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Pitfalls in Interpreting Umbilical Cord Blood Gases and Lactate at Birth

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2013

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DOCTORAL DISSERTATION

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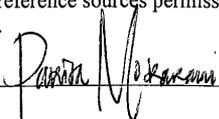the Department of Obstetrics and Gynecology,
Skåne University Hospital, Malmö

May 25, 2013 at 9.00 a.m.

Faculty opponent
Professor Ola Didrik Saugstad
Oslo University Hospital, University of Oslo, Norway

Organization	Document name
LUND UNIVERSITY	DOCTORAL DISSERTATION
Department of Obstetrics and Gynecology	Date of issue May 25, 2013
Institute of Clinical Sciences, Malmö, Medical Faculty, Lund University, SWEDEN	Sponsoring organization
Author Parisa Mokarami	

Title and subtitle

PITFALLS IN INTERPRETING UMBILICAL CORD BLOOD GASES AND LACTATE AT BIRTH

Abstract

Acid-base status in umbilical cord blood is an objective measure of the fetus' exposure to and ability to handle hypoxia. The objective of this thesis was to clarify some of the methodological pitfalls in interpreting umbilical cord blood gases and lactate values at birth. *Study I* pinpoints the methodological confounding in calculating base deficit (BD) with algorithms used in different brands of blood gas analyzers and reports the consequences in diagnosing metabolic acidosis (MA) at birth. Neonatal MA rates cannot be compared between maternity units or between scientific articles where different fetal compartments (blood or extracellular fluid) and different algorithms for calculating BD have been used. *Study II* addresses the issue of possible diagnostic discrepancies when acid-base parameter value decimals are rounded off. A drift of a dichotomy parameter value cutoff due to decimal rounding will result in a shift in distribution of negative and positive cases in a population sample. The findings warrant a discussion on standardization of round-off rule and the number of decimals for a specific analyte result. *Study III* demonstrates that delayed cord blood sampling with intact pulsations affects umbilical acid-base values and hematological parameters in both vaginal and cesarean deliveries. The changes were more marked after vaginal delivery. A change towards acidemia and lactemia can be explained by the hidden acidosis phenomenon, i.e. a surge into the central circulation of peripherally trapped acid metabolites when the newborn starts to breathe. *Study IV* shows that clinical characteristics have a significant influence on the distribution of veno-arterial and arterio-venous gradients (Δ values) in umbilical cord blood. Validation criteria based on fixed ΔpH and ΔpCO_2 values may then exclude correct samples of clinical outliers. Lactate cannot be used for validation of umbilical cord blood samples. A negative ΔpO_2 value indicates delayed cord blood sampling or mix-up of samples and is the only certain validation criterion.

Key words: Blood gases, metabolic acidosis, umbilical cord, round-off, delayed sampling, validation

Classification system and/or index terms (if any):

Supplementary bibliographical information: Parisa.Mokarami@med.lu.se	Language English

Distribution by (name and address)

Signature _____   Date __12 April 2013__

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Lund University, Malmö, Sweden, 2013

Pitfalls in Interpreting Umbilical Cord Blood Gases and Lactate at Birth

Parisa Mokarami

LUND
UNIVERSITY
Faculty of Medicine

To my mother

*"Now so much I know, that things just don't grow
if you don't bless them with your patience."*

First Aid Kit – Emmylou

Contents

Abstract

Acid-base status in umbilical cord blood is an objective measure of the fetus' exposure to and ability to handle hypoxia. The objective of this thesis was to clarify some of the methodological pitfalls in interpreting umbilical cord blood gases and lactate values at birth. *Study I* pinpoints the methodological confounding in calculating base deficit (BD) with algorithms used in different brands of blood gas analyzers and reports the consequences for diagnosing metabolic acidosis (MA) at birth. Neonatal MA rates cannot be compared between maternity units or between scientific articles where different fetal compartments (blood or extracellular fluid) and different algorithms for calculating BD have been used. *Study II* addresses the issue of possible diagnostic discrepancies when acid-base parameter value decimals are rounded off. A drift of a dichotomy parameter value cut-off due to decimal rounding will result in a shift in distribution of negative and positive cases in a population sample. The findings warrant a discussion on standardization of round-off rule and the number of decimals for a specific analyte result. *Study III* demonstrates that delayed cord blood sampling with intact pulsations affects umbilical acid-base values and hematological parameters in both vaginal and cesarean deliveries. The changes were more marked after vaginal delivery. A change towards acidemia and lactemia can be explained by the hidden acidosis phenomenon, i.e. a surge into the central circulation of peripherally trapped acid metabolites when the newborn starts to breathe. *Study IV* shows that clinical characteristics have a significant influence on the distribution of veno-arterial and arterio-venous gradients (Δ values) in umbilical cord blood. Validation criteria based on fixed ΔpH and ΔpCO_2 values may then exclude correct samples of clinical outliers. Lactate cannot be used for validation of umbilical cord blood samples. A negative ΔpO_2 value indicates delayed cord blood sampling or mix-up of samples and is the only certain validation criterion.

Abbreviations

ACOG   American College of Obstetricians and Gynecologists
AGA   appropriate-for-gestational age
AS   Apgar score
ATP   adenosine triphosphate
A-V   arterio-venous
BD   base deficit
BE   base excess
CD   cesarean delivery
CI   confidence interval
CP   cerebral palsy
CTG   cardiotocography
CLSI   Clinical and Laboratory Standards Institute
CV%   coefficient of variance in percent
DPG   diphosphoglycerate
ecf   extracellular fluid
H^+ hydrogen ion
Hb   hemoglobin
HbA   adult hemoglobin
HbF   fetal hemoglobin
HCO_3^- bicarbonate
H_2CO_3 carbonic acid
Hct   hematocrit
H_2O water
HIE   hypoxic-ischemic encephalopathy
LGA   large-for-gestational age
MA   metabolic acidosis
NAD   nicotinamide adenine dinucleotide

NADH	nicotinamide adenine dinucleotide, reduced form
OH⁻	hydroxide ion
pCO_2	partial pressure of carbon dioxide
pH	power of hydrogen
pO_2	partial pressure of oxygen
PRSR	Perinatal Revision South Register
RCT	randomized controlled trial
SD	standard deviation
SGA	small-for-gestational age
SID	strong-ion-difference
STAN	ST analysis
V-A	veno-arterial

Original Papers

This thesis is based on the following papers referred to in the text by their Roman numerals. The papers are appended at the end of the thesis.

I. Parisa Mokarami, Nana Wiberg & Per Olofsson
An overlooked aspect on metabolic acidosis at birth: blood gas analyzers calculate base deficit differently
Acta Obstetricia et Gynecologica Scandinavica
2012;91:574-9.

II. Per Olofsson, Parisa Mokarami, Karin Källén & Nana Wiberg
How mathematics warp biology: round-off of umbilical cord blood gas case value decimals distorts calculation of metabolic acidosis at birth
Acta Obstetricia et Gynecologica Scandinavica
2012;91:39-43.

III. Parisa Mokarami, Nana Wiberg & Per Olofsson
Hidden acidosis – an explanation of acid-base and lactate changes occurring in umbilical cord blood after delayed sampling
British Journal of Obstetrics and Gynaecology
Published Online 10 April 2013.

IV. Parisa Mokarami, Nana Wiberg, Karin Källén & Per Olofsson
An approach to validation of umbilical cord blood gases at birth
Submitted manuscript.

Permission for reprinting has been granted by the publishers.

Preface

For many years obstetricians and pediatricians have sought tools for evaluating the newborn's condition, and mainly the degree of asphyxia. In 1953, Virginia Apgar introduced the Apgar scoring system based on the subjective assessment of the newborns Appearance (skin color), Pulse (heart rate), Grimace (reflex irritability), Activity (muscle tone), and Respiration (breathing) (Apgar, 1953). The relation between umbilical cord blood gases and neonatal vitality was first described in 1958 (James *et al.*, 1958). In the early 1960s, Erich Saling of Berlin introduced fetal scalp blood sampling (Saling, 1968), and in the 1970s, physicians in Sweden started using this as an objective measurement to assess fetal hypoxia during labor. Since 1981 umbilical cord blood sampling has been routine in Malmö, and a few years later the procedure was adopted in Lund. Many maternity units in Sweden and worldwide use blood gas and lactate values in umbilical cord blood at birth to retrospectively evaluate the course of labor and management in individual cases, to judge malpractice in litigation cases, to assess the quality of care at a maternity unit, and in research as an objective perinatal outcome parameter.

In contrast to the Apgar scoring system, acid-base status in umbilical cord blood is an objective measure of the fetus' exposure to and ability to handle hypoxia. The intrauterine milieu is characterized by low oxygen partial pressure (pO_2) and a gradual development of acidemia, hypercapnia, and lactemia with advancing gestational age (Wiberg *et al.*, 2006a; Wiberg *et al.*, 2008a). During labor the fetus is exposed to hypoxic stress, but healthy fetuses can handle even severe oxygen reductions. Transient hypoxia and hypercapnia have little pathological significance, but if oxygen deficit is prolonged fetal metabolism switches to anaerobic, and blood gases and lactate are deranged more than normally expected. This leads to a decrease in pH, accumulation of lactate, and an increase in base deficit (BD). Metabolic acidosis (MA) in umbilical cord arterial blood at birth is commonly defined as pH <7.00 (or <7.05) and BD ≥12.0 mmol/L. Metabolic acidosis and low Apgar scores both correlate with increased morbidity and indicate that the newborn may need neonatal care. Unless severely affected, the correlation between these two parameters is poor, as is their association with short- and long-term morbidity.

Umbilical cord blood should be sampled from the vein and one of the arteries to enable evaluation of the degree and duration of an acidosis. The vein provides the fetus with oxygenated blood and nutrients from the mother and the arteries transport deoxygenated blood and waste products from the fetus to placenta. The newborn's acid-base status is therefore best reflected by arterial blood gas and lactate values while venous blood contents also depend on maternal acid-base status and the placental function. Immediate sampling of the umbilical cord blood makes the samples more reliable since blood gas and lactate values can change when the newborn starts to breath. Moreover, analyte values can change if the time from sampling to analysis is delayed. An interpreter of blood gas and lactate values should be aware of the many physiological and methodological factors that can influence the readings. This thesis deals with some of the pitfalls in interpreting acid-base and lactate values at birth, such as the choice of algorithm and fluid compartment for the calculation of BD and making the MA diagnosis, the influence of rounding off pH and BD value decimals when diagnosing MA, the physiology of acid-base changes at delayed cord blood sampling, and the association between clinical characteristics and validation criteria for distinguishing arterial and venous blood samples.

Introduction

Power of hydrogen (pH)

Hydrogen ion concentration [H⁺] in a solution is usually expressed as the power of H⁺ (pH). In 1909, the Danish chemist Sørensen described the influence of hydrogen ions on different biochemical reactions. He defined pH as the negative logarithm of the free concentration of H⁺. In the body, hydrogen ions are mainly bound to different buffers and the pH in blood should be regarded as an indicator of the relationship between the acid and base buffers. Free H⁺ are highly reactive and even small changes in pH can influence the body's physiological processes and lead to tissue damage. Buffer systems therefore aim to keep pH within a narrow range (Lentner, 1984a; Theodorsson & Malm, 2003). The fact that the relationship between [H⁺] and pH is logarithmic rather than linear is important to keep in mind when evaluating the degree of acidemia. A 0.10 unit decrease in pH from 7.00 to 6.90 results in a 2.2-fold increase in hydrogen concentration compared with a fall from 7.30 to 7.20 (Westgate *et al.*, 1994).

Acids and bases

Throughout history different definitions of acids and bases have been presented, contributing to the development of different models for the understanding of acid-base physiology. In the 1880s the Swedish chemist Arrhenius defined an acid as a substance that produces hydrogen ions when dissolved in water, causing [H⁺] to increase (Story, 2004). The Henderson-Hasselbalch equation for the calculation of pH was first described in 1916 (Siggaard-Andersen, 2006) expressing the relationship between pH, the weak carbonic acid (H_2CO_3)**,** and the base bicarbonate (HCO_3^-) as:

$$pH = pK + \log [HCO_3^- / H_2CO_3]$$

(Eq.1)

where pK is the equilibrium constant for the reaction:

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \qquad\qquad\qquad (Eq.2)$$

In blood plasma pK is about 6.1 and carbonic acid is:

$$H_2CO_3 = 0.03 \times pCO_2 \qquad\qquad\qquad (Eq.3)$$

The equation can be rewritten as:

$$pH = pK + \log[HCO_3^- / (0.03 \times pCO_2)] \qquad\qquad (Eq.4)$$
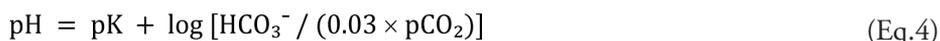
where 0.03 is the carbonic dissociation constant at 37°C.

In 1920, Van Slyke accepted the view presented earlier by Faraday and Naunyn regarding anions such as chloride, and cations such as sodium, as acid respectively base forming. This added the influence of electrolytes on acid-base status to the discussion (Van Slyke, 1934; Story, 2004). The Bronsted-Lowry definition, developed after World War I, described an acid (HA) as a proton (H+) donating substance and a base (A-) as a proton accepting substance (Theodorsson & Malm, 2003; Story, 2004):

$$HA \rightleftharpoons H^+ + A^- \qquad\qquad\qquad (Eq.5)$$
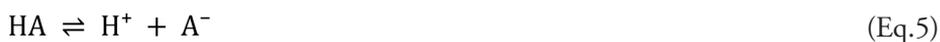
In water-based solutions such as plasma this equation can be rewritten as:

$$H_2O \leftrightarrow H^+ + OH^- \qquad\qquad\qquad (Eq.6)$$

and in a larger perspective, in the body as:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \qquad\qquad (Eq.7)$$
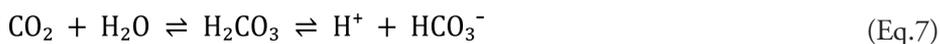
In the mid-1950s, focus was directed to bicarbonate, which until then had been thought of only as an indicator of acid-base status rather than a significant determinant. It was recognized that bicarbonate was produced not only because of the increase in the partial pressure of carbon dioxide (pCO_2), which causes the above equilibrium (Eq.7) to shift to the right, but also as a compensatory physiological response by the kidneys when pH is decreased. The respiratory component of the acid-base status can clearly be reflected by pCO_2 levels, but since bicarbonate is influenced by pCO_2 it cannot be considered solely a metabolic component. In their search for a measure of the metabolic component of the acid-base status, Singer and Hasting introduced the buffer base in 1948. This measure was defined as the

sum of weak acid (buffer) anions in plasma, such as albumin anions and bicarbonate (Story, 2004).

In the late 1950s, Siggaard-Andersen and Engel developed base excess (BE) as an alternative measure of the pCO_2 independent acid-base changes (Andersen & Engel, 1960). Base excess was defined as the amount of acid (in mmol) needed to shift pH in a liter of blood to 7.40 at 37°C, with pCO_2 at 5.3 kPa (40 mmHg), and oxygen saturation at its actual value. A nomogram was constructed for clinical use and later mathematically transcribed (the van Slyke equation) to be used in blood gas analyzers (Astrup *et al.*, 1966; Siggaard-Andersen, 1977). Base excess is usually calculated by using the linear association between pH and the logarithm for pCO_2. The Vans Slyke algorithm is still used for the calculation of BE_{blood}:

$$BE = \{[HCO_3^-] - 24.4 + (2.3 \times [Hb] + 7.7) \times (pH - 7.4)\} \times (1 - 0.023 \times [Hb])$$
$$\text{(Eq.8)}$$

where [Hb] is the hemoglobin concentration.

The 'great trans-Atlantic debate' started with the introduction of BE. Since plasma *in vivo* is in continuity with the less buffer rich extracellular fluid (ecf), an *in vitro* model of blood was considered inaccurate (Bunker, 1965). This argument lead to modification of BE, to fit the conditions in extracellular fluid. This model of ecf is based on one part of blood and 2 parts of its own plasma. In the algorithm this is achieved by assuming a [Hb] of 50 g/L (3.1 mmol/L). Moreover, the indirect influence of pCO_2 on BE was pointed out leading to changes in the nomogram and a correction factor in the algorithm. (Siggaard-Andersen, 1977; Theodorsson & Malm, 2003; Story, 2004). Base excess is based on the distribution of the adult fluid compartments and is widely used as a complement to pH for the evaluation of the metabolic component of the acid-base status. Base deficit (BD) is practical to use when BE is negative, i.e. when there is a deficit rather than an excess of bases.

In the late 1970s, Stewart presented a new approach to acid-base physiology based on the previous ideas from Arrhenius, Van Slyke, Singer, and Hasting (Stewart, 1978; Greenbaum & Nirmalan, 2005). This approach was based on the influence of strong-ion-difference (SID), weak acids, pCO_2, and the dissociation constant of water and strong acids on (H^+/ HCO_3^-). According to Stewart's physicochemical concept, the H^+ and OH^- ions act as reciprocal charge buffers. The $[H^+]$ in aqueous solutions depends on the dissociation of water into H^+ and OH^-, where just three parameters influence the dissociation: pCO_2 and the dissociation of "weak" and "strong" electrolytes. Strong electrolytes dissociate completely in water in contrast to weak electrolyte ions. The SID has a powerful influence on the dissociation of water because electrochemical neutrality must be maintained. In an SID negative

solution, the [H⁺] is always higher than the [OH⁻], and the converse is true for an SID positive solution (Figure 1). Most acute acid-base metabolic changes are a result of changes in the SID. Changing the water content of plasma will result in a drift of SID, with a change towards higher [H⁺] in case of dilution and a change towards higher [OH⁻] in case of draining. These changes are called dilutional acidosis and contraction alkalosis, respectively (Stewart, 1978, 1983; Stewart *et al.*, 2009).
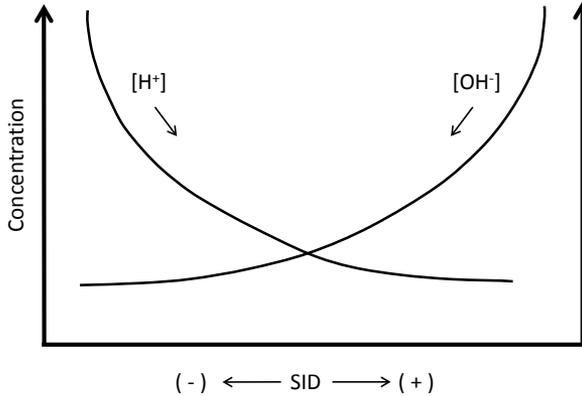
Figure 1. The strong-ion-difference (SID) system explaining the reciprocal relation between [H⁺] and [OH⁻]. As the [OH⁻] decreases the SID decreases, resulting in acidosis. Alkalosis is the consequence of an increased SID. Modified from Stewart (Stewart *et al.*, 2009).

Fetal metabolism

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. Glycolysis is the major pathway of glucose breakdown in which smaller energy units called adenosine triphosphate (ATP) are produced and utilized by the cells. In this pathway, glucose is first converted into pyruvate, and 2 ATP molecules are generated. Pyruvate can then be converted to Acetyl-CoA and enter the citric acid cycle, which is the case when energy generation is needed and oxygen is available (Figure 2).
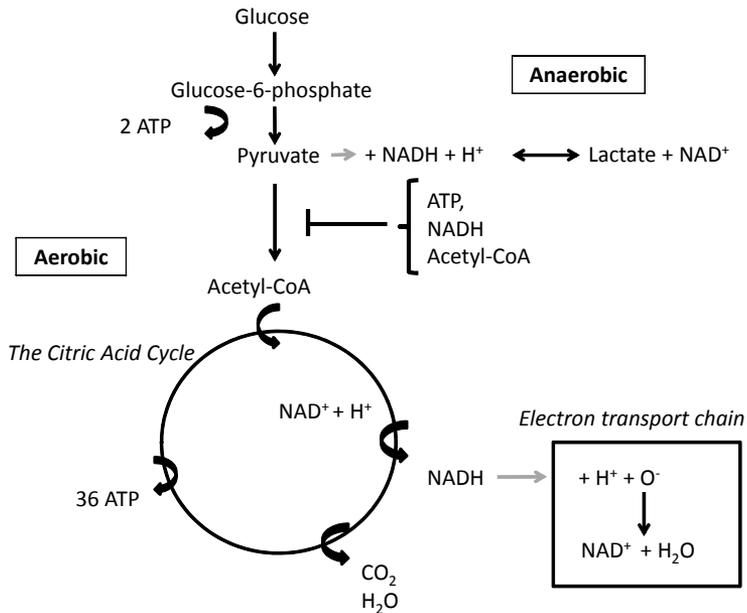
Figure 2. Schematic illustration of glycolysis during aerobic and anaerobic conditions. Modified from Despopoulos and Silbernagl (Despopoulos & Silbernagl, 1991).

The end product of the citric acid cycle is 36 ATP and nicotinamide adenine dinucleotide (NADH) which is formed by NAD^+ and H^+. Carbon dioxide is released as a waste product from these reactions. Some of the pyruvate is metabolized to lactate giving rise to NAD^+. Under aerobic conditions the reaction reverses and lactate is oxidized back to pyruvate. Lactate can then be re-created to glucose and glycogen through gluconeogenesis, but the major fate of pyruvate is to enter the citric acid cycle. In metabolic steady state, the relationship between pyruvate and lactate reflects the intracellular redox status. The ratio of lactate to pyruvate is normally 10-16:1 (Rooth, 1988; Chou *et al.*, 1998). During anaerobic conditions the accumulated NADH promote NAD^+ regeneration by increasing the pyruvate reduction to lactate. Anaerobic metabolism predominates during hypoxic stress, leading to the accumulation of lactate and subsequently a decrease in pH. Therefore, an increased lactate to pyruvate ratio is a sign of hypoxia. High concentrations of lactate could lead to necrosis and edema as well as toxic effects on the brain (Gluck *et al.*, 1977), though some suggest that lactate to a certain extent can have positive effects by supporting oxidative metabolism in cortical neurons (Pellerin *et al.*, 1998).

Oxygen and hemoglobin

Oxygen circulation can be divided into four steps: uptake, transport, supply, and tissue oxygenation. The partial pressure of oxygen (pO_2) is a measure of the uptake. The transport is measured by the total oxygen concentration, the saturation, or p50 that is the pO_2 value at a saturation of 50%. The supply is reflected by the difference between arterial and venous pO_2, the so called oxygen extraction rate. Finally, the lactate concentration is a measure of the tissue oxygenation since lactate increases during hypoxia (Theodorsson & Malm, 2003; Labmedicin, 2012).

Ninety-eight percent of oxygen is bound to Hb and only 2% is transported in its free form in plasma. The oxygen-hemoglobin dissociation curve is dependent on pH and pCO_2, as described by the Bohr effect (Figure 3). A decrease in pH and an increase in pCO_2 each cause a shift of the curve to the right. This so called Bohr effect facilitates the dissociation of O_2 in tissues were pH is relativly low and pCO_2 high. In the lungs where the gradients are the opposite, the oxygen-hemoglobin dissociation curve is shifted to the left and the oxygenation of Hb is thereby facilitated.
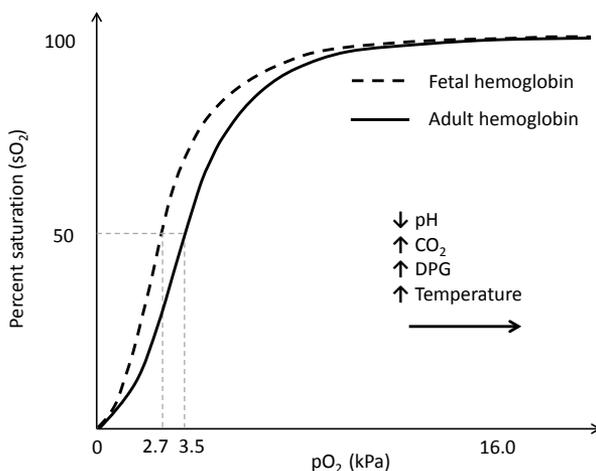
Figure 3. The oxygen-hemoglobin dissociation curve illustrating both fetal and adult hemoglobin. Decreased pH and increased CO_2, DPG, and temperature causes a shift to the right.

After some time, the Bohr effect is balanced by the decreasing concentration of 2,3-diphosphoglycerate (DPG), which is produced in the erythrocytes through the

anaerobic glycolytic pathway. The anion DPG binds to Hb and lowers the oxygen affinity of Hb. In other words, the deoxygenated form of Hb is stabilized when bound to DPG. By decreasing pH, DPG adds to the Bohr effect. This anion has a stronger binding to adult Hb (HbA) than to fetal Hb (HbF). This property of HbF is due to the structure of the Hb tetramer molecule which consists of γ-chains instead of the adult β-chains, in addition to the α-chains included in both molecules (Blechner, 1993; Brandis, 1997). Due to its lack of interaction with DPG, HbF has a greater oxygen affinity than HbA. This explains the steeper slope of the HbF oxygen dissociation curve and the shift to the left compared to HbA (Lentner, 1984b; Theodorsson & Landin, 2003). The oxygen diffusion is dependent on the pO_2, [Hb], type of Hb, oxygen saturation, and blood flow. The fetus has a higher [Hb], approximatley 170 g/L compared to the mothers' 120 g/L, and therfore a greater oxygen transport capacity (Brandis, 1997). In addition, the greater oxygen affinity of HbF and the lower fetal blood pH further facilitate oxygen uptake in the placenta.

Hemoglobin is also a buffer and accounts for approximately 35% of the fetus' total buffering capacity in blood (Blechner, 1993). Hemoglobin is a more effective buffer in its deoxygenated form and the buffering itself facilitates oxygen release. With advancing gestational age, HbF is replaced by HbA. The replacement is gradual, and near term about 20% of hemoglobins are HbA (Beaven *et al.*, 1951). Since Hb is a buffer and HbF and HbA have different oxygen carrying and oxygen delivering properties, a change in Hb type with advancing gestational age might alter both the fetal buffering capacity and the oxygen-hemoglobin dissociation curve.

Carbon dioxide and bicarbonate

Oxygen diffuses into fetal blood across the placental chorionic villus cell layers ('membranes'), while carbon dioxide passes in the opposite direction (Figure 4). Five percent of the CO_2 is transported freely and as carbonic acid in the plasma, 20% is bound to proteins, and 75% of CO_2 is transported as bicarbonate. In erythrocytes, CO_2 is converted to bicarbonate that enters plasma in exchange for chloride. This reversible reaction is catalyzed by carbonic anhydrase. Erythrocytes contain much carbonic anhydrase and have a high permeability for bicarbonate and CO_2. This makes the red blood cells the perfect place for rapid conversion of carbon dioxide and water to bicarbonate and protons according to Eq. 7.
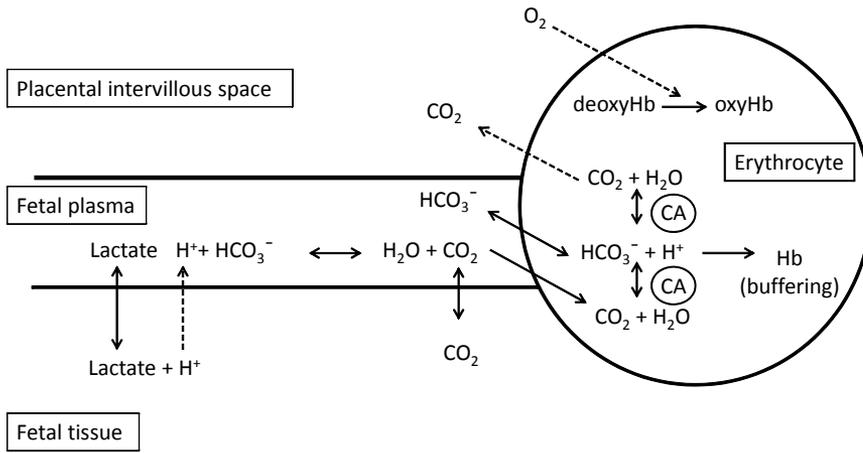
Figure 4. The main transport principles of CO_2, O_2, and H^+ in the feto-placental unit. CA denotes carbonic anhydrase. Modified from Despopoulos and Silbernagl (Despopoulos & Silbernagl, 1991).

The immediate buffering is mainly intracellular since the buffering capacity in the cells is larger than in the extracellular compartment. However, the extracellular bicarbonate-carbonic acid buffer system is of outmost physiological importance since it involves the volatile element CO_2. Carbon dioxide can be eliminated rapidly and in large amounts across the placenta when the uterine and umbilical blood flows are adequate. The bicarbonate-carbonic acid buffer system is the main buffer system in plasma, though proteins in plasma and the extracellular compartment also have important buffering capacities. Buffers are most effective when the concentration of the conjugate acid-base pairs are equal, in other words at their dissociation constant. The ratio between bicarbonate and its conjugate carbonic acid is 20:1. Despite the unfavorable physiological ratio of the bicarbonate-carbonic acid buffer system, it accounts for 35% of the fetal buffering capacity in blood (Blechner, 1993; Theodorsson & Malm, 2003).

Maternal and fetal regulation of acid-base equilibrium

The placenta acts as the fetus' lungs, kidneys, and guts allowing gas exchange, waste elimination, and nutrient uptake via the mother's blood supply. In the intervillous space, oxygenated blood from the mother's spiral arterioles flows around the fetal chorionic villi which contains deoxygenated blood from the two umbilical arteries. The intrauterine milieu is characterized by a low pO_2 and a gradual development of acidemia, hypercapnia, and lactemia with advancing gestational age (Wiberg *et*

al., 2006a; Wiberg *et al.*, 2008a). The load on the bicarbonate-carbonic acid buffer system increases with advancing gestational age, as reflected by a decrease in standard bicarbonate and an increase in BD (Wiberg *et al.*, 2006b). Maternal glomerular filtration rate increases with approximately 50% during pregnancy in order to handle the additional excretory burden (Gilstrap *et al.*, 1989; Blechner, 1993).

The physiological development of acidemia is mainly related to the availability of oxygen, the metabolic rate, the fetal buffering capacity, and the exchange of blood gases and acids between mother and fetus. The fetal acid-base regulation mechanisms are limited since gas exchange is impossible in the fetal lungs and the kidneys are immature. Maintenance of gradients between the maternal and fetal sides in the placenta is therefore central to the regulation of acid-base equilibrium (Figure 5). Various mechanisms contribute to the maternal-fetal gradient. The progesterone-induced hyperventilation during pregnancy reduces maternal pCO_2 and maintains a negative gradient over the placental membrane. A maternal respiratory alkalosis follows although the bicarbonate-to-CO_2 ratio and pH is rather constant. The fetal bicarbonate-to-CO_2 ratio is lower and therefore fetal pH is usually 0.1 units lower than the maternal pH. Carbon dioxide can easily diffuse to the maternal side causing a double Bohr effect and diversion of the HbA and HbF dissociation curves. Oxygen unload is facilitated on the maternal side and oxygen uptake on the fetal side of the placenta. In addition, the fetus has a 40% higher Hb concentration and HbF has a higher O_2 affinity. (Blechner, 1993; Brandis, 1997).

Uterine artery

pO_2: 13.3 kPa
sO_2: 98%
pCO_2: 4.2 kPa

Uterine flow: 600 mL/min
Maternal [Hb]: 120 g/L
P50 of HbA: 3.5 kPa

Maternal intervillous space

Uterine vein

pO_2: 5.3 kPa
sO_2: 75%
pCO_2: 6.0 kPa

Mean pO_2 gradient = 4.0 kPa

Fetal capillaries

Umbilical artery

pO_2: 2.4 kPa
sO_2: 45%
pCO_2: 7.3 kPa

Umbilical flow: 300 mL/min
Fetal [Hb]: 170 g/L
P50 of HbF: 2.7 kPa

Umbilical vein

pO_2: 3.7 kPa
sO_2: 70%
pCO_2: 5.3 kPa
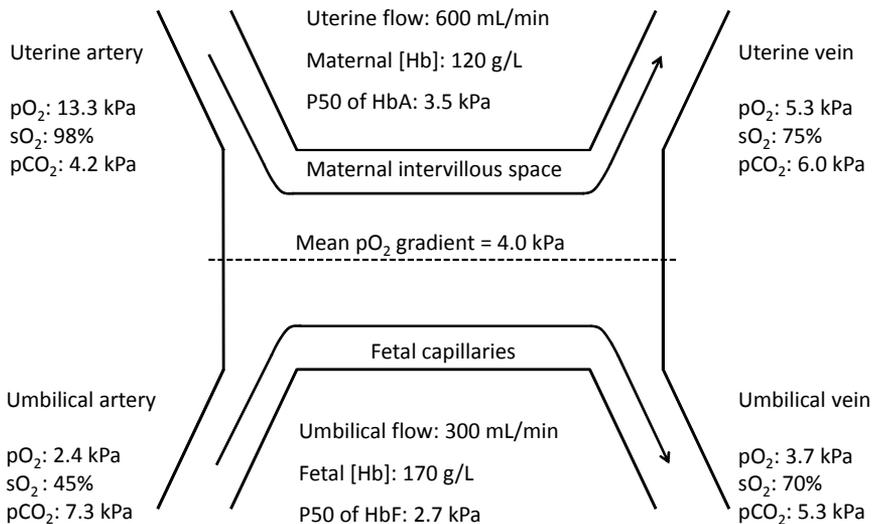
Figure 5. Gradients in the placenta. Arrows indicate flow direction. p50 of HbA and HbF is shown in the oxygen-hemoglobin curve in Figure 3. Modified from Brandis (Brandis, 1997).

Fetal coping with acidemia and acidosis

A healthy fetus with appropriate glycogen reserves can cope with mild-moderate hypoxia, but if oxygen deficit is prolonged there is a risk of acidosis followed by impairment of organ functions. The fetal heart rate increases in cases of hypoxia and hypercapnia in order to enhance oxygen availability in peripheral tissues and increase pCO_2 elimination and pO_2 extraction in the placenta. Hyperventilation is an essential tool with the same purpose but impossible during intrauterine life. Other compensatory mechanisms consist of increased pO_2 extraction thanks to the Bohr effect and decreased pO_2 consumtion by means of reduced physical activity, and in a longer perspective reduced fetal growth rate. A further defence mechanism used by the fetus is redistribution of the blood circulation to prioritize vital organs such as the brain, heart, and adrenals. A consequence of this centralization of the blood circulation, called 'brain-sparing', is hypoperfusion of peripheral tissues (Li *et al.*, 2006). As the hypoxia worsens, the organism adapts by switching to anaerobic metabolism. Lactate increases and contributes to metabolic acidosis (MA).

Acidosis at birth

Impairment of feto-placental gas exchange causes hypoxemia, i.e. reduced oxygen in the blood, and consequently an accumulation of CO_2 causing respiratory acidemia. Respiratory acidemia is often a part of short-term hypoxia, which the healthy fetus can handle. If the oxygen deficit is prolonged, i.e. the oxygen supply does not match the need in tissues, hypoxia will develop and the metabolic process is adapted to the hypoxic conditions. Anaerobic metabolism gives rise to lactic acid formation and therby MA (decreased pH in tissues) and eventually acidemia (decreased pH in the blood). The fetus has different defence mechanisms against hypoxia as mentioned above, but when these responses are insufficient there is a risk of organ failure.

Metabolic acidosis based on umbilical cord blood samples at birth is thus an objective measure of the fetus' exposure to and ability to resist hypoxia. Therefore, MA is widely used as a reliable fetal outcome parameter. Metabolic acidosis at birth is usually defined as a cord artery pH <7.00 and base deficit ≥12.0 mmol/L (ACOG, 2006). The pH threshold derives from studies by Gilstrap *et al.* and Goldaber *et al.* that showed an association between pH values <7.00 and increased frequency of low Apgar scores (AS ≤3), neonatal morbidity (early neonatal seizures), and mortality. However, two thirds of the newborns with pH values <7.00 showed no neurological impairments and did not need any additional treatment (Gilstrap *et*

al., 1989; Goldaber *et al.*, 1991). The BD cut-off recommendation is based on the result from a case-control study of term neonates with moderate to severe complications. Low *et al.* showed that among newborns with arterial BD ≥12.0 mmol/L, the incidence of encephalopathy and respiratory complications increased. It is not known which BD algorithm Low and co-workers used in their study (Low *et al.*, 1997).

Factors influencing acid-base balance in umbilical cord blood

There are several factors that may influence acid-base balance in umbilical cord blood. These can be divided into maternal and fetal factors, and those more directly related to the uterine wall, the placenta, or the umbilical cord. Diseases, drugs, or conditions during pregnancy and labor can all affect placental perfusion, energy and oxygen supply, and the maternal buffering capacity (Blechner, 1993). For example, regional anesthesia (spinal more than epidural) is associated with lower cord artery pH and higher lactate concentrations (Robson *et al.*, 1992; Roberts *et al.*, 1995; Ngan Kee *et al.*, 2000; Mercier *et al.*, 2001; Ngan Kee, 2010). Herbst *et al.* found labor with breech presentation, administration of oxytocin and pethidine, late decelerations, complicated variable decelerations, and cord entanglement to be associatied with umbilical cord artery acidemia (Herbst, 1997; Herbst *et al.*, 1997). Others confirm that oxytocin use and hyperactive uterine contractions are risk factors for acidemia (Bakker *et al.*, 2007; Jonsson *et al.*, 2008). Moreover, acid-base values in umbilical cord blood changes with gestational age as a result of fetal growth with increased metabolism and CO_2 load, and aging of the placenta with impaired gase exchange (Nicolaides *et al.*, 1989; Kitlinski *et al.*, 2003; Wiberg *et al.*, 2006b). Primiparity and duration of labor both correlate to lower arterial pH (Lauener *et al.*, 1983; Katz *et al.*, 1987; Nickelsen & Weber, 1987). Figure 6 summarizes factors influencing acid-base balance in umbilical cord blood.
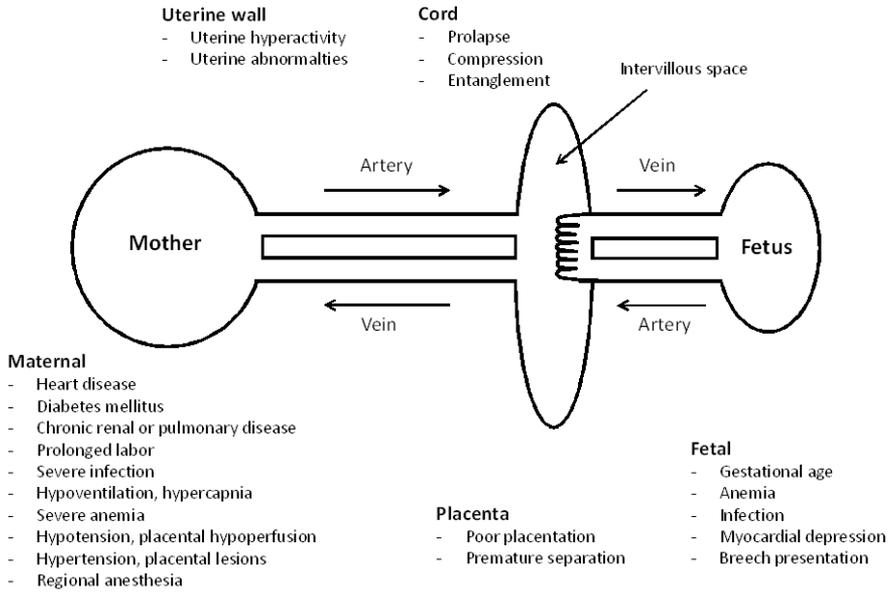
Figure 6. Factors influencing acid-base balance in umbilical cord blood. Modified from Westgate (Westgate, 1993).

Umbilical cord blood gases, Apgar score, and outcome

Assessment of both arterial and venous umbilical cord blood gives us valuable information about the duration, degree, and type of acidosis. The majority of cases with fetal acidosis are acute in onset. Large differences between cord acid-base values indicate that the hypoxic event has been of short duration and that the placenta has not had the time to equilibrate acid metabolites. Smaller differences are seen after more long-lasting hypoxia and imply saturation of the buffering capacity in placenta caused by a greater load of acids from the fetus. Since non-volatile acids such as lactate have a slower transfer over placenta than the volatile CO_2, respiratory acidosis will be corrected faster than MA. It is suggested that it is the degree rather than the type of acidosis that is more strongly correlated to asphyxia related morbidity (Herbst, 1997). According to Low both the duration and the degree of MA are important prognostic factors (Low, 1988). Others agree that it is the metabolic component of the acidosis that makes it significantly pathological, i.e. increases neonatal morbidity (Andres *et al.*, 1999).

Nelson and Ellenberg showed that AS at 1 and 5 minutes are poor predictors of long-term neurological outcome and that 75% of children with cerebral palsy (CP) have normal AS (Nelson & Ellenberg, 1981). Others have shown low correlation between AS and acid-base status at birth and that both of these evaluation parameters are poor predictors of perinatal brain damage (Sykes *et al.*, 1982; Ruth & Raivio, 1988). At birth, the newborn is in an arousal state caused by a catecholamine surge partially due to hypoxia. Catecholamines affect heart rate, respiration, reflex irritability, and tone, all of which are components of the AS. Therefore it should be no surprise that AS and acid-base values have a poor correlation unless they are severely affected. Apgar scores at 15 and 20 minutes have a good correlation with subsequent neurological dysfunction, and this reflects newborns who do not respond to resuscitation. Low scores at 15 minutes were associated with 53% mortality and among survivors 36% developed CP; the corresponding figures associated with low scores at 20 min were 60% and 57% respectively (Nelson & Ellenberg, 1981).

Asphyxia and neurological outcome

Fetal asphyxia is defined as hypoxemia and hypercapnia with a significant metabolic acidemia (Bax & Nelson, 1993; Low, 1997). To regard an intrapartum event severe enough to cause CP, the American College of Obstetricians and Gynecologists (ACOG., 2003) propose the following criteria:

1. Evidence of MA in fetal umbilical cord arterial blood obtained at delivery (pH <7.00 and base deficit ≥12.0 mmol∕L).
2. Early onset of severe or moderate neonatal encephalopathy in infants born at 34 or more weeks of gestation.
3. CP of the spastic quadriplegic or dyskinetic type.
4. Exclusion of other identifiable etiologies such as trauma, coagulation disorders, infectious conditions, or genetic disorders.

"Neonatal encephalopathy is a clinically defined syndrome of disturbed neurologic function in the term or near term infant during the first week after birth, manifested by difficulty with initiating and maintaining respiration, depression of tone and reflexes, altered level of consciousness, and often by seizures" (Leviton & Nelson, 1992).

The definition of CP has changed slightly over the years, and in 2005 Bax proposed the following definition: "CP is a group of disorders of the development of movement and posture causing activity limitation, that are attributed to non-

progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of CP are often accompanied by disturbances of sensation, cognition, communication, perception and/or behaviour and/or by a seizure disorder" (Bax *et al.*, 2005). The incidence of CP is 0.2% in Western countries and has remained quite unchanged although perinatal mortality has decreased over the years (Longo & Hankins, 2009; Oskoui *et al.*, 2013). There is an association between birth asphyxia and CP although the majority of newborns with asphyxia do not have poor outcome. Only 10-20% of the cases with CP in term newborns are related to asphyxia (Nelson & Ellenberg, 1986; Blair & Stanley, 1988). Prenatal factors such as intrauterine infections, intrauterine growth restriction, intrapartum bleeding, preterm birth, and genetic anomalies may explain 70-80% of cases with CP (Yudkin *et al.*, 1995; Badawi *et al.*, 1998; Bax *et al.*, 2006; Longo & Hankins, 2009). Some of these factors are also associated with asphyxia.

During sustained hypoxia the blood circulation is redistributed and the blood flow is increased in the brainstem, maintained in subcortical areas, but decreased in the cerebellum and cortex (Richardson *et al.*, 1989). This brainstem-sparing model protects the respiratory and vasomotor centers. Fetal heart rate is regulated from centers in the medulla oblongata, which in turn is affected by activity in the cerebral cortex and hypothalamus (Parer, 1997). Therefore an early sign of cerebral hypoxia may be loss of variability on cardiotocography due to decreased cortical blood flow (Westgate, 1993).

Hypoxic-ischemic events may result in several different brain injury patterns in the fetus and newborn. The severity of cerebral hypotension, the duration of the episode, and the maturity of the brain at the time of insult are all factors that influence the pattern of brain damage (Barkovich, 1997). Mild to moderate injuries as a result of acute asphyxia lead to hypotension and can affect areas in the brain with the lowest perfusion pressure. These areas are particularly at the borders between the supplies of the cerebral arteries, that is between the anterior, middle, and posterior cerebral arteries and so the injury will have a watershed distribution (Volpe *et al.*, 1985; Greisen, 1992; Cowan *et al.*, 1994). Severe events also affect deeper brain structures. During the first trimester there is a risk of malformations in the central nervous system; white matter injuries often occur between 23 and 32 weeks of gestation and after 34 weeks, damage in cortical and subcortical areas, or in the basal ganglia predominate (Bax *et al.*, 2006; Robinson *et al.*, 2009). Animal studies show that accumulation of lactate, cellular calcium uptake, and breakdown of phospholipids with a subsequent increase in free fatty acids are all factors in neuronal cell damage (Siesjö & Wieloch, 1985). Neuronal loss can be diveded into two phases:

1.   During the primary hypoxic episode due to a disturbed cellular steady state, with acidosis, ion distribution changes, and altered tissue perfusion.

2. During reperfusion and reoxygenation where metabolic and circulatory changes, as well as neurotoxicity are the damaging mechanisms.

The neurotoxicity in the second phase is caused by generation of oxygen-derived free radicals (Saugstad, 1996) and excitatory amino acids (Pulsinelli *et al.*, 1982). Early biochemical measurements of neuronal injury can be of great help in identifying newborns at risk of brain damage who may be helped by specific treatments such as cooling, i. e. systemic or cerebral hypothermia. Several biochemical markers have been the subject of studies, but none are yet established in clinical practice. These biochemical variables can be divided into two categories:

1. measurements of cellular metabolism and indicators of hypoxia, or
2. metabolites released due to tissue damage.

Measures of MA and purine metabolism are examples of the former category and excitatory amino acids and free radicals result from tissue damage. Hypoxanthine is a purine metabolite and the end product of ATP breakdown in most tissues except the liver and intestine where further oxidation occurs, finally resulting in uric acid (Saugstad, 2005). This marker accumulates during hypoxia and has been associated with pH, BD, lactate, and survival time in animal studies (Saugstad & Aasen, 1980; Thiringer *et al.*, 1980). Elevated hypoxanthine levels have been measured in hypoxic newborns (Saugstad, 1975; Thiringer, 1983; Saugstad *et al.*, 1986). Other studied biochemical markers are protein S100B, a fairly nerve tissue specific protein (Lackmann & Tollner, 1995; Beharier *et al.*, 2012), and lactate dehydrogenase (Reddy *et al.*, 2008; Karlsson *et al.*, 2010).

The severity of encephalopathy seems to be a good predictor of both short- and long-term outcome. Hypoxic-ischemic encephalopathy (HIE) is usually classified as mild, moderate, or severe (Sarnat & Sarnat, 1976). Mild HIE lasts less than 24 hours and is characterized by hyper-alertness, irritability, normal or hyperactive reflexes, and the absence of seizures. Moderate HIE includes lethargy, hypotonia, proximal muscular weakness, depressed primitive reflexes, and multifocal seizures. Newborns with severe HIE are stuporous, flaccid, lack primitive reflexes, and brain stem and autonomic functions are suppressed (Thornberg *et al.*, 1995). Cases with mild encephalopathy do relatively well, while the majority of newborns with moderate or severe forms either have persistent neurological impairments or die (Robertson & Finer, 1993; Thornberg *et al.*, 1995). The purpose of fetal surveillance during labor is to prevent asphyxia while the purpose of postpartum measurements such as AS and umbilical cord blood gases and lactate is to identify newborns that need neonatal intensive care to minimize morbidity and mortality.

Methodological factors influencing the interpretation of acid-base values in umbilical cord blood

Determination of cord blood gases and MA is important for various reasons: to retrospectively evaluate the course of labor and management in individual cases, to judge malpractice in litigation cases, to assess the quality of care at a maternity unit, and in research as an objective perinatal outcome parameter. There are many methodological confounding factors along the way, from sampling to analysis and interpretation of acid-base values in umbilical cord blood. Some of these factors are summarized in Figure 7.

- Prolonged storage

- *Algorithm and fluid compartment for BD calculation*

| **Sampling** | → | **Analysis** | → | **Interpretation of results** |

- *Delayed sampling*
- Venous , arterial or mixed sample
- Same vessel twice
- Incorrect labeling

- Incorrect input information
- Air bubbles
- Inhomogenous sample
- Coagulation
- Hemolysis
- Measurement imprecision

- *Arterio-venous gradient*
- *Decimal rounding and further calculations*

Figure 7. Methodological factors influencing the interpretation of blood gas and lactate values in umbilical cord blood. Factors in italics are the main issues of Studies I-IV in this thesis.

The time of sampling influences the levels of pH, BE, pO_2, pCO_2, and lactate in umbilical cord blood (Ullrich & Ackerman, 1972; Lievaart & de Jong, 1984; Wiberg *et al.*, 2008b). This is probably a consequence of the centralization of blood during labor and the following surge of 'trapped' metabolites into the central circulation when the newborn starts to move and breathe sufficiently. During the procedure of sampling there is a risk of confusing the cord vessels, sampling the same vessel twice, or of incorrect labeling. The umbilical vein is larger than the two arteries and usually the easiest to sample.

Blood gas samples should be analyzed whithin 30 min according to the manufacturer (Wennecke & Juel, 2011). Delaying the time from sampling to analysis can cause changes in blood gas and lactate values. Because of ongoing metabolism in leucocytes and reticulocytes in the sample, pCO_2 and lactate can increase and pH and pO_2 decrease, causing incorrect values (Armstrong & Stenson, 2006; Lynn & Beeby, 2007; Dessolle *et al.*, 2009; Wennecke & Juel, 2011).

When starting the analysis and introducing the blood sample into the analyzer, the input of the patient identification number and sample origin is done manually. In this step, there is again the risk of mixing up arterial and venous samples. Analysis can be affected by air bubbles in the sample which may increase the pO_2 values, or be interrupted because of coagulation, or result in inaccurate values due to inhomogeneous samples. Another factor is the measuring imprecision of the analyzer, often expressed as the coefficient of variance and reported as percent (CV%). Each measured parameter has its own CV% and it differs at different concentrations.

The fact that BD is not a measured but a calculated entity from pH and pCO_2 values is a problem, because different brands and models of blood gas analyzers use different algorithms to calculate BD. This results in significantly different BD values, although the pH and pCO_2 values are the same (Wiberg *et al.*, 2006b). Older blood gas analyzers include a fixed [Hb] value in the algorithm, whereas some modern blood gas analyzers measure the [Hb]. This introduces another methodological problem when comparing BD from different analyzers. In addition, BD can be calculated either in blood or in extracellular fluid which also affects the results (Kofstad, 2001). A puzzling fact is that the algorithms used by blood gas analyzers are based on the adult fluid compartment distribution while the fetus has a proportionally larger extracellular compartment representing a considerable buffering capacity.

To ensure the origin of a blood sample it is recommended that both arterial and venous samples are analyzed and that validation of the origin of samples is made by calculating the arterio-venous (A-V) or veno-arterial (V-A) gradient. There is no agreement on validation criteria but current recommendations are based on minimum accepted differences between V-A pH and A-V pCO_2. Venous pH and arterial pCO_2 is expected to be higher than the corresponding arterial and venous values.

Both in clinical practice and medical research, case values are deemed negative or positive relative to a predetermined cut-off. In a study with a certain cut-off for a variable, a case value may be deemed positive when not rounding off a decimal, but negative when rounding off. There is a risk of dichotomy drifts with the exactness of the analyte values reported by the analyzer, i.e. with the number of decimals. Of course, this comes as no surprise for those who attended math class in elementary school, but it is certainly an often overlooked factor in writing study protocols and analyzing test results. The diagnosis of MA is based on cut-off values of pH and BD. The analyzer reports pH values with three decimals. Since the pH value is included in the BD algorithm, rounding off the pH value decimals can influence both the pH and the BD value.

The measurement bias, for both pH and pCO_2, depends on the brand and type of analyzer, value level of the measured parameter, the amount of blood analyzed, and whether whole blood or capillary blood is analyzed (Radiometer, 2003). In whole blood the measurement bias for a pH of 7.00 is up to 0.004 in the Radiometer ABL 735. This suggests that the third pH decimal is an uncertain digit. Although there are no obvious mathematical obstacles to the use of pH values with three decimals in calculating BD and defining acidosis, the third decimal measurement imprecision indicates that we should continue to report pH values with no more than two decimals.

Many factors influence the fetus' ability to handle the relatively sour and hypoxic intrauterine milieu and the final challange: the birth. The duration and severity of hypoxemia, fetal cardiovascular responses and energy reserves, and obstetric surveillance are cruical for the outcome. "Given the complexity of this situation it is unlikely that any single variable will totally reflect fetal condition during labor" (Westgate, 1993).

Background and Aims of the Studies

Study I

During prolonged hypoxia, fetal metabolism switches to anaerobic glycogenolysis and the fetus eventually develops metabolic acidosis (MA). Metabolic acidosis in umbilical cord blood at birth is therefore an objective measure of the fetus' exposure to and ability to resist hypoxia. Consequently, MA is widely used as a reliable fetal outcome parameter. The diagnosis of MA is based on low cord artery pH and a high base deficit (BD, the negative value of base excess, BE).

Base deficit is not a measured entity but an artificial entity calculated from measured values of pH and the partial pressure of carbon dioxide (pCO_2). Due to different algorithms and different fetal compartments (i.e. blood or extracellular fluid) for the calculation of BD, significantly different BD values are obtained, even when based on the same pH and pCO_2 values (Wiberg *et al.*, 2006b). Some modern blood gas analyzers use the measured hemoglobin concentration [Hb] in the algorithm, whereas older blood gas analyzers include a fixed Hb value.

The hypothesis of *Study I* was that the prevalence of MA varies significantly with different algorithms, fetal fluid departments, and the use of fixed or actual [Hb] in the algorithm. The aim of *Study I* was to demonstrate the magnitude of these differences by calculating the rate of BD ≥12.0 mmol/L with algorithms from three brands of commonly used blood gas analyzers compared to standards recommended by the Clinical and Laboratory Standards Institute (CLSI, 2009). The study included calculation of BD in blood (BD_{blood}) with measured and fixed Hb concentrations, and BD in extracellular fluid (BD_{ecf}).

Study II

Rounding of decimals is common in everyday life. The number of decimals for different variables needs to be restricted for practical reasons. Most people probably round off decimals without considering potential consequences. Especially when variables included in equations and algorithms are rounded off in different steps, the results can be affected. When a certain cut-off for a variable is used, a case value may be deemed positive when not rounding off a decimal, but negative when rounding off. This can influence the interpretation of results. For example, if an umbilical cord artery pH value <7.05 at birth is defined as acidotic, the case value pH 7.049 implies acidosis, but when rounded off to 7.05 it does not. The Swedish Research Council suggested in a statement on a case of alleged misconduct in research that umbilical artery pH values should be reported with three decimals and not with two, in order to avoid the problem (Vetenskapsrådet, 2010). However, in the obstetric literature, pH values are generally given with only two decimals.

Study II aimed to illustrate the consequences of including two or three decimals for case values of pH in the algorithm to calculate BD on making a diagnosis of MA at birth. Furthermore, we wanted to illustrate possible differences when using two different mathematical round-off rules for decimals. The hypothesis was that rounding off pH and BD values would significantly change the prevalence of pH <7.05 and the diagnosis of MA (pH <7.05 plus BD >12.0 mmol/L).

Study III

Delayed clamping allows placental blood transfusion to the newborn as long as cord pulsations are present in an intact cord. The main transfusion occurs during the first 30-60 seconds after birth and ceases within 3 minutes (Yao *et al.*, 1968; McDonnell & Henderson-Smart, 1997). Uterine contractions and the position of the newborn relative to the placenta are two determinants of the blood transfusion (Yao *et al.*, 1969; Yao & Lind, 1969). An increased blood volume facilitates the newborn's cardiopulmonary transition to extrauterine life, improves hematological status, prevents anemia during the first 3-4 months, and is associated with reduced risk of intraventricular hemorrhage in preterm neonates (Yao *et al.*, 1968; Mercier *et al.*, 2001; Rabe *et al.*, 2004; Hutton & Hassan, 2007; Andersson *et al.*, 2011). Immediately after birth newborns have a 10-20% greater erythrocyte volume relative to body mass compared to adults, and when cord clamping is delayed this difference increases to 30-50% (Saigal *et al.*, 1972; Linderkamp *et al.*, 1974). In cesarean delivery (CD) the amount of transfused blood is less than after vaginal

delivery, but late cord clamping can still increase the preterm newborn's blood volume (Aladangady *et al.*, 2006).

When umbilical cord clamping is delayed after vaginal deliveries, a decrease in arterial pH and BE and an increase in the partial pressure of O_2 (pO_2), pCO_2, and lactate concentration have been reported (Ullrich & Ackerman, 1972; Lievaart & de Jong, 1984; Wiberg *et al.*, 2008b). The phenomenon called 'hidden acidosis' can explain the changes towards acidemia and lactemia. During uterine contractions, the umbilical cord blood flow is restricted and gas exchange temporarily impaired causing a centralization of the fetal circulation to prioritize vital organs. This occurs at the expense of perfusion of low-priority organs and peripheral tissues (Li *et al.*, 2006), leading to an accumulation of acid metabolites in peripheral tissues. The peripheral perfusion is restored when the newborn starts to move and breathe. 'Trapped' metabolites then surge into the central circulation and can after a few seconds be detected in umbilical cord blood (Wiberg *et al.*, 2008b). Hidden acidosis was, to the best of our knowledge, first described in an animal study from 1965, where induced aggregation of erythrocytes and severe capillary plugging, followed by resolution of the aggregate, showed a gradual release of hidden acidotic metabolites over a prolonged time (Litwin *et al.*, 1965). The phenomenon has also been demonstrated in animal studies at restoration of the peripheral circulation after provoked hypovolemic shock (Bergentz *et al.*, 1969; Strodel *et al.*, 1977), and in limb tourniquet ischemia-reperfusion experiments (Enger *et al.*, 1978; Szokoly *et al.*, 2006). Soon after volume expansion and reperfusion, a rapid drop in pH and an increase in lactate concentration are seen.

Our hypothesis was that hidden acidosis explains the blood gas and lactate changes occurring in the umbilical cord when sampling is delayed after vaginal deliveries (Figure 8). We further hypothesized that hidden acidosis should be less pronounced after CD compared to vaginal deliveries due to the absence of uterine contractions, and since newborns seldom show acrocyanosis after planned CD. By showing the difference in blood gas and lactate changes after the two delivery modes we aimed to further strengthen hidden acidosis as the explanation of the changes toward acidemia and lactemia at delayed sampling. The opening of peripheral vascular beds might result in changes in hemoconcentration in cord blood, and the aim of Study III were to investigate temporal changes, not only in blood gases and lactate concentration, but also in hematocrit (Hct) and [Hb].
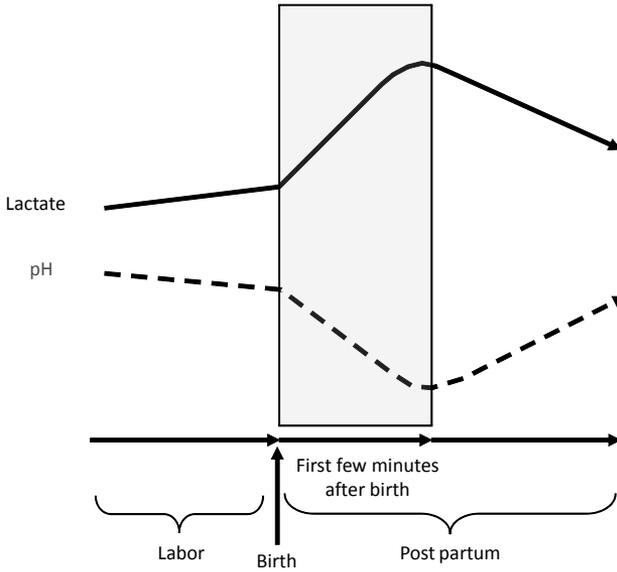
Figure 8. Schematic illustration of the hidden acidosis phenomenon. The grey box represents the first few minutes after birth, when a steep decrease in pH and increase in lactate concentration are first seen according to the hypothesis.

Study IV

Fetal metabolism is best represented by the arterial cord blood and to ensure the origin of a blood sample it is recommended that both arterial and venous samples are analyzed. Samples might erroneously be drawn twice from the same vessel, or arterial and venous blood samples might be confused. To differentiate arterial from venous samples, validation of the origin of the samples is made by calculating the arterio-venous (A-V) or V-A gradients, and samples not fulfilling certain validation criteria are questioned.

Using analyses of pH and pCO_2, the current validation criteria are based on probabilities that a sample originates from either the artery or vein (Westgate *et al.*, 1994; Kro *et al.*, 2010; White *et al.*, 2012), but there is no general agreement on validation criteria. Westgate *et al.* in 1994 settled the minimum accepted V-A pH gradient (ΔpH) to be represented by the 5th population ΔpH percentile, equal to 0.02 units, and the A-V ΔpCO_2 cutoff at 0.5 kPa represented by the 10th percentile (Westgate *et al.*, 1994). In a study of White *et al.*, the corresponding percentile

values were ΔpH 0.01 and ΔpCO_2 0.45 kPa (White *et al.*, 2012). Other used cut-offs are ΔpH 0.03 units (Huisjes & Aarnoudse, 1979) and ΔpCO_2 0.7 (Kro *et al.*, 2010) or 1.0 kPa (Sundström *et al.*, 2005).

Validation of arterial and venous samples in umbilical cord blood is a sort of quality assurance, although quality assurance is usually reserved for technical equipment while different procedures, processes, and activities are said to be validated. Validation can be described as an act demonstrating that a given process consistently leads to the expected results. In the case of validation of umbilical cord blood samples we do not entirely know what differences to expect between arterial and venous samples. The A-V or V-A differences (Δ values) of the acid-base parameters in umbilical cord blood are classified as positive, zero, or negative. The positive values are claimed to be correct, zero to represent cases where samples have been drawn twice from the same vessel, and negative values are thought to represent cases where arterial samples were identified as venous and vice versa. The influence of other factors, such as clinical characteristics, on the distribution of Δ values has not been investigated as far as we know. There are several confounding factors along the way from sampling to analysis results that can affect the interpretation of umbilical cord blood samples. Until we know more about the physiological differences between arterial and venous umbilical cord blood, we have to rely on the probability that a sample is arterial or venous.

The aim of *Study IV* were to explore the possibility of using distributions not only of ΔpH and ΔpCO_2, but also of ΔpO_2 and Δlactate to improve the certainty of identifying the origin of cord blood samples. The overall hypothesis was that in arterial cord blood pH and pO_2 should be lower, and pCO_2 and lactate higher, than in venous cord blood. Moreover, we wanted to investigate if there were any associations between Δ values and degree of cord blood acidemia and clinical parameters and we also aimed to describe percentiles for the Δ values in our material.

Material and Methods

Umbilical cord blood sampling

Determination of umbilical arterial and venous cord blood gases at birth has been routine at Skåne University Hospital in Malmö and Lund since the early 80s. The instructions to the midwifes have been that arterial and venous blood should be sampled in pre-heparinized 2 mL plastic syringes immediately after birth and before the newborn's first breath. The samples should be analyzed within 15 minutes. After vaginal deliveries the samples are drawn without clamping the cord in Lund, whereas in Malmö the cord is double-clamped. In cesarean deliveries the cord is double-clamped before sampling at both units.

Analysis of acid-base and lactate values

During the period 2001-2010, Radiometer ABL 735 blood gas analyzers (Radiometer A/S, Copenhagen, Denmark) were used. The ABL 735 analyzer is a 'mini-lab' with determination not only of blood gases, but with optional electrodes for determination of lactate, hemoglobin (Hb), electrolytes, etc. The analyzer measures pH and the partial pressure of carbon dioxide (pCO_2) by potentiometry, the partial pressure of oxygen (pO_2) and lactate by amperometry, and Hb by spectrophotometry at a temperature of 37°C.

Potentiometry is a measuring method based on the potential of an electrode chain that is an electrical circuit. In the Radiometer analyzer this chain consists of an electrode constituting the whole sensor unit, a reference electrode, a voltmeter, membranes, electrolyte solutions, and a sample. The elements in the electrode chain all contribute with an amount of voltage to the total potential recorded by the voltmeter. The difference between the total potential and the standard potential is then the sample potential which can be related to the concentration of the measured substance in the sample with the aid of the Nernst equation:

$$E = E_0 - 61.5 \times pH \qquad\qquad (Eq. 9)$$

where

E = potential difference across the glass membrane
E_0 = standard electrode potential
$61.5\,\dfrac{mV}{pH}$ = theoretical sensitivity of the pH electrode at 37℃

This principle is used for the measurement of pH, pCO_2, and electrolytes. However, the pCO_2 electrode is slightly different as it is a combined pH and Ag/AgCl reference electrode encased in a bicarbonate electrolyte. The covering membrane allows passage of uncharged CO_2, O_2, and N_2, but charged ions such as H^+ cannot pass. Consequently, CO_2 from the sample diffuses into the bicarbonate electrolyte until equilibrium is reached and the H^+ ions released changes the pH of the solution, causing a potential difference across the membrane. This potential change is converted into a pH value with the aid of the Nernst equation. The pCO_2 measurement is then given according to the Henderson-Hasselbalch equation (Eq. 4) with the measured pH included (Radiometer, 2003).

The pO_2 and lactate electrodes use amperometric measuring methods. The electrode chain consists of an anode and a cathode in an electrolyte solution separated from the sample by membranes. An amperometer and a voltage source is part of this chain. Selected molecules pass over the membranes and a redox reaction takes place, including oxidation at the anode and reduction at the cathode. The reaction gives rise to an electrical current proportional to the concentration of the reduced substance (Radiometer, 2003).

The analyzer also has an optical system for the measurement of Hb, oxygen saturation, bilirubin, etc. This system is based on a spectrophotometer connected to a combined hemolyzer and measuring chamber via an optical fiber. A small amount of the blood is ultrasonically hemolyzed in a cuvette that then is exposed to light. The absorption spectrum of the sample is proportional to the concentration of the compound and to the length of the light trough the sample according to Lambert-Beer's law (Radiometer, 2003).

The measurement imprecision can be expressed as the Coefficient of Variance in percent (CV%). The CV is the ratio between the standard deviation and the mean and shows the extent of variability (Radiometer, 2009). This ratio varies between the different parameters (pH, pCO_2, pO_2, and lactate) and at different concentrations. The reported CV% values in the Radiometer manual for ABL 800 (Table 1) is applicable for the ABL 700 (Labmedicin, 2012).

Table 1. The Coefficient of Variance in percent (CV%) for the pH, pCO_2 pO_2, and lactate at different levels.

pH		pCO_2 (kPa)		pO_2 (kPa)		Lactate (mmol/L)	
Level	CV%	Level	CV%	Level	CV%	Level	CV%
6.82	0.022	1.6	4.2	9.2	1.4	1.7	2.1
7.11	0.015	5.3	1.5	13.4	1.1	4.6	1.3
7.41	0.013	8.7	1.3	19.0	1.2	10.9	2.1

Base deficit (BD) is calculated from measurements of pH, pCO_2, and hemoglobin and can either be calculated in blood or in extracellular fluid depending on the analyzer settings. Total hemoglobin includes deoxy-, oxy-, carboxy- and methemoglobin. Hematocrit (Hct) is available as a derived parameter, calculated according to the algorithm:

$$Hct = 0.0485 \times Hb + 8.3 \times 10^{-3} \qquad (Eq.10)$$

Analyte results are printed on paper, but are also stored on the analyzer's hard drive where they can be identified by date of analysis and maternal personal identification numbers. The Radiometer analyzer reports pH values with three decimals, pCO_2 values with three digits, pO_2 with two decimals, and BD and lactate with one decimal. This means that for pCO_2 values ≤9.99kPa the second decimal is reported, but for values ≥10.00 the second decimal is always digit 0. The origin of sampling, i.e. "artery" or "vein", was entered manually for each sample at analysis. The data stored on the analyzer´s hard drive could be retrieved and transferred directly to a computerized statistical program. The analyzer is part of an accredited laboratory.

What is partial pressure?

A gas consists of a collection of moving particles that continuously collide with one another and with the wall of their container. The collision with the wall is so frequent that it causes a constant pressure which is expressed as a force per unit area. The SI unit (The International System of units) for pressure is pascal (Pa) which is defined as a pressure of 1 newton per square meter: 1 Pa = 1 N/m². The partial pressure of a gas is dependent on the activity of the molecules, the volume, and temperature. Each gas in a mixture has a partial pressure which represents the pressure that the gas would have if it occupied the volume of the mixture alone at the same temperature. The sum of all partial pressures in a mixture is the total pressure of a gas mixture. It is the partial presssure rather than the concentration of a gas in a gas mixture or in a liqiud that determines how the gas dissolves, diffuses, and reacts (Burton *et al.*, 1994).

Ethical Committee approvals

The Research Ethics Committee at the Faculty of Medicine at the University of Lund, in Sweden, approved *Study I* (reference number: LU 43998), *II* and *IV* (reference number 2009/222). *Study III* was approved by the Central Ethical Review Board, in Stockholm, Sweden (reference number Ö 50-200), and all included women gave their informed oral and written consent to participate in the study.

Perinatal Revision South Register

The Perinatal Revision South Register (PRSR) is a computerized database in southern Sweden, started in 1994. The register is based on data from 11 delivery units: the university hospital in Malmö and Lund, the central hospitals in Kristianstad, Helsingborg, Halmstad, Karlskrona, Växjö, and the county hospitals in Ystad, Ljungby, Trelleborg, and Ängelholm, of which the last three are now closed. In PRSR, clinical data regarding obstetric and neonatal care were collected from the units every 3 months for quality assurance of perinatal care (Molin, 1997). The region has a population of 1.6 million with an annual delivery rate of approximately 20 000.

Statistics

Statistics were performed with the aid of StatView® computer software 5.0 (SAS Institute, Cary, NC, USA) except for McNemar's test which was performed online at the Vassar College VassarStats' website for statistical computation (VassarStats). One-tailed statistics were performed for comparison of pH values before and after decimal round-off in *Study II*, whereas two-tailed statistics were performed for other comparisons and a *P* value of <0.05 was considered statistically significant.

Variables

The measurable attribute or characteristic of an object in a study population is called a variable. The variable should be able to assume different values, making comparisons between different subjects in a population possible. A variable can be quantitative, such as age, and assume numerical values, or qualitative, such as a diagnosis, and assume other values than numerical. Variables can also be con-

tinuous and assume any value within a range, such as pH, or discrete and only assume distinct values within an interval, such as parity. Depending on the level of measurement, variables are divided into different scales. Observations that can be categorized are on the nominal scale (qualitative) while ranked observations are on the ordinal scale (quantitative). Gender is an example of a nominal variable and small-, appropriate- and large-for-gestational age (SGA, AGA and LGA) are examples of ordinal variables. Quantitative variables can also be on interval or ratio scales. If each of the steps on a scale is equal to one another then the scale is known as interval. When observations have an absolute zero point and it is possible to express meaningful ratios with the measurement it fits the criteria for a variable on the ratio scale.

Descriptive statistics and distribution

In order to choose the most suitable statistical method it is crucial to be aware of the distribution of the data. To get a visual impression of the distribution, a histogram can be drawn. When data are normally distributed the sample is centralized around the mean value and equally distributed around it. This is called the Gaussian distribution. In this case, mean and median values of the data should coincide, which is another way of testing the distribution in a material. However, median and mean can also coincide in material with skew distribution. One way of testing if the study population is normally distributed is to run the Wilk-Shapiro test. In this test, the distribution of the test sample is compared with a normal distribution with the hypothesis that the test sample is normally distributed. If the calculated P value is small then the hypothesis that the test sample has a Gaussian distribution has to be rejected. In normally distributed populations the average and distribution of the data could be described as mean and standard deviation (SD). The SD defined as the measure of how much the values in a population differ from the mean value.

$$SD = \sqrt{\frac{\sum(x - \bar{x})}{n - 1}}$$ (Eq.11)

where

$\sum$ = sum of
x = the single values
$\bar{x}$ = mean value
n = number of observations

Normally distributed data are suitable for parametric statistical tests such as t-test, analysis of variance (ANOVA), or z-test. If the material is not normally distributed, it is not meaningful to compute mean values or SD, and non-parametric tests, such as the Mann-Whitney *U* test are preferable. Non-parametric statistical tests are based on fewer assumptions and are therefore more robust and appropriate to use when the distribution of data is skew. In these samples, the distribution is preferably expressed as median with the quartiles or percentiles, and when the population is small, minimum and maximum values can be reported. In large samples, minimum and maximum values should not be the only reported measurement of distribution. In order to give a detailed description of the skewness of the distribution, both the median and mean are reported in *Study IV*. Median and range or mean with SD or 95% confidence interval (CI), are reported in the studies and included in this thesis as appropriate.

Significance: probability and confidence

In 1925, Ronald Aylmer Fisher introduced the significance testing and the probability value (*P* value). The *P* value is a measurement of the probability of finding a result at least as extreme as the one observed if the null hypothesis is true. Fisher recommended a *P* value <0.05 (5 %) as a level for evidence against the hypothesis tested. This arbitrary level was not intended to become an absolute threshold for significance but rather a support in the interpretation of a result while taking all existing facts into consideration. Fisher simply chose the figure 0.05 because it was 'convenient'. This means that vital scientific questions from then on have been decided by an entirely arbitrary standard. In the 1960s, the weakness in Fisher's probability testing attracted attention when a 200-year-old mathematical formula known as Bayes's Theorem reappeared (Matthews, 1998). It is a formula which gives scientists a way to assess how much more plausible their hypotheses become as the data fill up. Thus, type of study, sample size, statistical analysis, and clinical relevance are some factors that need to be taken into consideration when interpreting a study result. The statistical concepts of CI should be regarded as a complement to the *P* value. The *P* value gives information about to what extent one can be sure that the null hypothesis can be rejected, but does not give any information on the size of e.g. the difference between groups. A *P* value is used for the probation of a hypothesis and does not indicate the direction of the study results found. In contrast, the CI appreciates the effect studied including the direction of the effect.

The CI is a given range within which the true value lies with a certain probability and it is dependent on the sample size, the average difference between the groups studied (if differences are compared), the chosen probability, and the standard error. A large sample size gives a more confident and narrow CI. The chosen prob-

ability is often 99 or 95% in medical research. High dispersion gives a wider CI and makes the result less certain (du Prel *et al.*, 2009). When differences are compared the CI can be expressed as:

$$CI = D \pm c \times SE \tag{Eq.12}$$
where
$$SE = \frac{SD}{\sqrt{n}}$$

D = average difference found in the observation
c = constant dependent on the chosen significance level
 (1.96 for significance at the 95% level)

Statistical tests used in this thesis

Simple linear regression analysis was used to describe the linear relationship between variable (x) and the dependent variable (y). This model can be written as:

$$y = a + b \times x + e \tag{Eq.13}$$

When the constant, a, is the intersection point (where the line crosses the y-axis), b is the slope of the line, and e is the residual that cannot be explained by variable x. The *P* value for the regression analysis describes the probability that there is no linear relationship, while the slope coefficient (b) reveals the degree of linear correlation. Simple linear regression is sensitive to outliers and when the distribution of variables is skewed the non-parametric alternative, e.g. the Spearman's rank correlation, is preferred. The correlation coefficient (r) can assume values from -1 to +1, depending on the degree of correlation and if the relationship is positive or negative. Simple linear regression was used in *Study I* and *IV*.

The *Chi-square test* could be used when comparing frequencies or proportions between two or more unrelated groups. When comparing binary outcomes in the case of paired observations, the *McNemar's test* is preferred. The McNemar's test was used in *Study I* and *Study II*, where the variables were binomial (nominal) and the proportions compared were based on the same sample. The Chi-square test was used in *Study IV* when comparing frequencies between three independent groups. Since the rounding of pH value decimals in *Study II* always resulted in a smaller number of acidotic values (values 7.045–7.049 were rounded to 7.05), one tailed statistics were performed for comparison of pH values before and after decimal round-off, whereas two-tailed statistics were performed for the other comparisons in *Study I, II* and *IV,* where the result could assume values in two directions. The

tests are based on contingency tables. The observed distribution is compared to the distribution that would be expected if the null hypothesis was true. In a table for critical values of the chi-square distribution, the minimum value of the test variable needed for statistical significance, at a specific degree of freedom and at the chosen significance level, can be determined.

The Mann-Whitney U test is a non-parametric test that can be used when comparing two unrelated groups. This means that no individual in the sample can have more than one measurement, and if that is the case, the measurements must first be summarized. The measurement level of the variables should at least be on the ordinal scale since the test is based on ranking of the observations. When observations have been ranked, the sum of the ranks and the mean rank can be calculated. The P value is then determined based on the difference of the mean rank in the two groups and the sample sizes. This test is robust in the presence of outliers and suitable if the sample size is small. The Mann-Whitney U test was used in *Study III* for comparison of continuous parameters between the vaginal and cesarean delivery group and in *Study IV* for comparisons between the different groups with regard to clinical data. The *Kruskal-Wallis one-way test of variance* is a similar test based on ranking that is used for comparisons between three or more groups. However when statistically significant results are received the Kruskal-Wallis test does not reveal where the differences occur. The Kruskal-Wallis test was used in *Study IV* for comparisons of clinical data between the subgroups with different delivery modes.

Wilcoxon's signed-ranks matched pairs test can be used when comparing matched samples, two related samples, or two repeated measurements on the same sample. The statistical calculation in this non-parametric test is based on differences in mean rank between the pairs. The data have to be on the interval scale to enable calculation of the differences, which can then be ranked. While there is a relation within the pairs, independence is assumed between the different pairs. In addition, the distribution of differences between the observations in each pair should be relatively symmetrical. This test suited the longitudinal comparisons in *Study III,* where repeated measurements on the same sample were compared.

In addition to the mentioned references, the following books were used when the section Statistics was compiled: (Dawson & Trapp, 1994; Bring & Taube, 2006; Björk, 2011).

Material and methods specified: Study I-IV

Study I

From 15th of March 2001 to 24th of September 2008, there were 29 122 deliveries at the University hospital in Malmö and Lund. Cord blood gas data were available in the analyzer hard drive in 85-90% of these cases. For a large number of samples the origin of sampling was missing in the analyzer's hard drive database. Therefore only 37 191 cord blood gas values marked with either "artery" (17 318) or "vein" (19 873) were left in the data base. Only singleton deliveries with both pH and pCO_2 values present from the artery and vein were included in the study. Cases with a venous to arterial pH gradient <0.020 were excluded since they may represent mixed-up samples or two samples drawn from the same vessel according to Westgate's validation criteria (Westgate *et al.*, 1994). The pCO_2 value was not used for validation because there was no risk of mixing up pH and pCO_2 values obtained from the same vessel during the electronic transfer of data from the analyzer to the statistical database. Further inclusion criteria were that complete arterial data on pH, pCO_2, BD, and the total Hb concentration were present. In addition to the BD values in blood obtained from the Radiometer analyzer, BD was calculated in extracellular fluid (ecf) according to the Radiometer analyzer manual (Radiometer, 2003). BD_{blood} and BD_{ecf} were also calculated *post hoc* with algorithms retrieved from two other blood gas analyzers and standards recommended by the Clinical Laboratory Standards Institute (CLSI, 2009). In the following descriptions of equations and algorithms, base excess (BE) is reported as its negative value BD. Values in millimetres of mercury for pCO_2 were converted to SI units according to the conversion equation, mmHg = 7.5 × kPa. The algorithms for calculation of BD values were:

Clinical and Laboratory Standards Institute (CLSI) calculations (CLSI, 2009)

BD in blood (BD_{blood}) = − (1 − 0.014 × ctHb) × [cHCO$_3^-$ − 24.8 + (1.43 × ctHb + 7.7)(pH − 7.40)] (Eq. 14)

where

$\log c$HCO$_3^-$ = pH + log(pCO_2 × 0.2325) − 6.095
BD in ecf (BD_{ecf}) = − [cHCO$_3^-$ − 24.8 + 16.2(pH − 7.40)]

The prefix c denotes concentration and ctHb total concentration of hemoglobin (deoxy-, oxy-, carboxy-, and methemoglobin).

Radiometer ABL 735 (Radiometer A/S) calculations (Radiometer, 2003)

BD was automatically calculated by the analyzer according to the following algorithm:

$$BD_{blood} = - [0.5 \times (8a - 0.919)/a + 0.5 \times [((0.919 - 8a)/a)^2 - 4 \times (24.47 - cHCO_3^-(5.33))/a]^{1/2}] \tag{Eq. 15}$$

where

$a = 4.04 \times 10^{-3} + 4.25 \times 10^{-4} \, cHb$

$cHCO_3^- (5.33) = 0.23 \times 5.33 \times 10^{[(pH \, (st) - 6.161)/0.9524]}$

$pH \, (st) = pH + \log (5.33/pCO_2) \times [(pH(Hb) - pH)/(\log pCO_2(Hb) - \log(7.5006 \times pCO_2)]$

$pH \, (Hb) = 4.06 \times 10^{-2} cHb + 5.98 - 1.92 \times 10^{(-0.16169cHb)}$

$\log pCO_2 \, (Hb) = - 1.7674 \times 10^{-2} cHb + 3.4046 + 2.12 \times 10^{(-0.15158cHb)}$

In the calculation of BD_{ecf}, cHb was replaced by a fixed value of 3.0 mmol/L according to the Radiometer manual (Radiometer, 2003).

Corning 178 (Ciba Corning Diagnostics, Halstead, UK) calculations (Westgate, 1993)

$$BD_{blood} = - (1 - 0.014 \times Hb) \times [HCO_3^- - 24 + (9.5 + 1.63 \times Hb)(pH - 7.4) \tag{Eq. 16}$$

where

$cHCO_3^- = 0.2325 \times pCO_2 \times 10^{(pH-6.1)}$

with

Hb fixed at 9.3 mmol/L (150 g/L)

$BD_{ecf} = - 0.9149 \times [0.2325 \times pCO_2 \times 10^{(pH - 6.1)} - 24.1 + 16.21 \times (pH - 7.4)]$

Roche cobas b 221 (Hoffmann-La Roche, Basel, Switzerland) calculations (Roche, 2009)

$$BD_{blood} = - (1 - 0.014 \times tHb) \times [(1.43 \times tHb + 7.7) \times (pH - 7.4) - 24.8 + cHCO_3^-] \tag{Eq.17}$$

where

$cHCO_3^- = 0.2303 \times pCO_2 \times 10^{(pH-6.105)}$

$BD_{ecf} = - [16.2 \times (pH - 7.4) - 24.8 + cHCO_3^-]$

The BD values from the three blood gas analyzers were compared with standards recommended by the CLSI (CLSI, 2009). In the BD_{blood} algorithms from Corning and Roche the Hb concentration is included at a fixed value of 9.3 mmol/L (150 g/L), whereas the Radiometer analyzer measures the Hb concentration and includes it in the algorithm. The BD_{blood} values from the Corning and Roche analyzers were also calculated with the measured Hb concentration.

In the following text, BD for CLSI is denoted BD_{CLSI}, for the Corning analyzer $BD_{Corning}$, for the Roche analyzer BD_{Roche}, and for the Radiometer analyzer $BD_{Radiometer}$. In the *post hoc* calculations of BD the pH and pCO_2 values were included with all reported decimals. The BD values were then rounded off to one decimal before the rates of metabolic acidosis (MA) were calculated. Rounding was done according to the 'round half to even' rule (see bankers' rule below). Base deficit ≥12.0 mmol/L combined with a pH <7.00 in arterial cord blood was the definition used for MA in this study (ACOG, 2006).

Study II

Cases with both arterial and venous pH and pCO_2 values were included. Validation of the samples was done according to Westgate's criteria, i.e. cases with a venous to arterial pH gradient of <0.020 were excluded (Westgate *et al.*, 1994). Thus, 18 831 cases with a complete blood gas profile were retrieved.

pH and pCO_2 were measured by the Radiometer ABL analyzer and BD_{ecf} was then calculated from these determinations by using the equation derived from the Siggaard-Andersen acid-base chart (Siggaard-Andersen, 1977), also called the Van Slyke equation:

$$- 0.9149 \times \left[0.23 \times pCO_2 \times 10^{(pH - 6.1)} - 24.1 + 16.21 \times (pH - 7.4) \right] \quad \text{(Eq. 18)}$$

Several studies use BD_{ecf} in the definition of MA. All reported digits for pCO_2 were included when calculating BD_{ecf}. The pH on the other hand was first included in the algorithm with three-decimal values and then with the third decimal rounded off. The impact on MA diagnosis by reducing decimals of BD_{ecf} was analyzed, for both calculations. Decimals were rounded off to the nearest tenth. Round-off of digit 5 was performed according to two common round-off rules called rule A and B in this article (Wikipedia, 2012). The rules are:

A. 'round half to even' or the 'bankers' rule' where digit 5 is rounded down when preceded by an even digit, and up when preceded by an uneven digit (e.g. 0.25 to 0.2 and 0.35 to 0.4).

B.   'round half-up' rule where digit 5 always rounded up (e.g. 0.25 to 0.3 and 0.35 to 0.4).

The pH was defined as acidotic when the crude three-decimal value was <7.050, and after rounding off the third decimal when <7.05. In keeping with previous studies (Westgate *et al.*, 1993; Amer-Wåhlin *et al.*, 2001; Luttkus *et al.*, 2004; Amer-Wåhlin & Rosén, 2006; Norén *et al.*, 2006; Norén *et al.*, 2007; Welin *et al.*, 2007; Westerhuis *et al.*, 2010), we defined MA in cord arterial blood as pH <7.05 plus BD_{ecf} >12.0 mmol/L, but in this study also as pH <7.050 plus BD_{ecf} >12.000 mmol/L.

Study III

In this study, 124 newborn singletons were included, and of these neonates 66 were born vaginally in cephalic presentation and 58 were delivered by planned cesarean section. All were at 36-42 gestational weeks according to the routine ultrasound dating in the early second trimester. The recruitment of women in the vaginal group was done at admission to the delivery ward, while women in the cesarean delivery (CD) group were asked a few hours before the operation. The indications for CD were breech presentation or maternal request and the abdominal deliveries were all planned.

Immediately after birth (time zero, T0) and at 45 seconds (T45) arterial and venous blood were sampled from unclamped umbilical cords with intact pulsations. Only newborns that were expected to have no need of immediate rescue procedures were included in the study. Otherwise the delayed cord clamping would have interfered with the need of immediate care.

General (N =6) and spinal (N =52) anesthesia was used in the CD group and all women in this group received prehydration. Bupivacaine and fentanyl was used for the spinal anesthesia and surgery was performed with the women in supine position and tilted 15° to the left. An intravenous infusion with 50 mg ephedrine in 500 mL sodium chloride solution was started and adjusted with the aim to maintain mean arterial blood pressure within 25% of its initial value. Drugs administered for general anesthesia were thiopental, suxamethonium, and sevoflurane. After cord clamping all women received oxytocin.

Babies delivered vaginally were placed on the abdomen of the mother, whereas babies born by cesarean section were placed between the maternal legs. The newborns were kept warm under a towel during cord blood sampling. The samples were taken and analyzed by one of the authors (N.W.) who was not involved in the obstetric care of the women. The procedure was meticulously prepared. The

first sample pair was taken from the artery and then within a few seconds, from the vein, at the same location on the cord. Forty-five seconds later the next sample pair of arterial and venous blood was taken from the cord, a few millimetres closer to the placenta. A 0.6 or a 0.9 mm needle was used for puncture and the samples were collected in 2 mL pre-heparinized plastic syringes. A minimum of 0.5 mL blood from each vessel was used for analysis in the blood gas analyzer (ABL 735, Radiometer A/S, Copenhagen, Denmark). The samples were analyzed within 15 min in chronological order.

During the second stage of labor, the women in labor were monitored with cardiotocography. Small-for-gestational age was defined as a birthweight below minus 2 SD from the gestational-age-adjusted mean value, AGA as a birthweight within mean ± 2 SD, and LGA as above mean + 2 SD (Marsál *et al.*, 1996).

As umbilical cord blood gas and lactate values change with advancing gestational age (Kitlinski *et al.*, 2003; Wiberg *et al.*, 2006a; Wiberg *et al.*, 2008a), comparisons between vaginal and cesarean delivery groups were also analyzed using cord arterial pH adjusted to a gestational age of 280 days according to the regression coefficient 0.00096 per day of gestational age (Kitlinski *et al.*, 2003).

Study IV

Analyte data stored in the Malmö and Lund analyzers' hard drives were electronically transferred to a computerized statistical program. A prerequisite for inclusion in the study was that each set of parameters, i.e. pH, pCO_2, pO_2, and lactate, was complete for both the cord artery and vein in each case and could be identified without doubt in the analyzers' hard drives by the unique maternal personal identification number, site of sampling (arterial, venous), place of origin (delivery ward), and time of analysis. In the computerized database each analyte parameter result is followed by a report of analysis quality and all cases where the quality check system reported error for any of the study parameters, e.g. poor calibration, temperature error, electrode instability, and air bubbles at the electrode, were ruthlessly excluded from the study. Using the personal identification numbers, laboratory data were paired with clinical data from the regional PRSR and only cases with available clinical data where included. Figure 9 is a flow chart showing the merged Malmö and Lund databases (80 770 blood samples) and the step-wise exclusion of cases until 27 233 newborns with ensured identifications and complete panels of clinical data, as well as paired blood gases and lactate determinations, remained.
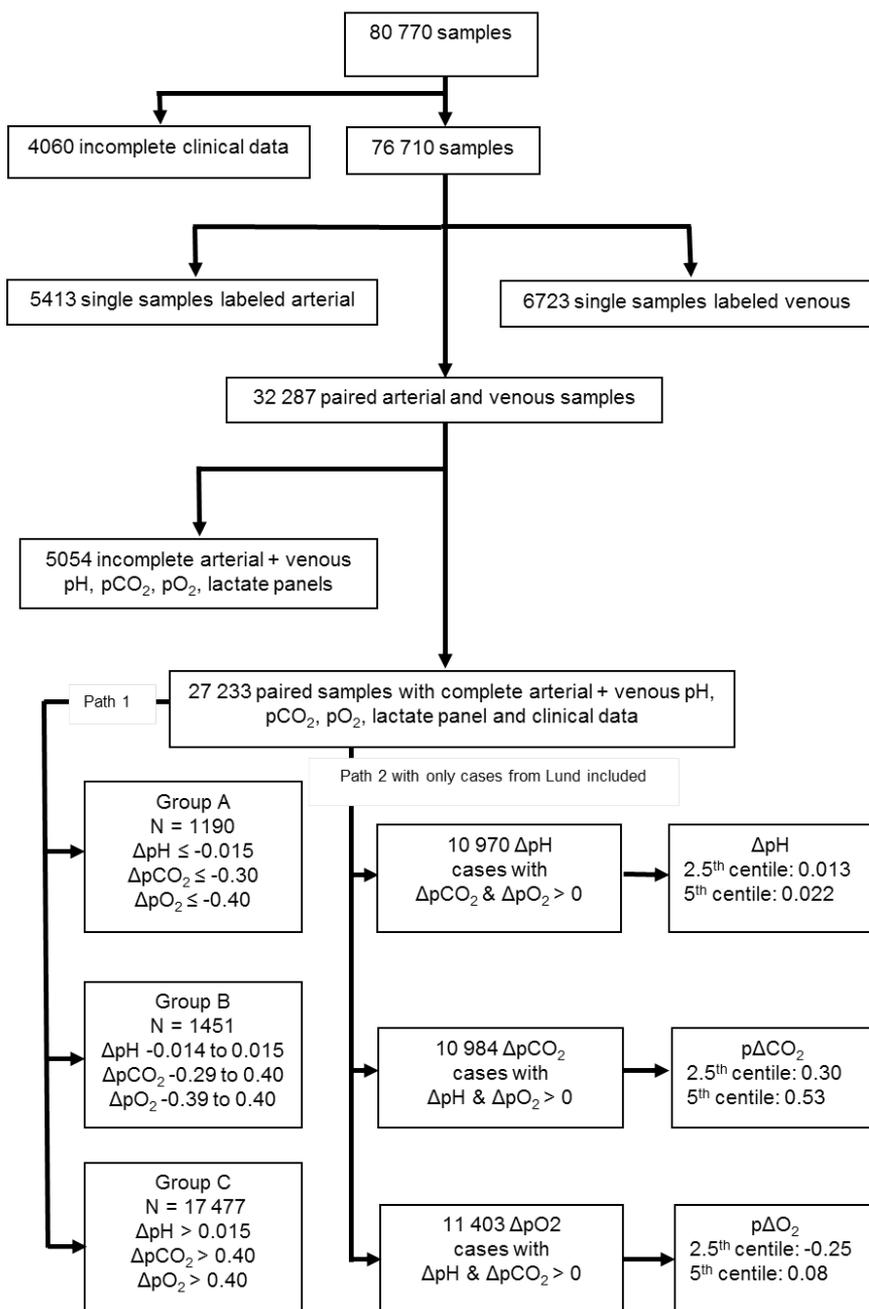
Figure 9. Flow chart showing number of samples with blood analysis values extracted from the blood gas analyzers' hard drives (80 770 blood samples) and exclusions due to incomplete data.

Results and Comments

Study I

Results

In *Study I*, 15 354 cord arterial blood gas determinations with complete data on pH, the partial pressure of carbon dioxide (pCO_2), base deficit ($BD^{Radiometer}$), and hemoglobin (Hb) concentrations were included. The numbers and prevalence of BD ≥12.0 mmol/L according to the algorithms from the Clinical and Laboratory Standards Institute (CLSI) and the blood gas analyzers are displayed in Table 2. The prevalence of BD_{blood} ≥12.0 mmol/L was significantly higher when calculated with the three different analyzer algorithms compared to the CLSI algorithm. Inter-analyzer comparisons showed significant differences in the prevalence of BD_{blood} ≥12mmol/L when calculated with both actual and fixed Hb. The prevalence of BD_{ecf} ≥12mmol/L was significantly different between all algorithms and highest when calculated with the Roche algorithm. All intra-analyzer and "intra-CLSI" comparisons also showed significant differences in the prevalence of BD_{blood}, with either actual or fixed Hb, and in BD_{ecf} ≥12mmol/L (P <0.0001). In inter-analyzer comparisons the greatest difference for BD_{blood} was between the Radiometer analyzer and CLSI, being 163% (ratio 2.63), and for BD_{ecf} the greatest difference was found between the Roche and Corning analyzers, at 210% (ratio 3.1). The corresponding figure for intra-analyzer comparisons was 426% (ratio 5.26) regarding actual Hb $BD_{blood}^{Corning}$ versus $BD_{ecf}^{Corning}$.

Table 2. Prevalence of base deficit (BD) ≥12.0 mmol/L in umbilical cord blood at birth calculated with four different BD algorithms in a population sample of 15 354 deliveries. BD was calculated in blood (BD_{blood}) and extracellular fluid (BD_{ecf}), using both the actual hemoglobin (Hb) concentration and a fixed Hb of 9.3 mmol/L (150 g/L) according to the algorithms described in the analyzer manuals. For description of blood gas analyzers and algorithms, see Material and Methods.

Algorithm / analyzer	BD_{blood} with actual Hb		BD_{blood} with Hb = 9.3 mmol/L		BD_{ecf}	
	N	%	N	%	N	%
CLSI	303[a]	1.97	–	–	267[c]	1.74
Radiometer	796[a]	5.18*	–	–	167[c]	1.09*
Corning	589[d]	3.84*	505[b]	3.29*	112[c]	0.73*
Roche	505[d]	3.29*	450[b]	2.93*	347[c]	2.26*

CLSI = Clinical and Laboratory Standards Institute
a) Original analyzer algorithm includes actual Hb for calculation of BD_{blood}
b) Original analyzer algorithm includes a fixed Hb of 9.3 mmol/L for calculation of BD_{blood}
c) For the Radiometer analyzer a fixed Hb of 3.0 mmol/L was used in the algorithm, for other analyzers Hb is not included in the BD_{ecf} algorithms
d) *Post hoc* calculations using actual Hb concentration
*) Comparisons to CLSI, McNemar test: *P* <0.000001

Regression analysis showed a scattering of values (Figure 10) due to the inclusion of actual Hb concentrations in both the BD_{blood}^{CLSI} and $BD_{blood}^{Radiometer}$ algorithm. A BD_{blood}^{CLSI} value of 12.0 mmol/L corresponded to a $BD_{blood}^{Radiometer}$ value of 14.1 mmol/L and a BD_{blood}^{Roche} value of 12.7 (figure not shown) calculated with the regression equations.
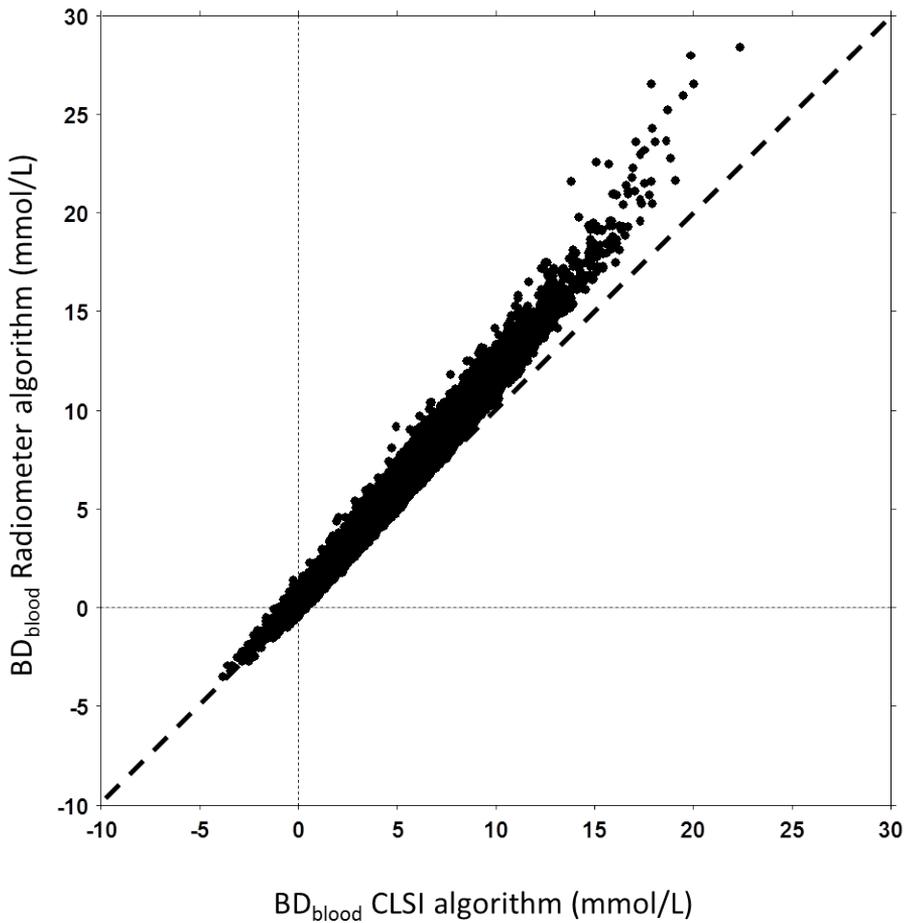
Figure 10. Relationship between base deficit in blood (BD_{blood}) calculated with the Clinical and Laboratory Standards Institute (CLSI) algorithm and the Radiometer algorithm, which both include the actual hemoglobin concentration. The correlation coefficient was 0.990 with simple linear regression analysis ($Y = 0.161 + 1.162 \times X$). The line of identity is indicated. A BD_{blood}^{CLSI} value of 12.0 mmol/L corresponded to a $BD_{blood}^{Radiometer}$ value of 14.1, and a $BD_{blood}^{Radiometer}$ of 12.0 to a BD_{blood}^{CLSI} of 10.2 mmol/L.

Of the 15 354 cases, 104 (0.68%) had a pH <7.00. The prevalence of MA (pH <7.00 and BD ≥12.0 mmol/L) with regard to the different BD algorithm are presented in Figure 11. Values obtained with the analyzers' BD_{blood} algorithms resulted in significantly higher prevalence of MA compared to the BD_{blood}^{CLSI} algorithms. At inter-analyzer comparisons, all MA prevalences were significantly different with the BD_{ecf} calculations, whereas only the $BD_{blood}^{Radiometer}$ calculation differed from the other BD_{blood} calculations. The differences between BD_{blood} and BD_{ecf} calcula-

tions were significant in all intra-analyzer comparisons; no statistical differences were found when calculating MA in blood with actual or fixed Hb.
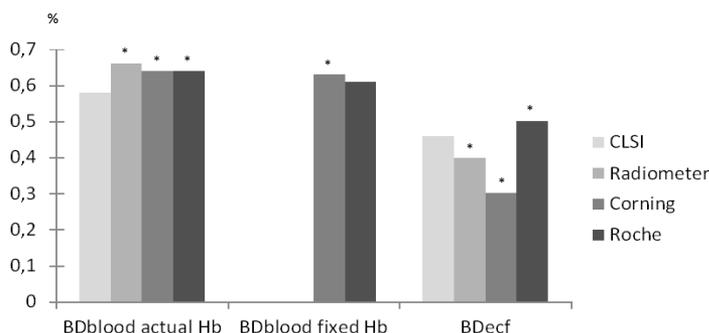
Figure 11. Prevalence of metabolic acidosis (pH <7.0 and BD ≥12.0 mmol/L) in arterial umbilical cord blood at birth calculated with four different BD algorithms in a population sample of 15 354 deliveries. *) Comparisons to CLSI, McNemar test: $P <0.02$; a) $P =0.06$.

Case description

A 27-year old para 0 had labor induced due to post term pregnancy. Cardiotocography (CTG) was initially non-reassuring and fetal surveillance with fetal ECG monitoring (ST analysis, STAN) was started early in labor. Labor progression was normal. At 7.27 pm the STAN system alarm went off because of an increase in the T/QRS ratio by 0.06, but since the managing obstetrician judged the CTG pattern normal no action was taken according to the CTG+STAN interpretation algorithm (Amer-Wåhlin *et al.*, 2001). At 9:01 pm a slack baby boy was born. He received Apgar scores (AS) 0-1-5 at 1, 5, and 10 minutes of age. Blood gases in arterial umbilical cord blood indicated a mixed metabolic and respiratory acidosis, with pH 6.9, pCO_2 14.3 kPa, and BD_{blood} 22.6 mmol/L, indicating MA as determined by the Radiometer ABL 735 blood gas analyzer. In venous blood, pH was 7.13, pCO_2 6.39 kPa, and BD_{blood} 15.3 mmol/L. The boy recovered well and after a few days in the hospital he was discharged home together with his mother. The parents blamed the boy's depressed vitality at birth on the obstetrician in charge and filed complaints to the Swedish National Medical Responsibility Board for disciplinary action because of negligence by not performing a cesarean section. In her utterance to the board, the obstetrician claimed the labor course was managed correctly and MA was not present. By recalculating the BD_{blood} value with the BD_{ecf} algorithm used in randomized controlled trials on CTG only versus CTG+STAN surveillance (Westgate *et al.*, 1993; Amer-Wåhlin & Rosén,

2006), i.e. the $BD_{ecf}^{Corning}$ algorithm described in *Study II*, the arterial BD_{blood} value of 22.6 mmol/L was transformed to a BD_{ecf} of 10.5 mmol/L, and the venous BD_{blood} of 15.3 mmol/L to a BD_{ecf} of 11.6 mmol/L. Since BD was then no longer ≥12.0 mmol/L, the MA diagnosis 'disappeared' in the recalculation. The Medical Responsibility Board judged that the delivery was managed correctly, and rejected the complaints.

Comments

An umbilical artery BD ≥12.0 mmol/L at birth is associated with increased risk of neonatal cerebral dysfunction (Low *et al.*, 1997) and this threshold together with a low pH is often used as the definition of MA (ACOG, 2006). Our study demonstrates that the number of values above this threshold significantly varies with different blood gas analyzers. The rate of BD_{blood} values ≥12.0 mmol/L was up to 163% higher when calculated with the analyzer algorithms compared with the CLSI standard, and for BD_{ecf} it varied between 42% lower and 30% higher. A BD_{blood}^{CLSI} value of 12.0 mmol/L corresponded to a $BD_{blood}^{Radiometer}$ value of 14.1 mmol/L and a BD_{blood}^{Roche} value of 12.7, which further demonstrates the impact of different algorithms. These differences call into question the current use of BD as a well-defined entity.

The different ways of calculating BD have so far attracted little attention in the obstetric literature. The BD calculations vary between blood gas analyzers due to the use of different solubility factors for CO_2, the slope of the CO_2 equilibration line for whole blood, the plasma bicarbonate concentration at reference pH, and the different standards for including the actual oxygen saturation in the calculation algorithm. In a paper from 2001, Kofstad calculated BD_{blood} and BD_{ecf} in 81 simulated clinical situations with acid-base disturbances in adults. The algorithms used in the study were from different blood gas analyzers and the maximum BD_{blood} difference, within the range of normal to acidotic pH values, was 1.2 mmol/L. For BD_{ecf}, the difference was 0.2 mmol/L. Kofstad concluded that these differences are clinically negligible (Kofstad, 2001). However, in our material, differences of 3-4 mmol/L at normal and moderately high BD_{blood} values were common, and at extremely high BD values the differences reached 8-9 mmol/L (Figure 10). These are very large differences that cannot be neglected in obstetric and neonatal practice. The differences might result in active neonatal treatment in one situation and conservative management in another. Gestational age, length of labor, fetal fluid compartment for calculation, and time of blood sampling after birth are other factors influencing BD values in umbilical cord blood (Wiberg *et al.*, 2006b, 2006b, 2008b).

The differences in prevalence of BD_{blood} and BD_{ecf} ≥12.0 mmol/L both had a significant impact on the diagnosis of MA. Calculation according to the CLSI standard resulted in 0.58% MA, defined as BD_{blood} ≥12.0 mmol/L and a pH <7.00 in arterial cord blood, which was significantly lower than the figures of 0.64-0.66% according to the blood gas analyzer algorithms. At the most the difference was 13 MA cases among 104 newborns with arterial pH <7.00. When replacing the fixed Hb value in the Corning and Roche analyzer algorithms with the actual Hb concentration, BD_{blood} changed significantly while the prevalence of MA stayed unchanged. The MA rate decreased markedly when based on BD_{ecf} instead of BD_{blood}: from 0.58 % to 0.46 % with the CLSI algorithm, and from 0.64-0.66 % to 0.30-0.50 % with the analyzer algorithms.

Rosén and Murphy have suggested that BD should be calculated in extracellular fluid rather than in blood with the argument that BD_{ecf} is influenced to a lesser degree than BD_{blood} by pCO_2 (Rosén & Murphy, 1991). Hypercapnia is common in newborns and results in respiratory acidosis, while BD should be a measure of the metabolic component of an acidosis. Furthermore, the proportionally large fetal extracellular compartment represents a considerable buffering capacity. According to Morgan et al. (Morgan et al., 2000), pCO_2 levels and low Hb has a minor effect on the BD_{blood} value. Because the BD algorithms are based on the adult fluid compartment distribution, Roemer (2011) suggested that BD_{ecf} should be used with correction to 100% oxygen saturation of fetal Hb (HbF). Roemer stated though that the correction seems clinically irrelevant. Since pH is more strongly associated with CTG pattern and Apgar score, the use of estimates of the metabolic component, i.e. BD can be questioned (Wiberg et al., 2006b; Roemer, 2011).

Determination of cord blood gases and MA is important for various reasons: to evaluate the course of labor and management in individual cases, to judge malpractice in litigation cases, to assess the quality of care at a maternity unit, and in research as an objective perinatal outcome parameter. A revealing example of how a scientific result can be altered by using different algorithms to calculate BD_{ecf} has been reported in the literature (Welin et al., 2007). The value of STAN for fetal surveillance in labor was evaluated in a Finnish randomized controlled trial (RCT) with CTG only versus CTG+STAN (Ojala et al., 2006). Base deficit in ecf was calculated with an algorithm identical to the Roche algorithm in the present study and the rate of MA was 1.7% in the group monitored with CTG+STAN and 0.7% in the CTG alone group. We discovered the discrepancy in BD algorithm between the Finnish RCT and the two previous RCTs by Westgate et al. (1993) and Amer-Wåhlin et al. (2001); when recalculated with the $BD_{ecf}^{Corning}$ algorithm used in most STAN studies (Westgate et al., 1993; Amer-Wåhlin et al., 2001; Luttkus et al., 2004; Amer-Wåhlin & Rosén, 2006; Norén et al., 2006; Norén et al., 2007; Welin et al., 2007) the rates decreased to 0.8% in the CTG+STAN and 0.6% in the CTG

group (Welin *et al.*, 2007). The considerable difference between BD algorithms was noticed also by Westerhuis *et al.* in a Dutch CTG versus CTG+STAN RCT, in which was found a significant reduction in MA when BD was calculated according to the $BD_{blood}^{Corning}$ algorithm but not with the $BD_{ecf}^{Corning}$ algorithm (Westerhuis *et al.*, 2010).

Thus, as analyzers use different algorithms for the calculation of BD, different values are reported even though they are derived from the same analyte. There is no evidence for which BD algorithm best reflects neonatal acid-base status or predicts short- and long-term outcomes, and neither is there any consensus on which algorithm to use. The difference in the number of abnormal BD values is large, as demonstrated in *Study I*, and this methodological confounding raises the question about BD as a solid measure.

Study II

Results

The result of the pH round-off procedure is displayed in Figure 12. Arterial pH was ≤7.049 in 339 cases (1.8%) of 18 831. In 25/339 (7.4%) pH was 7.049-7.046, rounded off to 7.05 when truncated to two decimals. In 2 cases pH was 7.045: with rule A (bankers' rule) the figure was rounded off to 7.04 and with rule B to 7.05. Thus, after round-off to two decimals the number of cases with pH remaining <7.05 was 314 with rule A (92.6 %) and 312 with rule B (92.0%) (crude vs. round-off values, P <0.000001).
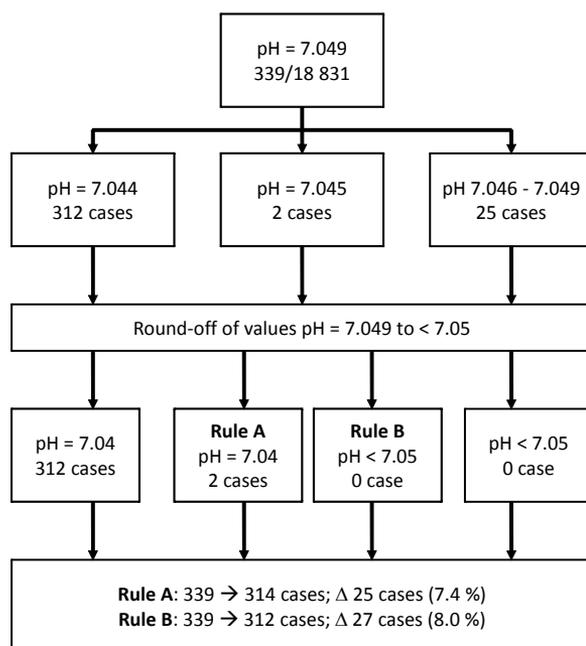
Figure 12. Flow chart showing round-off of umbilical artery pH values ≤7.049 in 339 newborns. Rule A denotes the 'round half to even' round-off rule and rule B the 'round half up' rule. The differences 339 versus 314 and 339 versus 312 cases were statistically significant (one-tailed McNemar test; P <0.000001).

Figure 13 shows the stepwise procedures when including two or three decimals in pH case values in the BD_{ecf} algorithm, and the resulting numbers of MA cases before and after decimal round-off.

Step 1: the number of BD_{ecf} >12.000 mmol/L was 131 when three-decimal pH values were used in the algorithm, and 134 when the pH case values included in the calculation were truncated to two decimals according to rule A and 136 with rule B. Neither the difference between crude and rounded off values nor the difference between rule A and B truncations were significant (P =0.06 for 131 vs. 136). Step 2: when defining MA as pH ≤7.049 plus BD_{ecf} >12.000 (left arm in Figure 13) or pH <7.05 plus BD_{ecf} >12.000 (right arm in Figure 13), the numbers of MA cases were 75 and 74 cases respectively. In the final step (step 3), rounding off pH values to two decimals in the left arm and BD_{ecf} values to one decimal in both arms, and defining MA as pH <7.05 plus BD_{ecf} >12.0 mmol/L, the numbers of MA cases

were reduced to 71 and 74. Discrepancies between crude and rounded off values to show MA were found in 8 of 75 cases (10.7%). Table 3 shows how MA appeared and disappeared in the 8 cases, depending on the number of decimals and which round-off rule was used. Cases no. 73 and 77 illustrate that it is necessary to know three decimals to make a correct truncation to one decimal.
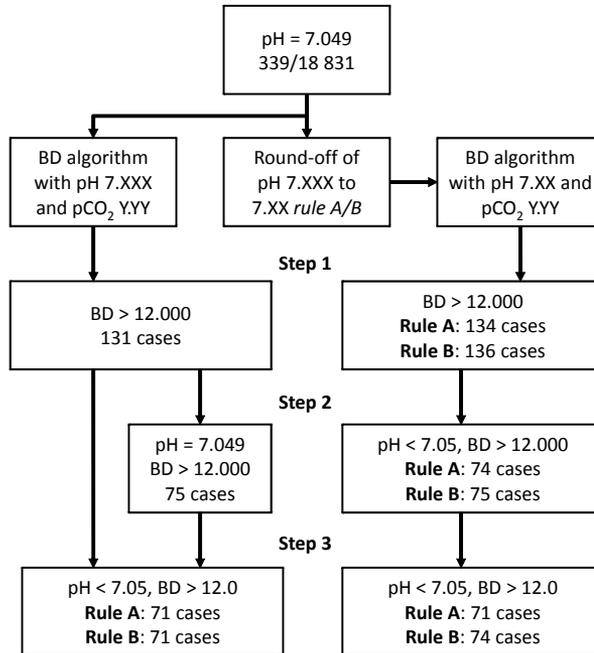
Figure 13. Flow chart showing the differences in calculation of base deficit (BD) and metabolic acidosis (MA, defined pH <7.05 plus BD >12.0 mmol/L) when including three pH decimals (7.XXX, left arm) or two decimals (7.XX, right arm) in the BD calculation algorithm. pCO_2 values were included with two decimals in both arms, denoted Y.YY. Rule A denotes the 'round half to even' round-off rule and rule B the 'round half up' rule. The differences 131 vs. 134 cases and 131 vs. 136 cases were statistically not significant (two-tailed McNemar test; P =0.2 and 0.06, respectively); the differences in MA (71 vs. 74 cases, etc.) were not significant ($P ≥0.2$).

Table 3. Eight cases with discrepancies in diagnosing metabolic acidosis (MA) among 339 cases with pH ≤7.049. Calculations of base deficit (BD) in extracellular fluid (BD_{ecf}) were made with regard to number of decimals of the pH value included in the BD calculation algorithm. For details, see Results and comments, *Study II*.

Case no.	pH with three decimals[a]			pH rounded off, rule A[b]				pH rounded off, rule B[c]			
	pH	BD_{ecf}	MA	pH	BD_{ecf}	BD_{ecf}^{d}	MA	pH	BD_{ecf}	BD_{ecf}^{d}	MA
23	6.866	12.044	Yes	6.87	11.819	11.8	No	6.87	11.819	11.8	No
73	6.966	12.257	Yes	6.97	12.047	12.0	No	6.97	12.047	12.1[e]	Yes*
77	6.971	11.995	No	6.97	12.047	12.0	No	6.97	12.047	12.1[e]	Yes*
107	6.990	12.040	Yes	6.99	12.040	12.0	No	6.99	12.040	12.0	No
166	7.013	11.995	No	7.01	12.148	12.2	Yes	7.01	12.148	12.2	Yes
314	7.045	11.833	No	7.04	12.085	12.1	Yes	7.05	11.580	11.6	No
322	7.046	17.636	Yes	7.05	17.488	17.5	No	7.05	17.488	17.5	No
332	7.048	12.148	Yes	7.05	12.049	12.0	No	7.05	12.049	12.1[e]	No

a. pH with three decimals included in the BD_{ecf} calculation algorithm.
b. pH rounded off to two decimals according to the 'round half to even' rule (rule A) before inclusion in the BD_{ecf} algorithm.
c. pH rounded off to two decimals according to the 'round half up' rule (rule B) before inclusion in the BD_{ecf} algorithm.
d. Crude BD value rounded off step by step, i.e. the third decimal determined the second decimal and then the second decimal determined the first decimal.
e. Rounded off value was 12.0 when all three BD value decimals were known and MA cases denoted (*) disappeared.

Comments

Decimal rounding affects all of us almost daily, for example when paying in the supermarket. Occasionally, it has drastic or even disastrous consequences: during the first Gulf War in 1990, the Patriot missile defense system failed to target an Iraqi Scud missile and 28 people were killed. The reason was chopping off 0.0001% of timing values in the system's computer and when the system continuously recycled itself the time error accumulated to 0.3422 seconds after running for 100 hours (Skeel, 1992).

The idea for *Study II* came up when a Swedish RCT on CTG versus CTG+STAN (Amer-Wåhlin *et al.*, 2001) was under investigation by the Swedish Research Council because of suspicion of misconduct. The result of the investigation showed inconsistent rounding of decimals in a few cases (Vetenskapsrådet, 2010). In medical practice and research, case analyte values are deemed negative or positive relative to a predetermined cut-off. In *Study II* we demonstrated the drifts of dichotomized variables with round-off of analyte values, i.e. the number of decimals reported by the analyzer. This is certainly an often overlooked factor in

writing study protocols and analyzing test results. In the present study, the pH cut-off <7.050 drifted from the limit 7.049/7.050 when three decimals were used to 7.045/7.046 (=7.04/7.05) with the 'round half to even' round-off rule (rule A) and to 7.044/7.045 (=7.04/7.05) with the 'round half-up' rule (rule B) at the two-decimal cut-off <7.05. Thus, with the 'round half-up' rule, 27 of 339 (8%) acidotic pH values disappeared when case values of 7.045–7.049 were rounded off to 7.05. Although the absolute number of MA cases did not vary much with the different ways of calculation and decimal round-off rules – at most it decreased from 75 to 71 cases – the overall round-off discrepancy among MA cases was considerable, 10.7%. This figure corresponded to 8 cases.

Study II demonstrates another mathematical principal: one needs to know at least two more decimals than the decimal one is rounding off to. This axiom is demonstrated by the round-off of BD_{ecf} to one decimal, which is common practice since it is how BD is reported by the blood gas analyzer. A BD_{ecf} value of, for example, 12.047 is first rounded off to 12.05 by both round-off rules; then to 12.0 according to the 'round half to even' rule but to 12.1 according to the 'round half-up' rule. Consequently, a case of MA might disappear with the former rounding rule but remain in the latter. Only with knowledge of the third decimal is it clear that the correct round-off value is 12.0 with rule A and 12.1 with rule B.

To limit the complexity of our article we did not discuss rounding of pCO_2 values. In this context the analyzer's measurement precision also needs to be considered. The measurement bias, for both pH and pCO_2, depends on the brand and type of analyzer, value level of the measured parameter, the amount of blood analyzed, and whether whole blood or capillary blood is analyzed (Radiometer, 2003). In whole blood, which was the case in the present study, at a pH of 7.00 the measurement bias for the Radiometer ABL 735 is up to 0.004. This suggests the third pH decimal is an uncertain digit. Although there are no obvious mathematical obstacles to the use of pH values with three decimals in calculating BD and defining acidosis, the third decimal measurement imprecision indicates that we should continue to report pH values with no more than two decimals. Similarly, the second pCO_2 value decimal is an uncertain digit (Radiometer, 2003).

Study III

Results

The characteristics of the study population are shown in Table 4. Gestational age at delivery was significantly lower and Apgar score at 1 min significantly higher

in the cesarean delivery (CD) group. One newborn had an AS of 4 at 1 min, but otherwise all scores at 1 min were ≥8 and at 5 and 10 min ≥9.

Table 4. Characteristics of the study population (N = 124).

	Vaginal delivery (N = 58)	Cesarean delivery (N = 66)
Maternal characteristics		
Duration of second stage of labor (min)	41 (5-234)	-
Duration of pushing (min)	24 (4-90)	-
Induction of labor	5 (7.6%)	-
Instrumental birth	9 (13.6%)	-
Drugs administered		
Pethidin	6 (9.1%)	-
Oxytocin	31 (47.0%)	-
Nitrous oxide	50 (75.8%)	-
Anesthesia		
Epidural	15 (22.7%)	-
Spinal	-	52 (90.0%)
General	-	6 (10.0%)
Newborn characteristics		
Gestational age (weeks)*	40+0 (36+0 – 42+0)	38+4 (36+4 – 40+3)
Birthweight (g)	3595 (2560-4405)	3535 (2516-5320)
SGA	3 (4.5%)	0
AGA	62 (93.9%)	47 (81.0%)
LGA	1 (1.5%)	11 (19.0%)
Apgar score		
1 min*	9 (4-10)	9 (8-10)
5 min	10 (8-10)	10 (7-10)
10 min	10 (9-10)	10 (9-10)
CTG		
Intermediate	13 (19.7%)	
Pathological	3 (4.5%)	

Values are median (range) or number of cases (%). CTG, cardiotocography. *The difference in gestational age and Apgar score at 1 min was statistically significant (Mann–Whitney U test; $P \leq 0.03$) between the two groups.

Serial blood samples were taken in all 124 cases, but four analyses at T0 (Time zero)(one vaginal delivery and three CDs) and 10 analyses at T45 (six vaginal deliveries and four CDs) failed because of instrument failure or blood clotting. For each parameter, only cases with valid measurements obtained at both T0 and T45 were included in the statistical analyses. Data for arterial and venous acid-base and hematological measurements are shown in Tables 5 and 6.

Table 5. Arterial blood gas, lactate concentration, hematocrit (Hct), and total hemoglobin concentration (Hb) median (range) values obtained immediately after birth (time T0) and 45 seconds later (T45) in unclamped umbilical cords with intact pulsations after vaginal delivery (VD) and cesarean delivery (CD).

			T0		T45		VD versus CD	
	VD	CD	VD	CD	VD	CD	Significance of difference (P)	
	N	N	Median (range)	Median (range)	Median (range)	Median (range)	T0	T45
pH	58	39	7.235 (7.008-7.379)	7.305 (7.162-7.397)	7.207 (7.005-7.384)	7.296 (7.116-7.424)	< 0.0001	< 0.0001
pCO_2 (kPa)	58	39	7.55 (5.24-11.6)	7.30 (5.86-9.56)	7.87 (5.94-11.8)	7.57 (5.56-10.4)	0.3	0.03
pO_2 (kPa)	57	39	2.31 (0.62-7.93)	1.99 (1.18-3.72)	2.66 (1.09-4.94)	2.28 (1.18-3.25)	0.1	0.02
Lactate (mmol/L)	56	37	4.8 (2.0-13.3)	1.8 (1.1-4.8)	5.5 (2.3-13.3)	2.2 (1.5-6.2)	< 0.0001	< 0.0001
Hct	57	38	0.507 (0.051-0.625)	0.452 (0.409-0.585)	0.514 (0.423-0.635)	0.460 (0.372-0.583)	< 0.0001	< 0.0001
Hb (g/L)	57	38	167 (134-205)	148 (133-191)	168 (138-208)	151 (121-191)	< 0.0001	< 0.0001

The Mann–Whitney U test was used for group comparisons.

Table 6. Venous blood gas, lactate concentration, hematocrit (Hct), and total hemoglobin concentration (Hb) median (range) values obtained immediately after birth (time T0) and 45 seconds later (T45) in unclamped umbilical cords with intact pulsations after vaginal delivery (VD) and cesarean delivery (CD)

			T0		T45		VD versus CD	
	VD	CD	VD	CD	VD	CD	Significance of difference (P)	
	N	N	Median (range)	Median (range)	Median (range)	Median (range)	T0	T45
pH	64	41	7.331 (7.068-7.471)	7.371 (7.320-7.479)	7.329 (7.470-7.474)	7.367 (7.318-7.469)	< 0.0001	< 0.0001
pCO_2 (kPa)	64	41	5.49 (3.91-9.70)	5.78 (4.37-7.46)	5.42 (4.05-9.54)	5.77 (4.69-7.54)	0.2	0.1
pO_2 (kPa)	63	41	3.57 (1.46-15.70)	3.46 (1.87-7.45)	3.68 (1.52-7.38)	3.46 (1.40-6.43)	0.6	0.9
Lactate (mmol/L)	60	40	4.6 (1.9-10.9)	1.5 (1.1-2.7)	4.7 (2.1-10.8)	1.6 (1.2-3.0)	< 0.0001	< 0.0001
Hct	63	38	0.515 (0.401-0.648)	0.455 (0.410-0.585)	0.513 (0.058-0.633)	0.456 (0.389-0.590)	< 0.0001	< 0.0001
Hb (g/L)	64	39	168 (131-212)	148 (133-191)	168 (126-208)	149 (127-193)	< 0.0001	< 0.0001

The Mann–Whitney U test was used for group comparisons.

Longitudinal changes in arterial blood gases, lactate concentration, hematocrit (Hct), and total Hb concentration (Hb) are illustrated in Figure 14. With the exception of pCO_2 in the CD group (P =0.4), all blood gas and lactate parameters changed significantly. Acid-base changes in venous blood were in the same directions as in arterial blood, although in the vaginal group only the lactate increase was significant (P =0.001) and in the CD group only the pH decrease (P =0.03) and lactate increase (P <0.0001) were significant (not shown in Figure 14). Hct and Hb increased significantly in the artery in both groups, whereas venous values decreased significantly in the vaginal group (P ≤0.04) and remained unchanged in the CD group (P ≥0.2).
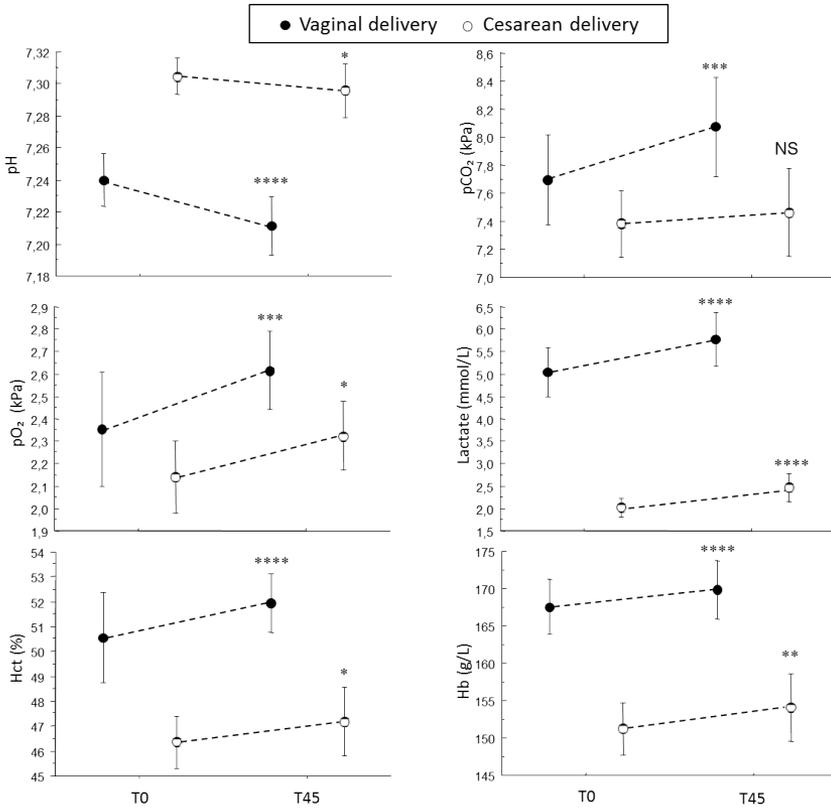
Figure 14. Measurements of arterial umbilical cord blood gases, lactate concentration, hematocrit (Hct), and total hemoglobin concentration (Hb) obtained immediately after birth (Time zero, T0) and 45 seconds later (T45) in unclamped umbilical cords with intact pulsations after vaginal and cesarean deliveries. The figure shows mean values and 95% confidence intervals. The Wilcoxon signed-ranks test was used to compare values at T0 and T45. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. NS, not significant.

When longitudinal arterial pH, lactate, and pCO_2 changes were compared between groups, the decrease in pH and increase in pCO_2 were found to be significantly greater in the vaginal group ($P \leq 0.04$), but there was no statistically significant difference between the groups regarding the increase in lactate concentration from T0 to T45 ($P = 0.9$). Adjusting pH for the difference in gestational age between the groups did not change the results.

Neonates born by the vaginal route had significantly lower pH and higher lactate concentration, Hct, and Hb concentration at T0 and T45 in both the artery and the vein compared with neonates delivered by cesarean section (Tables 5 and 6). At T45, pCO_2 and pO_2 in the artery in the vaginal group were also significantly higher. Neonates in the CD group with spinal anesthesia (N = 52) had lower pH and higher pCO_2 and lactate concentration at T0 compared with neonates in the general anesthesia group (N = 6), but only the difference in lactate concentration was statistically significant ($P = 0.03$).

Comments

Study III showed significant changes in acid-base and hematological parameters in umbilical cord blood when sampling was delayed by 45 seconds, being more marked for pH and pCO_2 in the vaginal delivery group. The similar increase in lactate concentration in the two groups indicate that considerable hidden acidosis was also present in the CD group.

The lack of change in venous pCO_2 indicates that placental perfusion and gas exchange were maintained during the first 45 seconds, after both delivery modes. Thus, the temporal increase in arterial pCO_2 must be a result of CO_2 inflow from the newborn and not from the placenta. Moreover, the significant pO_2 increase indicates a rapid establishment of functional pulmonary ventilation, which would result in escape of CO_2 and lowering of the pCO_2 unless there was a considerable ongoing contribution from the newborn. As it is unlikely that the CO_2 contribution was a result of a sudden rise in neonatal metabolism, a washout of CO_2 from peripheral tissues is the most plausible explanation for this finding.

At T0 the pO_2 was similar in the vaginal and CD groups, but at T45 it was significantly higher in the vaginal group as a result of a steeper increase. This demonstrates the protective role of vaginal delivery, with more effective release of lung surfactant and alveolar expansion, absorption of pulmonary fluid, and rapid circulatory transition to extrauterine life. At 45 seconds, alveolar clearance of fluid and alveolar expansion are the most important processes (Jain & Dudell, 2006). In a fetal lamb model, Berger *et al.* showed that in lambs treated with lung liquid volume reduction before birth the arterial blood gases and acid-base status improved quicker than

in lambs not treated (Berger *et al.*, 1996). The mechanisms of fluid absorption are not yet fully understood and the traditional explanations with "Starling forces" and "vaginal squeeze" can only account for a fraction of the absorption. Regulated by developmental changes of endogenous hormones and Na^+ channel expression when the pregnancy approaches term, and enhanced by labor contractions, osmotic gradients created by active solute transport by alveolar epithelial cells account for the bulk of fluid clearance in the newborn (Jain & Dudell, 2006).

After 45 seconds, arterial blood showed a small but significant hemoconcentration and venous blood a hemodilution in the vaginal group. A relevant question is then whether these concentration changes could have influenced the temporal acid-base and lactate changes. According to Stewart's physicochemical concept, a change towards alkalosis should occur during hemoconcentration as dehydration results in a higher $[OH^-]$ (Stewart, 1983). The body's normal state is on the alkaline side of neutral, and dehydration and hemoconcentration then concentrates the alkalinity (contraction alkalosis) and hydration dilutes the alkaline state towards neutral (dilutional acidosis). In the present study, the changes in hemoconcentration paralleled changes towards acidosis in the artery, indicating that the temporal acetous change was not a result of the hemoconcentration.

The study was performed in cases in which minimal neonatal assistance was expected to be required, and only two newborns in the vaginal group and none in the CD group had an umbilical artery pH <7.10 in the first samples. Both these newborns had a pathological CTG. One newborn was vigorous immediately, whereas the other was initially moderately depressed. The Apgar scores, blood gas, and lactate values are shown in Table 7. Interestingly, in the vigorous newborn the blood gas and lactate values deteriorated further to 45 seconds of age while in the depressed newborn the values remained mainly unchanged. These observations support the hypothesis that hidden acidosis is a physiological phenomenon, occurring in newborns with a rapidly established circulation.

Table 7. The Apgar scores, blood gas, and lactate values of the two newborns with pH <7.10 at T0 (Time 0) and T45.

Neonate	Apgar score at 1, 5 and 10 min	T0				T45			
		pH	pCO_2 (kPa)	BD_{ecf}* (mmol/L)	Lactate (mmol/L)	pH	pCO_2 (kPa)	BD_{ecf}* (mmol/L)	Lactate (mmol/L)
Vigorous	8-9-10	7.06	10.0	12.7	12.2	7.02	10.5	15.3	12.9
Moderately depressed	4-8-10	7.01	11.2	14.9	13.3	7.01	11.8	14.4	13.3

* BD_{ecf} calculated in Radiometer ABL 735 blood gas analyzer (Radiometer A/S, Copenhagen, Denmark).

It was not expected that the hidden acidosis phenomenon would occur so clearly in neonates born by cesarean section, as these neonates were not exposed to hypoxic stress by uterine labor contractions. However, it is well known that fetal/neonatal effects occur during regional anesthesia for planned CD. Despite precautions in terms of prehydration and vasopressor administration, spinal anesthesia in particular (Ngan Kee, 2010) is frequently associated with maternal hypotension and lower umbilical cord arterial pH (Robson *et al.*, 1992; Roberts *et al.*, 1995; Ngan Kee *et al.*, 2000; Mercier *et al.*, 2001). Vasopressor substances can cross the placenta (Alahuhta *et al.*, 1992; Ngan Kee *et al.*, 2001; Ngan Kee *et al.*, 2008; Ngan Kee, 2010; Habib, 2012) and the maternal supine wedged position during CD frequently results in fetal heart rate changes due to occult aortocaval compression (Preston *et al.*, 1993). Consequently, Doppler ultrasound has shown affection of the uteroplacental circulation after spinal blockade (Lindblad *et al.*, 1988; Alahuhta *et al.*, 1992; Robson *et al.*, 1992; Karinen *et al.*, 1995). In concordance with these findings, the present study showed higher lactate values in the spinal anesthesia group than in the general anesthesia group. It seems that even with the most modern techniques for spinal anesthesia, this side effect is difficult to avoid (Jain *et al.*, 2012).

Even small blood gas changes can affect the interpretation of a newborn's status and lead to a false diagnosis of acidosis, as we have previously demonstrated (Wiberg *et al.*, 2008b). Hypoxic fetuses are expected to have a more pronounced circulatory centralisation and to develop hidden acidosis and, as they already have lower pH, an additional decrease is more likely to tip them below the lower limit of the reference interval. It would be difficult to create reliable normal reference intervals taking late cord blood sampling into account because, as discussed above, vigorous newborns would show changes towards acidemia, lactemia, and hypercapnia whereas depressed newborns would show small changes.

Study IV

Results

Population sample characteristics

Characteristics of the study population and the total population are presented in Table 8. Of the 76 710 samples with clinical data (Figure 9), 12 136 (15.8%) were single vessel blood samples labeled either artery (5413, 7.1%) or vein (6723, 8.8%). The single vessel samples group had significantly higher pH and lower pCO_2 and lactate in both the artery and vein compared with the paired arterial

and venous samples group ($P \leq 0.0005$). In the single arterial samples group pO_2 was lower, but it was higher in the single venous samples group compared with the paired samples group ($P < 0.0001$). Single vessel samples were significantly more common in Malmö (41.5%) than in Lund (32.6 %) ($P < 0.0001$).

Table 8. Demographic characteristics of the study population (N =27 233) and the total population in Lund and Malmö, 2001-2010, (N =75 793).

	Study population	Total population
Characteristics	**Number (%) or median (range)**	**Number (%) or median (range)**
Maternal age (years)	31 (14-48)	31 (13-57)
Singleton pregnancy#	26 668 (97.9)	73 384 (96.6)
Severe preeclampsia	183 (0.7)	579 (0.8)
Pre-existing maternal diabetes	257 (0.9)	657 (0.9)
Gestational diabetes	827 (3.0)	2316 (3.0)
Mode of delivery		
Spontaneous vaginal delivery	21 082 (77.4)	58 831 (77.4)
Instrumental delivery (ventouse, forceps)	1559 (5.7)	3961 (5.2)
Cesarean section	4268 (15.7)	12 272 (16.2)
Gestation		
Preterm delivery (< 37+0 w)	1656 (6.1)	5682 (7.5)
Term delivery (37+0 – 41+6 w)	24 087 (88.5)	66 115 (87)
Postterm delivery (≥ 42+0 w)	1432 (5.3)	3934 (5.2)
Birthweight		
SGA (< mean – 2 SD)	853 (3.1)	2124 (2.8)
AGA (mean ± 2 SD)	24 874 (91.3)	70 652 (93)
LGA (> mean + 2 SD)	15 065 (5.5)	2773 (3.6)
Five-minute Apgar score		
0-3	48 (0.2)	539 (0.7)
4-6	210 (0.8)	863 (1.1)
≥7	26 922 (98.9)	74 352 (97.9)
Umbilical artery blood gas values		
pH	7.269 (6.646-7.551)	7.26 (6.51-7.7)
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 (0.04-13.8)
Lactate (mmol/L)	4.4 (0-25.0)	-

SGA, small-for-gestational age; AGA, appropriate-for-gestational age; LGA, large-for-gestational age. #For twin pregnancies, data from only the first twin were included in the study. In the Perinatal Revision South Register (PRSR), arterial pH values were available in 71%, pCO_2 in 66%, and pO_2 in 19% of the total population. pH and pCO_2 values were both available in 66%, and pH, pCO_2, and pO_2 in 19%. Lactate is not available in the PRSR for the study period and pO_2 is registered from 2005 and forward.

Distribution of delta values

The distributions of positive, zero, and negative V-A (pH and pO_2) or A-V (pCO_2 and lactate) Δ values are illustrated in Table 9. Positive values (i.e. in agreement with the hypothesis) were found in 86.1% of ΔpH, 84.8% of ΔpCO_2, 79.8% of ΔpO_2, and 64.9% of $\Delta lactate$ values. Overall, 52 % (14 223/27 233) had positive Δ values for all variables. Of the 86.1% with positive ΔpH, negative values for ΔpCO_2, ΔpO_2 and $\Delta lactate$ was found in 2.6 %, 13.3%, and 19.5%, respectively. Of the 84.8% with positive ΔpCO_2, negative values for ΔpH, ΔpO_2, and $\Delta lactate$ were found in 1.0 %, 12.0%, and 19.8%, respectively.

Table 9. Distribution of veno-arterial ΔpH and ΔpO_2 values and arterio-venous ΔpCO_2 and $\Delta lactate$ values. Values for ΔpH were calculated with three decimals reported by the blood gas analyzer, ΔpCO_2 and ΔpO_2 with two decimals, and $\Delta lactate$ with one decimal. Shaded squares show values in agreement with the hypothesis.

			ΔpCO_2 A-V			ΔpO_2 V-A			$\Delta Lactate$ A-V		
			+	0	-	+	0	-	+	0	-
		N 27233	23086	76	4071	21721	112	**5400**	**17680**	**2352**	**7201**
ΔpH V-A	+	**23455**	22799	50	606	20273	58	3124	17071	1810	4574
	0	**172**	55	9	108	88	6	78	39	63	70
	-	**3606**	232	17	3357	1360	48	2198	570	479	2557
ΔpCO_2 A-V	+					20269	48	2769	16754	1757	4575
	0					34	4	38	22	18	36
	-					1418	60	2593	2590	577	904
ΔpO_2 V-A	+								14506	1851	5364
	0								44	21	47
	-								3130	480	1790

Figure 15 shows distribution histograms of Δ values. For ΔpH and ΔpCO_2 (Figure 15a and 15b), three sub-populations could be discerned by curve intersections: one negative Δ values group (group A), one with values around zero (group B), and one with positive values (group C). For ΔpO_2 (Figure 15c), group A could not be discerned and for $\Delta lactate$ (Figure 15d) only one population was seen.
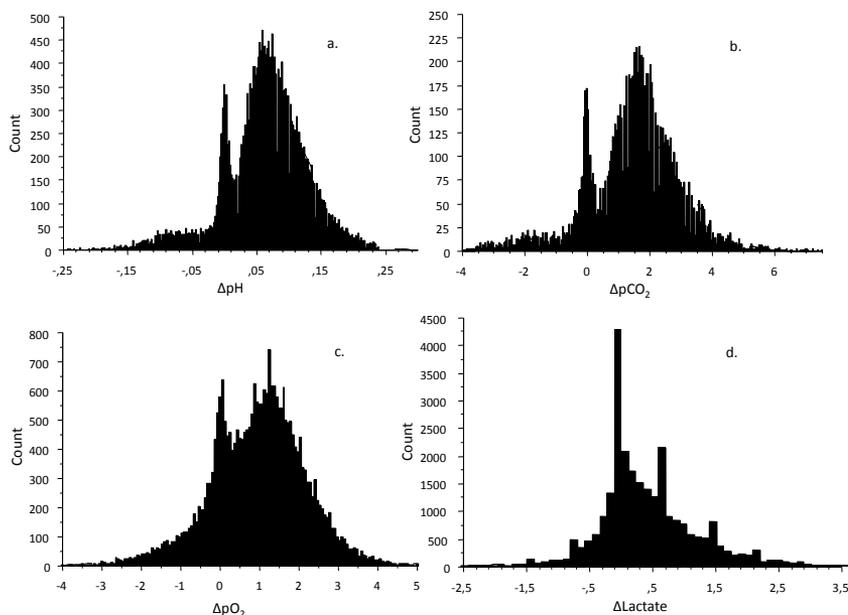
Figure 15. Distribution of veno-arterial ΔpH (panel a) and ΔpO_2 (panel c) values, and arterio-venous ΔpCO_2 (panel b), and Δlactate (panel d) values.

When comparing Δ values between the two maternity units, ΔpH, ΔpCO_2, and ΔpO_2 were lower and Δlactate higher among newborns in Malmö (P <0.0001). Negative ΔpH, ΔpCO_2, ΔpO_2, and Δlactate values were found in 21.1, 24.0, 32.2, and 27.1%, respectively, of vaginal deliveries in Malmö, compared with 4.4, 4.9, 7.5, and 30.4% of vaginal deliveries in Lund.

Delta values and clinical data

To investigate the relations between Δ values and clinical parameters, the intersections of the distribution curves of the sub-population groups were identified by magnifying the individual histograms on the computer screen. The intersection values between curves in groups A and B, and between B and C, are shown in Figure 9 (path 1) and the footnote to Table 10. No intersection A-B value could be identified for ΔpO_2; the intersection value B-C was 0.40 kPa and the A-B intersection value was therefore set to -0.40 kPa.

In group C (N =17 477), negative correlations were found between arterial pH and ΔpH (r = -0.54, P <0.0001) and arterial pO_2 and ΔpO_2 (r = -0.20, P <0.0001),

and positive correlations between arterial pCO_2 and ΔpCO_2 (r =0.69, P <0.0001) and arterial lactate and Δlactate (r =0.52, P <0.0001).

Table 10 shows 20 118 cases where group A contains cases with ΔpH, ΔpCO_2, and ΔpO_2 intersection values all smaller than the respective A-B intersection value (N =1190), group B contains all three Δ values between intersection A-B and B-C values (N =1451), and group C contains all three Δ values larger than the B-C intersection values (N =17 477). Significant differences between the groups were found regarding pushing time, birthweight, arterial and venous pH, and mode of delivery. The differences remained when comparing clinical data between the two vaginal delivery groups (P ≤0.007). In addition, there was a significant difference for gestational age (P ≤0.01). Comparisons of clinical data between groups A, B, and C within the cesarean delivery groups showed significant differences in arterial and venous pH (P <0.0001). In the emergency cesarean delivery group birthweight differed (P =0.02) as well.

Table 10. Comparisons of clinical data between groups A, B, and C. For definition of groups, see text and table. The Mann-Whitney U test was used for group comparisons of continuous variables, and the Chi-square test for comparisons of categorical variables.

	Population (N =20 118)			Significance of difference (P) Mann-Whitney U-test		
	A[a] (N =1190) Median (range) *Mean (SD)*	B[b] (N =1451) Median (range) *Mean (SD)*	C[c] (N =17 477) Median (range) *Mean (SD)*	A vs. B	A vs. C	B vs. C
Gestational age (days)	279 (178-306) *278 (13)*	279 (180-301) *277(13)*	279 (167-303) *277 (13)*	0.1	0.1	0.5
Duration of pushing (minutes) Only vaginally delivered (N =964, 1276, 14061 respectively)	17 (0-164) *23 (20)*	19 (0-146) *25 (22)*	15 (0-178) *20 (19)*	0.1	<0.0001	<0.0001
5-min Apgar score	10 (3-10) *9.7 (0.9)*	10 (4-10) *9.8 (0.6)*	10 (0-10) *9.8 (0.7)*	0.07	0.5	0.07
Birthweight (g)	3508 (690-5600) *3504 (560)*	3440 (520-5240) *3431 (567)*	3565 (584 -6360) *3551 (570)*	0.002	0.0009	<0.0001
Arterial pH	7.338 (6.908-7.520) *7.327 (0.073)*	7.328 (6.960-7.551) *7.319 (0.077)*	7.270 (6.646-7.508) *7.259 (0.075)*	0.006	<0.0001	<0.0001
Venous pH	7.254 (6.864-7.452) *7.242 (0.080)*	7.327 (6.967-7.551) *7.317 (0.077)*	7.348 (6.781-7.569) *7.349 (0.064)*	<0.0001	<0.0001	<0.0001

	N (%)	N (%)	N (%)	Significance of difference (P) Chi-square test		
				A vs. B	A vs. C	B vs. C
Spontaneous vaginal delivery N =15 371	885 (5.8%)	1189 (7.7%)	13 297 (86.5%)			
Instrumental vaginal delivery N =930	79 (8.5%)	87 (9.4%)	764 (82.2%)			
Planned cesarean delivery N =1861	90 (4.8%)	57 (3.1%)	1714 (92.1%)	<0.0001	<0.0001	<0.0001
Emergency cesarean delivery N =1772	122 (6.9%)	96 (5.4%)	1554 (87.7%)			

a. Group A: $\Delta pH \leq$ -0.015, $\Delta pCO_2 \leq$ -0.30 kPa and $\Delta pO_2 \leq$ -0.40 kPa
b. Group B: ΔpH -0.014 to 0.015, ΔpCO_2 -0.29 to 0.40 kPa and ΔpO_2 -0.39 to 0.40 kPa
c. Group C: ΔpH >0.015, ΔpCO_2 >0.40 kPa, and ΔpO_2 >0.40 kPa

In the single-vessel group pushing time was shorter, spontaneous vaginal delivery more common, and birthweight lower (P <0.001) compared with the paired samples group.

Planned cesarean delivery was the mode of delivery with the highest rate of "correctly" identified blood samples, i.e. this mode of delivery had the proportionally largest group C (92.1%).

Since the negative ΔpO_2 group (group A) was much smaller in Lund than in Malmö (7.5% versus 32.2%, indicating delayed blood sampling in Malmö, see DISCUSSION), only data from Lund were used to calculate percentiles of Δ value distributions. The distribution of ΔpH after exclusion of ΔpCO_2 and ΔpO_2 values ≤ 0 is shown in Table 11, with ΔpH ranging from -0.104 to 0.495 units. ΔpO_2 and ΔpCO_2 distributions were calculated according to the same principle, i.e. only cases with positive Δ values for the two other variables were included (Table 11). According to this model, 0.5% of the ΔpH values remained negative when negative Δ values of the other variables were excluded. For ΔpCO_2 and ΔpO_2 the corresponding figures were 0.6% and 4.2 %.

Table 11. Statistical terms of distribution when for each Δ blood gas parameter the Δ values ≤ 0 of the other two parameters were excluded. Only newborns from Lund were included.

	Mean (SD)	Median (range)	2.5th centile	5th centile	10th centile	90th centile	95th centile	97.5th centile
ΔpH	0.084 (0.049)	0.74 (-0. 104 – 0.495)	0.013	0.022	0.032	0.148	0.179	0.207
ΔpCO_2 kPa	1.982 (1.099)	1.81 (-4.50 – 10.04)	0.30	0.53	0.80	3.40	1.99	4.59
ΔpO_2 kPa	1.493 (1.262)	1.41 (-21.16 – 22.95)	-0.25	0.08	0.37	2.68	3.17	3.65

Table 12 shows the distributions of ΔpH values ≥ 0.02, corresponding to the 5th percentile reported in Table 11, of $\Delta pCO_2 \geq 0.5$ kPa, which was the 5th percentile for ΔpCO_2, and of the two cut-offs in combination. When applying both validation criteria, 76.5% (21 542/27 233) of the cases remained. The corresponding figures for the Westgate (Westgate *et al.*, 1994) and White (White *et al.*, 2012) materials are also shown in Table 12. There was a significant difference between Malmö and Lund in the proportion of approved "double validated" cases, 66.5% and 89.0% respectively (*P* <0.0001). When calculating values for Malmö and Lund separately, adding the cut-off $\Delta pCO_2 \geq 0.5$ to $\Delta pH \geq 0.02$, resulted in exclusion of an additional 3.3% (496/15 135) and 1.8% (220/12 098) of values.

Table 12. Frequencies of single ($\Delta pH \geq 0.02$) and double ($\Delta pH \geq 0.02$ and $\Delta pCO_2 \geq 0.5$ kPa) validated cases in our material and in the study populations of Westgate *et al.* (Westgate *et al.*, 1994) and White *et al.* (White *et al.*, 2012).

	Malmö & Lund N=27 233	Malmö N =15 135	Lund N =12 098	Westgate (Westgate *et al.*, 1994) N =1798	White N =29 874
	N %	N %	N %	N %	N %
$\Delta pH \geq 0.02$	21 541 79.1	10 560 69.8	10 981 90.8	1589 88.4%	27 022 90.5%
$\Delta pCO_2 \geq 0.5$ kPa	21 066 77.4%	10 190 67.3%	10 976 89.9%	?	?
$\Delta pH \geq 0.02$ & $\Delta pCO_2 \geq 0.5$ kPa	20 825 76.5%	10 064 66.5%	10 761 89.0%	1448 80.5	25 378 85.0

Comments

The ΔpH and ΔpCO_2 sub-populations with negative Δ values, called group A (Figure 15a and 15), is claimed to represent cases where arterial samples were identified as venous and vice versa, the sub-population with values around zero (group B) cases where samples were drawn twice from the same vessel, and the sub-population with positive Δ values (group C) cases where samples were identified correctly (Westgate *et al.*, 1994; White *et al.*, 2012). If the validation issue was only a matter of correct identification of blood samples, one would expect equal blood gas values and clinical characteristics in the three groups, but this was not found: duration of active pushing, mode of delivery, birthweight, and blood gas values differed significantly between the groups. There was also a tendency of differences in Apgar scores at 5 min. This, and the fact that only 52% had positive Δ values for all parameters, indicate that validation of umbilical cord blood samples is not only about excluding cases with incorrect origin, but also that A-V gradients depend on clinical factors. The group with 'approved' Δ values (group C in the present study) will then not represent a random population sample but a selected sample, and

clinical outliers will be excluded incorrectly. Uniform validation criteria then seem not applicable to all newborns.

In cases of severe fetal distress it may be difficult to obtain both arterial and venous blood samples because the cord contains little blood. White *et al.* (White *et al.*, 2012) obtained single-vessel samples in 13.7% of term deliveries and the corresponding figure in the present study was 15.8%. In our study, pH was significantly higher and pCO_2 and lactate significantly lower in both the arterial and venous single-vessel groups compared with the corresponding artery/vein in the paired samples group, suggesting no over-representation of hypoxic newborns in the single-vessel groups.

Blood gases and lactate change towards acidemia, hypercapnia, and lactemia when blood sampling is delayed (Wiberg *et al.*, 2008b; Mokarami *et al.*, 2013). This phenomenon, called hidden acidosis (Mokarami *et al.*, 2013), might distort the Δ values. When the newborn starts to breathe, the arterial pO_2 increases in an accelerated manner until the pulsations cease, whereas venous pO_2 shows a peak at 45 seconds and then a downward curve (Wiberg *et al.*, 2008b). In delayed blood sampling the upward arterial curve occasionally intersect with the downward venous curve, resulting in negative V-A ΔpO_2 values (Figure 16) (Wiberg *et al.*, unpublished data). Negative V-A ΔpO_2 values cannot occur when blood is correctly sampled immediately after birth because the fetus cannot produce oxygen. The impact of delayed clamping is illustrated by the different blood sampling techniques used in vaginal delivery at our two maternity units: in Lund the routine was puncture of the cord vessels immediately after birth, whereas in Malmö the cord was double-clamped and blood sampled later. Immediate double-clamping does not allow a net blood transfusion to the newborn. The rate of 32.2% negative ΔpO_2 values in Malmö compared with only 7.5% in Lund suggests that the midwives in Malmö deliberately delayed cord clamping to favor neonatal blood transfusion. Likewise, the higher frequency of single-vessel samples in Malmö indicates delayed sampling since persistent pulsations in the umbilical cord eventually leave the cord almost empty of blood. In addition, the rates of negative ΔpH and ΔpCO_2 values were markedly higher among cases from Malmö. Therefore we included only cases from Lund when calculating the percentiles presented in Table 11. In planned cesarean delivery the cord was double-clamped and cut without delay and there were optimal conditions to correctly identify and sample the cord vessels. As expected, this resulted in a larger group C and smaller groups A and B in comparison with vaginal deliveries.
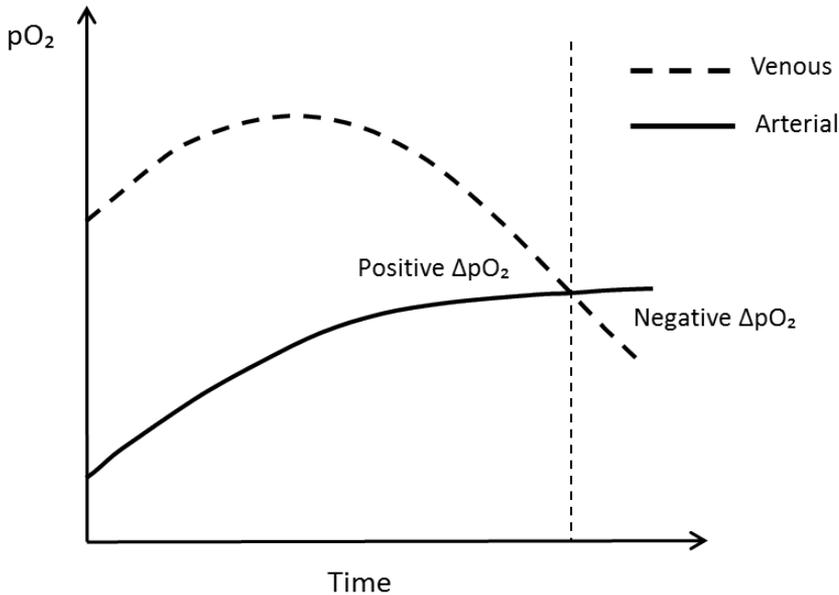
Figure 16. Theoretic illustration of how arterial and venous pO_2 changes with time in an unclamped pulsating umbilical cord after birth. At delayed sampling the upward arterial curve may intersect with the downward venous curve, resulting in a negative veno-arterial ΔpO_2 value.

The Δlactate distribution histogram displayed no contours of sub-populations and only one peak, at approximately zero. Thirty-five percent of the values were zero or negative (Table 9, Figure 15d). Previous studies have not been conclusive as to whether arterial or venous concentrations of lactate are normally highest (Burd *et al.*, 1975; Soothill *et al.*, 1986a; Soothill *et al.*, 1986b; Piquard *et al.*, 1990). Burd *et al.* (Burd *et al.*, 1975) found in a fetal sheep experiment mean lactate concentrations higher in the umbilical vein than in the artery. They estimate that 25% of fetal oxidative metabolism is based on lactate provided by the placenta. Soothill *et al.* (Soothill *et al.*, 1986a; Soothill *et al.*, 1986b) found in fetoscopy and cordocentesis studies that venous lactate concentrations were equal to arterial values in the second trimester, but higher in the third trimester. A rise of venous but not arterial lactate concentration during late pregnancy suggests a net transfer of lactate to the fetus. However, other authors argue that there is a positive A-V gradient in umbilical cord blood and that the lactate concentration is higher on the fetal side than on the maternal side of the circulation (Piquard *et al.*, 1990). The placental transport of lactate is dependent not only of the feto-maternal lactate gradient but also on facilitated diffusion coupled with proton transfer. This means that lactate is transported to the compartment of the placenta with the lowest hydrogen ion concentration. As maternal pH is generally higher than fetal pH, the direction of

transport is in general from fetus to mother. Data from Piquard et al. (Piquard et al., 1990) support this statement, though the net transfer of lactate varies with maternal and fetal acid-base status. In a large population-based study we found the difference between mean cord arterial and venous lactate at birth was only 0.3-0.5 mmol/L and the SD range 1.1-2.5 mmol/L (Wiberg et al., 2008a). In analogy with the literature, this suggests that both positive and negative A-V Δlactate values are physiological. From the distribution data of Δlactate in the present study it can be concluded that Δlactate cannot be used for validation of umbilical cord blood samples.

When excluding all negative ΔpH and ΔpCO_2 values in the series from Lund, which had the best cord blood sampling technique, the 2.5[th] ΔpO_2 percentile had a value of -0.25 kPa. As discussed above, negative ΔpO_2 values are physiologically not possible when blood sampling is performed correctly; then, exclusion of negative pH and pCO_2 Δ values still allowed inclusion of erroneous samples in the material remaining for validation. An unanswered question is whether negative ΔpH and ΔpCO_2 values are unphysiological. In the series from Lund, ΔpH ranged -0.104 to 0.495 units after exclusion of zero and negative ΔpCO_2 and ΔpO_2 values. The 2.5[th] and 5th ΔpH percentiles were 0.013 and 0.022, respectively, rounded off to 0.01 and 0.02. Interestingly, our 2.5[th] percentile value of 0.01 corresponds to the 5th percentile value of 0.01 reported by White et al. (White et al., 2012), whereas our 5th percentile value of 0.02 is the same as the 5th percentile in the material of Westgate and co-workers (Westgate et al., 1994). Moreover, the 5th ΔpH percentile in our material corresponded to the groups B-C intersection value of the total population (N=27 233), just like the 5th percentile in Westgate's material was the same as their intersection value (Westgate, 1993). The ΔpCO_2 values, calculated with only positive ΔpH and ΔpO_2 values, showed a range from -4.50 to 10.04 kPa and the 2.5[th] and 5th percentiles were 0.30 and 0.53 kPa. Then, the rounded-off 5th percentile value of 0.5 kPa corresponds to the 10th percentile value of 0.5 kPa calculated by Westgate and co-workers (Westgate et al., 1994).

Strengths, Weaknesses and Prospects

There is currently no evidence for which base deficit (BD) algorithm best reflects neonatal acid-base status or best predicts short- and long-term outcomes, and in addition there is no consensus on which algorithm to use. In *Study I* it would have been of clinical value to demonstrate which BD algorithm and fluid compartment best reflects the metabolic component of the newborn's acid-base status and which one has the strongest association with relevant clinical parameters. Correlating the different BD values to the concentration of lactate and to clinical parameters such as Apgar score and need of neonatal intensive care could help us to answer this question. On the other hand we found in a previous study that lactate has a stronger association with a 5-min Apgar score <7 than $BD_{blood}^{Radiometer}$ (Wiberg *et al.*, 2010). When combining different parameters the gestational age-adjusted pH and lactate values were slightly better than the age-adjusted pH and $BD_{blood}^{Radiometer}$ values. Lactate may be an alternative for the estimation of the fetal 'oxygen debt' as it is the end-product of anaerobic metabolism. In contrast to BD, lactate is a measured value and not an estimate calculated from other acid-base parameters. Lactate production is the major contributor to a BD increase during hypoxia, and a recent animal study indicates that the lactate production during severe hypoxia represents an accurate assessment of the fetal metabolic acidosis status (Frasch *et al.*, 2009).

One might argue that the discrepancy of eight cases, or 27 cases, in the population of 18 831 in *Study II* is much ado about nothing. However, in a randomized controlled trial applying dichotomous statistics, such a discrepancy might warp the result. The direct clinical impact of our results may seem minor, but implementation of research conclusions affected by round-off errors can influence important clinical decisions. Researchers should be aware of this bias, which has not attracted appropriate attention in the literature. This study is therefore unique and relevant not only in obstetric research but in research in general.

One of the strengths of *Study III* is that the repeated blood samplings were performed by an experienced obstetrician and analyzed within 15 minutes in chronological order. Sampling and measurement errors were thereby minimized. The inclusion of only newborns presumed to be vigorous however made extrapolation to asphyxiated newborns problematic. Before the start of *Study III* a methodological try-out was performed in a small pilot study. The umbilical arteries and vein were identified with certainty and correctly punctured in all cases, and there were no mix-up of vessels or samples because all samples were taken and analyzed by the same investigator (N.W.). Consequently, we found no reason to validate our data against data from studies performed much less cautiously. If we were to redo this study we would include more cases with general anesthesia to enable more certain comparisons with the cesarean delivery group receiving spinal anesthesia. In addition simultaneous sampling from the mother could provide us with knowledge about the maternal influence on fetal acid-base status, but that would have been another study.

Within the panel of the four acid-base variables in *Study IV* it was not possible to enter an erroneous parameter value since values were coupled and transferred electronically. To minimize the influence of decimal round-off, we included all analyte value decimals reported by the analyzer in our calculations. To eliminate analysis errors, all cases where the analyzer reported error in any of the panel parameter analyses were ruthlessly excluded. Many cases were excluded due to lack of certain identifications of sample origin, personal identification number, or a full panel of four analyses in both the cord artery and vein. Despite these exclusions, the population sample was as large as 27 233 cases and the tabulation of demographic characteristics showed the population sample was not a selected sample of the whole population. The unknown timing of sampling is clearly a weakness, but then again that reflects clinical reality. It would be of value to redo this study knowing the sampling time and also to investigate how the Δ values change when sampling is delayed.

Overall, the large number of cases in studies *I*, *II*, and *IV* was a great strength. As values were transferred electronically from the blood gas analyzer's hard drive to the statistical database, the errors connected with manual transfer of data, i.e. from blood gas analyzer paper printout via the obstetric database to the study database, were eliminated. In this way we handled analyses from more than 200 000 blood samples.

Summary and Conclusions

Study I pinpointed the methodological confounding in calculating base deficit (BD) with algorithms used in different brands of blood gas analyzers and the consequences for diagnosing metabolic acidosis (MA) at birth. The inter-analyzer rate of BD ≥12.0 mmol/L differed by up to 163% for BD_{blood} and 210% for BD_{ecf}. The intra-analyzer rate difference for BD_{blood} versus BD_{ecf} was up to 426%. As a result, there were statistically significant differences in the rates of MA diagnosis between analyzers. The clinical and scientific implications of these findings are obvious: neonatal MA rates cannot be compared between maternity units or between scientific articles where different fetal compartments (blood or extracellular fluid) and different algorithms to calculate BD have been used. In a computerized milieu it would be easy to calculate a defined BD value *post hoc*, like in *Study I*, but further studies are needed to clarify which are the best cord blood parameters to indicate hypoxia and MA in the human fetus and newborn.

Study II addressed the issue of possible diagnostic discrepancies when acid-base parameter value decimals are rounded off. Rounding off the third case value decimals reported by the analyzer resulted in an 8% shift of pH values from acidotic to normal. Likewise, round-off of pH and BD_{ecf} decimals resulted in a 10.7% discrepancy of making an MA diagnosis. A round-off of the third pH decimal always resulted in fewer cases having a value <7.05, but rounding of BD_{ecf} decimals resulted in values both greater and less than 12.0 mmol/L. Thus, in some cases an MA diagnosis appeared, and in others it disappeared after decimal round-off. A drift of a dichotomy parameter value cut-off due to decimal round-off will result in a shift in distribution of negative and positive cases in a population sample. This phenomenon has previously not attracted any attention in the literature, and the findings warrant a discussion on standardization of the number of decimals a specific analyte result should be reported with, and by which round-off rule case value decimals should be rounded off.

Study III aimed to show the difference in acid-base and lactate changes at delayed cord blood sampling between newborns delivered vaginally and by cesarean section. The study demonstrated that delayed cord blood sampling with intact pulsations affected umbilical acid-base values and hematological parameters in both vaginal and cesarean deliveries, but the changes were more pronounced after vaginal delivery. A change towards acidemia and lactemia can be explained by the hidden acidosis phenomenon, i.e. a surge into the central circulation of peripherally trapped acid metabolites when the newborn starts to breathe sufficiently. A small hemoconcentration occurred in arterial blood and hemodilution in venous blood, but these changes could not explain the changed acid-base status. Several authors promote late cord clamping since it can be beneficial for the newborn due to placental blood transfusion. *Study III* did not address that issue, but to obtain both a relevant cord blood acid-base status and placental blood transfusion, in both vaginal and cesarean deliveries, the blood should be sampled from the unclamped cord a few seconds after birth. This study and our clinical experience show that it is feasible.

Study IV aimed to explore the possibility of using distributions not only of veno-arterial (V-A) ΔpH and A-V ΔpCO_2 values to ensure the origin (validate) of umbilical cord blood samples, but also of V-A ΔpO_2 and A-V $\Delta lactate$, and to investigate associations between Δ values and clinical characteristics. The numeric distributions of ΔpH and ΔpCO_2 values displayed three sub-populations. Delayed blood sampling at birth, as revealed by a considerable portion of negative ΔpO_2 values, and clinical characteristics had significant influences. This indicates that Δ values vary with clinical conditions and circumstances and that validation criteria based on fixed Δ values may exclude correct samples of clinical outliers. Neither we nor other authors have to our knowledge been able to reveal the 'true' distribution of pH and pCO_2 Δ values since the tails of the three subgroup histograms overlap. There is a considerable discordance between percentile values in different studies; this may reflect differences in population demographics, blood sampling techniques, round-off of case value decimals, etc. One cannot with certainty claim what is correct in determining cut-off values for validation of umbilical cord blood gas values. Based on our and other extensive studies, cut-off values can be set to 0.01 or 0.02 for ΔpH and any value between 0.3 and 0.5 kPa for ΔpCO_2. Delta lactate is an inappropriate parameter for validation of umbilical cord blood gases. Physiologically, the only certain validation criterion is the exclusion of negative ΔpO_2 values. The main conclusions of the study are presented in Table 13.

Table 13. Main conclusions of *Study IV*.

1. Clinical characteristics have a significant influence on the distribution of Δ values and validation criteria based on fixed Δ values may then exclude correct samples of clinical outliers.

2. Cases with single-vessel samples had less acidemia, hypercapnia, and lactemia than cases with paired samples. Cases with paired samples will then not represent a random population sample.

3. Double parameter validation using cut-offs for both ΔpH and ΔpCO_2 had a minor influence on remaining cases with approved blood samples in comparison with single parameter validations.

4. Exclusion of negative pH and pCO_2 Δ values will still result in inclusion of erroneous blood samples. It is an unanswered question whether negative ΔpH and ΔpCO_2 values are unphysiological.

5. Positive and negative Δlactate values are both physiological. Thus, Δlactate cannot be used for validation of umbilical cord blood samples.

6. The only certain validation criterion is the exclusion of negative ΔpO_2 values.

Summary in Swedish
Populärvetenskaplig sammanfattning på svenska

Förlossnings- och barnläkare har i många år sökt efter ett instrument för utvärdering av det nyfödda barnets hälsotillstånd. Virginia Apgar introducerade 1953 ett poängsystem som bygger på en subjektiv bedömning av barnets hudfärg, hjärtfrekvens, reflexer/retbarhet, muskeltonus och andning. Koncentrationen i blodet av olika gaser, syror och baser speglar också det nyfödda barnets tillstånd. Relationen mellan påverkade blodgaser och det nyfödda barnets vitalitet uppmärksammades för första gången 1958 av James m.fl. Mätning av pH i blodprov från fosterskalpen under förlossningen introducerades av Erich Saling från Berlin på 1960-talet. Metoden introducerades i Sverige på 1970-talet och har sedan använts för bedömning av syrebrist (hypoxi) hos fostret under förlossningen. Som första klinik i landet införde man i Malmö 1981 rutinmässig mätning av blodgaser i navelsträngsblod när barnet var fött. Några år senare infördes rutinen även i Lund. Numera använder många kliniker, både i Sverige och utomlands, mätning av blodgaser och mjölksyra (laktat) i navelsträngsblod. Blodgaser och laktatnivåer i navelsträngsblod används som ett viktigt instrument för att retrospektivt utvärdera förlossningsförloppet i det enskilda fallet, för att värdera kvaliteten på förlossningsvården, vid undervisning samt som utfallsparameter i vetenskapliga artiklar.

Syra-bas-balansen i navelsträngsblod är till skillnad från Apgar-poäng ett objektivt mått på barnets tillstånd vid födelsen och reflekterar inte bara den hypoxi som fostret exponerats för utan även fostrets förmåga att bemästra denna syrebrist. Fostret utvecklar med ökande graviditetslängd en blandad metabolisk och respiratorisk acidemi, dvs. sjunkande pH pga. ansamling av sura metaboliter och koldioxid. Under förlossningen utsätts fostret för en hypoxisk provokation. Det innebär bl.a. att pH sjunker och laktat stiger i navelsträngsblod. Övergående hypoxi och koldioxidansamling (hyperkapni) är ofarligt, men om fostret inte klarar av att upprätthålla en aerob metabolism måste den anaeroba metabolismen utnyttjas och blodgaser och laktat ändras då mer än vad som förväntas. Detta leder till laktatansamling, acidos och ett större underskott av baser (base deficit, BD). Metabolisk acidos (MA) i navelsträngsblod indikerar att fostrets utsatts för betydande hypoxi och definieras

som ett pH-värde <7,00 (eller <7,05) plus ett BD-värde ≥ 12,0 mmol/L i arteriellt navelsträngsblod vid födelsen. Både en uttalad fetal acidos och låga Apgar-poäng korrelerar med en ökad risk för sjuklighet och är därför ett observandum som ofta leder till att det nyfödda barnet förs till neonatal intensivvård. Lyckligtvis klarar sig de flesta barn med låga Apgar-poäng eller acidos utan kvarstående men, vilket talar för att dessa parametrar är trubbiga instrument för utvärdering av det nyfödda barnets hälsotillstånd och fortsatta utveckling. Endast då dessa mått är kraftigt påverkade ses en tydlig koppling till kvarstående sjuklighet. Apgar-poäng och navelsträngsprover kan dock komplettera varandra och är idag fortfarande det bästa som finns att tillgå för bedömning av det nyfödda barnets tillstånd.

Blodproverna bör tas från både venen och en av de två artärerna i navelsträngen för att man bäst ska kunna bedöma allvarlighetsgraden och durationen av en eventuellt genomgången syrebrist. Venen innehåller det blod som förs från moderkakan till fostret och artärerna det blod som förs tillbaka till moderkakan från fostret. Därför reflekterar arteriellt blod bäst det nyfödda barnets syra-bas-status medan det venösa blodet dessutom återspeglar moderns syra-bas-balans och moderkakans funktion. Proverna bör tas direkt efter födelsen, då försening kan leda till förändringar av blodgaser och laktat i blodet. Detta sker då barnet börjar andas och sprattla och blodcirkulationen ställs om till livet utanför livmodern. Dessutom kan innehållet i proverna ändras om tiden från provtagning till analys förlängs. För att man ska kunna lita på provsvaren är det viktigt att den som tolkar navelssträngs-prover är medveten om de många fysiologiska och metodologiska faktorer som kan påverka syra-bas-värden. Denna avhandling handlar om fallgropar vid mätning och tolkning av blodgaser och laktat i navelsträngsblod. De faktorer som utgör fokus för de olika delarbetena i avhandlingen är:

I. val av algoritm och vätskerum (blod eller extracellulärvätska) vid uträkning av BD och diagnos av MA,
II. betydelsen av decimalavrundning av pH- och BD-värden då diagnosen MA ställs,
III. senareläggning av navelsträngsprovtagningen och hur det påverkar syra-bas-värdena, och
IV. sambandet mellan kliniska faktorer och valideringskriterier för urskiljning av det arteriella respektive venösa provet.

Studie I redovisar förekomsten av MA beräknat med olika formler (algoritmer) för BD. Base deficit är en artificiell variabel som inte mäts av blodgasapparaten utan beräknas utifrån uppmätta värden av pH, partialtrycket av koldioxid (pCO_2) och hemoglobin (Hb). I vissa blodgasapparater används ett bestämt Hb-värde på 9,3 mmol/L (150 g/L) för att beräkna BD, medan andra apparater använder ett uppmätt värde. Dessutom skiljer sig algoritmerna för beräkning av BD i blod res-

pektive extracellulärvätska (ecf). I denna studie uppmärksammar vi kliniker och forskare på att MA-diagnosen, som baseras på pH <7,00 plus BD ≥12,0 mmol/L, kan bero på vilken algoritm för uträkning av BD som används i blodgasapparaten. Beräkningar gjordes utifrån pH, pCO_2 och Hb-värden i 15 354 navelsträngsblod-prover. Algoritmer för uträkning av BD hämtades dels från Clinical and Laboratory Standards Institute i USA och dels från tre olika blodgasapparater. Frekvensen MA varierade signifikant mellan de olika blodgasapparaterna beroende på sättet att be-räkna BD. Ingen skillnad påvisades om man använde ett uppmätt eller bestämt Hb-värde i algoritmerna. Med tanke på att skillnaderna i förekomst av BD ≥12.0 mmol/L var upp till 426% mellan algoritmer för blod respektive extracellulärvät-ska är det inte förvånande att man i andra studier, som uppmärksammat våra fynd, funnit skillnader i frekvens av MA beroende på val av vätskerum. Det är ingen tvekan om att vald algoritm för beräkning av BD har stor klinisk och vetenskaplig betydelse och om BD fortsatt ska användas som ett utfallsmått bör val av algoritm standardiseras.

Studie II inspirerades av ett utlåtande från Vetenskapsrådet om misstänkt oredlig-het i forskning. Inkonsekvent avrundning vid beräkning av MA skulle undvikas genom att ange samtliga tre decimaler i pH-värdet i vetenskapliga artiklar. Vi syfta-de till att illustrera inverkan av pH- och BD-decimalavrundning på diagnosen MA vid födelsen och inkluderade 18 831 navelsträngsblodprover. Blodgasapparaten vi använde (Radiometer ABL 735) rapporterade pH med 3 decimaler. Vi fann att vid avrundning av pH-värden mellan 7,045 och 7,049 med avrundningsregeln "halva mot jämnt" (bankers' rule, 5 avrundas uppåt om den föregående siffran är udda och nedåt om den är jämn) försvann 25 av 339 (7,4%) värden med pH ≤7,049. Då avrundningsregeln "halv uppåt" (round half-up, 5 avrundas alltid uppåt) användes försvann 27 av 339 (8,0%) värden. Således avrundades upp till 8% av acidotiska pH-värden bort. BD beräknades sedan dels med alla 3 decimalerna i pH-värdena medtagna i algoritmen, dels med avrundning av tredje decimalen utifrån de två olika avrundningsreglerna. pH ≤7,049 plus BD >12,000 mmol/L (75 fall) jämför-des sedan med olika sätt att avrunda till pH <7,05 plus BD >12,0 mmol/L (71-74 fall beroende på avrundningsregel). Den maximala diskrepansen var 8 fall (10,7%) där diagnosen MA försvann eller uppkom beroende på olika avrundningar. På grund av den redan komplexa redogörelsen för avrundning av pH och BD-värden tog vi inte med avrundning av pCO_2-värden i kalkylerna. Eftersom tredje deci-malen i pH-värdena och andra decimalen i pCO_2-värdena är osäkra p.g.a. teknisk mätosäkerhet bör man fortsätta att i kliniskt arbete använda två decimaler för pH och en för pCO_2. Studien visade alltså att ända upp mot 10.7% av värdena kan byta förtecken mellan metabolisk acidos och icke acidos. Samma sak gäller sanno-likt även andra biokemiska substanser. I vetenskapliga beräkningar bör man vara medveten om att det på grund av matematisk avrundning av decimaler kan bli ett

skifte mellan "positiva och negativa" diagnoser när vi definierar värden över och under ett fastställt gränsvärde som normalt eller icke normalt.

Studie III beskriver hur blodgas- och laktatvärden i navelsträngsblod ändras när man väntar med att avnavla barnet. Det finns studier som visar att blodvolym och järndepåer ökar hos det nyfödda barnet om avnavling sker först då pulsationer i navelsträngskärlen avstannat. Den extra blodvolym som shuntas från moderkakan till barnet kan underlätta omställningen till livet utanför livmodern. Detta gynnar framför allt för tidigt födda barn. Vi har tidigare visat att pH sjunker medan pCO_2, partiellt syrgastryck (pO_2), BD och laktat ökar vid sen avnavling efter vaginal förlossning. Baserat på observationer från djurstudier lade vi fram hypotesen att dessa förändringar beror på inflöde av sura metaboliter från perifer vävnad då det nyfödda barnet börjar andas. Under värkarbete sker en centralisering av cirkulationen till prioriterade organ såsom hjärta, hjärna och binjurar. Strax efter födelsen öppnas kärlbäddar till dåligt försörjda organ upp och sura metaboliter strömmar in i den centrala cirkulationen. Förekomsten av detta fenomen, kallat "hidden acidosis", ville vi demonstrera genom att jämföra blodgas- och laktatförändringar vid vaginal förlossning och planerade kejsarsnitt. Hypotesen var att hidden acidosis förekom i mindre utsträckning vid planerade kejsarsnitt eftersom dessa barn ej utsatts för den syrebrist som orsakas av kompression av navelsträngen under livmodersammandragningar. Vi inkluderade 66 vaginalt förlösta nyfödda och 58 nyfödda efter planerat kejsarsnitt. Utöver blodgas- och laktatvärden undersöktes även Hb och andelen röda blodkroppar (hematokrit) i blodet för att utesluta att förändringar i blodets sammansättning orsakade fenomenet. Vår hypotes bekräftades, men det var överraskande att signifikanta förändringar av blodgas- och laktatvärden inträffade även i kejsarsnittsgruppen. Detta kunde förklaras av att ryggbedövning påverkar foster negativt och 90% av de kejsarsnittade kvinnorna i vår studie hade fått ryggbedövning. Studien kunde bara göras på fall där man förväntade sig ett friskt barn och därför hade endast två barn ett arteriellt pH <7,10. Det ena barnet var omedelbart piggt och alert och som förväntat sjönk pH ytterligare och laktat och BD steg inom 45 sekunder. Det andra barnet hade svårt att komma igång med andning och cirkulation och hos detta barn var blodgaser och laktatvärden oförändrade under de första 45 sekunderna. Dessa observationer ger ytterligare stöd för att hidden acidosis är ett fysiologiskt snarare än patologiskt fenomen hos nyfödda. Konklusionen från studien var att vid sen avnavling, inklusive planerade kejsarsnitt, förändras blodgaser och laktat så att initialt normala navelsträngsvärden kan komma att bli onormala och diagnosen MA ställas på felaktiga grunder. Det torde bli omöjligt att definiera adekvata normalvärden för blodprov tagna vid sen avnavling eftersom alerta barn visar förändringar mot acidemi, hyperkapni och laktatemi, medan slappa och medtagna barn inte visar samma förändringar. För att erhålla adekvata provsvar utan att störa blodtransfusion till det nyfödda barnet bör

provtagningen ske från en icke-klampad navelsträng strax efter födelsen. Denna studie samt vår kliniska erfarenhet visar att det är möjligt.

Studie IV syftade till att värdera etablerade valideringskriterier för blodgaser i navelsträngsblod. Syftet med att validera blodgaser tagna från navelsträngen är att försäkra sig om att provet kommer från en artär och inte venen eftersom artärerna för blod från fostret till moderkakan (placenta) och därmed bäst representerar fostrets metabola status. För att utesluta att man förväxlat artär med ven, räknar man ut den veno-arteriella (V-A) gradienten för pH och A-V gradienten för pCO_2. Dessa värden, Δ-värden, ska teoretiskt vara positiva om provet är rätt taget. Är värdet negativt anser man att proverna är förväxlade och är värdet kring 0 anser man att båda proverna kommer från samma blodkärl. Etablerade valideringskriterier är ΔpH ≥0,02 enheter och ΔpCO_2 ≥0,5 kPa, men även andra kriterier har föreslagits. Vårt material bestod av 27 233 parade blodprover. I histogrammen för ΔpH och ΔpCO_2 urskildes tre subpopulationer, vilka i tur och ordning enligt etablerade valideringskriterier då borde representera 1) förväxlade prover (negativa Δ-värden), 2) upprepade prov från samma kärl (värden kring 0) och 3) korrekt identifierade prover (positiva Δ-värden). Vid jämförelser av kliniska parametrar mellan dessa tre grupper fann vi signifikanta skillnader för krysttid, födelsevikt, arteriellt och venöst pH, samt förlossningssätt. Detta talar för att inte bara korrekt identifiering av proverna spelar roll för distributionen av Δ-värden utan att även kliniska parametrar har en påverkan. Bland ΔpO_2-värdena var 19,8% negativa, jämfört med 13,2% negativa ΔpH-värden och 14,9% negativa ΔpCO_2-värden. Negativa ΔpO_2-värden är en fysiologisk omöjlighet för fostret kan inte producera syrgas. I tidigare studier med sen avnavling hade vi observerat att pO_2 kurvan stiger brant och accelererande i artären efter framfödandet av barnet men i venen stiger den de första 45 sekunderna för att sedan minska. Negativa ΔpO_2 värden tyder alltså inte bara på felaktig identifiering av proverna utan även på sen avnavling. Då vi jämförde vaginala förlossningar i Lund och Malmö fick vi stöd för att sen avnavling i många fall är orsaken till negativa ΔpO_2-värden: i Lund tas blodproverna från den pulserande navelsträngen så snart det går medan navelsträngen dubbelklampas i Malmö innan provtagning. Frekvensen negativa ΔpO_2-värden bland vaginalt förlösta var 7,5% (Lund) respektive 32,2% (Malmö). Detta talar starkt för att provtagningsrutinerna har haft stor betydelse och att proverna från Malmö tagits senare jämfört med proverna från Lund. När vi sållade bort alla negativa Δ-värden för pO_2 och pCO_2 då ΔpH beräknades, och negativa ΔpO_2 och ΔpH då ΔpCO_2 beräknades, fann vi att 5:e percentilen för ΔpH var 0,022 enheter och ΔpCO_2 0,53 kPa räknat på Lundamaterialet. Histogrammet för Δlaktat visade endast en population varav 26,4% hade negativa värden och 8,6% 0-värden. Laktatkoncentrationen i navelsträngsblod är avhängigt en komplex samverkan mellan foster, placenta och moder, där bl.a. syra-bas-balans och laktatproduktionen i placenta har betydelse. Vår studie bekräftade tidigare studier, där somliga visat positiva och andra negativa

Δ-laktatvärden. Sammanfattningsvis visade vår studie att även om valideringskriterierna delvis överensstämde med tidigare rapporterade kriterier är de beroende av kliniska karakteristika. Man riskerar därför att med valideringskriterier utesluta fall med korrekt tagna blodprover men med kliniska särdrag. Laktat kan inte användas för validering av navelsträngsprover. Det enda säkra valideringskriteriet är negativa ΔpO_2 som talar för sen avnavling alternativt förväxling av arteriella och venösa prover.

Acknowledgments

This thesis was carried out at the Department of Obstetrics and Gynecology, Skåne University Hospital in Malmö. During the years of work that have resulted in this thesis, many people have contributed in one way or another. I am very grateful to all of you, and although words are not enough to express my feelings I want to direct special thanks to some of you:

First of all I would like to thank my tutor and dear friend, *Per Olofsson*, for his guidance, patience and optimism. Your many ideas, enthusiasm, and interest in teaching have been the best support. I appreciate our fruitful research discussions, but mostly the talks we have had about everything else. Thanks to your wisdom and sense of humor it was never boring at our meetings. Thank you for encouraging me to keep on with my research when I was about to give up. I hope you forgive me for choosing another specialty.

Thanks to my co-tutor, *Nana Wiberg,* for all the wise comments and critical contributions to the studies. Thank you for generously sharing knowledge and data. I really appreciate the pep talks and I will never forget the advice you gave me in Milan.

Karin Källén, my co-tutor for all statistical help, precious advice, and patience when I hardly knew what I was doing.

Jesper Petersson, head of the Department of Neurology at Skåne University hospital where I am currently a resident physician. Thank you for your understanding and support which has made it possible for me to write my thesis at another department. From now on, I am fully devoted to neurology.

Thanks to *Maria Nilsson* at the blood flow lab for her kindness and help.

My colleagues but mostly my dear friends *Petrea Frid* and *Sara Halldén.* Thanks to *Petrea* for her amazing support both when I was feeling down but also when I was hyper and no one else could catch up with me. Thank you for being so talented

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