations of Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) and Glial fibrillary acidic protein (GFAP) in neonates developing hypoxic-ischemic encephalopathy

It has been shown that UCH-L1 and GFAP are elevated in the serum of neonates with moderate to severe HIE [144, 145, 161], however, biomarkers in umbilical cord blood obtained immediately at birth have been studied only occasionally. This study aimed to compare UCH-L1 and GFAP concentrations in umbilical cord blood collected directly at birth of neonates who developed moderate or severe HIE (stage II or III) to healthy neonates, and to relate the serum concentrations to the severity of neurological symptoms and long-time outcomes. The hypothesis was that stage II–III HIE would be associated with higher concentrations of UCH-L1 and GFAP.

Results

Table 19 shows the clinical data from 34 identified HIE cases. In 16 HIE cases, stored umbilical cord sera were found in the serum bank of the Maternity Unit of Skåne University Hospital in Malmö.

Significant differences were found for mode of delivery, low Apgar score, cord pH values and cord blood acidosis between HIE cases and controls (Table 20). The UCH-L1 and GFAP analyses failed in one HIE case (stage III) and one control, so the number of HIE cases with valid UCH-L1 and GFAP values was 15 (seven cases with HIE stage II, eight cases with stage III) and the number of controls was 31.

Comparisons of UCH-L1 and GFAP concentrations between controls and HIE cases

The concentration of UCH-L1 was statistically not different between cases (mean 33.5, SD 51.9, median 16.0, range 2.3–195.1 ng/mL) and controls (mean 20.3, SD 25.6, median 14.0, range 1.1–137.9 ng/mL; P =0.61); the lack of statistical difference remained after exclusion of two cases and one control with values >100 ng/mL (P =0.96).

Comparisons of UCH-L1 and GFAP concentrations between controls and HIE cases with and without cord blood acidemia

Umbilical cord blood pH was determined in all 16 HIE cases and in 27 of 32 controls. Cord blood pH and Apgar scores in the HIE group are displayed in Table 19. Acidemia

was significantly more common among HIE cases than among controls (Table 20). All controls had Apgar scores 9–10–10 at 1, 5 and 10 minutes.

There were no significant differences in UCH-L1 and GFAP concentrations between controls and HIE cases with and without cord blood acidemia (K–W test: UCH-L1, GFAP, $P \geq 0.21$).

Comparisons of UCH-L1 and GFAP concentrations between controls and cases with HIE stage II and III

There was no significant difference in neither UCH-L1 nor GFAP concentrations when we compared controls to cases with HIE stage II (N =7) and III (N =8; K–W test: UCH-L1, GFAP, $P \geq 0.75$; Figures 22 and 23).

Comparison of UCH-L1 and GFAP concentrations between controls and HIE cases with and without sequelae

There was no significant difference in UCH-L1 and GFAP concentration when we compared controls (N =31) to HIE cases without (N =8) and with (N =7) sequelae/death (K–W test: UCH-L1, GFAP, $P \geq 0.70$).

Table 19:
Characteristics of 34 neonates with moderate (stage II) or severe (stage III) hypoxic-ischemic encephalopathy (HIE) (Sarnat & Sarnat, 1976). For classification of aEEG, see text.

Nr	Serum	HIE	Apgar[a]	Artery pH	Seizures	aEEG pattern	Sequelae[b]	UCH-L1	GFAP
1	Yes	II	4-7-9	7.06	Yes[c]	Continuous	No	195.051	0.137
2	Yes	II	1-3-4	6.63	Yes[c]	Continuous	No	16.010	0.206
3	No	II	1-3-5	7.29	Yes[c]	Discontinuous	No	-	-
4	No	II	2-4-6	6.85	Yes[c]	Continuous	No	-	-
5	Yes	II	2-3-7	6.97	Yes	Discontinuous, seizures	No	4.525	0.356
6	Yes	II	1-3-9	7.36	Yes	Continuous, seizures	No	11.234	0.096
7	No	II	4-6-8	7.17	Yes	Continuous, seizures	No	-	-
8	Yes	II	1-5-7	7.26	Yes	Continuous, seizures	No	5.542	0.507
9	No	II	1-6-9	6.66	Yes	Burst-supression, seizures	No	-	-
10	Yes	III	1-1-1	7.12	Yes	Low-voltage, saw-tooth	Deceased 4th day	[e]	[e]
11	No	III	3-5-5	6.87	Yes	Continuous, seizures	No	-	-
12	Yes	II	1-6-7	6.94	Yes	Continuous, seizures	No	44.960	0.066
13	No	II	2-3-5	6.71	Yes	Discontinuous, seizures	Mental retardation, mother died	-	-
14	Yes	III	0-5-5	6.69	Yes	Discontinuous, seizures	Yes (hyperactivity)	3.108	0.205
15	No	II	5-7-7	6.83	Yes	Continuous, siezures	No	-	-
16	No	III	0-2-3	6.72	Yes	Low-voltage, seizures	Deceased 1st day	-	-
17	Yes	II	2-4-6	7.14	Yes	Discontinuous, seizures	EP, mental retardation	2.268	0.232
18	No	II	8-8-8	7.19	Yes	Discontinuous, seizures	CP, A cerebri media infarction	-	-
19	Yes	III	0-2-3	6.80	Yes	Burst-suppression, seizures	Deceased 3rd day	37.635	0.242
20	No	II	1-3-5	6.95	Yes	Discontinuous, seizures	No	-	-
21	No	III	0-3-5	6.74	Yes	Burst-suppression, seizures	CP	-	-
22	No	III	0-3-5	6.80	Yes	Discontinuous, seizures	No	-	-
23	No	III	1-3-4	6.92	Yes	Burst-suppression, seizures	No	-	-
24	No	III	1-6-8	7.16	Yes	Burst-suppression, seizures	No	-	-
25	Yes	III	5-7-8	7.20[d]	Yes	Continuous, seizures	No	10.510	0.455
26	Yes	III	8-9-9	6.84	Yes	Discontinuous, seizures	No	106.568	0.290
27	No	III	1-3-4	7.14	Yes	Burst-suppression, seizures	No	-	-
28	Yes	III	3-7-9	7.01	Yes	Continuous, saw-tooth	N. facial paralysis	19.760	0.207
29	Yes	III	7-8-9	7.09	Yes	Burst-suppression, seizures	CP	21.395	0.084
30	Yes	III	9-10-10	7.11	Yes	Continuous, seizures	CP	17.098	0.298
31	Yes	III	1-3-6	6.89[d]	Yes	Low-voltage, seizures	CP, EP	6.951	0.098
32	No	III	2-4-5	6.79	Yes	Low-voltage, seizures	CP, EP	-	-
33	No	III	1-1-3	7.17	Yes	Isoelectric	Deceased 2nd day	-	-
34	No	III	1-3-4	6.49	Yes	Low-voltage, seizures	Deceased 1st day	-	-

aEEG = continuous amplitude-integrated electroencephalogram; EP = epilepsy; CP = cerebral palsy; ICH = intracranial hemorrhage
[a] Apgar scores at 1, 5 and 10 minutes of age
[b] Follow-up to age 6-7 years
[c] Hyper-excitability and twitching understood as a single seizure, no sustained seizures confirmed by aEEG
[d] Venous blood pH, arterial pH missing
[e] Analysis failed

Table 20:
Demographic data and outcome in HIE stage II-III neonates and controls. Figures are number of cases or mean ± SD (median; range)

	HIE neonates (N =16)	Controls (N =32)	Significance of difference (*P*)
Maternal age (years)	30.0 ± 5.7 (28.5; 21–39)	28.1 ± 4.9 (27; 19–40)	0.3
Nulliparae	13	17	0.07
Gestational weeks at delivery[a]	40.0 ± 1.5 (40; 37–42)	40.0 ± 1.4 (40; 37–42)	1.0
Storage of cord sera (months)[a]	202.6 ± 36.1 (204.5; 142–258)	203.9 ± 35.5 (206; 142–258)	0.8
Spontaneous delivery	1	32	<0.000000[b]
Operative vaginal delivery	9	0	-
Cesarean section	6	0	0.000001[c]
Female gender[a]	7	14	1.0
Birthweight (g)	3524 ± 588 (3313; 2990–4785)	3629 ± 439 (3670; 2670–4310)	0.2
Apgar score <7 at 5 min	10	0	0.000001
Umbilical artery pH	7.00 ± 0.21 (7.01; 6.63–7.36)	7.26 ± 0.07 (7.25; 7.13–7.42)	<0.0001
Umbilical vein pH	7.14 ± 0.16 (7.17; 6.80–7.39)	7.35 ± 0.05 (7.34; 7.23–7.50)	<0.0001
Umbilical artery pH <7.00	7/16	0/27	0.0004
Umbilical artery pH <mean minus 2 SD	9/16	0/27	0.00002
HIE II (of whom sequelae/dead)	7 (1)[d]	-	-
HIE III (of whom sequelae/dead)	9 (7)[d]	-	-
Dead among stage II/stage III[e]	0/2[f]	-	-
Survivors with sequelae at age 6–7 years	6/14	-	-

Statistics performed with the Mann–Whitney *U* test, Chi-square test or Fisher's exact test.
[a] Matching criterion.
[b] Spontaneous vaginal delivery versus other.
[c] Cesarean section versus other.
[d] HIE stage II versus stage III for sequelae or dead: *P*= 0.04.
[e] Among the originally 34 HIE neonates, 5 died and all dead had HIE stage III (Table 19).
[f] HIE stage II versus stage III: *P*= 0.5.

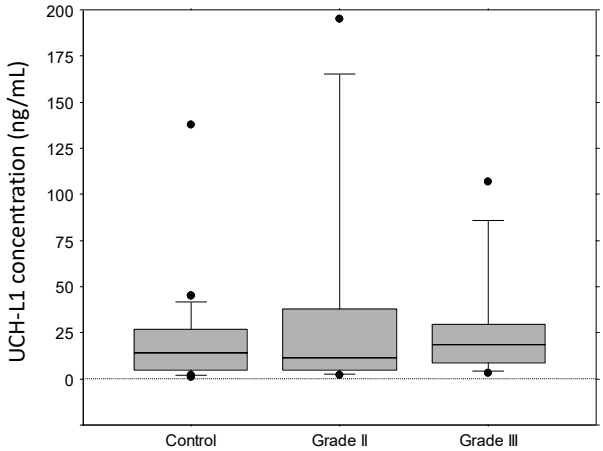

Figure 22:
Umbilical cord serum concentration of Ubiquitin carboxy- terminal hydrolase L1 (UCH-L1) in seven neonates with hypoxic- ischemic encephalopathy (HIE) stage II and eight neonates with HIE stage III compared to 31 healthy matched control neonates. No statistically significant differences were found between controls and HIE cases (Mann–Whitney *U* test, *P* =0.61) or between controls, HIE stage II and HIE stage III (Kruskal–Wallis test, *P* =0.75).

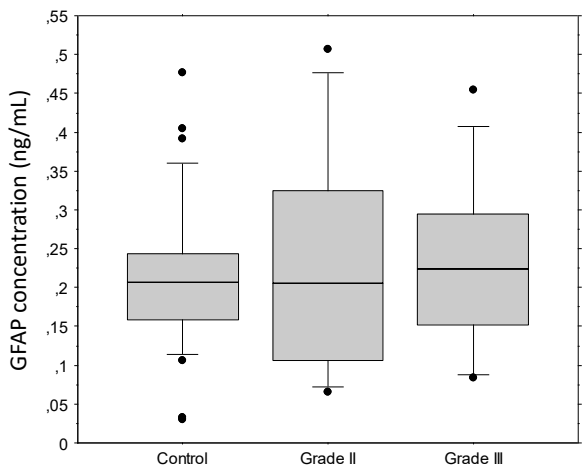

Figure 23:
Umbilical cord serum concentration of Glial fibrillary acidic protein (GFAP) in seven neonates with hypoxic-ischemic encephalopathy (HIE) stage II and eight neonates with HIE stage III compared to 31 healthy matched control neonates. No statistically significant differences were found between controls and HIE cases (Mann–Whitney *U* test, *P* =0.80) or between controls, HIE stage II and HIE stage III (Kruskal– Wallis test, *P* =0.87).

Comments

This study showed that perinatal asphyxia leading to moderate (stage II) or severe (stage III) HIE was not significantly associated with increased levels of UCH-L1 or GFAP in umbilical cord blood at birth. Likewise, there was no association between the biomarker concentrations and the severity of disease, i.e. cord blood acidemia at birth, pathology of the aEEG pattern, stage of HIE or whether the condition developed into a permanent or fatal injury.

Previous studies indicate that both UCH-L1 and GFAP are elevated already in the early postpartum period in HIE stage II–III neonates [140, 144-146, 161, 178], but our results are not necessarily in conflict with these studies. We analyzed cord blood sampled immediately at birth, which has been done in only one previous study [145]. Ennen et al. [145] compared HIE stage II–III neonates and controls and found no difference in GFAP concentration in cord blood, whereas in the second batch of blood samples obtained at admission to the neonatal intensive care unit up to 6 hours after birth, the HIE neonates showed significantly higher GFAP concentrations compared to the controls. A single HIE neonate had an abnormal brain MRI and that neonate showed no higher GFAP value than the HIE neonates with a normal MRI. In accordance with our results, the observations indicate that GFAP in cord blood might not be elevated even in neonates developing structural brain lesions. Regarding UCH-L1, we have found no previous study comparing cord blood; Douglas-Escobar et al. [140] collected cord blood from controls but not from HIE neonates.

No study has serially followed the natural courses of GFAP or UCH-L1 in HIE neonates over days [145]; during cooling therapy, however, the early high GFAP level remains for days and may even increase, but the UCH-L1 concentration declines after some hours [140, 145, 146, 161, 178]. A decline in UCH-L1 could occur also in neonates who eventually die [144]. The magnitude of surge is for both biomarkers associated with the initial severity of disease [144, 146], and both correlate with the extent of brain injury and impaired neurodevelopmental outcome [140, 145, 146, 161, 178]. It seems that the best discriminatory ability of UCH-L1 is obtained by a blood sample taken early in the disease course, whereas GFAP seems to have a later point of time [140, 161]. Rat experiments have shown that an increase in GFAP could continue for several days and even up to a month after an ischemic brain lesion [179, 180]. In neonatology, a rising value is an ominous sign indicating ongoing secondary injury [145, 161].

Summary

This study examined UCH-L1 and GFAP concentrations in umbilical cord blood taken at birth of neonates that later went on to develop moderate/severe HIE, and compared them to healthy controls. Our findings did not suggest any association between neuronal injury and biomarker concentrations. This, however, is not necessarily in conflict with previous studies showing opposite results since a rapid increase in neuronal biomarker concentrations may occur during the very first hours of life in affected neonates and may not be detectable at the time of birth. Our study was small and further research is needed to elucidate the role of GFAP and UCH-L1 in umbilical cord blood as biomarkers for neonatal HIE.

Paper V: Protein S100B in umbilical cord blood as a potential biomarker of hypoxic- ischemic encephalopathy in asphyxiated newborns

Hypoxic ischemic encephalopathy (HIE) is a devastating condition resulting from the sustained lack of oxygen during birth. The interest in identifying a relevant biomarker of HIE has thrown into limelight the role of protein S100B as a clinical diagnostic marker of hypoxic brain damage in neonates. This study aimed to evaluate the diagnostic value of protein S100B, measured in umbilical cord blood immediately after birth, as a useful biomarker in the diagnosis of HIE stages II-III as well as a marker for long-term mortality and morbidity.

Results

For S100B analyses, cord blood serum was retrieved from 13 HIE cases and 21 controls. Six cases were diagnosed with HIE stage II and 7 cases with HIE stage III (Table 21).

Significant differences between HIE cases and controls were found for mode of delivery, low Apgar score, cord pH values and cord blood acidosis (Table 22). Two controls showed outlying values (S100B concentration 5.27 and 1.94 μg/L, respectively) (Fig. 24). Spontaneous vaginal delivery occurred in both, gestational ages were 39 and 40 weeks, birthweights were 3460 g and 3770 g, Apgar scores were in both 9 at 1 min and 10 at 5 and 10 min, and cord artery pH was 7.33 and 7.22, respectively. Both neonates were discharged home healthy.

Comparison of S100B concentration between HIE cases and controls

The difference in S100B concentration was marginally statistically significant between cases (median 0.55 µg/L, range 0.23–3.22) and controls (median 0.32 µg/L, range 0.18–5.27) (M-W test, P = 0.056) (Fig. 24); the difference was significant after exclusion of the two control outliers mentioned above (P = 0.013).

A significant ordinal association was found between S100B concentration and severity of HIE (controls vs. HIE II vs. HIE III; Kendall rank correlation, P = 0.027), but the K-W test was not significant (P = 0.16).

S100B concentration relative to cord blood acidosis

Cord blood pH and Apgar scores in cases are displayed in Table 21. Acidosis was significantly more common among HIE cases than among controls (Table 22). A significant negative relation was found between S100B and cord artery pH with Kendall rank correlation (tau –0.27, P = 0.046) but not with simple linear regression (P = 0.57).

Table 21:
Characteristics of 13 neonates with moderate (stage II) or severe (stage III) hypoxic-ischemic encephalopathy (HIE) [114] with protein S100B determined in umbilical cord blood at birth. For classification of aEEG, see text.

Nr.	HIE	Apgar[a]	Artery pH	Seizures	aEEG Pattern	Sequelae[b]	Protein S100B (ug/L)
1	II	4-7-9	7.06	Yes[c]	Continuous	No	0.38
2	II	1-3-4	6.63	Yes[c]	Continuous	No	0.82
3	II	2-3-7	6.97	Yes	Discontinuous, seizures	No	0.33
4	II	1-5-7	7.26	Yes	Continuous, seizures	No	0.46
5	II	1-6-7	6.94	Yes	Continuous, seizures	No	2.15
6	II	2-4-6	7.14	Yes	Discontinuous, seizures	EP, mental retardation	0.52
7	III	1-1-1	7.12	Yes	Low-voltage, saw-tooth	Deceased 4th day	0.55
8	III	0-2-3	6.80	Yes	Burst-suppression, seizures	Deceased 3rd day	0.58
9	III	5-7-8	7.20[d]	Yes	Continuous, seizures	No	0.32
10	III	8-9-9	6.84	Yes	Discontinuous, seizures	No	1.27
11	III	3-7-9	7.01	Yes	Continuous, saw-tooth	N. facial paralysis	3.22
12	III	7-8-9	7.09	Yes	Burst-suppression, seizures	CP	0.23
13	III	9-10-10	7.11	Yes	Continuous, seizures	CP	0.77

aEEG = continuous amplitude-integrated electroencephalogram; EP = epilepsy; CP = cerebral palsy; ICH = intracranial hemorrhage
[a] Apgar scores at 1, 5 and 10 minutes of age.
[b] Follow-up to age 6-7 years.
[c] Hyper-excitability and twitching understood as a single seizure, no sustained seizures confirmed by aEEG.
[d] Venous blood pH, arterial pH missing.

Table 22:
Demographic data and outcome in HIE stage II-III neonates and controls. Figures are number of cases or mean ± SD (median; range).

	HIE neonates N=13	Controls N=21	Significance of difference (*P*)
Maternal age (years)	29.3 ± 5.6 (28; 21-39)	28.5 ± 4.5 (28; 21-40)	0.79
Nulliparae	2	10	0.075
Gestational weeks at delivery[a]	39.8 ± 1.5 (40; 37-42)	39.9 ± 1.2 (40; 37-42)	0.85
Storage of cord sera (months)[a]	201.7 ± 36.8 (204; 142-258)	209.0 ± 35.5 (207; 142-258)	0.51
Spontaneous vaginal delivery	1	21	<0.00000[b]
Operative vaginal delivery	8	0	-
Cesarean section	4	0	-
Female gender[a]	5	10	0.73
Birthweight[a] (grams)	3580 ± 636 (3270; 2990-4785)	3601 ± 462 (3640; 2670-4310)	0.40
Apgar score <7 at 5-minutes	7	0	0.0003
Umbilical artery pH	7.00 ± 0.17 (7.03; 6.63-7.26)	7.24 ± 0.06 (7.25; 7.13-7.33)	<0.0001
Umbilical vein pH	7.14 ± 0.14 (7.17; 6.80-7.37)	7.34 ± 0.06 (7.34; 7.23-7.50)	<0.0001
Umbilical artery pH <7.00	5/12	0/16	0.008
Umbilical artery pH < mean minus 2SD	8/13	0/21	0.00007
HIE II (of whom sequelae/dead)	6 (1)[c]	-	-
HIE III (of whom sequelae/dead)	7 (5)[c]	-	-
Dead among stage II/stage III	0/2[d]	-	-
Survivors with sequelae at age 6-7 years	4/11	-	-

Statistics performed with the Mann-Whitney *U* test, Chi-square test or Fisher´s exact test.
[a] Matching criterion.
[b] Spontaneous vaginal delivery versus operative vaginal delivery and caesarean section.
[c] HIE stage II versus stage III for sequelae or dead: $P = 0.10$.
[d] HIE stage II versus stage III: $P = 0.46$

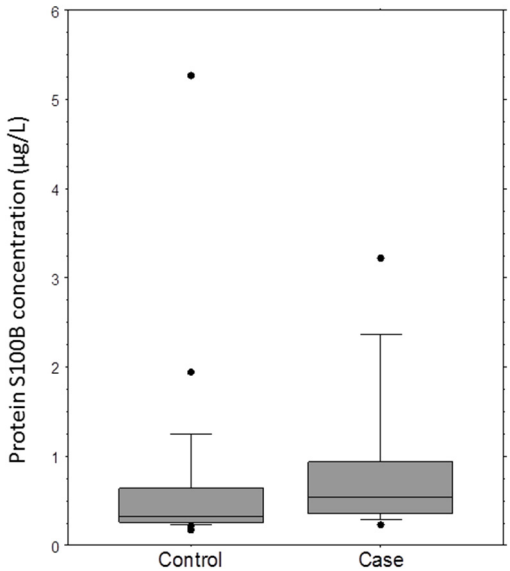

Figure 24:
Comparison of protein S100B concentration in umbilical cord blood at birth between 13 neonates affected by hypoxic-ischemic encephalopathy Sarnat stage II-III and 21 healthy controls. The difference is statistically marginally significant (Mann-Whitney *U* test, *P* = 0.056); exclusion of two outlying controls (S100B 5.27 and 1.94 µg/L, respectively) resulted in a significant difference (*P* = 0.013).

S100B concentration relative to permanent sequelae/death

Among the six neonates with HIE stage II, only one infant suffered from sequelae at follow-up (Table 21: neonate nr. 6 had epilepsy and mental retardation); among the seven neonates with HIE stage III, only two were without sequelae at follow-up (Table 22: cases nr. 9 and 10). Since only three cases were discordant, the stage of HIE and development of a permanent sequela were closely related (McNemar test, *P* = 1.0). A significant ordinal association was found between S100B concentration and sequelae (controls vs. no sequelae vs. sequelae; Kendall rank correlation, tau = 0.27, *P* = 0.027), but the K-W test was not significant (*P* = 0.16).

Comments

An association between elevated neonatal S100B concentrations and development of encephalopathy has been established previously in several studies [135, 181, 182], with elevated S100B levels proportional to the severity of disease [129, 130, 134, 183-189]. Thorngren-Jerneck et al. [190] showed a significant relationship between S100B levels and severity of HIE in blood obtained from an umbilical catheter after admission to the neonatal

intensive care unit some hours after birth, and Massora et al. [191, 192] examined S100B levels during hypothermia treatment of infants with encephalopathy and concluded that higher S100B levels were associated with poor neurodevelopmental outcome at 15 months of age. Our study supports those findings, but since S100B has a half-life of about 30 min [193], the optimal detection of an intrapartum hypoxic injury might be in samples obtained early in the neonatal course. Thus, serial sampling starting immediately after birth may give additional information about the extent and course of the injury.

The identification of biomarkers that could identify neonates who would benefit from neuroprotective treatments such as hypothermia is presently a hot topic in perinatal medicine. Summanen et al. [194] recently showed in a prospective case-control study that S100B levels were not significantly raised in asphyxiated neonates when taken from the umbilical cord artery immediately after birth. However, their diagnostic criteria for birth asphyxia were relatively milder as compared to other studies, including the present study.

Even though our study showed a relationship between S100B and HIE II-III, it was not possible to outline a cut-off value and evaluate the performance of cord blood S100B as a binary predictive test for HIE. This was because of a considerable overlap of values between cases and controls, but also because of two outliers occurring in the control group.

As mentioned earlier, HIE is a rare condition and collecting appropriate clinical material is challenging. This can be illustrated by the fact that during the 12-year study period only 34 neonates with HIE II-III were identified in our registers, corresponding to an incidence of 0.09% (34/36 550 total births). Furthermore, with advancing perinatal care the incidence of birth asphyxia is declining [195].

The retrospective design of our study, in which we used serum samples stored frozen at −20 °C, might be a limitation, although we found no influence of storage time and we were vigilant to rule out a possible effect of hemolysis. In contrast, Müller et al. [196] found a significant increase of the S100B concentration in a cohort of 31 serum samples first analyzed in 1997 and then stored frozen at −20 °C over 6 years until 2003 when the samples were analyzed again. These findings are in conflict with other published data [197, 198] and it is paradoxical since in cell-free serum there should be no de novo synthesis or release of proteins from hemolysis over time. The sample handling, which is critical for S100B analysis [198], was not described by Müller et al. [196], nor was the evaluation of hemolysis and statistical test used.

Summary

This study shows an association between umbilical cord blood S100B concentrations in asphyxiated newborns and risk for development of moderate to severe HIE elucidated in relation to the severity of diagnostic signs and development of neurological handicaps and death. However, outlying values in the control group suggest that S100B may be high for other reasons than brain injury caused by intrapartum hypoxia. This may be a limitation for using protein S100B as a single predictor of HIE developing to neurodevelopmental handicap.

As discussed by other authors [135, 182, 190] adding brain injury biomarkers to the arsenal of clinical signs, laboratory data and imaging techniques may enhance the diagnosis and better predict the risk of permanent handicap, but the biomarker analysis must be point-of-care to enable early initiation of therapies to prevent and restrict brain damage. The small sample sizes, statistically indefinite results, and uncertain conclusions in this and previously published articles call for further studies in multicentre settings to reveal the true diagnostic potential of protein S100B.

5. Conclusions

This thesis highlighted the value of umbilical cord blood sampling at birth and investigated the possible connection between different analytes within cord blood, to the risk of death or neurodevelopmental outcome later on in life. Based on the findings from the original studies included in this thesis, the following conclusions were made:

I. Both umbilical cord arterial and venous pH decreased linearly with increasing gestational age. Median 5-minute Apgar score was <10 before 31 weeks of gestation.

II. Considering cord acidemia as a proxy for intrapartum hypoxia, large-for-gestational age (LGA) fetuses showed no impaired ability to produce lactate during hypoxia. Maternal diabetes did not hamper the ability of LGA fetuses to produce lactate during hypoxia.

III. Umbilical cord arterial pH and BE did not change significantly with advancing gestational age in extremely preterm newborns. Intrapartum hypoxia was not associated with an increased risk for death or neurodevelopmental outcome by 6.5 years of age.

IV. No significant differences in cord blood concentrations of UCH-L1 and GFAP were found between HIE neonates and controls. Furthermore, biomarker concentrations did not correlate to the severity of encephalopathy and the risk for permanent neurodevelopment outcome or death.

V. Protein S100B in neonates suffering from moderate-severe hypoxic ischemic encephalopathy (HIE) appeared to be elevated in umbilical cord blood at birth. The S100B concentrations were positively associated to the severity of disease and the risk of suffering from neurodevelopmental sequelae and even death.

6. Methodological considerations

Study I: Gestational age-related reference values for Apgar score and umbilical cord arterial and venous pH in preterm and term newborns

The ABL Radiometer is a mini-laboratory that determines not only blood gases but also has optional electrodes for the determination of hemoglobin, lactate, different electrolytes etc. The same model of the Radiometer (Model 735) was used from 2001-2010 at both Malmö and Lund maternity units with trained bioanalytical experts in charge of machine calibration and maintenance.

Starting with over 200 000 samples, after meticulous sorting (details below), we were left with 27 175 paired cord blood samples with corresponding clinical data. Despite the extensive sorting, the relatively large sample size was considered a major strength of the study. All samples that did not fulfil the blood gas analyzers' quality check system were discarded. This included the rejection of all analyses where not only the pH electrode but also electrodes for pO_2, pCO_2 and lactate showed poor calibration and instability. Such electrode errors are usually not shown on the paper printouts from the analyzer but are only detected when the analyzer's hard drive is explored. In addition, the pH values were transferred directly to the statistical computer program, thus eliminated the possibility of human error in retrieving the data.

The pH values were calculated using three decimal places, as reported by the blood gas analyzers, since rounding-off decimals would introduce bias in the statistical calculations [199]. However, both the second and third decimal values are uncertain since the standard drift tolerance of the pH potentiometer electrode is ± 0.020 [147]. Nevertheless, in a substantially large sample, upward and downward drifts would neutralize one another.

Since the aim of the study was to estimate "normal" pH reference values, efforts were made to sort out newborns with adverse outcomes. This was performed with the exclusion of all newborns with a 5-minute Apgar score less than the median score according to gestational week at birth as well as all operative deliveries. This step ensured the inclusion of only "normal", vaginal deliveries in our calculations of the pH reference values. However, despite the large series, we could not create reference values for premature newborns born <34 gestational weeks due to too few cases to perform

robust statistics. It can be argued that an overly strict inclusion criteria may have resulted in this low power, but one must also consider than a sizable portion of preterm infants are often growth restricted and delivered by cesarean section [89]. Inclusion of these infants would thus not reflect "true" changes in acid-base balance since mode of delivery was found to associate significantly with the probability of cord arterial pH being < -2SD percentile.

In conclusion, the strict inclusion criteria ensured high data quality wherewith the aims of the study were reported with added conviction.

Study II: Assessment of lactate production as a response to sustained intrapartum hypoxia in large-for-gestational-age (LGA) newborns

The same crude database of umbilical cord blood samples was used as in Study I. After sorting, over 17 000 paired samples of arterial and venous cord blood we included in the study of which only 8 LGA cases were identified with cord blood acidemia in the group of maternal diabetes. This low number may reflect extra precautions in place at the delivery units, ensuring close intra-partum monitoring and earlier interventions in this high-risk group of women. According to Mundry & Fisher [151], this number was still sufficient to perform the non-parametric Mann-Whitney *U* test, but it can still be noted as a limitation when evaluating hypoxic LGA fetuses of diabetic mothers.

In some of our previous studies, we discovered a relatively large difference between the tied and the untied *P* values. Spearman's rho and Kendall's tau are often used when simple linear regression analysis cannot be performed for the comparison of variables. Spearman's rho is a more commonly used test to show associations between variables but this test can be handicapped by the large influence of ties. We thus used Kendall's tau instead of Spearman's rho for the comparison of variables.

Pre-gestational and gestational diabetes were merged into one group in the statistical analyses, with the reasoning that glycemic control might vary just as much within one of the groups as between the two groups. In addition, we did not adjust for certain factors that have been found to influence acid-base status at birth, such as epidural analgesia and length of labor [200]. We performed numerous statistical tests, being aware of the risk of type 1 error, nevertheless, all our findings consistently pointed to the intact lactate-producing capacity of LGA fetuses during hypoxia.

Study III: Lack of associations between umbilical cord blood gases in extremely preterm newborns and neurodevelopmental outcomes at 6.5 years of age

The Extremely Preterm Infants in Sweden Study (EXPRESS) included data from over 1000 infants born in Sweden between gestational ages 22+0 to 26+6. Of these, 705 infants were live-born and cord arterial pH were available in 322 cases (45.7%). The EXPRESS was not explicitly designed from the view point to collect and analyze blood gas data and this can explain why cord blood gases were available in less than half of the population. We were therefore unable to comment on the quality of the cord blood samples, procedure techniques used etc because of this reason. In any case, cord blood sampling is an established routine at most maternity centres in Sweden and in all University Hospitals since many years.

Of the 322 cases included in the study, just 6 (1.9%) infants were survived birth at 22 gestational weeks and 31 (9.6%) during gestational week 23. Due to the low number of samples in these weeks, we merged gestational week 22 with 23 to obtain reasonable power in our statistics.

In the study, BE_{ecf} (i.e. standard BE) was calculated post hoc from measured values of pH and pCO_2 in order avoid any mix-up between 'actual BE' and 'standard BE' values from the different hospitals. One source of possible error was thus eliminated. However, the sample population was unique since establishing a reasonable statistical power is challenging in this high-risk group of infants considering that the overall incidence of extremely preterm birth (< 27 completed gestational weeks) was only 3.3 per 1000 births in Sweden during the study period [89].

When correlating low blood gases to the risk for impaired neurodevelopmental outcome or death, a cord arterial pH of < 10^{th} percentile (pH < 7.08) was used as a cut-off value, which was higher than the mean -2SD cut-off of 7.02. This higher cut-off value improved the power in our statistics. We compared the distribution of pH values in the extremely preterm group with reference values in term infants and found significantly higher values in the former group (Figure 21). This finding could possibly be explained by the population sizes, being 322 newborns in the extremely preterm group and 24 390 in the historic term group. With a larger cohort, the variance range would be narrower [177] along with the fact that cord blood gases are gestational age dependent [40].

Study IV: Umbilical cord blood concentrations of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) in neonates developing hypoxic-ischemic encephalopathy

From 1969-2001, mixed arterial and venous cord blood was collected and serum extracted for storage at Skåne University Hospital in Malmö. Considering the samples were stored at -20 °C and not at the recommended temperature of -80 °C [198], one could question the quality of samples, especially with regard to the long storage time. However, we found no statistically significant relation between UCH-L1 and GFAP concentrations and the storage time. In addition, free Hb (due to hemolysis in the samples) can possibly affect protein analysis in various immunoassay calibrator machines. However, no significant difference in hemolysis was found between cases and controls.

UCH-L1 and GFAP analysis were not available in Sweden when the study was performed. The serum samples were therefore packaged in dry ice and sent for analysis at Banyan Biomarkers, Alachua, Florida USA. Care was taken to ensure that the samples would remained frozen during the transport process.

The large inter-sample variations in biomarker concentrations were challenging to explain since certain infants with moderate HIE had higher biomarker concentrations than infants with severe HIE (Table 19). The timing of biomarker release or gaps in clinical documentation can be possible explanations.

Thankfully, moderate and severe HIE are rare conditions and therefore published series are small with low statistical power. Some researchers might not be aware that to evaluate the 5% level of significance, there must be a certain minimum number of observations. The studies series comprised 15 HIE neonates and 31 controls, which were sufficient to perform group comparisons with the Mann–Whitney *U* test, and Spearman's rank correlation coefficient in the control group.

Study V: Protein S100B in umbilical cord blood as a potential biomarker of hypoxic-ischemic encephalopathy in asphyxiated newborns

Of the 15 HIE cases identified in *Study IV*, there was enough serum left from 13 HIE cases and 21 controls for S100B analysis. The S100B analysis was available at the Department of Laboratory Medicine, Skåne University Hospital in Malmö and the samples were not shipped elsewhere, like in *Study IV*.

The concentration of free Hb in the serum samples varied from 0.05 to 0.83 g/L among controls, and from 0.05 to 2.21 g/L among HIE cases, which was well below the value of >10 g/L for interference with the S100B analysis in the Roche Cobas instrument [162].

Hemolysis was thus no confounder of S100B analyses. The length of storage in the freezer varied from 142 to 258 months among both HIE cases and controls. We found no significant relation between S100B concentrations and the storage time in controls. The use of mixed cord blood may be a possible limitation of *Study IV* and *V*, since the possible clearance of the proteins in question (via placental passage) is unknown.

S100B was marginally significant (M-W test, $P = 0.056$) in the 13 neonates affected by moderate to severe HIE vs 21 matched controls. Exclusion of two outlying controls with high S100B concentration of 5.27 and 1.94 µg/L, respectively (Figure 24), resulted in a significant difference ($P = 0.013$). Re-examination of obstetric records may provide a clue as to why these seemingly normal controls had abnormally high neurological biomarker concentrations.

The relatively small sample sizes in *Study IV* and *V* increased the risk of type 1 errors and a well-designed, multicentre, prospective study can be recommended to explore the relevance of these biomarkers even further.

7. Future perspectives

Fetal life conditions have a significant impact on the individual's development and health later in life. For example, growth restriction in utero increases the risk of developing heart disease and atherosclerosis during adulthood. Similarly, LGA infants are at increased risk for metabolic syndrome later on in life. Antenatal check-ups during pregnancy and surveillance during delivery help to discover deviations from normal fetal development. However, little is known how these deviations influence the individual's health later in life.

With regard to fetal hypoxia and metabolic acidosis, it has been shown that newborns with a pH <7.00 in the umbilical cord artery and an Apgar score <4 at 5 minutes, run a 50% risk of developing cerebral palsy (CP) later in life. The strong association between low cord pH and perinatal mortality was confirmed in several other studies. However, these are crude statistics and little is known about different degrees of cord blood acidosis at birth and the risk of developing not only neurological problems but also other diseases and handicaps in the long-term. *Study III* could not show any association between cord blood gases of extremely preterm infants and the risk for death or neurodevelopmental outcome up to 6.5 years. However, this study was in a small and very specific patient group and a larger cohort study with long term follow-up is the need of the hour.

Using our databases, we can perform such a study. At the maternity unit in Malmö, umbilical cord pH determinations have been a routine since August 1981 and we have maternal personal numbers for over 102 000 women who have delivered infants at our maternity centres during this time. In combination with data from the other registers, including: Patient Register (inpatient + outpatient), Cause of Death Register, Medicine Register and the Medical Birth Register; we have the possibility to correlate cord blood gases to the development of disease and handicap up to 38 years of age.

Our study would give invaluable information about the long-term consequences of having acidosis or pre-acidosis at birth. This would help neonatologists to target preventive measures to limit the extent of injury caused by hypoxia, and help them realistically counsel the parents of affected newborns. It will also give insight to the long-term effects of perinatal asphyxia and enable obstetricians to make more informed decisions when monitoring mothers intrapartum. The study would also clarify the on-going debate of routine vs. selective cord blood gas determinations.

8. Populärvetenskaplig sammanfattning på svenska

Avhandlingen består av fem delarbeten. Den röda tråden i avhandlingen är ”Informative Fetal Blood”, vilket innebär att vi har analyserat olika ämnen i navelsträngsblod vid födseln och jämfört deras koncentrationer med olika utfall vid förlossningen eller sjukdom senare i livet. Ämnena vi undersökt är dels s.k. hjärnskademarkörer, dels blodgaser och laktat. Två av studierna bygger på data hämtade från register, medan tre studier bygger på material som insamlats prospektivt.

Ämnesomsättningen (metabolismen) är beroende av tillgång till syrgas och energikällor. Fostrets huvudsakliga energikälla är glukos. Ett normalt foster bygger upp energidepåer av glukos i form av glykogen, som lätt omvandlas till glukos igen vid aerob metabolism.

Den intrauterina miljön är syrefattig men fostret har väl utvecklade försvarsmekanismer för att klara även aggraverad syrefattigdom, åtminstone under en begränsad tidsperiod. Vaginal förlossning med livmodersammandragningar innebär att syretillförseln från modern stängs av tillfälligt under sammandragningarna, med risk för att utveckla akut syrebrist om sammandragningarna är långa och täta, eller om fostret redan ligger på gränsen till syrebrist. Fostret utsätts för kraftig hypoxisk stress under förlossningen och aldrig senare i livet har en människa så höga nivåer av stresshormoner som när man föds. Under perioder av syrebrist, d.v.s. då tillgången till syrgas är lägre än behovet, inträder ett tillstånd av anaerob metabolism. Vätejoner (H^+) bildas då i ökad mängd och pH sjunker samtidigt som laktat stiger. Om syrebristen är allvarlig och ihållande kan fostret utvecklar en ”syraförgiftning” eller acidos som kan drabba bl.a. fostrets hjärna och leder till permanent skada.

En metabolisk acidos manifesterar sig genom en kombination av lågt pH och lågt basöverskott (base excess) samt högt laktatvärde. Att mäta pH eller laktat i skalpblodprov från fostret under förlossningen är sedan länge ett viktigt kliniskt instrument för att identifiera de foster som klarar förlossningen sämre, för att i tid hinna ingripa och undvika bestående hypoxisk skada. En allvarlig syrebrist under förlossningen kan påvisas genom att mäta pH i arteriellt navelsträngsblod, vilket man vid Kvinnokliniken i Malmö gjort rutinmässigt sedan 1981. Dessa mätningar är viktiga

för att utvärdera handläggningen före och under förlossningen, samt för kvalitetsuppföljning och klinisk forskning.

Vissa foster är särskilt känsliga, såsom t.ex. de tillväxthämmade, vid maternell diabetes, de tillväxtaccelererade (stora barn) och de prematura. En viktig fråga är då om dessa foster på samma sätt som de ”normala” fostren kan mobilisera tillräckligt med energi för att vidmakthålla den mycket energikrävande anaeroba metabolismen. Vid anaerob metabolism ökar laktatproduktionen. I en tidigare studie har vi påvisat att tillväxthämmade foster lika bra som normalstora foster klarar av att producera laktat som tecken på syrebrist, och i den föreliggande avhandlingen har vi nu undersökt hur de tillväxtaccelerade stora fostren klarar av samma situation. Kvinnor med diabetes föder oftare stora barn och vi undersökte därför foster till mödrar med eller utan diabetes hur de klarade av att producera laktat vid syrebrist.

En annan grupp med risk för skador är de extremt för tidigt födda barnen, d.v.s. de födda graviditetsvecka 22 till 26. Här undersökte vi ett samband mellan acidos vid födseln och utvecklande av neurologiska utvecklingshandikapp under barndomen.

Syrebrist under förlossning är inte ovanligt, ca 7% drabbas, men endast 0,2% riskerar utveckla en så svår syrebrist att det leder till en hypoxisk skada på den nyföddas hjärna, vilket benämns ”hypoxisk ischemisk encefalopati” (HIE). HIE indelas i tre grader, lätt, moderat och svår (HIE I-III). HIE utvecklas i två faser, där den första fasen inträffar under förlossningen och den andra fasen timmarna efter förlossningen. Den första fasen ska i bästa fall upptäckas och förhindras via fosterövervakning, men det sker tyvärr inte alltid. Under de första 6 timmarna omedelbart efter förlossningen kan man sätta in behandling med nedkylning av barnet för att lindra förloppet av den andra fasen av skada och att HIE II-III utvecklas till en permanent hjärnskada. Barnläkarna har emellertid vissa problem med att särskilja de barn som skulle kunna ha nytta av kylbehandling och de som inte skulle ha det, eftersom neurologiska tecken på hjärnskada många gånger inte uppträder förrän efter mer än 6 timmar. Forskare har sedan länge letat efter biomarkörer som är prediktiva för hjärnskada, d.v.s. proteiner som frisätts från vävnader och som kan påvisas i kroppsvätskor. I avhandlingen har vi undersökt tre olika hjärnskademarkörers förekomst i navelsträngsblod vid födseln och deras prediktiva värde för att senare i livet utveckla hjärnskada och handikapp.

Det prediktiva värdet av acidos vid födseln är omdebatterat och den vetenskapliga litteraturen är härvidlag sparsam. En förutsättning för att kunna använda syra-basstatus i navelsträngsblod som ett prediktivt instrument är att vi vet vad som är normalt. Vi har tidigare publicerat referensvärden för pH i arteriellt navelsträngsblod, men även om studien var stor (24 000 nyfödda) så hade den vissa brister. Arteriellt navelsträngsblod speglar fostrets situation bättre än venöst blod och i avhandlingen har vi på ett säkrare

sätt än tidigare kunnat säkerställa att det är arteriellt respektive venöst blod vi undersökt. Vi har ställt mycket höga krav på både metodologisk och teknisk kvalitet på proverna och sorterat bort de som inte var av toppkvalitet. Vi vill påstå att någon annan motsvarande stor databas, med närmare 19 000 par av arteriella-venösa prover i avhandlingsstudien, inte finns. I studien kalkylerade vi normalvärden inte bara för arteriellt pH för fullgångna nyfödda, utan även för prematura barn och för venöst pH.

I avhandlingen ingår fem delarbeten:

I. I det ***första delarbetet*** har vi beräknat referensområden för pH i både arteriellt och venöst navelsträngsblod från graviditetsvecka 33 till 42. I studien ingick navelsträngsprov från 27 175 förlossningar. Vi har rapporterat fördelningen av Apgarpoäng vid 5 minuters ålder för nyfödda i graviditetsveckorna 28 till 42. Studien ligger till grund för jämförelser mellan vad som är normalt och onormalt, både i kliniken och i forskningsstudier.

II. I det ***andra delarbetet*** görs en jämförelse mellan laktatproduktionen hos stora foster och normalstora foster under förlossning med syrebrist. I studien ingick 17 358 nyfödda barn. Som proxy för syrebrist använde vi lågt pH och högt basöverskott. Studien visade att stora foster producerar laktat i samma nivå som normalstora foster, och vi kunde inte påvisa någon skillnad mellan stora foster för mödrar med eller utan diabetes. Därmed har vi fastställt att laktat kan användas även vid förlossning av stora barn och barn födda av diabetiker.

III. Barn födda före graviditetsvecka 27 anses extremt prematura. Åren 2004 till 2007 utfördes i Sverige en nationell studie, ”Extremely premature infant study in Sweden” (EXPRESS), där omfattande data om graviditet och förlossning prospektivt registrerades i en databas och där uppföljning avseende neurologisk utveckling har gjorts vid 2,5 och 6,5 års ålder. 705 barn föddes levande och blodgaser i arteriellt navelsträngsblod mättes i 46 % av fallen. I det ***tredje delarbetet*** jämfördes syra-basstatus vid födseln (pH och baseöverskottet) med neurologiska handikapp påvisade i barndomen, men något samband mellan acidos och mortalitet eller handikapp kunde inte påvisas. Detta tyder på att syrebrist under förlossningen inte är den viktigaste faktorn för att överleva och att överleva intakt, om man är född extremt prematurt.

IV. I ***delarbete IV*** (och ***V***) användes serum från navelsträngsblod samlat från förlossningar i Malmö. Denna ”serumbank” etablerades redan 1969. Studierna är fall-kontrollstudier, där barn som utvecklat HIE av måttlig (HIE II) eller svår grad (HIE III) identifierades via olika register och databaser. Dessa barn har följts på Barnkliniken i Malmö och uppgifter om deras hälsotillstånd

inhämtades från journalerna. Matchade kontroller valdes ut via förlossningsliggarna, två kontroller för varje fall. I serumbanken förvaras proverna nedfrusna och de identifierades genom barnets födelsedag och moderns personnummer. I 16 av de 34 registrerade fallen av HIE II/III hittades serumprover. I studie ***IV*** analyserades hjärnskademarkörerna ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) och glial fibrillary acidic protein (GFAP). UCH-L1 är ett cytoplasmatiskt enzym specifikt förekommande i neuron och neuroendokrina celler, och GFAP är ett cytoskelettalt protein specifikt för astrocyter. Anledningen till att just dessa två ämnen analyserades är att laboratorieanalyserna gjordes vid Banyan Biomarkers laboratorium i Florida och den kunskap som fanns där. Banyanlaboratoriet drev forskningsprojekt åt US Army för att identifiera olika prognostiska hjärnskademarkörer att användas då amerikanska soldater skadades vid insatser utomlands. Vår studie kunde inte påvisa någon skillnad i koncentrationer av UCH-L1 och GFAP mellan fall och kontroller.

V. I ***delarbete V*** analyserades det hjärnskadeprotein som är mest undersökt av alla, protein S100B. Efter det att analyserna för UCH-L1 och GFAP gjorts, fanns det serum kvar för analys av S100B hos 13 fall och 21 kontroller. Detta antal var tillräckliga för våra statistiska jämförelser. Vi fann i studien högre S100B koncentrationer hos de skadade barnen än hos kontrollerna, samt en korrelation med hur allvarlig hjärnskadan var, vilket stödjer tanken att använda S100B som en markör för risken att utveckla hjärnskada.

Nyhetsvärde

De register och biologiska material vi använt för våra studier är unika i flera avseenden. Databasen med blodgasvärden som vi använde i ***delarbete I*** omfattade mer än 27 000 förlossningar med blodgaser från både artär och ven i navelsträngen. Vi vågar påstå att databasen håller högsta möjliga kvalitet eftersom provtagning har gjorts rutinmässigt och standardiserat sedan 1981, att databasen skapades genom att data överfördes elektroniskt utan mänsklig mellanhand direkt från blodgasapparaterna till databasen, samt att vi sorterade bort alla värden där det fanns minsta tvivel om analyskvalitet och identitet.

Referensvärden för arteriellt pH i navelsträngen har tidigare publicerats av oss för fullgångna graviditeter, men nyhetsvärdet med ***delarbete I*** är att vi nu även presenterar referensvärden för venösa prover och för prematurfödda barn från graviditetsvecka 34 och framåt. Vi presenterar dessutom hur Apgarpoängen vid 5 minuters ålder fördelar sig i förhållande till graviditetsveckor, räknat från vecka 28.

I ***delarbete II*** undersökte vi huruvida stora foster kan producera laktat i samma nivå som normalstora foster när de utsätts för hypoxisk stress under förlossningen. Vi fann ingen skillnad i laktatproduktion. Såvitt vi känner till var det första gången en sådan studie gjordes.

I ***delarbete III*** undersökte vi sambandet mellan blodgaser i navelsträngsblod hos extremt prematurfödda barn och utveckling av neurologiska handikapp upp till 6,5 års ålder. EXPRESS är unik genom att materialet är stort och uppföljningen med neurologiska tester lång och omfattande. Vi kunde inte hitta något samband mellan blodgaser och handikapp, vilket talar för att det är andra faktorer än hypoxi som är viktigare för dessa barns utveckling. Såvitt vi känner till har ingen motsvarande studie gjorts tidigare.

I ***delarbetena IV och V*** användes serum från navelsträngsblod samlat vid förlossningar under många år. På så sätt är vår serumbank unik. Även om vårt material var litet så var det ändå stort, för det är mycket svårt att få ihop ett stort material eftersom både HIE II och III är sällsynta (tack och lov!). Nyhetsvärdet med studierna är att vi undersökte förutom S100B också UCH-L1 och GFAP, samt att proverna representerar födelseögonblicket och inte timmarna efter. Vår studie talar för att S100B, men inte de båda andra hjärnskademarkörerna, kan ha ett kliniskt värde för att bedöma prognosen för barn som är medtagna vid förlossningen p.g.a. syrebrist.

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References

1. William J. Human embryology. Second edition. Philadelphia: Elsevier/Churchill Livingstone; 1997.
2. Cochard LR. Netter's Atlas of Human Embryology. 1st Edition. New Jersey: Icon Learning Systems; 2002.
3. Gill RW, Kossoff G, Warren PS, Garrett WJ. Umbilical venous flow in normal and complicated pregnancy. Ultrasound Med Biol. 1984;10(3):349–63.
4. Weissman A, Jakobi P, Bronshtein M, Goldstein I. Sonographic measurements of the umbilical cord and vessels during normal pregnancies. J Ultrasound Med. 1994;13:11–4.
5. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY. Williams Obstetrics. 23rd ed. New York: McGraw Hill Medical; 2010.
6. Hanretty K. Obstetrics Illustrated. Edinburgh: Churchill Livingstone Elsevier; 2010.
7. Predanic M. Sonographic assessment of the umbilical cord. Ultrasound Rev Obstet Gynecol 2005;5:105–10.
8. ACOG Committee on Obstetric Practice. ACOG Committee Opinion No. 348, November 2006: Umbilical cord blood gas and acid-base analysis. Obstet Gynecol. 2006;108(5):1319-22.
9. Malin GL, Morris RK, Khan KS. Strength of association between umbilical cord pH and perinatal and long-term outcomes: systematic review and meta-analysis. BMJ. 2010;340:c1471.
10. Armstrong L, Stenson BJ. Use of umbilical cord blood gas analysis in the assessment of the newborn. Arch Dis Child Fetal Neonatal Ed. 2007;92:F430–4.
11. Cantu J, Szychowski JM, Li X, Biggio J, Edwards RK, Andrews W, et al. Predicting fetal acidemia using umbilical venous cord gas parameters. Obstet Gynecol. 2014;124:926–32.
12. Armstrong L, Stenson B. Effect of delayed sampling on umbilical cord arterial and venous lactate and blood gases in clamped and unclamped vessels. Arch Dis Child Fetal Neonatal Ed 2006;91(5): F342-5.
13. Lievaart M, de Jong PA. Acid-base equilibrium in umbilical cord blood and time of cord clamping. Obstet Gynecol 1984; 63(1):44-7.

14. Wiberg N, Källén K, Olofsson P. Delayed umbilical cord clamping at birth has effects on arterial and venous blood gases and lactate concentrations. BJOG 2008;115:697-703.
15. Kirshon B, Moise KJ. Effect of heparin on umbilical arterial blood gases. J Reprod Med. 1989;34(4):267-9.
16. Riley RJ, Johnson JWC. Collecting and analyzing cord blood gases. Clin Obstet Gynecol. 1993;36(1):13-23.
17. Bobrow CS, Soothill PW. Causes and consequences of fetal acidosis. Arch Dis Child Fetal Neonatal Ed. 1999; 80: F246–9.
18. Siggaard-Andersen O, Engel K. A new acid-base nomogram: an improved method for calculation of the relevant blood acid-base data. Scand J Clin Lab Invest 1960;12:177-86.
19. Dunlop W. Normal pregnancy: Physiology and endocrinology. In: Edmonds DK, editor. Dewhurst's Textbook of Obstetrics and Gynecology for Postgraduates. 6th ed. United Kingdom, London: Blackwell Sciences Ltd; 1999: 76–90.
20. Blechner JN. Maternal-fetal acid-base physiology. Clinic Obstet Gynecol. 1993;36:3-12.
21. Mushambi MC. Pinnock C, Lin T, Smith T. Physiology of pregnancy, Fundamentals of Anaesthesia. 2002; London: Greenwich Medical Media Ltd: 511-27.
22. Suresh MS, Segal BS, Preston RL, Fernando R, Mason CL. Shnider and Levinson's Anesthesia for Obstetrics, Fifth Edition. Publisher LWW. 2012.
23. Siggaard-Andersen O. The Van Slyke equation. Scand J Clin Lab Invest 1977;37:15-20.
24. American College of Obstetricians and Gynecologists (ACOG) and American Academy of Pediatrics (AAP). Neonatal encephalopathy and neurologic outcome, Second Edition. ACOG; Washington, DC 2014.
25. Victory R, Penava D, Da Silva O, et al. Umbilical cord pH and base excess values in relation to adverse outcome events for infants delivering at term. Am J Obstet Gynecol. 2004;191:20-1.
26. Low JA, Lindsay BG, Derrick EJ. Threshold of metabolic acidosis associated with newborn complications. Am J Obstet Gynecol. 1997;177(6):1391-4.
27. Wiberg N, Källén K, Olofsson P. Base deficit estimation in umbilical cord blood is influenced by gestational age, choice of fetal fluid compartment, and algorithm for calculation. Am J Obstet Gynecol. 2006;195(6):1651-6.
28. Low JA. Determining the contribution of asphyxia to brain damage in the neonate. J Obstet Gynaecol Res. 2004;30(4):276-86.

29. Chauhan SP, Hendrix NW, Magann EF, et al. Neonatal organ dysfunction among newborns at gestational age 34 weeks and umbilical arterial pH<7.00. J Matern Fetal Neonatal Med. 2005;17(4):261-8.
30. Mokarami P, Wiberg N, Olofsson P. An overlooked aspect on metabolic acidosis at birth: blood gas analyzers calculate base deficit differently. Acta Obstet Gynecol Scand 2012;91(5):574-9.
31. Olofsson P. Determination of base excess in umbilical cord blood at birth: Accessory or excess? Am. J. Obstet. Gynecol. 2015;213:259–61.
32. Hendricks CH. Amniotic fluid pressure recording. Clin Obstet Gynecol. 1966;9:535–53.
33. Fleischer A, Anyaegbunam AA, Schulman H, Farmakides G, Randolph G. Uterine and umbilical artery velocimetry during normal labor. Am J Obstet Gynecol. 1987;157:40–3.
34. Brar HS, Platt LD, DeVore GR, Horenstein J, Medearis AL. Qualitative assessment of maternal uterine and fetal umbilical artery blood flow and resistance in laboring patients by Doppler velocimetry. Am J Obstet Gynecol. 1988;158:952–6.
35. Olofsson P, Thuring-Jönsson A, Marsál K. Uterine and umbilical circulation during the oxytocin challenge test. Ultrasound Obstet Gynecol. 1996;8:247–51.
36. Hagelin A, Leyon J. The effect of labor on the acid-base status of the newborn. Acta Obstet Gynecol Scand. 1998;77:841–4.
37. Zaigham M, Källén K, Olofsson P. Gestational age-related reference values for Apgar score and umbilical cord arterial and venous pH in preterm and term newborns. Submitted manuscript.
38. Li H, Gudmundsson S, Olofsson P. Acute centralization of blood flow in compromised human fetuses evoked by uterine contractions. Early Hum Dev. 2006;82 (11):747-52.
39. Yeh P, Emary K, Impey L. The relationship between umbilical cord arterial pH and serious adverse neonatal outcome: analysis of 51,519 consecutive validated samples. BJOG. 2012;119(7):824-31.
40. Kitlinski ML, Källén K, Marsál K, Olofsson P. Gestational age-dependent reference values for pH in umbilical cord arterial blood at term. Obstet Gynecol. 2003;102:338-45.
41. WHO. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. Acta Obstet Gynecol Scand. 1977;56:247–53.
42. Naegele FC. Erfahrungen und Abhandlungen aus dem Gebiethe der Krankheiten des Weiblichen Geschlechtes. Nebst Grundzügen einer Methodenlehre der Geburtshülfe. Mannheim: Loeffler, 1812:280–1.

43. Creinin MD, Keverline S, Meyn LA. How regular is regular? An analysis of menstrual cycle regularity. Contraception. 2004;70(4):289-92.
44. Committee on Obstetric Practice. Committee Opinion No. 688: Management of Suboptimally Dated Pregnancies. Obstet Gynecol. 2017;129(3):e29-32.
45. Persson PH, Weldner BM. Reliability of ultrasound fetometry in estimating gestational age in the second trimester. Acta Obstet Gynecol Scand. 1986;65:481–3.
46. Hagenfeldt K, Axelsson O, Blenow M, Bojo¨ F, Bygdeman M, Crang-Svalenius E, et al. Rutinmässig ultraljudsundersökning under graviditet. Chapter 4. SBU report no. 139. Stockholm, Sweden: The Swedish Council on Technology Assessment in Health Care; 1998:47–54. Available at: http://www.sbu.se. Accessed January 5 2019.
47. Apgar V. A proposal for a new method of evaluation of the newborn infant. Curr. Res. Anesth. Analg. 1953;32(4):260-7.
48. Finster M, Wood M. The Apgar score has survived the test of time. Anesthesiology. 2005;102(4):855–7.
49. Skolnick AA. Apgar quartet plays perinatologist's instruments. JAMA. 1996;276:1939-40.
50. JS, Kennedy C, Schwarz BK. The Apgar score as an index of neo- natal mortality: a report from the Collaborative Study of Cerebral Palsy. Obstet Gynecol. 1964;24:222-30.
51. American Academy of Pediatrics. The Apgar score. Pediatrics. 2006;117:1444–7.
52. The Apgar score. Committee Opinion No. 644. American College of Obstetricians and Gynecologists. Obstet Gynecol 2015;126:52–5.
53. Gilstrap LC III, Hauth JC, Hankins GDV, Beck AW. Second-stage fetal heart rate abnormalities and type of neonatal acidemia. Obstet Gynecol. 1987;70:191-5.
54. ACOG Committee on Obstetric Practice, American Academy of Pediatrics Committee on Fetus and Newborn. Use and abuse of the Apgar score. Pediatrics. 1996;98:141-2.
55. Casey BM, McIntire DD, Leveno KJ. The continuing value of the Apgar score for the assessment of newborn infants. N Engl J Med. 2001;344:467–71.
56. Iliodromiti S, Mackay DF, Smith GCS, Pell JP, Nelson SM. Apgar score and the risk of cause-specific infant mortality: a population-based cohort study. Lancet. 2014:384:1749–55.
57. Rao PN, Shashidhar A, Ashok C. In utero fuel homeostasis: lessons for a clinician. Indian J Endocrinol Metab. 2013;17:60-8.
58. Hay WW Jr. Placental-fetal glucose exchange and fetal glucose metabolism. Trans Am Clin Climatol Assoc. 2006;117:321–39.
59. Hay WW, Sparks JW. Placental, fetal and neonatal carbohydrate metabolism. Clin Obstet Gynecol. 1985;28:473–85.

60. Despopoulos A, Silbernagl S. Color Atlas of Physiology. 4th Edition. Stuttgart: Thieme Verlag: 369; 1991.
61. Nordström L, Marcus C, Persson B, Shimojo N, Westgren M. Lactate in cord blood and its relation to pH and catecholamines in spontaneous vaginal deliveries. Early Hum Dev. 1996;46:97-104.
62. Morton SU, Brodsky D. Fetal Physiology and the Transition to Extrauterine Life. Clin Perinatol. 2016;43(3):395-407.
63. Sadiq F, Holtzclaw L, Chundu K, Muzaffar A, Devaskar S, Muzzafar A. The ontogeny of the rabbit brain glucose transporter. Endocrinology. 1990;126:2417-24.
64. Cowett RM, Farrag HM. Selected principles of perinatal-neonatal glucose metabolism. Semin Neonatol. 2004;9:37–47.
65. Pedersen J. The Pregnant Diabetic and Her Newborn. Problems and Management. Copenhagen: Munksgaard; 1967.
66. Persson B. Neonatal glucose metabolism in offspring of mothers with varying degrees of hyperglycemia during pregnancy. Semin Fetal Neon Med. 2009;14:106-110.
67. The HAPO Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations with neonatal anthropometrics. Diabetes. 2009;58:453-9.
68. Foxdahl P, Sjödin B, Rudstam H, Ostman C, Ostman B, Hedenstierna GC. Lactate concentration differences in plasma, whole blood, capillary finger blood, and erythrocytes during submaximal graded exercise in humans. Eur J App Physiol. 1991;61:218-22.
69. Wiberg N, Källén K, Herbst A, Åberg A, Olofsson P. Lactate concentration in umbilical cord blood is gestational age-dependent: a population-based study of 17 867 newborns. BJOG. 2008;115:704-9.
70. Beard RH, Morris DE. Fetal and maternal acid-base balance during normal labour. J Obstet Gynaecol Br Comnwlth. 1965;72:496–506.
71. Nickelsen C., Thomasen S.G., Weber T. Continuous acid-base assessment of the human fetus during labour by tissue pH and transcutaneous carbon dioxide monitoring. Br J Obstet Gynecol. 1985;92:220–05.
72. Nordström L, Achanna S, Naka K, Arulkumaran S. Fetal and maternal lactate increase during active second stage of labour. BJOG. 2001;108:263-8.
73. Holzmann M, Cnattingius S, Nordström l. Lactate production as a response to intrapartum hypoxia in the growth-restricted fetus. BJOG. 2012;119:1265-9.
74. Najmi RS. Distribution of birthweights of hospital born Pakistani infants. J Pak Med Assoc. 2000;50(4):121–4.
75. Xu H, Simonet F, Luo ZC. Optimal birth weight percentile cut-offs in defining small- or large-for-gestational-age. Acta Paediatr. 2010;99(4):550-5.

76. Boulet SL, Alexander GR, Salihu HM, Pass M. Macrosomic births in the united states: determinants, outcomes, and proposed grades of risk. Am J Obstet Gynecol. 2003;188(5):1372-8.
77. Alexander GR, Himes JH, Kaufman RB, et al. A United States national reference for fetal growth. Obstet Gynecol. 1996;87(2):163-8.
78. Chauhan SP, Grobman WA, Gherman RA, et al. Suspicion and treatment of the macrosomic fetus: a review. Am J Obstet Gynecol. 2005;193(2):332-46.
79. Hamilton BE, Martin JA, Osterman MJ, et al. Births: Final Data for 2014. Natl Vital Stat Rep. 2015;64:1-8.
80. Surkan PJ, Hsieh CC, Johansson AL, Dickman PW, Cnattingius S. Reasons for increasing trends in large for gestational age births. Obstet Gynecol. 2004;104(4):720–6.
81. Vinter CA, Jensen DM, Ovesen P, Beck-Nielsen H, Jørgensen JS. The LiP (Lifestyle in Pregnancy) study: a randomized controlled trial of lifestyle intervention in 360 obese pregnant women. Diabetes Care. 2011;34(12):2502-7.
82. Spellacy WN, Miller S, Winegar A, Peterson PQ. Macrosomia-maternal characteristics and infant complications. Obstet Gynecol. 1985;66(2):158-61.
83. Groenendaal F, Elferink-Stinkens PM, Netherlands Perinatal Registry. Hypoglycaemia and seizures in large-for-gestational-age (LGA) full-term neonates. Acta Paediatr. 2006;95(7):874-6.
84. Lackman F, Capewell V, Richardson B, et al. The risks of spontaneous preterm delivery and perinatal mortality in relation to size at birth according to fetal versus neonatal growth standards. Am J Obstet Gynecol. 2001;184(5):946-53.
85. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379:2162–72.
86. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L., Moller AB, et al. Born too soon: the global epidemiology of 15 million preterm births. Reprod Health. 2012;10(1):S2.
87. Stephansson O. Statistik om graviditeter, förlossningar och nyfödda barn 2016 (Internet). 2018. Accessed January 5, 2019. Available at: https://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/20807/2018-1-6.pdf.
88. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet. 2016;388(10063):3027-35.
89. Fellman V, Hellstrom-Westas L, Norman M, Westgren M, Kallen K, Lagercrantz H, Marsal K, Serenius F, Wennergren M. One-year survival of extremely preterm

infants after active perinatal care in Sweden. JAMA J Am Med Assoc. 2009;301(21):2225–33.

90. Serenius F, Ewald U, Farooqi A, et al. Extremely Preterm Infants in Sweden Study Group. Neurodevelopmental Outcomes Among Extremely Preterm Infants 6.5 Years After Active Perinatal Care in Sweden. JAMA Pediatr. 2016;170:954–63.
91. Marlow N, Wolke D, Bracewell MA, Samara M: Neurologic and developmental disability at six years of age after extremely preterm birth. N Engl J Med. 2005; 352:9-19.
92. Doyle LW, Ford G, Davis N. Health and hospitalistions after discharge in extremely low birth weight infants. Semin Neonatol. 2003;8:137-145.
93. Greenough A. Long term respiratory outcomes of very premature birth (<32 weeks).Semin Fetal Neonatal Med.2012;17:73-6.
94. Mwaniki MK, Atieno M, Lawn JE, Newton CR. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet. 2012;379:445-52.
95. Serenius F, Källén K, Blennow M, Ewald U, Fellman V et al. Neurodevelopmental outcome in extremely preterm infants at 2.5 years after active perinatal care in Sweden. JAMA. 2013;309:1810-20.
96. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics. 2010;126(3):443-56.
97. Mercier CE, Dunn MS, Ferrelli KR, Howard DB, Soll RF, Vermont Oxford Network ELBW Infant Follow-Up Study Group. Neurodevelopmental outcome of extremely low birth weight infants from the Vermont Oxford network: 1998-2003. Neonatology. 2009;97(4):329-38.
98. Randolph DA, Nolen TL, Ambalavanan N, et al. Outcomes of extremely low birthweight infants with acidosis at birth. Arch Dis Child Fetal Neonatal Ed. 2014;99(4):F263-8.
99. Andersson CB, Flems C, Kesmodel US. The Danish national quality database for births. Clin Epidemiol. 2016;25:595–9.
100. Jørgensen JS. Author's reply re: AGAINST: fetal scalp blood sampling in conjunction with electronic fetal monitoring reduces the risk of unnecessary operative delivery. BJOG. 2017;124(9):1453-4.
101. Bax M, Nelson KB. Birth asphyxia: a statement. World Federation of Neurology Group. Dev Med Child Neurol. 1993;35(11):1022-4.
102. Badawi N, Kurinczuk JJ, Keogh JM, et al. Antepartum risk factors for newborn encephalopathy: The Western Australian case-control study. BMJ. 1998;317(7172):1549-53.

103. Badawi N, Kurinczuk JJ, Keogh JM, et al. Intrapartum risk factors for newborn encephalopathy: The Western Australian case-control study. BMJ 1998;317(7172):1554-8.

104. Martinez-Biarge M, Diez-Sebastian J, Wusthoff CJ, et al. Antepartum and intrapartum factors preceding neonatal hypoxic-ischemic encephalopathy. Pediatrics. 2013;132(4):e952-9.

105. Volpe JJ. Perinatal brain injury: from pathogenesis to neuroprotection. Ment Retard Dev Disabil Res Rev. 2001;7(1):56-64.

106. Tan S, Zhou F, Nielsen VG, et al. Sustained hypoxia-ischemia results in reactive nitrogen and oxygen species production and injury in the premature fetal rabbit brain. J Neuropathol Exp Neurol. 1998;57(6):544-53.

107. Wu YW, Backstrand KH, Zhao S, et al. Declining diagnosis of birth asphyxia in California: 1991-2000. Pediatrics 2004; 114(6):1584-90.

108. Rei M, Ayres-de-Campos D, Bernardes J. Neurological damagde arising from intrapartum hypoxia/acidosis. Best Pract Res Clin Obstet Gynaecol. 2016;30:79-86.

109. Thornberg E, Thiringer K, Odeback A, Milsom I. Birth asphyxia: incidence, clinical course and outcome in a Swedish population. Acta Paediatr.1995;84(8):927-32.

110. Lee AC, Kozuki N, Blencowe H, et al. Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. Pediatr Res. 2013;74(1):50-72.

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

112. Gluckman PD, Pinal CS, Gunn AJ. Hypoxic-ischemic brain injury in the newborn: pathophysiology and potential strategies for intervention. Semin Neonatol. 2001;6:108-26.

113. Fatemi A, Wilson MA, Johnston MV. Hypoxic-ischemic encephalopathy in the term infant. Clin Perinatol. 2009;36:835-58.

114. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol 1976; 33(10): 696-705.

115. Ferriero DM. Neonatal brain injury. N Engl J Med. 2004;351(19):1985-95.

116. Robertson C, Finer N. Term infants with hypoxic-ischemic encephalopathy: outcome at 3.5 years. Dev Med Child Neurol. 1985;27(4):473-84.

117. Robertson CM, Finer NN, Grace MG. School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. J Pediatr. 1989;114(5):753-60.

118. Finer NN, Robertson CM, Richards RT, et al. Hypoxic-ischemic encephalopathy in term neonates: perinatal factors and outcome. J Pediatr. 1981;98:112.

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

120. van Handel M, Swaab H, de Vries LS, Jongmans MJ. Long-term cognitive and behavioral consequences of neonatal encephalopathy following perinatal asphyxia: a review. Eur J Pediatr. 2007;166(7):645-54.

121. Tagin MA, Woolcott CG, Vincer MJ, et al. Hypothermia for neonatal hypoxic ischemic encephalopathy: an updated systematic review and meta-analysis. Arch Pediatr Adolesc Med. 2012;166(6):558-66.

122. Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev. 2013;1(1):CD003311.

123. EhrensteinV. Association of Apgar scores with death and neurologic disability. Clin Epidemiol. 2009;1:45-53.

124. East CE, Leader LR, Sheehan P, Henshall NE, Colditz PB. Intrapartum fetal scalp lactate sampling for fetal assessment in the presence of a non-reassuring fetal heart rate trace. Cochrane Database Syst Rev. 2010;(3):CD006174.

125. Rørbye C, Perslev A, Nickelsen C. Lactate versus pH levels in fetal scalp blood during labor: using the Lactate Scout System. J Matern Fetal Neonatal Med. 2016;29(8):1200-4.

126. White CR, Doherty DA, Henderson JJ, Kohan R, Newnham JP, Pennell CE. Accurate prediction of hypoxic-ischaemic encephalopathy at delivery: a cohort study. J Matern Fetal Neonatal Med. 2012;25(9):1653–9.

127. Moore BW, McGregor D. Chromatographic and Electrophoretic Fractionation pf Soluble Proteins of Brain and Liver. J Biol Chem. 1965;240:1647-53.

128. Cocchia D, Michetti F, Donato R. S100 antigen in normal human skin. Nature. 1981;294:85-7.

129. Michetti F, Gazzolo D. S100B protein in biological fluids: a tool for perinatal medicine. Clin Chem.2002; 48:2097-104.

130. Gazzolo D, Pluchinotta F, Bashir M, et al. Neurological abnormalities in full-term asphyxiated newborns and salivary S100B testing: the 'Cooperative Multitask against Brain Injury of Neonates' (CoMBINe) international study. PLoS ONE. 2015;10:e0115194.

131. de Kruijk JR, Leffers JR, Menheere PP, Meerhoff S, Twijnstra A. S100B and neuron-specific enolase in serum of mild traumatic brain injury patients. A comparison with healthy controls. Acta Neurol Scand. 2001;103:175-9.

132. Anderson RE, Hansom LO, Nilsson O, Dijlai-Merzoug R, Settergren G. High serum S100B levels from trauma patients without head injuries. Neurosurgery. 2001;48:1255-8.

133. Gazzolo D, Vinesi P, Bartocci M, Geloso MC, Bonacci W, Serra G, et al. Elevated S100 blood level as early indicators of intraventricular hemorrhage in preterm infants. Correlation with cerebral Doppler velocimetry. J Neurol Sci. 1999;170:32-5.

134. Gazzolo D, Di Iorio R, Marinoni E, Masetti P, Serra G, Giovannini L, et al. S100B Protein is increased in asphyxiated term infants developing intraventricular hemorrhage. Crit Care Med. 2002;30:1356-60.

135. Nagdyman N, Komen W, Ko HK, Muller C, Obladen M. Early biochemical indicators of hypoxic-ischemic encephalopathy after birth asphyxia. Pediatr Res. 2001;49:502-6.

136. Thompson RJ, Doran JF, Jackson P, Dhillon AP, Rode J. PGP 9.5 - a new marker for vertebrate neurons and neuroendocrine cells. Brain Res. 1983;278:224-8.

137. Day INM, Thompson RJ. UCHL1 (PGP 9.5): Neuronal biomarker and ubiquitin system protein. Progr Neurobiol. 2010;90:327-62.

138. Jackson P, Thompson RJ. The demonstration of new human brain-specific proteins by high-resolution two-dimensional polyacrylamide gel electrophoresis. J Neurol Sci. 1981;49:429– 38.

139. Gong B, Leznik E. The role of ubiquitin C-terminal hydrolase L1 in neurodegenerative disorders. Drug News Perspect. 2007;20:365–70.

140. Douglas-Escobar MV, Heaton SC, Bennett J, Y oung LJ, Glushakova O, Xu X, Yasova Barbeau D, Rossignol C, Miller C, Old Crow AM, Hayes RL, Weiss MD. UCH-L1 and GFAP serum levels in neonates with hypoxic-ischemic encephalopathy: a single center pilot study. Front Neurol. 2014;5:273.

141. Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000). Neurochem Res. 2000;25:1439-51.

142. Mir IN, Chalak LF. Serum biomarkers to evaluate the integrity of the neurovascular unit. Early Hum Dev. 2014;90:707-11.

143. Middeldorp J, Hol EM. GFAP in health and disease. Prog Neurobiol. 2011;93:421–43.

144. Douglas-Escobar M, Yang C, Bennett J, Shuster J, Theriaque D, Leibovici A, Kays D, Zheng T, Rossignol C, Shaw G, Weiss MD. A pilot study of novel biomarkers in neonates with hypoxic-ischemic encephalopathy. Pediatr Res. 2010;68:531-6.

145. Ennen CS, Huisman TAGM, Savage WJ, Northington FJ, Jennings JM, Everett AD, Graham EM. Glial fibrillary acidic protein as a biomarker for neonatal hypoxic-ischemic encephalopathy treated with whole-body cooling. Am J Obstet Gynecol. 2011;205:251.e1-7.

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

147. ABL700 Series Reference Manual. Copenhagen: Radiometer Medical ApS. 2009. Accessed January 8, 2019. Available online at: http://biomed.au.dk/fileadmin/www.biomed.au.dk/faenotypering/Pdf/Radiometer-ABL-700-serie.pdf.

148. J Molin. A regional perinatal database in southern Sweden - a basis for quality assurance in obstetrics and neonatology. Acta Obstet Gynecol Scand. 1997;164:37-9.

149. Raju TN, Mercer BM, Burchfield DJ, Joseph GF. Periviable birth: executive summary of a Joint Workshop by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, Society for Maternal-Fetal Medicine, American Academy of Pediatrics and American College of Obstetricians and Gynecologists. J Perinatol. 2014;34:333-42.

150. World Health Organization. The World Health Report 2005: make every mother and child count. http://www.who.int/whr/2005/whr2005_en.pdf. Accessed January 5, 2019.

151. Mundry R, Fischer J. Use of statistical programs for nonparametric tests of small samples often leads to incorrect P values: examples from animal behavior. Anim Behav. 1998;56:256-9.

152. Kirkwood BR. Essentials of medical statistics. 2nd edition. Blackwell Science Ltd; 2003.

153. Cox DR. The regression analysis of binary sequences (with discussion). J Roy Stat Soc B. 1958; 20(2):215–42.

154. Mokarami P, Wiberg N, Källén K, Olofsson P. Arterio-venous blood gas Δ values for validation of umbilical cord blood samples at birth are biased not only by sample mix ups but also affected by clinical factors. Acta Obstet Gynecol Scand. 2019;98(2):167-75.

155. Marsál K, Persson P-H, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasound estimated foetal weights. Acta Paediatr. 1996;85:843-8.

156. Anderberg E, Källén K, Berntorp K, Frid A, Åberg A. A simplified oral glucose tolerance test in pregnancy: compliance and results. Acta Obstet Gynecol Scand. 2007;86:1432-6.

157. Wechsler D. Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV). San Atonio, TX: The Psychological Corporation; 2003.

158. Rosenbaum P, Paneth N, Leviton A, et al. A report: the definition and classification of cerebral palsy. Dev Med Child Neurol.2007;109:8–14.

159. Uppföljningsprogram för cerebral pares. CPUP, Follow-up program for cerebralpalsy. Accessed January 2019. Available from: https://cpup.se/.

160. Toet MC, Hellström-Westas L, Groenendaal F, Eken P, de Vries LS. Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed. 1999;81:F19–23.

161. Massaro AN, Jeromin A, Kadom N, et al. Serum biomarkers of MRI brain injury in neonatal hypoxic ischemic encephalopathy treated with whole-body hypothermia: a pilot study. Pediatr Crit Care Med. 2013;14:310–7.

162. Medicine Service Clinical Chemistry, S-1 100 B på Cobas (NPU19781). Accessed January 5, 2019. Available from: http://www.skane.se/Upload/Webbplatser/Labmedicin/Verksamhetsomr%C3%A5den/Klinisk%20kemi/Analyser/Skane/S-S%20100%20B%20p%C3%A5%20Cobas%20(NPU19781).pdf

163. Harboe M. A method for determination of hemoglobin in plasma by near-ultraviolet spectrophotometry. Scand J Clin Lab Invest. 1959;11:66-70.

164. Lowry R. VassarStats: website for statistical computation. Accessed January 5, 2019. Available from: http://vassarstats.net/.

165. Lopriore E, van Burk GF, Walther FJ, de Beaufort AJ. Correct use of the Apgar score for resuscitated and intubated newborn babies: questionnaire study. BMJ. 2004;329:143-4.

166. Pakkanen M, Lindblom B, Otterblad Olausson P, Rosén M. Stora regionala skillnader i förlossningsvårdens resultat [Large regional differences in perinatal outcome]. (In Swedish. English abstract available). Läkartidningen. 2004;101:3320-5.

167. Nicolaides KH, Economides DL, Soothill PW. Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses. Am J Obstet Gynecol. 1989;161:996–1001.

168. Helwig JT, Parer JT, Kilpatrick SJ, Laros RK Jr. Umbilical cord blood acid-base state: what is normal? Am J Obstet Gynecol. 1996;174:1807–12.

169. Herbst A, Wölner-Hanssen P, Ingemarsson I. Maternal fever in term labour in relation to fetal tachycardia, cord artery acidaemia and neonatal infection. BJOG. 1997;104:363-6.

170. The American College of Obstetricians and Gynecologists Neonatal encephalopathy and neurologic outcome, second edition. Obstet Gynecol. 2014;123:896–901.

171. Intrapartum care. Care of healthy women and their babies during childbirth. National Collaborating Centre for Women's and Children's Health. Commissioned by the National Institute for Health and Clinical Excellence. RCOG Press, 2007. Accessed January 8, 2019. Available online at: http://www.perinatalandalucia.es/file.php?file=%2F20%2FIntrapartum_Care_2007_NICE.pdf.

172. Wiberg N, et al. Relation between umbilical cord blood pH, base deficit, lactate, 5-minute Apgar score and development of hypoxic ischemic encephalopathy. Acta Obstet Gynecol Scand. 2010;89:1263–9.

173. Hafström M, Ehnberg S, Blad S, Norén H, Renman C, Rosén KG, et al. Developmental outcome at 6.5 years after acidosis in term newborns: a population-based study. Pediatrics. 2012;129:1501–7.

174. Dennis J, Johnson A, Mutch L, Yudkin P, Johnson P. Acid–base status at birth and neurodevelopmental outcome at four and one-half years. Am J Obstet Gynecol 1989; 161: 213-20.

175. Svirko E, Mellanby J, Impey L. The association between cord pH at birth and intellectual function in childhood. Early Hum Dev 2008; 84: 37-41.

176. Fiorica V. A table for converting pH to hydrogen ion concentration [H+] over the range 5-9. United States Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine. AM 68-23, 1968. Accessed January 8, 2019. Available online at: https://www.faa.gov/data_research/research/med_humanfacs/oamtechreports/1960s/media/am68-23.pdf.

177. Wellek S, Lackner KJ, Jennen-Steinmetz C, Reinhard I, Hoffmann I, Blettner M. Determination of reference limits: statistical concepts and tools for sample size calculation. Clin Chem Lab Med. 2014;52:1685–94.

178. Jiang S-H, Wang J-X, Zhang Y-M, Jiang H-F. Effect of hypothermia therapy on serum GFAP and UCH-L1 levels in neonates with hypoxic-ischemic encephalopathy. Chin J Contem Pediatr 2014;1 6: 1193–6. [Article in Chinese].

179. Valentim LM, Michalowski CB, Gottardo SP, et al. Effects of transient cerebral ischemia on glial fibrillary acidic protein phosphorylation and immunocontent in rat hippocampus. Neuroscience. 1999;91:1291–7.

180. Bidmon H-J, Jancsik V, Schleicher A, et al. Structural alterations and changes in cytoskeletal proteins and proteoglycans after focal cortical ischemia. Neuroscience. 1998;82:397–420.

181. Nagdyman N, Grimmer I, Scholz I, Muller C, Obladen M. Predictive value of brain-specific proteins in serum for neurodevelopmental outcome after birth asphyxia. Pediatr Res. 2003;54:270–5.

182. Beharier O, Kahn J, Shusterman E, Sheiner E. Review Article. S100B - a potential biomarker of early detection of neonatal brain damage following asphyxia. J Matern Fetal Neonatal Med 2012; 25: 1523-8.

183. Gazzolo D, Abella R, Marinoni E, Di Iorio R, Livolti G et al. New markers of neonatal neurology. J Matern Fetal Neonatal Med. 2009;22:57–61.

184. Gazzolo D, Marinoni E, Di Iorio R, Bruschettini M, Kornacka M et al. Measurement of urinary S100B protein concentrations for the early identification of brain damage in asphyxiated full-term infants. Arch Pediatr Adolesc Med. 2003;157:1163–8.

185. Michetti F, Corvino V, Geloso MC, Lattanzi W, Bernardini C et al. The S100B protein in biological fluids: more than a lifelong biomarker of brain distress. J Neurochem. 2012;120:644–59.

186. Michetti F, Massaro A, Russo G, Rigon G. The S-100 antigen in cerebrospinal fluid as a possible index of cell injury in the nervous system. J Neurol Sci. 1980;44:259–63.

187. Gazzolo D, Bruschettini M, Lituania M, Serra G, Bonacci W et al. Increased urinary S100B protein as an early indicator of intraventricular hemorrhage in preterm infants: correlation with the grade of hemorrhage. Clin Chem. 2001;47:1836–8.

188. Gazzolo D, Marinoni E, Di Iorio R, Bruschettini M, Kornacka M et al. Urinary S100B protein measurements: A tool for the early identification of hypoxic-ischemic encephalopathy in asphyxiated full-term infants. Crit Care Med. 2004;32:131–6.

189. Gazzolo D, Frigiola A, Bashir M, Iskander I, Mufeed H et al. Diagnostic accuracy of S100B urinary testing at birth in full-term asphyxiated newborns to predict neonatal death. PLoS One. 2009;4:e4298.

190. Thorngren-Jerneck K, Alling C, Herbst A, Amer-Wahlin I, Marsal K. S100 protein in serum as a prognostic marker for cerebral injury in term newborn infants with hypoxic ischemic encephalopathy. Pediatric Research. 2004;55:406-12.

191. Massaro AN, Chang T, Kadom N, et al. Biomarkers of brain injury in neonatal encephalopathy treated with hypothermia. J Pediatr. 2012;161:434–40.

192. Massaro AN, Chang T, Baumgart S, et al. Biomarkers S100B and NSE predict outcome in hypothermia-treated encephalopathic newborns. Pediatr Crit Care Med. 2014;15:615-22.

193. Schulte S, Podlog LW, Hamson-Utley JJ, Strathmann FG, Strüder HK. A systematic review of the biomarker S100B: implications for sport-related concussion management. J. Athl. Train. 2014;49:830–50.

194. Summanen M, Seikku L, Rahkonen P, Stefanovic V, Teramo K, Andersson S, et al. Comparison of Umbilical Serum Copeptin Relative to Erythropoietin and S100B as Asphyxia Biomarkers at Birth. Neonatology. 2017;112:60–6.

195. Wu YW, Backstrand KH, Zhao S, Fullerton HJ, Johnston SC Declining diagnosis of birth asphyxia in California: 1991-2000. Pediatrics. 2004;114(6):1584–90.

196. Müller K, Elverland A, Romner B, et al. Analysis of protein S-100B in serum: a methodological study. Clin Chem Lab Med. 2006;44:1111–4.

197. Ikeda Y, UmemuraK. Analysis of reference values of serum S100B concentrations of Japanese adults [in Japanese]. Rinsho Byori. 2005;53:395–9.

198. Djukanovic D, Hofmann U, Sucker A, Schadendorf D. Melanoma tumour markers S100B and MIA: evaluation of stability in serum and blood upon storage and processing. Br. J. Dermatol. 2001;145:1030–1.

199. Olofsson P, Mokarami P, Kállén K, Wiberg N. How mathematics warp biology: round-off of umbilical cord blood gas case value decimals distorts calculation of metabolic acidosis at birth. Acta Obstet Gynecol Scand. 2012;91:39-43.

200. Beneventi F, Locatelli E, Cavagnoli C, et al. Effects of uncomplicated vaginal delivery and epidural analgesia on fetal arterial acid–base parameters at birth in gestational diabetes. Diabetes Res Clin Pract. 2014;103:444-51.

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Informative Fetal Blood

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The findings of the five original studies included in this thesis help highlight the value of umbilical cord blood sampling at birth and elucidate the possible diagnostic value of specific constituents within cord blood to the risk of death or neurodevelopmental outcome later on in life.

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