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Cerebral oxygenation in newborn infants at risk

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Cerebral Oxygenation in Newborn Infants at Risk

Elisabeth Anna Verhagen

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1

General introduction and outline of the thesis

Elise A. Verhagen

Since the beginning of modern neonatology in the 1960s, neonatal intensive care and medical technology have improved considerably.[1] This resulted in a marked decline in the mortality of early and late preterm infants as well as sick term-born infants.[1-6] Morbidity, however, be it short-term or long-term, has not declined, on the contrary, it is rather high.[7,8] Many pathophysiological mechanisms and the extent of their effect are still unknown. Moreover, risk factors in the neonatal period that may potentially harm the integrity and development of the young brain abound.[1,8,9] Perinatal brain damage with adverse neurodevelopmental outcome affects a considerable number of infants. This is reflected in subtly or overt disturbed cognitive, motor, and behavioral development.[8,9] The chief targets of modern neonatology, therefore, are prevention of and early intervention in major neurodevelopmental problems due to brain damage.[10]

Brain development

During embryogenesis the development of the brain starts as soon as the neural tube is formed during the first weeks of pregnancy.[2] This is followed by the prosencephalic growth, neuronal proliferation and migration that take place during fetal development.[2] From 20 weeks' gestation onwards, organization of the neurons commences and continues for many years after birth.[2] The same holds true for myelination. It starts in the second trimester of pregnancy and continues for years after birth.[2] This means that at birth the young brain is still very much in development. As a consequence the preterm newborn brain is particularly vulnerable since, depending on an infant's gestational age at birth, neuronal organization takes place outside the womb from one to three months extra. There is much neuronal activity during this period with neurons migrating to the cerebral white matter and cerebral cortex. Furthermore, the preterm infant is exposed to a variety of risk factors that may influence its short-term or long-term outcome or both. Not only is the brain of the preterm newborn immature at birth, the preterm-born infant's cardiovascular system has not yet developed fully either.[11,12] Transitional adaptations from the intrauterine to extrauterine environment might, therefore, be insufficient, disturbed, or delayed, possibly leading to oxygenation problems in the preterm brain.

Transitional phase

The transition from intrauterine to extrauterine life depends on many physiological mechanisms that require rapid adaptation after birth. During intrauterine life, the fetus depends on the placenta for gas exchange and nutrition. The placenta has a very low vascular resistance.[12] Contrastingly, fetal lungs have a very high vascular resistance because of alveolar fluid, which results in a right-to-left shunt.[12] Blood flows from the right atrium to the left atrium through the foramen ovale and it flows from the pulmonary artery through the ductus arteriosus to the aorta, almost totally bypassing the fetal lungs in so doing.[12] Oxygen tension is very low in the fetus, whereas tissue oxygenation is adequate due to the presence of fetal hemoglobin (Hb) that has a higher oxygen affinity compared to maternal Hb.[13] The higher oxygen affinity allows oxygen to be transported across the placenta. This yields an oxygen saturation that is sufficient to meet the needs of the fetus, which due to its lower metabolism also has a lower consumption of oxygen. Moreover, blood flow is also distributed differently. The vital organs, e.g. the heart and brain, receive the most highly oxygenated blood.[12]

At birth the alveolar fluid needs to be cleared, the infant's lungs need to expand, and circulation needs to adapt to increase lung perfusion and to diminish the right-to-left shunts. Most newborn infants adapt to this transition from the intrauterine to the extrauterine environment without complications. There are, however, several risk factors that may influence the success of the transition.[14] One such risk is preterm birth. An infant born at a gestational age of less than 37 weeks is considered preterm.[15] This occurs in 7% to 8% of all pregnancies in the Netherlands.[15] Infants are considered very preterm when they are born at a gestational age of less than 32 weeks.[15] Preterm infants are at risk of neonatal complications due to physiological immaturity of, for example, the respiratory and cardiovascular system. This may result in respiratory distress syndrome, apnea of prematurity, persistence of the ductus arteriosus, and possibly a lack of cerebrovascular autoregulation. If these complications occur in the preterm infant they may pose the risk of disturbed cerebral oxygenation.

Cerebral oxygenation

Cerebral oxygenation depends on several parameters like oxygen delivery and oxygen demand and consumption.[16] In physiologically stable conditions, oxygen delivery and oxygen demand and consumption are balanced. Oxygen delivery is determined by the arterial oxygen content based on Hb concentration, the available red blood cells, and their oxygen binding and carrying capacity as well as cerebral blood flow (CBF).[16]

If oxygen delivery is insufficient, the brain is exposed to hypoxia. This can be categorized into three types: hypoxic hypoxia, anemic hypoxia, and ischemic hypoxia.[16] Hypoxic hypoxia results from low arterial oxygen saturation. This causes a decrease in oxygen delivery unless CBF increases. Anemic hypoxia results from low Hb concentration or insufficient availability or capacity of red blood cells. Preterm infants are at risk of low Hb concentrations due to frequent blood sampling as well as their immature hematopoietic system.[17] An apparently stable anemic preterm infant may be in a clinically unrecognized high cardiac output stage, which exposes the infant to the risk of disturbed cerebral oxygenation.[18] Ischemic hypoxia is the result of lower CBF. CBF is determined by the infant's cardiac output and cerebrovascular resistance. It is kept constant over a broad range of cerebral perfusion pressures due to cerebrovascular autoregulation. Increased perfusion pressure results in arteriolar vasoconstriction and decreased perfusion pressure results in vasodilation. The upper and lower limits of cerebrovascular autoregulation in preterm infants remain unclear (Figure 1).[19]

In case of disturbed cerebrovascular autoregulation, variability of blood pressure causes changes in CBF, which in turn leads to either cerebral underperfusion (low blood pressure) or hyperperfusion (high blood pressure). For the newborn infant this may pose the risk of brain damage. In order to keep oxygen consumption stable, in the case of cerebral underperfusion, the brain can extract more oxygen from the perfusing blood, or CBF could increase.[19] In preterm infants Kissack *et al*. found that a reduced cerebral oxygen supply on the first day after birth leads to increased oxygen extraction.[20] In the mature brain CBF is tightly coupled to cerebral metabolism. Wong *et al*. demonstrated uncoupled cerebral perfusion and metabolism in normotensive preterm infants.[21] Greisen, however, assumed that preterm and term born infants do have CBF-metabolism coupling.[22] This means that CBF increases if neurons are more active and require more oxygen, leading to unchanged fraction of oxygen extracted by brain tissue. At

the point where CBF reaches its maximum, however, oxygen extraction also has to increase to maintain oxygen consumption. Once this also reaches its maximum, oxygen consumption eventually decreases. Fluctuations in cerebral oxygenation may reflect disturbances in cerebral perfusion. Such disturbances are considered important pathogenic factors in the development of cerebral pathology.[22,23-25]

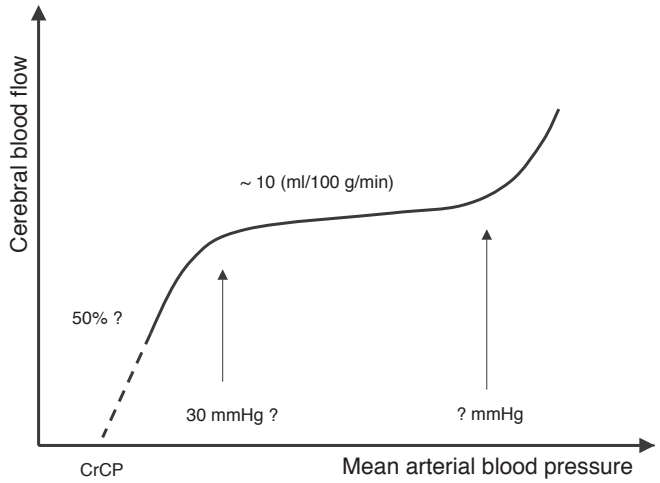

FIGURE 1. Cerebral blood flow versus mean arterial blood pressure of normal cerebral circulation in the preterm infant Adapted from G. Greisen.[19] CrCP indicates critical closing pressure of the arteries.

Cerebral pathology

Hypoxic-ischemic encephalopathy is a pathophysiological mechanism that commonly occurs in the newborn brain and results from disturbed oxygenation.[2] In the preterm infant, hypoxic-ischemic encephalopathy is often accompanied by periventricular leukomalacia (PVL) or germinal matrix hemorrhages-intraventricular hemorrhages (GMH-IVH).[2] The most serious form of PVL is cystic PVL, which is nearly always preceded by echodensities in the periventricular white matter.[26] Periventricular echodensities that persist for more than seven days are considered to be low grade PVL (PVL grade I).[26] There are strong indications that cerebral ischemia and hypoxia are involved in the development of periventricular white matter injury.[27]

For GMH-IVH, the primary lesion is hemorrhaging of small vessels in the germinal matrix.[2] The germinal matrix region is richly vascularized due to active cellular proliferation.[2] During the last three months of pregnancy this region gradually dissolves, but it is still present in preterm-born infants. These infants are, therefore, at risk of developing GMH-IVH. A GMH-IVH may be limited to the germinal matrix region (grade I) or it may rupture and extend into the adjacent ventricular system (grade II or grade III, depending on the extent of blood in the lateral ventricle).[28] A periventricular hemorrhagic infarction (PVHI), formerly described as grade IV GMH-IVH, is a complication of GMH-IVH.[2] Although the pathophysiological mechanism of GMH-VH is multifactorial, high, low, and fluctuating CBF have been reported as being associated with GMH-IVH.[2,24,29-32]

Neonatal parameters of perfusion and cerebral perfusion in particular

In current practice, the clinical status of infants is clearly and extensively monitored. In this way neonatologists are continuously informed about vital signs such as arterial oxygen saturation, blood pressure, heart rate, and respiratory parameters.[33-35] Several of these parameters, for example, blood pressure, pCO_2, and heart rate serve as indicators of systemic perfusion and cerebral perfusion.[34,35,36] Despite all the monitoring done, none of the parameters satisfactorily reflect actual cerebral perfusion or cerebral functioning.[37,38] Since the incidence of neurodevelopmental problems as a result of cerebral damage have not declined, there is an urgent need for a non-invasive, bedside tool that reflects cerebral perfusion, helps to identify pathologies, and in so doing might help to predict or eventually prevent adverse outcomes.

Research in neonatal neurology

Given the variety of perinatal and neonatal risk factors and the need of clinical tools that enable us to measure cerebral perfusion reliably, neonatal neurology has two main research focus areas.[2] The one area of focus relates to the availability of non-invasive diagnostic tools to assess brain function in the neonatal period up to several months of life. These tools are the assessment of the quality of general movements (GMs)[39,40], the analysis of electrocerebral activity through a cerebral function monitor to determine the amplitude-integrated electro encephalogram (aEEG)[41,42], and the assessment of cerebral tissue oxygenation by means of near-infrared spectroscopy (NIRS).[43] The validity and usefulness of these non-invasive diagnostic methods in seriously ill preterm and term-born neonates are investigated to determine the influence of several perinatal risk factors on the integrity and development of the young brain.

The other area of focus relates to a thorough follow-up examination up to school age and beyond of the motor, cognitive, and behavioral functions of children at risk.[44] By integrating these two branches of research, the prognostic value of the non-invasive techniques for later neurological and developmental findings for children exposed to specific risk factors can be determined.

Focus of this thesis

This thesis focuses mainly on NIRS, a non-invasive tool used to assess cerebral tissue oxygenation. In 1977, Jöbsis introduced NIRS as a way of monitoring cerebral oxygenation and hemodynamics.[45] The technique is based on the fact that biological tissue is relatively transparent to near-infrared light, i.e. wavelengths between 700 to 1000 nanometers (nm). Different chromophores will partly absorb one part of this near-infrared light.[45] A second part of the light will be scattered and a third part will be reflected.[46] The brain contains three chromophores: oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (HbR), and cytochrome oxydase.[16] Hb absorbs strongly and a deeper look at wavelengths below 700 nm is prevented due to the scattering of the light.[45,47] Above 1000 nm, water increasingly absorbs the near-infrared light.[47] The absorption by cytochrome oxydase is less than 10%.[47,48]

NIRS was first applied in neonates by Brazy *et al.* in 1985.[49] They measured changes in HbO_2 and total hemoglobin (HbT). Nevertheless, biological, technical, and practical limitations remained due to

movement artifacts and extracranial contamination thus limiting the measurements to differences in HbO_2, HbR, and HbT. The method did not provide absolute values.

Towards the end of the 1990s, spatially resolved spectroscopy (SRS) was introduced.[50,51] It provides absolute values within certain margins. In addition, SRS is less sensitive to movement and easier to use because the optical detector and receiver are combined in one sensor. SRS allows absorption of light by two or more detectors.

Debate is on-going about the usefulness and reliability of SRS-derived cerebral oxygenation values[43] because e.g. the precision of NIRS measurements depends on tissue homogeneity.[52] Moreover, replacement of the sensor gives a repeatability of NIRS values of about 5% to 8%.[47,53,54] Comparisons between different NIRS monitors in adults showed similar baseline values but relatively large interindividual and intraindividual differences.[55] This might be a probe-dependent artifact.[56,57]

Current use of near-infrared spectroscopy

Since its introduction in the 1970s, NIRS has been considered as a promising technique to measure cerebral perfusion. Because an indirect way of measuring CBF is to measure cerebral tissue oxygenation since one function of blood flow is to supply oxygen to tissue. Currently, NIRS is generally believed to be a valuable trend monitor in the individual patient and it is useful for comparing different groups of infants exposed to a variety of risk factors.[43,58-78]

Several different NIRS devices are currently available: FORE-SIGHT, INVOS, NIRO, InSpectra, O2C, OM-220, OxiplexTS, T.Ox, and TRS-20.[46,47] These devices use different near-infrared light sources (laser or LED), wavelengths, optode distances, and algorithms to calculate cerebral oxygen saturation.[48] The INVOS and NIRO devices use spatially resolved spectroscopy. All the studies reported on in this thesis were conducted with the INVOS 4100-5100 (Somanetics, Troy, Michigan, USA) in combination with pediatric SomaSensors. The optical SomaSensor measures the quantity of reflected light photons as a function of two wavelengths in the near-infrared region (730 and 805 nm), and determines the spectral absorption of the underlying tissue. NIRS differentiates oxygenated Hb from deoxygenated Hb, each of which has a distinct absorption spectrum (Figure 2). The ratio of oxygenated Hb to total Hb reflects the regional oxygen saturation of tissue.

The SomaSensor has two detectors, at a distance of 3 centimeters (cm) and 4 cm from the near-infrared optode (Figure 3A and B). The detector placed at 3 cm from the optode receives light scattered predominantly from the scalp and skull. The detector placed at 4 cm receives light scattered from the scalp, skull, and cerebral tissue. Thus, by subtraction the two detectors measure the oxygen saturation in the underlying cerebral tissue. A distance of more than 5 cm between optode and detector is not ideal because not enough light will be detected, unless the light intensity is increased, but this may be dangerous.[47]

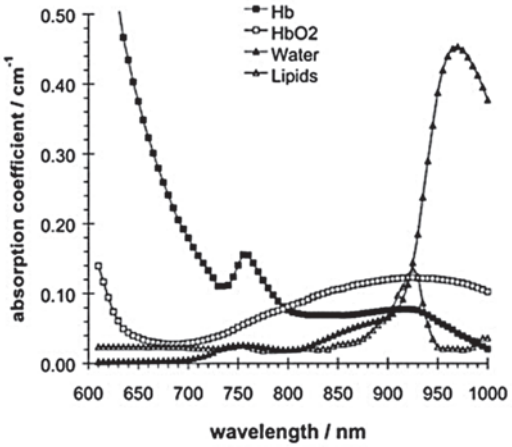

FIGURE 2. Oxygenated (HbO_2) and deoxygenated (Hb) hemoglobin absorption spectra
From: http://www.somanetics.com/our-technology/nirs-technology

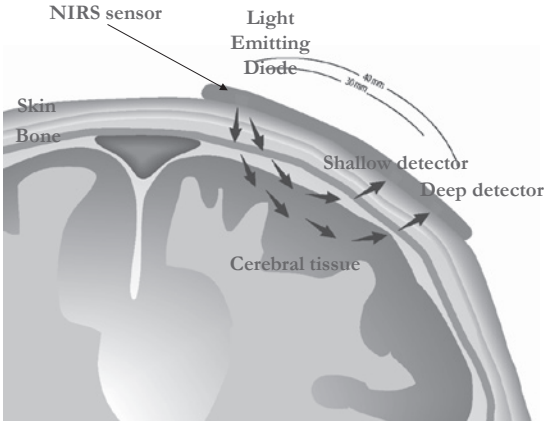

FIGURE 3A. Drawing of the SomaSensor applied to the head
Adapted from: http://www.somanetics.com/our-technology/nirs-technology

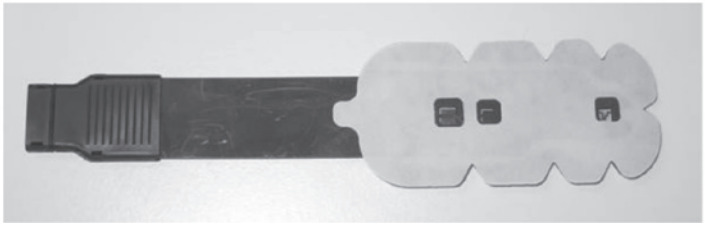

FIGURE 3B. The SomaSensor
Photo by E.A. Verhagen

The INVOS device measures regional cerebral tissue oxygen saturation (r_cSO_2) and the NIRO device measures the tissue oxygenation index (TOI). These measures are thought to reflect the oxygen saturation in a mixed vascular bed dominated by venules and serve as indicators of cerebral hypoxic hypoxia. About 70% to 80% comes from venous blood, 5% from the capillary compartment, and 20% to 25% comes from arterial blood.[79,80] Both TOI and r_cSO_2 have been compared to and used as surrogate measures of oxygen saturation in central venous or jugular venous blood.[81-83] Although a good correlation is found between jugular venous saturation and TOI or r_cSO_2, the absolute values are different. This means that one should be cautious when comparing studies reporting on either TOI or r_cSO_2.

When simultaneously measuring arterial oxygen saturation (SpO_2) by pulse oximetry, fractional tissue oxygen extraction (FTOE) can be calculated on the basis of r_cSO_2 and SpO_2 values: $FTOE = (SpO_2 - r_cSO_2)/SpO_2$.[20,61] FTOE has been validated in piglets.[84] FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen consumption.[20] As stated above, hypoxic-ischemic encephalopathy is a common pathophysiological condition of the newborn brain and results from disturbed oxygenation in which CBF seems to play a major role.[2] Since FTOE reflects the balance between cerebral oxygen supply (cerebral perfusion) and cerebral oxygen consumption, it serves as an indicator of cerebral ischemic hypoxia.[16] On the one hand, low FTOE might be explained by lower oxygen extraction or an increased oxygen supply while oxygen consumption remains constant. On the other hand, high FTOE is explained either by a low oxygen supply while oxygen consumption remains constant or by increased oxygen consumption. Thus, increased FTOE may indicate diminished cerebral perfusion.

Aims

In neonatal intensive care the need is felt for a practical, non-invasive clinical tool that reflects cerebral perfusion, helps to identify pathologies, and might in so doing contribute towards predicting or possibly preventing adverse outcomes. The primary aim of this thesis was, therefore, to determine the clinical value of monitoring cerebral oxygenation by means of NIRS. Besides, r_cSO_2 and FTOE values of preterm infants during the first weeks after birth are largely unknown. The secondary aim addressed in this thesis was, therefore, to determine the course of cerebral oxygen saturation and extraction during the first weeks after birth in newborn infants at risk of disturbed cerebral oxygenation.

Outline

The thesis consists of three parts:

1) neonatal risk factors for disturbed cerebral oxygenation,

2) maternal risk factors for disturbed cerebral oxygenation, and

3) other techniques to determine the (prognostic) value of NIRS.

The first part focuses on neonatal risk factors for disturbed cerebral oxygenation. In *Chapter 2*, we present a study on the relationship between mean arterial blood pressure (MABP) and FTOE in 25 preterm infants for 24 hours during the first 72 hours after birth. Our aim was to explore clinical parameters that might predict absence of cerebrovascular autoregulation. We assumed that a statistically significant negative correlation between MABP and FTOE reflects absence of cerebrovascular autoregulation, since

the extraction of oxygen will be higher in case of diminisched CBF. In *Chapters 3* and *4* we present two prospective, longitudinal observational studies on the course of r_cSO_2 and FTOE during the first two weeks after birth in preterm infants with and without cerebral lesions. *Chapter 3* deals with transient periventricular echodensities (TPE), since TPEs that persist for more than seven days are considered to be low grade PVLs and are quite common in preterm infants. In *Chapter 4* we compare r_cSO_2 and FTOE in preterm infants with GMH-IVH to preterm infants without GMH-IVH. In *Chapter 5* we studied infants who had possibly been exposed to anemic hypoxia. We determined whether Hb concentrations before red blood cell transfusion were associated with r_cSO_2 and FTOE, and whether red blood cell transfusions were associated with r_cSO_2 and FTOE during the 24-hour period thereafter.

There are many neonatal factors that possibly influence cerebral oxygenation, especially in preterm-born infants. Maternal pathophysiological conditions or maternal habits or both that may result in preterm birth could also contribute significantly to cerebral oxygenation once the infant is born. Part 2 focuses on two of these maternal risk factors that may pose a risk for the infant of disturbed cerebral oxygenation. In *Chapter 6* we address the influence of antihypertensive drugs on FTOE in preterm infants whose mothers were treated with antihypertensive drugs during pregnancy. As a result of the fact that these drugs cross the placenta, the fetus and newborn infant are at risk of developing hypotension, a potentially harmful condition because it could affect the supply of oxygen to cerebral tissue. In *Chapter 7* we describe the effect of tobacco exposure during pregnancy on rcSO2 and FTOE in preterm infants.

In Part 3 we describe two other techniques that help to determine the (prognostic) value of NIRS. In *Chapter 8* we studied the relationship between r_cSO_2 and FTOE and the amplitude-integrated electro encephalogram (aEEG). In *Chapter 9* we assess the predictive value of NIRS for neurodevelopmental outcome on the basis of an extensive follow-up examination. We studied a cohort of preterm infants between 2 to 3 years of age and established whether the cognitive, motor, neurological, and behavioral outcome of these infants was associated with r_cSO_2 and FTOE as measured during the first two weeks after birth.

Chapter 10 is a general discussion of the findings presented in the thesis and we explore some future perspectives. *Chapter 11* is a summary of the findings in English and Dutch.

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Part 1

Neonatal risk factors for disturbed cerebral oxygenation

Chapter 2

Near-infrared spectroscopy to detect absent cerebrovascular autoregulation in preterm infants

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Abstract

Objective Cerebrovascular autoregulation (CAR) may be impaired in preterm infants. This could lead to brain damage particularly if cerebral blood flow (CBF) is disturbed. An indirect way of measuring CBF is to measure cerebral oxygenation with near-infrared spectroscopy (NIRS). Our aim was to explore clinical parameters that might predict absence of CAR assessed by a negative relationship between mean arterial blood pressure (MABP) and fractional tissue oxygen extraction (FTOE) in preterm infants during 24 hours within the first three days after birth.

Design Prospective clinical observational cohort study.

Setting Third level neonatal intensive care unit.

Patients We included preterm infants (gestational age (GA) < 32 weeks). Within 72 hours after birth, we recorded infants' NIRS parameters and MABP for a 24-hour period. Fractional tissue oxygen extraction (FTOE) was calculated.

Interventions None.

Main outcome measures For each infant we calculated Spearman rank (rho) correlations. A statistically significant negative correlation between MABP and FTOE indicated that CAR was absent. We related absence of CAR to clinical parameters.

Results Ten (40%) out of 25 infants (median GA 29.1 weeks, range 25.4 - 31.7, birth weight 1245 grams, 560 - 1780) had a statistically significant negative correlation between MABP and FTOE (rho -0.432 to -0.156), suggesting absence of CAR. None of the clinical variables predicted absence of CAR.

Conclusions We were unable to predict absence of CAR in terms of clinical variables. Nevertheless, we found a statistically significant negative correlation between MABP and FTOE using NIRS, suggesting absence of CAR in almost half of the preterm infants studied.

Introduction

Cerebrovascular autoregulation (CAR) is a protective mechanism of the brain. Within limits cerebral vessels adapt to changes in blood pressure in order to maintain a constant cerebral blood flow (CBF).[1] There is conflicting evidence on whether this mechanism is present in preterm infants from birth onwards or whether it develops during the first days after birth.[2-7] Some studies suggested that the ability to effectively autoregulate may fluctuate over time.[8,9] In case of impaired CAR, changes in blood pressure cause changes in CBF, which in turn, leads to either cerebral underperfusion (low blood pressure) or hyperperfusion (high blood pressure). This could pose a risk for the preterm infant of developing brain damage.[10]

In the neonatal period CBF can be estimated by near-infrared spectroscopy (NIRS). NIRS measures regional cerebral tissue oxygenation saturation (r_cSO_2).[11,12] A study in newborn lambs reported that cerebral tissue oxygen saturation correlates well with changes in CBF.[13] Fractional tissue oxygen extraction (FTOE) is calculated by combining r_cSO_2 values with arterial oxygenation (SpO_2) values.[14,15] FTOE reflects the balance between oxygen supply and oxygen consumption. In baboons, oxygen extraction was measured invasively, and found to be inversely correlated with CBF.[17] In case of diminished CBF, more oxygen is extracted from the blood to meet the needs of cerebral metabolism. In case of high CBF, less oxygen needs to be extracted from the blood. In case CAR is intact and CBF is not disturbed, FTOE will remain constant.[18]

Our aim was to explore clinical parameters that might predict absence of CAR assessed by a negative relationship between mean arterial blood pressure (MABP) and fractional tissue oxygen extraction (FTOE) in preterm infants during 24 hours within the first three days after birth. We assumed that a statistically significant negative correlation between MABP and FTOE reflects absence of CAR, since the extraction of oxygen will be higher in case of diminished CBF. We hypothesised that we would find infants with and without CAR on the basis of their clinical situation, e.g. in younger, smaller, and sicker infants.[8,19,20]

Patients and methods

We studied preterm infants born after < 32 weeks' gestational age (GA) and admitted to our NICU. The attending neonatologist decided on NIRS monitoring on clinical grounds. Infants were included consecutively only if they were less than 72 hours of age when NIRS measurements commenced. We only included infants with an indwelling arterial catheter for constant blood pressure measurements. Exclusion criteria were major chromosomal or major congenital malformations.

Near-infrared spectroscopy

We used an INVOS 4100-5100 near-infrared spectrometer (Somanetics Corporation, Troy, Michigan, USA) in combination with the paediatric SomaSensor to measure r_cSO_2 values. The SomaSensor was placed on the left frontoparietal side of the infant's head and kept in place by an elastic bandage. A more detailed description of the method was published previously.[12]

At same time as measuring r_cSO_2, we measured SpO_2 by pulse oximetry and we calculated FTOE with the equation $FTOE = (SpO_2 - r_cSO_2)/SpO_2$.[14,15]

Study design

We performed a prospective clinical observational cohort study in preterm infants in whom r_cSO_2 and SpO_2 and invasive MABP measurements were performed simultaneously for 24 hours during the first 72 hours after birth. We measured MABP with an indwelling arterial catheter placed in the umbilical or radial artery. Clinical variables with probable or known associations with CAR such as birth weight[8,19], GA[8,19,20], haemoglobin concentration[21], glucose concentration[22], the presence of brain lesions[3,10,23,] CRIB-score[19,24], mortality[19], and blood gas values3 were collected, as well as the infants' postnatal age in hours, Apgar scores at 1, 5, and 10 minutes after birth, treatment for hypotension during NIRS measurement, mechanical ventilation, or the presence of a patent ductus arteriosus.

We determined haemoglobin concentration, glucose concentration, and blood gas values just before or during the 24-hour recording period. We defined hypotension as an MABP of less than the GA in weeks. In case of hypotension, the attending neonatologist decided whether or not treatment was required. The presence of brain lesions, e.g. intraventricular haemorrhage (IVH), periventricular leucomalacia (PVL), or transient periventricular echodensities (TPE) were diagnosed with cranial ultrasound using standard diagnostic criteria.[1,25,26]

Statistics

We used SPSS 18.0 software for Windows (SPSS Inc., Chicago, Illinois, USA) for the statistical analyses. NIRS data, as well as MABP measurements, were stored off-line for analysis. For further analyses we used the r_cSO_2, SpO_2, FTOE, and MABP values sampled every five minutes. Mean values for FTOE and MABP, along with the other variables, were calculated for the 24-hour recording periods. The recording period entailed a complete 24-hour period closest to the infant's birth. We determined the level of variability of MABP by calculating the coefficient of variation of MABP in each infant. We visually inspected the data for normality with Q-Q plots. In case of non-normal distribution, we used non-parametric exact tests.

We determined the correlation between MABP and FTOE values, sampled every five minutes for 24-hours, in each individual infant with the Spearman's rank correlation test. Correlations found between MABP and FTOE were categorised into two groups: those with a statistically significant negative correlation and those without a statistically significant negative correlation. Differences in FTOE and MABP values between these groups were analysed with the Mann-Whitney test.

To test the possible association of FTOE values with clinical variables we used the Spearman rank correlation test. Where appropriate, differences in proportions of categorical data were tested by Fisher's exact test or the χ^2-for-trend test. We performed univariate logistic regression analyses to investigate the influence of clinical variables on the presence or absence of CAR and univariate linear regression analyses to investigate the influence of clinical variables on the extent to which CAR is impaired. For the latter we used the correlation coefficients. A P value of $< .05$ was considered significant.

Results

Patient characteristics

We included 25 preterm infants with a median gestational age of 29.1 weeks (range 25.4 - 31.7), median birth weight of 1245 grams (range 560 - 1780), median postnatal age of 23.4 hours (range 2.7 - 63.4) and median CRIB-score of 1 (range 0 - 9). During the 24-hour recording period, eight infants received treatment for circulatory failure or signs thereof, seven of whom were treated with volume expansion and/or dopamine (n = 5) and/or dobutamine (n = 1). Three infants received red blood cell transfusions during NIRS recording. Brain ultrasonography revealed TPE in eight infants and IVH in eight infants. None of the infants had PVL. After NIRS measurement, three infants died before they were discharged from the NICU; one infant died on the fourth day, one on the tenth day, and one on the seventeenth day. All three infants died due to necrotizing enterocolitis followed by multi-organ failure. Table 1 shows the main clinical characteristics as identified per infant.

The relationship between MABP and FTOE

Lowest MABP measured during the 24-hour recording period was median 30 mmHg (range 16 - 35) and highest MABP median 49 (range 35 - 61). Median MABP per infant over the 24-hour period was 36 mmHg (range 24 - 45). Median coefficient of variation of MABP was 9.15 (range 5.99 - 18.37). Six infants had at least one episode of hypotension. Median FTOE was 0.23 (range 0.09 - 0.37).

We found ten infants (40%) with a statistically significant negative correlation (Spearman's rho -0.432 to -0.156, $P = .008$ to $< .001$), and fifteen infants without a statistically significant negative correlation between MABP and FTOE over the 24-hour period (Spearman's rho -0.104 to 0.396). There were no differences in MABP or FTOE between the infants with a statistically significant negative correlation or the infants without a statistically significant negative correlation. Figure 1 shows an example of decreasing MABP with simultaneously increasing FTOE in one of the preterm infants which suggested lack of CAR.

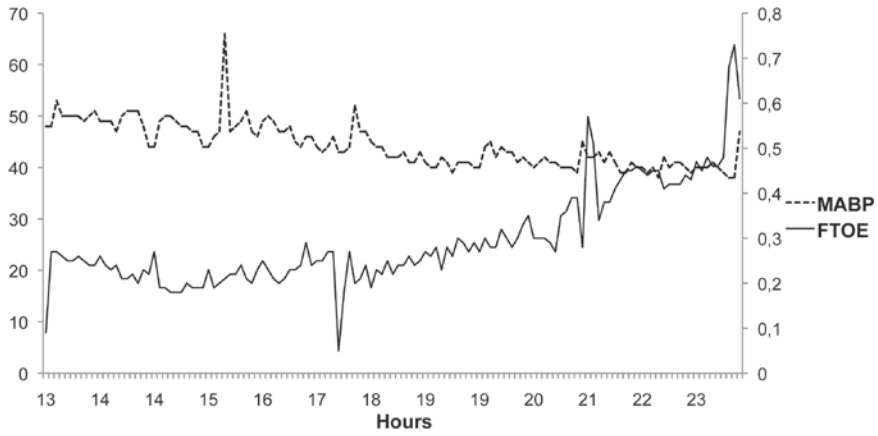

FIGURE 1. The course of MABP and FTOE in a single preterm infant, suggesting the absence of cerebrovascular autoregulation

TABLE 1. Patient characteristics

Infant	BW	GA	PNA	MABP	FTOE	hypo-tension	Spear-man's rho	*P* value	CRIB-score	cranial ultrasound	Hb	pCO_2	mini-mum glucose
1	1315	29+1	5.5	42	.32	-	-.432	.000	1	-	11.9	5.6	3.1
2	1200	30+3	28.6	36	.16	-	-.421	.000	3	-	11.0	6.0	4.6
3	560	29+6	30.5	34	.18	-	-.364	.000	7	-	9.3	5.0	3.5
4	750	27+0	11.5	30	.09	yes	-.287	.000	-	TPE	8.8	5.2	8.1
5	1280	29+6	62.0	45	.22	-	-.229	.000	1	IVH1	9.9	6.6	-
6	1645	31+0	42.5	39	.22	-	-.227	.000	1	-	11.2	5.2	3.9
7	1430	31+5	18.1	38	.31	-	-.181	.003	0	-	8.8	4.2	3.9
8	900	25+6	7.8	32	.28	yes	-.174	.004	3	TPE	8.5	4.5	6.3
9	1455	28+3	32.3	42	.27	-	-.165	.006	0	-	8.1	4.7	3.0
10	1414	29+2	6.1	32	.25	-	-.156	.008	1	-	9.2	5.5	5.2
11	1245	30+6	14.9	32	.18	yes	-.104	NS	-	-	10.1	6.6	3.0
12	1500	28+5	23.4	34	.32	-	-.090	NS	0	IVH2, TPE	7.7	4.9	3.4
13	1780	31+1	2.7	38	.37	yes	-.025	NS	5	TPE	9.7	4.7	4.0
14	1610	29+3	44.6	41	.26	-	-.012	NS	1	IVH1	7.6	4.0	-
15	1195	28+4	17.0	39	.33	-	.007	NS	2	TPE	8.7	3.5	2.6
16	1165	27+0	63.4	35	.30	-	.021	NS	9	IVH3	7.5	6.4	4.9
17	1149	30+3	7.3	33	.22	yes	.034	NS	1	-	7.5	5.7	3.0
18	1340	29+0	36.0	35	.17	-	.061	NS	1	TPE	8.8	5.4	4.4
19	1365	31+2	27.5	36	.21	-	.095	NS	1	IVH1	8.9	4.9	3.4
20	1500	30+2	23.7	43	.23	-	.120	.046	1	TPE	7.6	4.6	5.7
21	1240	27+0	14.6	39	.18	-	.131	.027	4	IVH1	7.9	5.8	2.8
22	980	25+4	19.2	31	.19	-	.156	.018	2	IVH1	8.8	4.8	3.4
23	900	25+3	12.7	36	.23	-	.282	.000	3	TPE	6.7	5.6	4.1
24	676	25+5	56.5	24	.32	yes	.337	.000	9	-	6.7	9.3	8.3
25	850	26+3	23.4	39	.24	-	.396	.000	9	IVH2	6.9	5.2	3.9

BW indicates birth weight, GA; gestational age, PNA; postnatal age, MABP; mean arterial blood pressure, FTOE; fractional tissue oxygen extraction, Hb; haemoglobin concentration, pCO_2; carbon dioxide TPE; transient periventricular echodensities, IVH; intraventricular haemorrhage (grade), NS; not significant.

The relationship between the investigated clinical variables and cerebrovascular autoregulation

Using logistic regression analyses, we found no relationship between the clinical variables and the presence of CAR, except for haemoglobin concentration ($P = 0.019$) (Table 2). For every 1 mmol/l increase of haemoglobin the odds for absence of CAR increased with an odds ratio of 3.53.

We found the same results with the linear regression analyses, i.e. no relationship between the clinical variables and the extent to which CAR is impaired, except for haemoglobin concentration ($P < .001$). Of the three infants who died before discharge, only the infant who died on the seventeenth

day after NIRS measurement appeared to have absence of CAR. The other two infants, who died on the fourth and tenth day after NIRS measurement appeared to have intact CAR.

TABLE 2. Logistic regression analyses clinical data to predict the absence of cerebrovascular autoregulation.

Variable	Median value	*P* value	B exp	CI interval
Birth weight	1245 (560-1780)	0.761	1.00	0.997 to 1.002
Gestational age	29.1 (25.4-31.7)	0.322	1.245	0.807 to 1.921
Apgar score at 5 minutes	8 (5-10)	0.690	1.178	0.527 to 2.630
Postnatal age at start NIRS measurement	23 (2-48)	0.860	0.996	0.950 to 1.044
Haemoglobin concentration	8.8 (6.7-11.9)	0.019*	3.527	1.227 to 10.138
Lowest glucose concentration per infant	3.9 (2.6-8.3)	0.400	1.271	0.727 to 2.223
Highest glucose concentration per infant	5.3 (3.4-10.8)	0.884	1.031	0.686 to 1.549
Lowest pCO_2 per infant	4.6 (3.2-6.7)	0.649	0.801	0.326 to 2.011
Highest pCO_2 per infant	5.9 (4.0-9.3)	0.592	0.865	0.508 to 1.472
CRIB-score	1 (0-9)	0.300	0.817	0.557 to 1.198
Mechanical ventilation (n)	13	0.870	0.875	0.176 to 4.341
Persistent ductus arteriosus (n)	14	0.742	1.312	0.259 to 6.643

* indicates $P < 0.05$, B exp; unstandardised coefficient, CI; confidence interval.

Discussion

This study demonstrated that CAR appeared to be lacking in 40% of the relatively healthy preterm infants we studied. This is based on the statistically significant negative correlation between MABP and FTOE as measured with NIRS. We expected to identify infants with and without CAR on the basis of a variety of clinical conditions. Except for haemoglobin concentration, however, no clinical data seemed to relate to the presence or absence of CAR. We stress the fact that our 24-hour recording periods with NIRS allowed us to differentiate between infants who were able to autoregulate and who were not, regardless of the lack in relationship between clinical data and CAR.

In recent years, NIRS devices have improved technically and spatially resolved spectroscopy has become available.[27] The technique was validated partially in piglets in order to determine CAR.[28] Several clinical studies have also been published on determining the presence or absence of CAR in preterm infants with spatially resolved spectroscopy.[18,29] In contrast to most studies that determined the relation between MABP and r_cSO_2 or tissue oxygenation index, we determined the correlation between MABP and FTOE. We decided to do this to eliminate the influence of changes in the arterial oxygen saturation.

The correlation coefficients we found ranged from -0.432 to -0.156 and were, therefore, not particularly strong. Other studies determined coherence between blood pressure and cerebral oxygen saturation. They defined absence of CAR as a coherence value of > 0.5.[19,23] Coherence eliminates the effect of time-lag between changes in MABP and cerebral oxygenation measurements. This might

explain these higher values compared to our correlation coefficients. Another possible explanation is the higher median birth weight and lower CRIB-scores of the infants in our study compared to those in the study by Wong *et al.*[19] In our case, the attending neonatologist decided on NIRS monitoring on clinical grounds. Perhaps NIRS monitoring was not applied to the smallest, youngest, and sickest infants in our unit.

As was suggested by Soul *et al.*, CAR might fluctuate over time.[8] In their study, CAR was impaired in only 31.7% of the hypotensive episodes. Since we measured continuously for 24 hours, our measurements possibly included episodes in which CAR was absent, as well as episodes in which CAR was present, which may have leveled out the strength of the correlations in doing so. Additionally, since we sampled FTOE and MABP values every five minutes we might have missed subtle adaptations of FTOE to MABP. It was suggested that FTOE responds slowly to changes in CBF.[13] This is another possible explanation for the smaller negative correlation coefficients in comparison to the findings reported by others.[19,23] Nevertheless, we did find infants with statistically significant negative correlations, suggesting absence of CAR. These infants might have been the infants in whom CAR was lacking during most of the study period.

Although it is well known that pCO_2 influences CBF[3] and cerebral fractional oxygen extraction[14,30,31], we did not find a relationship between pCO_2 and FTOE or the presence or absence of CAR. This is not surprising considering there were no continuous pCO_2 measurements and, moreover, most values being within the normal range. Furthermore, even in the smallest and youngest infants in our study group CAR could be present.

We only found higher haemoglobin concentration to be associated with the absence of CAR. Recently, we reported on the influence of anaemia on cerebral oxygenation and our finding that haemoglobin concentration correlates with FTOE.[32] In the present study haemoglobin concentration did not correlate with FTOE, although the haemoglobin concentrations were not as low as in our previous study. It is difficult to explain why the infants lacking CAR had higher haemoglobin concentrations. A report on stable preterm infants found that the width of the autoregulatory plateau is influenced by anaemia and red blood cell transfusion.[21] Blood transfusion led to an extension of the plateau, thus increased haemoglobin concentration led to increased autoregulatory capabilities. This is contradictory with our findings. It might be that in the present study we kept haemoglobin levels higher in sicker infants, but this was not reflected by the number of blood transfusions given. Further studies need to be performed to determine whether or not our finding on the relation with haemoglobin levels was based on chance.

CAR is supposed to be present in a certain range of blood pressures, the limits of which are largely unknown.[1,33] Previously, it has also been suggested, however, that absence of CAR might also occur in infants with blood pressures within the 'normal' range.[8] This observation is confirmed by our study. MABP between infants with and without CAR did not differ during our 24-hour recording period and ranged from 24 to 45 mmHg.

We recognize some limitations of this study. Statistically significant negative correlations were indirect measures to determine the presence or absence of CAR. Moreover, NIRS has not yet been

completely validated. Another limitation was the small number of infants in our study sample.

Our study might have clinical implications. In practice, it is difficult for neonatologists to make decisions regarding treatment to improve cerebral circulation. We believe that NIRS could be a helpful tool in this decision-making process since it is a non-invasive technique and continuously available at the bedside, having value for measuring the presence or absence of CAR with the lack of a relationship with clinical data. Whether this technique could help us prevent cerebral complications in future needs to be investigated in detail.

Conclusions

We conclude that we were unable to predict absence of CAR in terms of clinical variables. Nevertheless, by using NIRS we found a statistically significant negative correlation between MABP and FTOE in 40% of the relative healthy preterm infants studied, which suggests absence of CAR. NIRS could be a helpful tool to assess the presence or absence of CAR.

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Chapter 3

Cerebral oxygen saturation and extraction in preterm infants with transient periventricular echodensities

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Abstract

Objective Our aim was to determine regional cerebral tissue oxygen saturation and fractional tissue oxygen extraction in preterm infants with transient periventricular echodensities. We hypothesized that as a result of reduced cerebral perfusion, regional cerebral tissue oxygen saturation will be lower and fractional tissue oxygen extraction will be higher during the first days after birth.

Patients and Methods This was a prospective, observational study of 49 preterm infants (gestational age median: 30.1 weeks [26.0 - 31.8 weeks]; birth weight median: 1220 grams [615 - 2250 grams]). We defined transient periventricular echodensities as echodensities that persisted for > 7 days. Regional cerebral tissue oxygen saturation was measured on days 1 - 5, 8, and 15 after birth. Fractional tissue oxygen extraction was calculated as (transcutaneous arterial oxygen saturation - regional cerebral tissue oxygen saturation)/transcutaneous arterial oxygen saturation.

Results Transient periventricular echodensities were found in 25 of 49 infants. During the first week we found no difference between the 2 groups for cerebral tissue oxygen saturation and fractional tissue oxygen extraction values. On day 15 after birth, cerebral tissue oxygen saturation was lower in preterm infants with transient periventricular echodensities (66%) compared with infants without echodensities (76%) ($P = .003$). Fractional tissue oxygen extraction in infants with transient periventricular echodensities (0.30) was higher than fractional tissue oxygen extraction in infants without transient periventricular echodensities (0.20) ($P < .001$). The differences could not be explained by confounding variables.

Conclusions Persistent transient periventricular echodensities may be associated with increased cerebral oxygen demand after the first week after birth, which is contrary to our hypothesis. Cerebral oxygenation may be involved in the recovery of perinatal white matter damage.

Introduction

Periventricular white matter injury is one of the most common types of brain lesions suffered by preterm infants.[1] The most serious form is cystic periventricular leukomalacia (PVL), which is nearly always preceded by echodensities in the periventricular white matter.[2,3] In a minority of infants, however, periventricular echodensities develop into cystic lesions.[4] More often, echodensities persist for several weeks without evolving into cystic lesions. Periventricular echodensities that persist for more than 7 days are considered to be low-grade PVL (PVL grade 1)[2], also known as prolonged flares or transient periventricular echodensities (TPE). A wide range of risks for abnormal neurodevelopmental outcome in preterm infants with TPE have been reported.[5-9]

There are strong indications that cerebral ischemia and hypoxia are involved in the development of periventricular white matter injury.[10] It is difficult to measure cerebral ischemia and oxygenation with a non-invasive technique. A new, non-invasive method that could be useful is near-infrared spectroscopy (NIRS).[11] NIRS measures regional cerebral tissue oxygen saturation (r_cSO_2).[12-15] This measure is thought to reflect the oxygen saturation in a mixed vascular bed dominated by venules. Fractional tissue oxygen extraction (FTOE) is calculated on the basis of the values for r_cSO_2 and transcutaneous arterial oxygen saturation ($tcSaO_2$).[13,15-17] FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen extraction and, thus, may serve as an indicator of cerebral hypoxia and ischemia.[18,19]

The values for r_cSO_2 and FTOE during the first weeks after birth in preterm infants with and without TPE are unknown. The aim of this prospective, longitudinal, observational study, therefore, was to determine the course of r_cSO_2 and FTOE during the first 2 weeks after birth in preterm infants with and without TPE. We hypothesized that as a result of reduced cerebral perfusion, r_cSO_2 will be lower and FTOE will be higher in infants with TPE during the first days after birth.

Materials and methods

Patient population

For the purpose of this study we initially selected 53 preterm infants who had been admitted to the NICU of the University Medical Center Groningen between May 2006 and July 2007. The selection criterion was a gestational age of < 32 weeks. Infants suffering major chromosomal or congenital abnormalities were excluded from the study group. After primary inclusion, we excluded infants from further analysis if they had developed a germinal matrix hemorrhage grade 3, periventricular hemorrhagic infarction, or cystic PVL, all of which are conditions that may interfere with cerebral oxygenation. In this way, 4 infants had to be excluded: 3 because of a germinal matrix hemorrhage grade 3 and 1 because of a periventricular hemorrhagic infarction. Therefore, the final study group consisted of 49 preterm infants. Written informed parental consent was obtained in all cases. The study was approved by the review board of the University Medical Center Groningen.

Cranial ultrasonography

Cranial ultrasound scans were made of all infants within 72 hours after birth and, subsequently, at weekly intervals. The scans were made through the anterior fontanel by means of a real-time mechanical sector scanner (Siemens Sonoline Antares, Siemens AG, Erlangen, Germany) equipped with a 7.5-MHz transducer. All scans were assessed by 2 experts (Drs Bos and Martijn) to determine if periventricular echodensities were present. Periventricular echodensity is defined as increased echodensity in the periventricular region that is present on both sagittal and coronal planes and just as bright or brighter than the choroid plexus. The duration of the periventricular echodensities was also determined. TPE were defined as periventricular echodensities that persisted for more than 7 days.[2]

Near-infrared spectroscopy

We used an INVOS 4100 near-infrared spectrometer (Somanetics Corporation, Troy, Michigan, USA) in combination with the pediatric SomaSensor to measure the r_cSO_2 values. The r_cSO_2 is thought to reflect the oxygen saturation in a mixed vascular bed dominated by venules. This technology is based on the fact that biological tissues are relatively transparent to near-infrared (600 to 900 nm wavelength) light. The optical sensor measures the quantity of reflected light photons as a function of 2 wavelengths (730 and 805 nm) and determines the spectral absorption of the underlying tissue.[17,20] NIRS differentiates oxygenated hemoglobin from deoxygenated hemoglobin, which have distinct absorption spectra. The ratio of oxygenated hemoglobin to total hemoglobin reflects the regional oxygen saturation of tissue. The SomaSensor has 2 detectors at a distance of 3 and 4 cm from the near-infrared optode. The detector placed 3 cm from the optode receives light scattered predominantly from the scalp and skull. The detector placed at 4 cm receives light scattered from the scalp, skull, and cerebral tissue. Thus, by subtraction, the 2 detectors measure the oxygen saturation in the underlying cerebral tissue. From previous studies it was estimated that the depth of the signal is at least between 15 and 20 mm, enough to reach the white matter of the infants.[21,22]

R_cSO_2 was measured within the first 24 hours of birth and subsequently on the 2nd, 3rd, 4th, 5th, 8th, and 15th days. On these days, the r_cSO_2 was measured over a 2-hour period. Fifteen minutes were allowed for stabilization of the measurement. The optical sensor was placed to the left frontoparietal side of the infant's head and held in place by elastic bandaging. We marked the location of the sensor to ensure that the sensor was placed in the same position for each measurement.

Simultaneously, we measured $tcSaO_2$ by pulse oximetry. We calculated FTOE with the equation $FTOE = (tcSaO_2 - r_cSO_2)/tcSaO_2$.[15,17] FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen extraction.[19]

Clinical variables

Prospectively, we collected details on perinatal and neonatal characteristics that might influence hemodynamics. These details included gestational age, birth weight, birth asphyxia, early and late-onset sepsis, signs of circulatory failure, ventilatory status, and medication. Patency of the ductus arteriosus was routinely determined by echocardiography on the third to fifth days after birth if the infant was

ventilated, needed continuous positive airway pressure, or had other clinical signs suggestive of a patent ductus arteriosus. Maternal and pregnancy-related variables included medication and/or intoxications, intrauterine growth restriction, preeclampsia, and signs of maternal intrauterine infection. The presence of premature rupture of membranes (> 24 hours) and histologic characteristics of the placenta for signs of inflammation were also noted.

An Apgar score of < 5 at 5 minutes and/or resuscitation (external heart massage and/or use of epinephrine) and/or umbilical cord pH (arterial pH < 7.10) indicated birth asphyxia. Early-onset sepsis was diagnosed by a positive blood culture result and/or clinical signs within the first 48 hours after birth. Late-onset sepsis was diagnosed from 48 hours after birth. Circulatory failure was defined as hemodynamic instability and scored by the need for fluid resuscitation and/or the use of inotropes during the first 24 hours after birth. Intrauterine growth restriction was scored if birth weight was below the 10th centile according to Dutch intrauterine growth standards.[23] Maternal intrauterine infection was based on clinical signs such as fetal tachycardia and maternal fever (> 38° C), often combined with the mother taking antibiotics.

At the same time as r_cSO_2 and $tcSaO_2$ were measured, the infants' heart rate, respiratory rate, blood pressure, blood gas values, and hemoglobin concentration were recorded.

Statistical analysis

SPSS 14.0 software for Windows (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analyses. The mean values for r_cSO_2 and FTOE, along with the other variables, were calculated during the 2-hour period of measurement. The Spearman rank-order correlation test (2-tailed) was used to determine correlations between the clinical and NIRS parameters during the first 2 weeks after birth in infants with and without TPE. The mean values were analyzed by the Mann-Whitney *U* test because of the non-normal distribution. When appropriate, proportions of categorical data were tested by Fisher's exact test or χ^2-for-trend test. A *P* value of < .05 was considered significant.

Results

TPE were present in 25 of the 49 infants. Perinatal and neonatal characteristics were the same for infants with and without TPE (Table 1). Of the 24 infants without TPE, 9 infants were diagnosed with resolving periventricular echodensities within the first days after birth, and 15 infants never had echodensities. Five infants died before the 15th day after birth; 2 of them had periventricular echodensities, and 3 did not. One of the infants with periventricular echodensities died on the 13th day of combined respiratory and circulatory failure. The other infant died of massive lung bleeding on the 10th day. Of the 3 infants who did not show periventricular echodensities, 1 died because of asphyxia on the 4th day, the second died of combined respiratory and circulatory failure on the 6th day, and the third infant died of circulatory failure on the 11th day.

TABLE 1. Perinatal and neonatal characteristics

	No TPE	TPE
Number	N=24	N=25
Gestational age (weeks)	30.3 (28.2-31.1)	29.9 (28.3-30.3)
Birth weight (grams)	1250 (1015-1610)	1188 (1030-1470)
Female/Male	12/12	16/9
Apgar score at 5 minutes	7 (6-9)	8 (7-9)
Umbilical cord pH	7.24 (7.14-7.35)	7.20 (7.15-7.29)
Intrauterine growth restriction	n=6	n=3
Intrauterine infection	n=2	n=2
Early-onset sepsis	n=0	n=1
Late-onset sepsis	n=7	n=9
Ventilatory support	n=13	n=18
Duration (days)	1 (0-2)	1 (0-5)
Circulatory failure		
Fluid resuscitation	n=8	n=11
Inotropics	n=4	n=1
Intracranial hemorrhage grades 1-2	n=1	n=3

Data are expressed as median (p25 - p75) or as numbers unless otherwise specified. Differences between groups $P < 0.05$ are marked by *.

The course of r_cSO_2, FTOE, and $tcSaO_2$ in the 2 groups

During the first week we found no difference in r_cSO_2 and FTOE between infants with TPE and without TPE (Figure 1 A and B) except for the value of r_cSO_2 on the fifth day (median 77% versus 82%, TPE versus no TPE; Mann-Whitney *U* test, $P = .02$). When we repeated the analysis without the infants who had died before the 15th day after birth, we again found no difference between infants with and without TPE. On the 15th day, r_cSO_2 was lower in preterm infants with TPE in comparison to the infants without TPE (median 66% versus 76%; $P = .003$). FTOE in infants with TPE was higher than in infants without TPE (median 0.30 versus 0.20; $P < .001$).

In the group of infants with TPE, r_cSO_2 decreased from a median of 79% on the 1st day to 66% on the 15th day (Spearman's $\rho = -0.234$; $P = .04$). FTOE increased from a median of 0.16 to 0.30 ($\rho = 0.326$; $P < .001$). In the group of infants without TPE, r_cSO_2 decreased from a median of 82% on the 1st day to 76% on the 15th day ($\rho = -0.201$; $P = .004$). FTOE increased from a median of 0.13 to 0.20 ($\rho = 0.199$; $P = .02$).

$TcSaO_2$ did not differ between the 2 groups (Figure 1C). In the group of infants with TPE, $tcSaO_2$ increased slightly from a median of 93% on the 1st day to 97% on the 15th day (Spearman's $\rho = 0.216$; $P = .008$). In the group of infants without TPE, median $tcSaO_2$ was 95% on the 1st day and 95% on the 15th day.

During the first postnatal week, r_cSO_2, FTOE, and $tcSaO_2$ measured in infants with resolving periventricular echodensities within the first days after birth did not differ from infants without echodensities. This was also the case in infants with persistent echodensities, thus classified as TPE. When we repeated these analyses excluding the infants who had died before the 15th day after birth, again no differences were found during the first week after birth between groups with and without echodensities. The variation in r_cSO_2, FTOE, and $tcSaO_2$ remained equally broad.

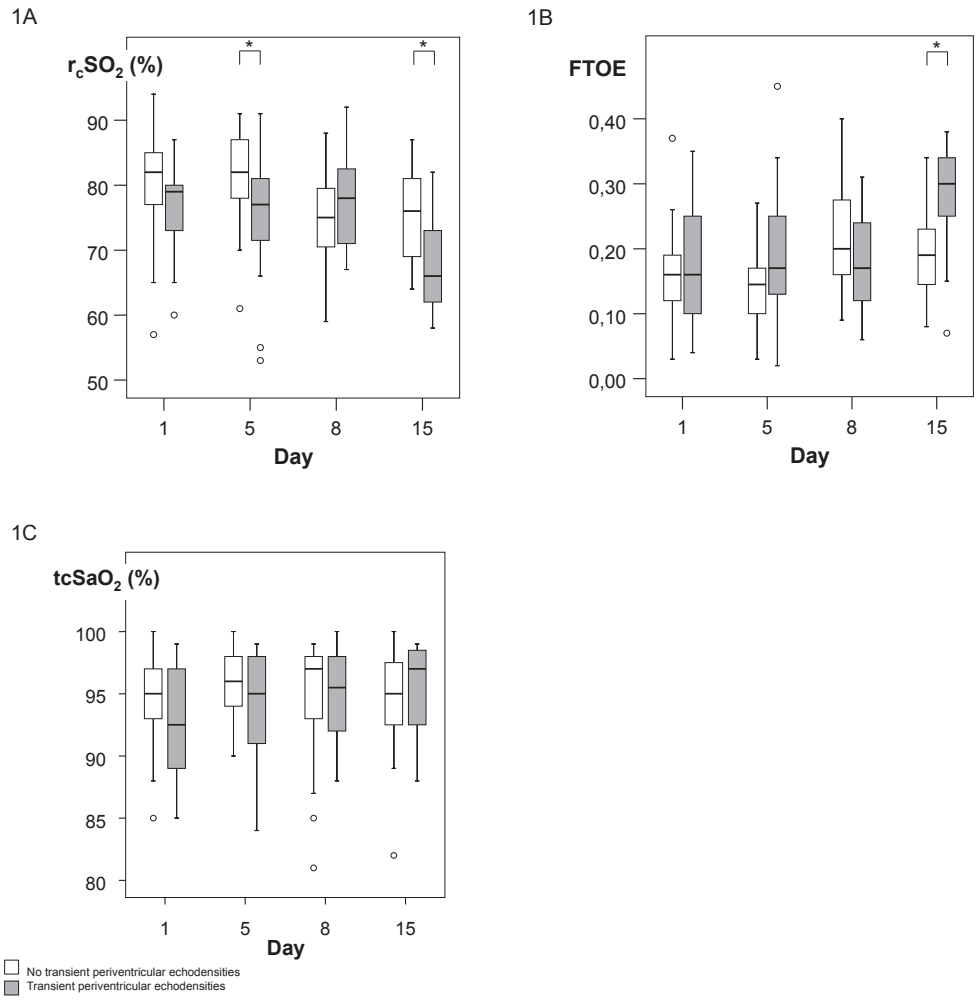

FIGURE 1. The course of the values for r_cSO_2 (A), FTOE (B), and $tcSaO_2$ (C) during the first 2 weeks after birth in infants with and without TPE

Differences between the 2 groups are marked with * ($P < .05$, TPE versus no TPE).

The relationship between r_cSO_2 and FTOE, and the clinical variables

Because several clinical conditions may influence cerebral hemodynamics and oxygenation, we investigated whether these conditions confounded the differences found in r_cSO_2 and FTOE. We checked blood pressure, $PaCO_2$, hemoglobin concentration, respiratory rate, and ventilatory status during the 2-hour period of measurement. The presence of a patent ductus arteriosus, based on clinical signs and confirmed by echocardiography, was not different between the groups. Mean blood pressure and $PaCO_2$ did not correlate with the simultaneously measured r_cSO_2 and FTOE. We found no differences between the 2 groups on any of the measurements except for the number of infants ventilated on the 8th day and a trend for a higher $PaCO_2$ ($P = .07$) in the group of infants without TPE on the 15th day (Table 2). This was also the case when we excluded the infants from the analysis who had died.

TABLE 2. Clinical variables on days 1 to 5, day 8 and day 15 in infants with and without TPE

	Days 1 - 5		Day 8		Day 15	
	No TPE	TPE	No TPE	TPE	No TPE	TPE
(Lowest) mean blood pressure (mmHg)	34	34	33	34	36	41
(Lowest) arterial pCO_2 (kPa)	4.4	4.4	6.0	5.7	6.6	5.7
Highest arterial pCO_2 (kPa)	6.0	5.8				
Ventilation (N)	12	15	0	5*	2	4
Respiratory rate	47	48	44	44	46	43
Lowest hemoglobin concentration (mmol/l)	8.4	7.9	8.3	7.8	7.6	8.4
Presence of patent ductus arteriosus	11	12	5	3	3	3

Differences between groups are marked by * ($P < 0.05$ versus no TPE).

Discussion

Our study demonstrates that TPE in preterm infants were associated with lower r_cSO_2 and higher FTOE on the 15th day after birth in comparison to infants without TPE. During the first week, up to the eighth day after birth, r_cSO_2 and FTOE were similar for the 2 groups, except for r_cSO_2 on the fifth day. This was contrary to our hypothesis, on the basis of which we expected lower r_cSO_2 and higher FTOE during the first days after birth in infants with TPE, but no longer on the 15th day.

There are several possible explanations for our findings. Because FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen consumption, increased FTOE can be explained either by a lower supply of oxygen or increased oxygen consumption.[18,24,25] A lower supply of oxygen (e.g. as a result of lower cerebral blood flow) is possible but not very likely. If this were the case, we would have expected reduced cerebral blood flow during the first days after birth but not as late as 2 weeks after birth. However, it could be that 2 weeks after birth, cerebral blood flow is lower than demanded in the infants with TPE because of the slightly lower PCO_2. Increased oxygen consumption (e.g. as a result of a higher metabolism) is another possibility. Normally, this would result in an increased cerebral blood flow

as a result of the coupling of flow and metabolism. This coupling could be disturbed in infants with TPE, leading to a higher FTOE.

When we analyzed the data for the presence or absence of echodensities during the first week only, the values of r_cSO_2 and FTOE were similar for infants with and without echodensities. These findings were in line with previous studies in infants developing cystic PVL. In these infants, cerebral oxygen extraction during the first 3 days after birth is not different in comparison to controls[26,27], which suggests that cerebral blood flow is not affected during the first days after birth in infants developing cystic PVL. In the second week, cerebral blood flow increases in infants not developing cystic PVL but not in infants developing cystic PVL.[27] Our data indicate that in infants with TPE, cerebral blood flow also did not increase during the second week after birth in comparison to controls.

It is also possible that cerebral blood flow is actually lowered during the first days after birth, which leads to higher FTOE on the one hand, while on the other hand the damaged cerebral tissue uses less oxygen, which results in lower FTOE.[28,29] The net effect would yield no difference in FTOE between infants with and without TPE. The higher FTOE on the 15th day could then be explained by increased cerebral metabolism and oxygen consumption to repair the damage that occurred.[30,31] This is only possible when the neurovascular flow-metabolism coupling is disturbed, which might be true in infants with TPE. This explanation, however, is highly speculative.

There were no differences between the 2 groups with regard to clinical variables, including inflammatory variables. Therefore, it is unlikely that confounders could be held responsible for the differences in r_cSO_2 and FTOE between the 2 groups. Only on the eighth day did we find the ventilatory status between the groups to be different. Previously, Lemmers *et al.*[17] found no difference in cerebral oxygenation measured by means of NIRS between ventilated and nonventilated preterm infants, although they suggested that cerebral autoregulation may be impaired when infants are ventilated. We found no differences in blood pressure between the 2 groups. However, no actual hypotension was found in any of the infants, which led us to think that the infants in this group might not have been susceptible to low cerebral perfusion as a result of impaired autoregulation. Another confounder that we considered was the influence of PCO_2 on r_cSO_2 and FTOE. Recently, a significant correlation was found between transcutaneous PCO_2 and FTOE during the first days of life.[32] We did not find any difference in $PaCO_2$ between groups, although there was a trend for elevated $PaCO_2$ in the group of infants without TPE on the 15th day. Therefore, we believe that the contribution of $PaCO_2$ to our findings was limited.

It was shown recently that preterm infants with patency of the ductus arteriosus have significantly lower r_cSO_2 and higher FTOE than controls.[33] We did not find a difference between our groups with regards to patency of the ductus arteriosus. It is possible that we may have missed an asymptomatic patent ductus arteriosus. Especially when the left-to-right shunt is modest, clinical signs can be unreliable. However, echocardiography was performed on most infants, and it is unlikely that undiagnosed patency of the ductus arteriosus was solely responsible for the lower r_cSO_2 and higher FTOE in the infants with TPE.

To our knowledge, the present study is the first to demonstrate the course of r_cSO_2 and FTOE during the first 2 weeks after birth. Several other studies were limited to the first 36 to 72 hours after birth. They demonstrated either stable or increased cerebral oxygen saturation, especially from the first to the

second day.[13,17,25,26,34] The present study indicates that r_cSO_2 decreased and FTOE increased during the second week after birth. This was the case both in infants with and without TPE. It is known that cerebral blood flow increases during the first week after birth[35-37] and thereafter.[27] It was found that cerebral metabolic rate increases more than twofold during the first 1 to 2 weeks after birth.[38,39] Thus, we could explain the course of r_cSO_2 and FTOE in the present study by increased cerebral blood flow during the first days after birth, followed by a steady increase of cerebral metabolic rate during the next few weeks.

The values we found for r_cSO_2 and FTOE showed a wide range. This finding is confirmed by various other studies[17,26,36] and points to large interindividual variation. We stress the fact that we did not identify any clinical variables related to this variation. One study showed a mean r_cSO_2 value of 66% in 10 preterm infants older than 7 days while receiving mechanical ventilation.[40] Compared with this study, the r_cSO_2 values of the infants in our study, without TPE, were somewhat higher but, nonetheless, within the same range.

In our study, the incidence of increased periventricular echodensities was rather high when compared with that reported from some other studies. Caution should be taken, however, when comparisons regarding TPE are made between studies, because the definition of echodensities, the equipment used, and the inclusion of infants with other ultrasound abnormalities differs somewhat.[5-8] In the present study, we classified moderately increased echogenicity as abnormal.

One of the limitations of our study is that we measured r_cSO_2 only during a 2-hour period. Although clinically unlikely in our study group, it could be that important decreases in r_cSO_2 occurred between the measurements. Another limitation is that we did not perform MRI on these infants; therefore we cannot confirm the presence of white matter lesions in the infants with TPE. The possibility exists that cranial ultrasonography failed to reveal genuine white matter abnormalities. TPE could also reflect venous congestion or reflection of neuronal tracts.[2,41] Another possible limitation was that we included infants with low grade intraventricular hemorrhages in our study. Nevertheless, these infants were equally distributed between the 2 groups.

Conclusions

TPE in preterm infants were associated with lower r_cSO_2 and higher FTOE values on the 15th day after birth in comparison to infants without TPE. During the first week, up to the eighth day after birth, r_cSO_2 and FTOE were similar for both groups. Our data suggest that persistent TPE were associated with an increased demand for cerebral oxygen after the first week after birth. Cerebral oxygenation may be involved in the recovery of perinatal white matter damage.

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Chapter

4

Cerebral oxygenation in preterm infants with germinal matrix-intraventricular hemorrhages

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Abstract

Background and Purpose Preterm infants are at risk of developing germinal matrix hemorrhages-intraventricular hemorrhages (GMH-IVH). Disturbances in cerebral perfusion are associated with GMH-IVH. Regional cerebral tissue oxygen saturation (r_cSO_2), measured with near-infrared spectroscopy, and fractional tissue oxygen extraction (FTOE) were calculated to obtain an indication of cerebral perfusion. Our objective was to determine whether r_cSO_2 and FTOE were associated with GMH-IVH in preterm infants.

Methods This case-control study included 17 preterm infants with grade I to III GMH-IVH or periventricular hemorrhagic infarction (median gestational age, 29.4 weeks; range, 25.4 to 31.9 weeks; birth weight, 1260 grams; range, 850 to 1840 grams). Seventeen preterm infants without GMH-IVH, matched for gestational age and birth weight, served as control subjects (gestational age, 29.9 weeks; range, 26.0 to 31.6 weeks; birth weight, 1310 grams; range, 730 to 1975 grams). R_cSO_2 and transcutaneous arterial oxygen saturation were measured during 2 hours on days 1 to 5, 8, and 15 after birth. FTOE was calculated as FTOE = (transcutaneous arterial oxygen saturation - r_cSO_2)/transcutaneous arterial oxygen saturation.

Results Multilevel analyses showed that r_cSO_2 was lower and FTOE higher in infants with GMH-IVH on days 1, 2, 3, 4, 5, 8, and 15. The largest difference occurred on day 5 with r_cSO_2 median 64% in infants with GMH-IVH versus 77% in control subjects and FTOE median 0.30 versus 0.17. R_cSO_2 and FTOE were not affected by the grade of GMH-IVH.

Conclusions Preterm infants with GMH-IVH had lower r_cSO_2 and higher FTOE during the first 2 weeks after birth irrespective of the grade of GMH-IVH. This suggests that cerebral perfusion is decreased persistently for 2 weeks in infants with GMH-IVH, even in the presence of mild hemorrhages.

Introduction

Preterm infants are at risk of developing germinal matrix hemorrhages-intraventricular hemorrhages (GMH-IVH). The primary lesion is a bleeding from small vessels in the germinal matrix.[1] The hemorrhage may be limited to the germinal matrix region (grade I) or it may rupture and extend into the adjacent ventricular system (grade II or III, depending on the extent of blood in the lateral ventricle).[2] A complication of GMH-IVH is a periventricular hemorrhagic infarction (PVHI), formerly described as grade IV GMH-IVH.[1] The pathogenesis of GMH-IVH is multifactorial.[1,3] Some studies hinted at high cerebral blood flow (CBF) being associated with GMH-IVH[4,5], whereas others reported that low CBF is associated with increased risk of GMH-IVH.[6-9] The latter studies, which use different methods to measure cerebral perfusion, seem to indicate that the occurrence of GMH-IVH is preceded by a period of low CBF.[6,7,9]

An indirect way of measuring CBF is to measure cerebral tissue oxygenation because one function of blood flow is to supply oxygen to tissue. A study in newborn lambs found that cerebral tissue oxygen saturation correlates well with changes in CBF.[10] A non-invasive method used to assess cerebral oxygenation is near-infrared spectroscopy (NIRS).[11] It measures regional cerebral tissue oxygen saturation (r_cSO_2).[12-15] This measure reflects the oxygen saturation in a mixed vascular bed dominated by venules. Fractional tissue oxygen extraction (FTOE) is calculated on the basis of r_cSO_2 and transcutaneous arterial oxygen saturation ($tcSaO_2$) values.[14-17] R_cSO_2 serves as an indicator of cerebral hypoxic hypoxia. FTOE reflects the balance between cerebral oxygen supply (cerebral perfusion) and cerebral oxygen consumption and thus serves as an indicator of cerebral ischemic hypoxia.[16-19]

Our objective was to determine whether r_cSO_2 and FTOE were associated with GMH-IVH in preterm infants. The majority of hemorrhages evolve within the first 72 hours of postnatal life.[1] We hypothesized that as a result of low cerebral perfusion, r_cSO_2 will be lower and FTOE will be higher in infants with GMH-IVH during the first days after birth. We were particularly interested in FTOE, but we also examined r_cSO_2 because oxygen saturation is what we actually measured.

Patients and Methods

Patient population

We performed a longitudinal case-control study. We included 17 preterm infants with grade I to III GMH-IVH, or PVHI. Seventeen preterm infants without GMH-IVH, matched for gestational age and birth weight, served as control subjects. Both sets of infants were selected for post hoc use from a larger cohort of 81 infants in which we measured r_cSO_2 and FTOE during the first days after birth. The selection criterion was a gestational age of < 32 weeks. Infants with major chromosomal or congenital abnormalities were not included in the study. Infants who developed cystic periventricular leukomalacia were not included either because this condition may interfere with cerebral oxygenation in a different way compared with GMH-IVH.[20] The infants had all been admitted to the neonatal intensive care unit of the University Medical Center Groningen between May 2006 and February 2008. The study was approved by the institutional review board of the University Medical Center Groningen. Written, informed parental consent was obtained in all cases.

Cranial ultrasonography

Cranial ultrasound scans were made of all the infants within 72 hours after birth and subsequently at weekly intervals. In case of intracranial hemorrhages, 2 to 3 cranial ultrasound scans were made during the first week. The scans were made through the anterior fontanel by means of a real-time mechanical sector scanner equipped with a 7.5-MHz transducer. All scans were assessed by 2 experts (A.F.B., A.M.), who were unaware of the NIRS data. They determined the presence of GMH-IVH or PVHI as well as the grade and localization (left/right) of the GHM-IVH or PVHI.[1,2] In addition, they noted the presence of post hemorrhagic ventricular dilatation, defined as a lateral ventricle size of -0.33 according to Evans index (the right and left lateral horn width divided by the maximum internal skull width).[21]

Near-infrared spectroscopy

We used an INVOS 4100 monitor (Somanetics Corporation, Troy, Michigan, USA) in combination with a pediatric SomaSensor to measure r_cSO_2 values. The optical sensor measures the quantity of reflected light photons as a function of 2 wavelengths (730 and 805 nm) and determines the spectral absorption of the underlying tissue.[14,22] Because oxygenated hemoglobin and deoxygenated hemoglobin have distinct absorption spectra, NIRS can differentiate between the two. The ratio of oxygenated hemoglobin to total hemoglobin reflects the regional oxygen saturation of cerebral tissue.

R_cSO_2 was measured within the first 24 hours after birth and subsequently on the second, third, fourth, fifth, eighth, and 15th days over a 2-hour period. The measurement was allowed some time to stabilize and therefore the first 15 minutes were not analyzed. The optical sensor was placed on the left frontoparietal side of the infant's head and held in place with elastic bandaging. We marked the location of the sensor to ensure that the sensor was placed at the same position for each measurement.

Simultaneously, we measured $tcSaO_2$ by means of pulse oximetry. We calculated FTOE as $FTOE = (tcSaO_2 - r_cSO_2)/tcSaO_2$.[14-17] FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen consumption.[16-18] FTOE serves better as a marker for ischemic hypoxia than r_cSO_2 alone because it is more independent of changes in arterial oxygen saturation.[19]

Clinical variables

Prospectively, we collected details on perinatal and neonatal characteristics that might influence hemodynamics. These included gestational age, birth weight, Apgar score, umbilical cord pH, birth asphyxia, early-onset and late-onset sepsis, signs of circulatory failure, ventilatory status, including mean airway pressure, patency of the ductus arteriosus, and medication. Maternal and pregnancy related variables included medication, intoxications, intrauterine growth restriction, premature rupture of membranes (> 24 hours), pre-eclampsia, and signs of maternal intrauterine infection. The placenta was examined for histological characteristics of inflammation.

The infant's heart rate, respiratory rate, mean arterial blood pressure, blood gas values, blood glucose, and hemoglobin concentration were recorded simultaneously with the r_cSO_2 and $tcSaO_2$ measurements.

Statistical analysis

We used SPSS 16.0 software for Windows (SPSS Inc., Chicago, Illinois, USA) for the statistical analyses. The mean and median values for r_cSO_2 and FTOE, along with the other variables, were calculated for the 2-hour recording periods. For the cross sectional analyses between groups, median values were analyzed by the Mann-Whitney *U* test for nonnormal distributions. The Spearman rank order correlation test (2-tailed) was used to determine correlations between the clinical and NIRS parameters during the first 2 weeks after birth. Where appropriate, we tested proportions of categorical data with Fisher exact test or the χ^2-for-trend test.

We categorized the infants into 3 groups: no hemorrhages (controls), mild hemorrhages (grade I to II), or severe hemorrhages (grade III, PVHI). To determine the relation between the categories of grading of the hemorrhages and the NIRS parameters, we built a multilevel model[23] into the statistical program MLwiN 2.15 (University of Bristol, Bristol, UK). This multilevel analysis allowed more accurate statistical testing than the standard repeated measures analysis of variance approach because it allows unequal numbers of observations per individual and it does not assume equality of group variances.[24] First, we constructed models in which NIRS measurements (level 1) were nested within subjects (level 2) thereby taking into account dependency between measurements and in which the intercept was measured on day 1 in the group without hemorrhages. The terms were a combination of the levels of the factors day of measurement and hemorrhage category. This led to 7 x 3 = 21 terms. Second, to arrive at a model that was both simpler and easier to interpret, each model was simplified by removing one by one those terms that were not included in a higher-order interaction term. This was done on the basis of the criterion that the coefficient of a term did not reach statistical significance ($P < 0.05$; backward model selection). We used these simplified models to test differences between weighted means. We used a *t* test to test for differences between 1 estimated mean and the intercept.[23] To test for differences between 2 estimated means, we tested the contrast of the sum of the parameters from which each estimate is derived using a χ^2 test with 1 degree of freedom.

Data were summarized as mean values +/- SD, as median values and ranges, or as weighted mean difference and a 95% CI where appropriate. A probability value of < 0.05 was considered statistically significant.

Results

Of the 17 infants with GMH-IVH, 10 had grade I GMH-IVH, 3 had grade II GMH-IVH, 2 had grade III GMH-IVH, and 2 infants had PVHI (Table 1). PVHI was secondary to GMH-IVH in both infants (1 grade II and 1 grade III). GMH-IVH or PVHI was seen in all infants on the first cranial ultrasound scans that were made within 72 hours after birth. In 1 infant, progression of grade I to grade II GMH-IVH was seen on cranial ultrasound scans between the third and the fifth days. No progression of the grade of GMH-IVH was seen in any other hemorrhages of the other infants on subsequent cranial ultrasound scans. Four infants had developed post hemorrhagic ventricular dilatation on subsequent cranial ultrasound scans: 1 had grade I GMH-IVH, 1 grade II GMH-IVH, and 2 infants had grade III GMH-IVH. One of the infants with grade

III GMH-IVH received several therapeutic lumbar taps allowing 10 to 15 ml/kg liquor drainage each time.

The perinatal characteristics of the infants with GMH-IVH or PVHI and the control subjects (Table 2) were similar. Two infants died before the 15th day after birth; 1 infant with grade III GMH-IVH died of multi-organ failure on the eighth day and 1 infant in the control group died of massive lung bleeding on the tenth day after birth.

TABLE 1. Distribution of GMH-IVH or PVHI

Grade	Number	Left	Right	Left+Right
I	10	5	4	1
II	3	1	1	1
III	2			2
PVHI	2	1	1	

Data are expressed as numbers.

TABLE 2. Perinatal Characteristics

	GMH-IVH or PHVI	Controls
Number	17	17
GA, weeks	29.4 (25.4-31.9)	29.9 (26.0-31.6)
BW, grams	1260 (850-1840)	1310 (730-1975)
Female/Male	9/8	9/8
Apgar score at 5 minutes	8 (5-9)	7 (3-9)
Umbilical cord pH	7.31 (7.05-7.44)	7.31 (7.17-7.41)
Head circumference, cm	27.0 (23.0-29.5)	27.5 (23.0-30.0)
Circulatory failure		
Fluid resuscitation	9 (53)	9 (53)
Inotropes	3 (17)	2 (12)
Small-for-gestational age, (%)	1 (6)	0 (-)
Maternal pre-eclampsia, (%)	2 (12)	3 (17)
Intrauterine infection, (%)	5 (29)	2 (12)
Premature rupture of membranes, (%)	6 (35)	5 (29)
Early-onset sepsis, (%)	0 (-)	2 (12)

Data are expressed as median (range) or as numbers unless otherwise specified. There were no significant differences between groups. GA indicates gestational age, BW; birth weight. Circulatory failure was defined as hemodynamic instability and scored by the need for volume administration or the use of inotropes or both during the first 24 hours after birth. Maternal intrauterine infection was based on clinical signs such as fetal tachycardia and maternal fever (> 38° C), often combined with the mother taking antibiotics. Early-onset sepsis was diagnosed by a positive blood culture or clinical signs or both within the first 48 hours after birth.

The effect of GMH-IVH or PVHI on r_cSO_2, FTOE, and $tcSaO_2$

Cross-sectionally, we found lower r_cSO_2 in preterm infants with GMH-IVH or PVHI on days 1, 2, 3, 4, 5, and 8 (Mann-Whitney *U* test, $P < 0.05$), but not on day 15 (Figure 1A). The largest difference appeared on day 5 with r_cSO_2 of 64% (median) in infants with GMH-IVH or PVHI versus 77% in control subjects (Mann-Whitney *U* test, $P < 0.001$). FTOE was higher in preterm infants with a GMH-IVH or PVHI on days 1, 2, 3, 4, 5, and 8 ($P < 0.05$), but not on day 15 (Figure 1B). The largest difference also appeared on day 5 with FTOE of 0.30 (median) in infants with GMH-IVH or PVHI versus 0.17 in control subjects ($P < 0.001$; Figure 2). $TcSaO_2$ did not differ between the 2 groups (Figure 1C). After excluding the infants that had died, we found the same results; that is, significant differences on days 1, 2, 3, 4, 5, and 8 for r_cSO_2, FTOE, and $tcSaO_2$. The 4 infants that subsequently developed post hemorrhagic ventricular dilatation could not be identified during the first 2 weeks after birth on the basis of their r_cSO_2 or FTOE values.

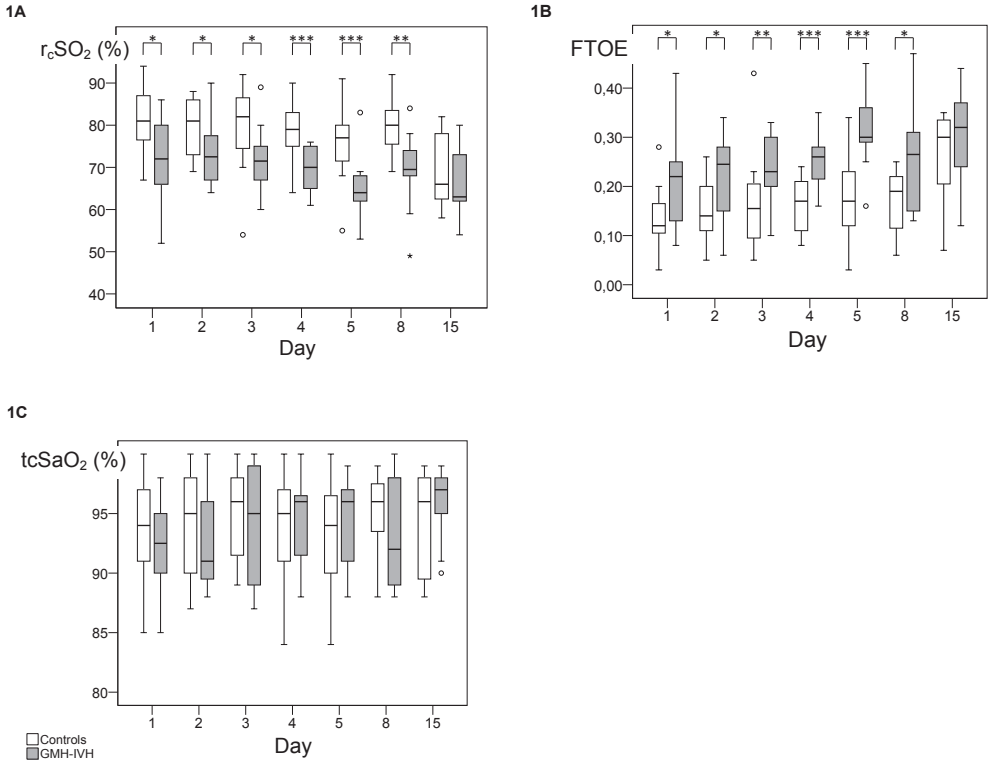

FIGURE 1. The course of r_cSO_2 (A), FTOE (B), and $tcSaO_2$ (C) in preterm infants with GMH-IVH or PVHI versus a preterm control group

Data are shown in box and whisker plots. Dots and stars represent outliers. Significant differences between the 2 groups are marked in the top of the figure by asterisks (* ≤ 0.05, ** ≤ 0.005, *** ≤ 0.001, GMH-IVH or PVHI versus control subjects).

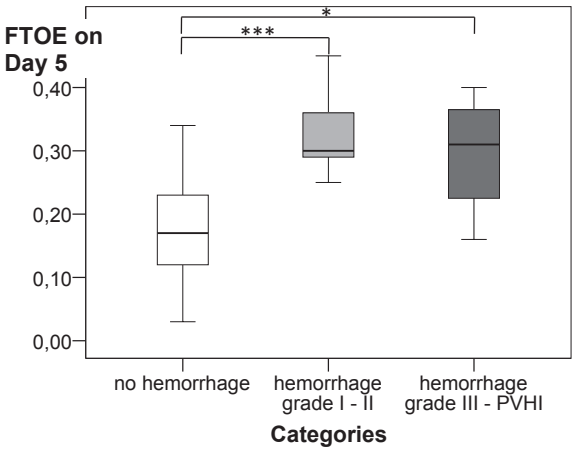

FIGURE 2. FTOE in preterm infants with no, mild (grade I to II), or severe (grade III, PVHI) hemorrhages on the fifth day after birth

Differences are marked by asterisks (* ≤ 0.05, *** ≤ 0.001).

The relationship between the grade of GMH-IVH or PVHI and r_cSO_2 and FTOE

In comparison to the control subjects, we found significantly lower r_cSO_2 on all days in infants with mild hemorrhages. This was established by multilevel analyses taking into account the longitudinal study design, the individual infant, and the differences between the days of measurement. The differences were largest on day 5 (weighted mean difference -12%; 95% CI, -18 to -6; $P < 0.001$). R_cSO_2 was also significantly lower in infants with severe hemorrhages (weighted mean difference -6%; 95% CI, -9 to -3; $P < 0.03$; Table 3). Compared with control subjects, we found a significantly higher FTOE on all days in infants with mild hemorrhages, the differences being largest on day 5 (weighted mean difference 0.14; 95% CI, 0.08 to 0.20; $P < 0.001$). FTOE was also significantly higher in infants with severe hemorrhages (weighted mean difference 0.07; 95% CI, 0.05 to 0.10; $P = 0.05$). We did not find a statistically significant difference in r_cSO_2 or FTOE between mild hemorrhage and severe hemorrhage on days 1, 2, 3, 4, 8, and 15. On day 5, there was a trend toward lower r_cSO_2 ($P = 0.1$) and higher FTOE ($P = 0.08$) in the infants with mild hemorrhages compared with the infants with severe hemorrhages.

TABLE 3. Simplified multilevel models for r_cSO_2 and FTOE

	r_cSO_2 (%)			FTOE		
Predictor term	estimate (%)	*t* ratio	*P* value	estimate	*t* ratio	*P* value
Intercept	79.2			0.16		
Day 5	-2.9	1.91	0.03	0.027	1.72	0.04
Day 15	-10.1	7.22	0.000	0.107	7.39	0.000
Mild hemorrhage	-8.1	3.96	0.000	0.08	4.24	0.000
Mild x day 5	-4.2	2.76	0.05	0.057	2.13	0.02
Severe hemorrhage	-5.9	1.61	0.03	0.073	2.13	0.05

In this simplified model, the intercept represents r_cSO_2 or FTOE in control group at days 1, 2, 3, 4, 8. The effects day 5, day 15, mild hemorrhage, and severe hemorrhage are added effects compared with the intercept. The interaction effect mild x day 5 is the added effect compared to the intercept taking the effects of day 5 and mild hemorrhage into account. The weighted average in r_cSO_2 for mild hemorrhage on day 5 is calculated as follows: 79.2% (intercept) – 2.9% (day 5) – 8.1% (mild hemorrhage) – 4.2% (mild x day 5) = 64.0%.

The relationship between the localization of the GMH-IVH or PVHI and r_cSO_2 and FTOE

We found no difference in left or right localization in any GMH-IVH or PVHI and r_cSO_2 or FTOE. Additionally, we found no difference in left or right localization in each grade of GMH-IVH or PVHI and r_cSO_2 or FTOE.

The relationship between r_cSO_2 and FTOE and perinatal and neonatal characteristics in both groups

To investigate whether other variables during the perinatal and neonatal period had confounded our findings on cerebral hemodynamics, we checked maternal medication and other intoxications, pre-eclampsia, premature rupture of membranes, signs of placental inflammation, and birth asphyxia. We found no differences between the groups nor did we find differences between the groups with regard to the presence of a patent ductus arteriosus based on clinical signs and confirmed by echocardiography. In addition, we checked mean arterial blood pressure, $PaCO_2$, blood glucose, hemoglobin concentration, heart rate, respiratory rate, and ventilatory status during the 2-hour period of measurement (Table 4). Mean arterial blood pressure, $PaCO_2$, hemoglobin concentration, heart rate, and respiratory rate did not correlate with r_cSO_2 and FTOE. On day 1, more infants with GMH-IVH or PVHI received ventilatory support through continuous positive airway pressure than control subjects (7 [50%] versus 2 [12%]; $P = 0.044$). No other relationships were found between the 2 groups and type of ventilatory support. Mean airway pressure was not different between the 2 groups either. Furthermore, we checked whether there was a relationship between mean airway pressure and cerebral oxygenation. We found this not to be the case except on day 4 when we found a negative correlation between mean airway pressure and FTOE (Spearman $\rho = -0.578$, $P = 0.049$). Blood glucose was higher on day 4 in infants that developed GMH-IVH or PVHI ($P = 0.007$).

TABLE 4. Neonatal characteristics

	Days 1 - 5		Day 8		Day 15	
	GMH-IVH versus control subjects		GMH-IVH versus control subjects		GMH-IVH versus control subjects	
Hemoglobin concentration, mmol/l	8.7†	7.9†	8.7	8.5	9.2	8.1
$PaCO_2$, kPa	4.5†	4.6†	5.4	6.0	5.8	6.2
Mean arterial blood pressure, mmHg	34†	37†	40	37	37	41
Blood glucose, mmol/l	2.8†	3.5†	5.7	4.6	4.1	5.3
Heart rate, beats per minute	150‡	149‡	155	152	155	158
Patent ductus arteriosus	6	6	2	4	1	3
Respiratory rate, breaths per minute	49‡	47‡	49	40	48	44
Ventilatory support						
Mechanical ventilation	8§	14§	4	4	5	3
CPAP	7§	2§*	7	3	2	3
Low flow	0§	1§	5	5	3	3

Data are expressed as median or as numbers unless otherwise specified. Differences between groups are marked by asterisks ($P < 0.05$ GMH-IVH or PVHI versus control subjects). † indicates median lowest value over days 1-5, ‡; Median value over days 1 through 5, §; number on day 1. Patency of the ductus arteriosus was routinely determined by echocardiography on the third to fifth days after birth if the infants were artificially ventilated, needed continuous positive airway pressure (CPAP), or had other clinical signs suggesting a patent ductus arteriosus.

Discussion

Our study demonstrated that preterm infants with GMH-IVH or PVHI had lower r_cSO_2 and higher FTOE during the first 2 weeks after birth in comparison to infants without GMH-IVH. Lower r_cSO_2 and higher FTOE occurred irrespective of the grade of GMH-IVH. These findings were not in line with our hypothesis. We expected r_cSO_2 to be lower and FTOE to be higher only during the first days after birth in infants with GMH-IVH or PVHI due possibly to lower cerebral perfusion. Instead, we found differences in cerebral oxygenation lasting at least for the first 2 weeks after birth.

Because FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen consumption, increased FTOE can be explained either by a lower supply of oxygen or by increased oxygen consumption.[16-18] A lower supply of oxygen could be the result of a lower CBF. A previous study found that CBF measured during the first 24 hours after birth was lower in 7 infants who developed a GMH-IVH compared with 17 infants without GMH-IVH.[7] The infants with severe GMH-IVH in particular had the lowest CBF.[7] Pryds *et al*. found that 10 infants, who subsequently developed GMH-IVH grade III or PVHI, had a 20% lower CBF compared with 38 infants with normal cranial ultrasound scans.[6] In a larger cohort of 254 preterm infants, a low CBF on the first day after birth was the dominant independent risk factor for the emerging GMH-IVH.[9] In these studies, CBF was measured in different ways using 131Xenon clearance[6], NIRS[7], and Doppler measurements on superior vena cava flow.[9] They all reported on CBF during the first days after birth but not later.

In the present study, we found lower r_cSO_2 and higher FTOE in infants with GMH-IVH or PVHI as compared with infants without GMH-IVH or PVHI from days 1 to 15. This suggests that CBF is persistently lower in infants with GMH-IVH, even in the presence of mild hemorrhages. One explanation could be that those infants who have low CBF, but still within the normal range, are at increased risk of acquiring GMH-IVH. Another explanation could be that circumstances leading to a lower CBF, which poses the infant at risk for GMH-IVH, remain present for a longer period than just the first few hours. Finally, the lower CBF could also be secondary to the presence of the hemorrhage itself as was suggested by Ment *et al*.[8]

Our study did not reveal whether lower r_cSO_2 and higher FTOE resulted from a lower CBF at the specific location of the hemorrhage itself or from a generally lower CBF. The germinal matrix receives its main arterial supply from the anterior cerebral artery, the middle cerebral artery, and the internal carotid artery.[1] We measured r_cSO_2 on the left frontoparietal side. We marked the location of the sensor to ensure that the sensor was placed in the same position each time we measured r_cSO_2. The location of GMH-IVH or PVHI in our study was evenly distributed in the group of infants with GMH-IVH or PVHI. Børch *et al*. found a lower CBF to the white matter of the affected hemisphere and a higher variation in CBF to the gray matter of both hemispheres and to the basal ganglia in 6 preterm infants with an intracerebral hemorrhage during the first days after birth.[25] This supports the idea that the GMH-IVH may be the result of an overall lower CBF. An overall lower CBF could explain why we found lower r_cSO_2 and higher FTOE in infants with GMH-IVH or PVHI in both the right and left hemispheres when we placed the SomaSensor on the left frontoparietal side. Previously, symmetrical cerebral oxygen saturation and extraction were found in preterm infants during the first 3 days after birth, including infants with grade I GMH, especially

in stable arterial oxygen saturation conditions.[26] Our data suggest that a difference in r_cSO_2 and FTOE between both hemispheres also does not occur in infants with more severe hemorrhages.

Previously, relationships between the severity of GMH-IVH or PVHI and cerebral oxygenation were found.[20,27,28] In these cases, however, cerebral oxygenation was only measured during the first days after birth. Contrary to the findings in these studies, we found no difference in r_cSO_2 or FTOE in the infants with mild hemorrhages compared with the infants with severe hemorrhages, except on the fifth day when, in the infants with mild hemorrhages, we surprisingly found a trend toward lower r_cSO_2 and higher FTOE.

Although information on the exact timing of the hemorrhages was unavailable, all the infants in our study already showed GMH-IVH or PVHI on the first cranial ultrasound scans that had been made within the first 72 hours after birth. We checked whether progression of GMH-IVH was visible on cranial ultrasound scans made after the first scan. This was not the case. The largest difference in r_cSO_2 and FTOE on the fifth day after birth could, therefore, not be attributed to a progression of GMH-IVH. The strength of our study lies in the fact that we followed these infants during the first 2 weeks after birth. Using multilevel analyses that allowed more accurate statistical testing, we found lower r_cSO_2 and higher FTOE from the first day onward until the 15th day. No confounders were identified that could otherwise explain this finding.

A lower supply of oxygen could also be the result of a lower concentration of hemoglobin as a result of the hemorrhage or lower arterial oxygen content. Hemoglobin levels, however, were the same in both groups, ruling out the possibility of a lower oxygen supply related to differences in hemoglobin levels. Furthermore, lower arterial oxygen content was not likely because $tcSaO_2$ did not differ between groups. We also found no differences between the 2 groups with regard to other clinical variables. Thus, we believe that these variables did not account for the differences in r_cSO_2 and FTOE between the 2 groups.

A final explanation for higher FTOE is increased oxygen consumption, but this is not very likely. Recently, increased oxygen consumption was found in late preterm and term infants with a large variety of brain injuries.[29] Possibly, these findings extend to our findings in very preterm infants, although this is purely speculative.

The values we found for r_cSO_2 and FTOE showed a wide range. This finding is confirmed by various other studies[14,15,20] and points to large interindividual variation. Sorensen *et al*. found a mean r_cSO_2 of 79% in preterm infants during the first day after birth.[30] This is in line with our study. Several other studies found r_cSO_2 values < 70% and FTOE values of approximately 0.30 during the first days after birth.[13,20,28] Another study showed a mean r_cSO_2 value of 66% in 10 preterm infants > 7 days of age at the time of receiving mechanical ventilation.[31] Compared with these studies, the r_cSO_2 values of the infants in our study were somewhat higher. Nonetheless, we did find differences of approximately 10% (r_cSO_2) and approximately 0.10 (FTOE) between infants with and without GMH-IVH or PVHI.

We recognize several limitations to our study. In this study, we did not measure CBF directly. Cranial ultrasound scans that measure Doppler flow velocity in cerebral arteries, before and after measuring r_cSO_2, might have yielded more information about CBF. Previous Doppler ultrasound studies, however, revealed conflicting results regarding the association between cerebral perfusion and risk of GMH-IVH.[4,32]

In addition, Doppler flow velocities correlated poorly with CBF measured by 131Xenon clearance.[33] Another limitation was the limited number of severe hemorrhages. Nevertheless, we even found differences in r_cSO_2 and FTOE in infants with a mild GMH-IVH, or even grade I GMH. Although it might be that the lower cerebral oxygenation in preterm infants who had a mild GMH-IVH is a random observation due to the post hoc nature of our study design, this finding is intriguing and requires further study. We have yet to find out whether this is also relevant for neurobehavioral outcome. A recent study did report that extremely low birth weight infants with grade I and II GMH-IVH have a poorer neurodevelopmental outcome at 20 months corrected age compared with infants with normal cranial ultrasound scans.[34]

Conclusion

Preterm infants with GMH-IVH or PVHI had lower r_cSO_2 and higher FTOE during the first 2 weeks after birth irrespective of the grade of GMH-IVH. This suggests that cerebral perfusion is decreased persistently for 2 weeks in infants with GMH-IVH, even in the presence of mild hemorrhages.

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Chapter 5

Cerebral tissue oxygen saturation and extraction in preterm infants before and after blood transfusion

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Abstract

Objective Preterm infants often need red blood cell (RBC) transfusions. The aim of this study was to determine whether haemoglobin levels before transfusion were associated with regional cerebral tissue oxygen saturation (r_cSO_2) and fractional tissue oxygen extraction (FTOE) and whether RBC transfusions were associated with r_cSO_2 and FTOE during the 24-hour period thereafter.

Design Prospective observational cohort study.

Setting Third level neonatal intensive care unit.

Patients Thirty-three preterm infants (gestational age 25-34 weeks, birth weight 605-2080 grams) were included.

Interventions None.

Main Outcome Measures R_cSO_2 was measured during a 1-hour period, before, 1 hour after and 24 hours after a 15 ml/kg RBC transfusion in 3 hours. Using r_cSO_2 and transcutaneous arterial oxygen saturation ($tcSaO_2$) values, FTOE was calculated: $FTOE = (tcSaO_2 - r_cSO_2)/tcSaO_2$. Results Forty-seven RBC transfusions were given. R_cSO_2 and FTOE correlated strongly with haemoglobin before transfusion ($\rho = 0.414$ and $\rho = -0.462$, respectively, $P < 0.005$). $TcSaO_2$ did not correlate with haemoglobin before transfusion. 24 hours after transfusion, r_cSO_2 increased from a weighted mean of 61% to 72% and FTOE decreased from a weighted mean of 0.34 to 0.23. The decrease in FTOE was strongest in the group with haemoglobin below 6.0 mmol/l (97 g/l). The decrease in FTOE was already present 1 hour after transfusion and remained unchanged at 24 hours after transfusion.

Conclusion Following RBC transfusion, cerebral tissue oxygen saturation increases and FTOE decreases. The data suggest that cerebral oxygenation in preterm infants may be at risk when haemoglobin decreases under 6 mmol/l (97 g/l).

Introduction

There is considerable controversy in neonatology as when to transfuse preterm infants with anaemia with red blood cells (RBC). As a result of frequent blood sampling and an immature haematopoietic system, preterm infants become progressively anaemic.[1] In an effort to limit the risks associated with RBC transfusion, many neonatal units have adopted more restrictive guidelines for transfusing preterm infants.[1] Two recently published randomised clinical trials both found that patients received fewer RBC transfusions when using restrictive guidelines.[2,3] The first study randomly assigned 100 preterm infants with birth weights of 500-1300 grams to either a restricted transfusion group, with haematocrit thresholds between 0.22 and 0.34, or a liberal transfusion group, with haematocrit thresholds between 0.30 and 0.46.[2] In each group, transfusion thresholds decreased with improving clinical status. The authors reported a decrease in transfusions in the restricted group: from 5.2 ± 4.5 (mean ± SD) in the liberal group to 3.3 ± 2.9 in the restricted group. The second study[3] randomly assigned 451 preterm infants below 1000 grams to either a restricted transfusion group, with thresholds between 68 and 115 g/l (consistent with haematocrit between 0.23 and 0.35)[4], or a liberal transfusion group, with thresholds between 77 and 135 g/l (consistent with haematocrit between 0.26 and 0.41). Transfusion thresholds decreased with improving clinical status and advancing postnatal age. The authors reported little evidence of clinical benefit (death or major morbidity) for either approach, with the low threshold group receiving fewer transfusions (mean 4.9 versus 5.7).[3] However, both studies produced conflicting results with regards to certain major neurological adverse events.[4] In the first study, adopting relatively high cut-off points for haematocrit levels in the control group, the incidence of cerebral haemorrhage and cystic periventricular leukomalacia increased significantly in the restrictedly transfused group.[2] In the other study no such differences were found, but adverse outcomes (death or major morbidity) were as frequent as 70% in both groups, and the difference regarding haemoglobin levels between the liberal and restricted groups was minimal.[3] Previously, it was demonstrated that apparently stable anaemic preterm infants may be in a clinically unrecognised high cardiac output state.[5] In this state, cerebral oxygen delivery may be at risk. There are some indications that insufficient oxygen delivery to the brain might be a mechanism involved in cerebral haemorrhage and periventricular leukomalacia.[2,6]

It is difficult to measure cerebral oxygenation non-invasively. A new, non-invasive method that could be useful is near-infrared spectroscopy (NIRS).[7] NIRS measures regional tissue oxygen saturation.[8-11] This measure is thought to reflect the oxygen saturation in a mixed vascular bed dominated especially by venules. The fractional tissue oxygen extraction (FTOE) is calculated on the basis of the values for the regional tissue oxygen saturation and the transcutaneous arterial oxygen saturation ($tcSaO_2$).[9,11-14] FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen consumption, and may thus serve as an indicator of cerebral hypoxia and ischaemia.[15,16] In the case of anaemia, the supply of oxygen may be impaired, resulting in a lower tissue oxygen saturation and a higher FTOE.

The values for the regional cerebral tissue oxygen saturation (r_cSO_2) and FTOE in preterm infants with anaemia are largely unknown. The aim of this study was therefore to determine whether r_cSO_2 and FTOE were associated with the haemoglobin level, and if so, at which level. Second, we were interested to see if an RBC transfusion influenced the values for r_cSO_2 and FTOE during the 24-hour period thereafter. We

hypothesised that as a result of reduced cerebral oxygen supply, r_cSO_2 will be lower and FTOE will be higher in infants before RBC transfusion, and that the transfusion will lead to a higher r_cSO_2 and lower FTOE.

Methods

Patients

For the purpose of this study we consecutively selected 33 preterm infants, who had been admitted to the neonatal intensive care unit of the University Medical Center Groningen between December 2006 and August 2007. The selection criteria were a gestational age of less than 35 weeks and need for an RBC transfusion, according to our local guidelines (Table 1). We excluded infants with major chromosomal or congenital abnormalities. The cut-off levels for haemoglobin in our guidelines range from 5.0 to 8.0 mmol/l (81 - 129 g/l). They are close to the cut-off levels reported in the restricted groups of previous randomised controlled studies: Bell *et al.*[2] reported cut-off levels between 4.6 and 7.0 mmol/l (74 - 113 g/l), and Kirpalani *et al.*[3] reported cut-off levels between 4.8 and 7.2 mmol/l (77 - 116 g/l). For blood transfusions on our unit, a single donor approach was used. The RBC aliquots were stored for a maximum of 2 weeks, had a haematocrit of 0.60 and were preserved with saline, adenine, glucose and mannitol. The administered RBC transfusion volume was 15 ml/kg in 3 hours. The guidelines were followed as closely as possible, but the final decision for an RBC transfusion was made by the attending neonatologist. Informed parental consent was obtained in all cases. The study was approved by the local institutional review board.

TABLE 1. Indications for RBC transfusion in relation to clinical variables

Threshold level	Clinical variables
Haemoglobin < 8 mmol/l (129 g/l)	First 24 hours postpartum
	Ventilator dependency
	Cardiorespiratory instability
Haemoglobin < 7 mmol/l (113 g/l)	Cardiorespiratory problems with stable clinical condition
Haemoglobin < 6 mmol/l (97 g/l)	Clinical symptoms of anaemia (such as tachycardia, tachypnea, apnoeas/ bradycardias, poor weight gain) in an otherwise stable neonate
Haemoglobin < 5 mmol/l (81 g/l)	Stable neonate, > 4 weeks post-term

Near-infrared spectroscopy

We used the INVOS 4100 monitor (Somanetics Corporation, Troy, Michigan, USA) in combination with the paediatric SomaSensor to obtain r_cSO_2 values. This technology is based on the fact that biological tissues are relatively transparent to near-infrared light (600 - 900 nm wavelength). The optical sensor measures the quantity of reflected light photons as a function of two wavelengths (730 and 805 nm), and determines the spectral absorption of the underlying tissue.[14,17] NIRS differentiates oxygenated

haemoglobin from deoxygenated haemoglobin, which each have distinct absorption spectra. The ratio of oxygenated haemoglobin to total haemoglobin reflects the regional oxygen saturation of tissue. The SomaSensor has two detectors, at 3 cm and at 4 cm distance from the near-infrared optode. The detector placed at 3 cm from the optode receives light scattered predominantly from the scalp and skull. The detector placed at 4 cm receives light scattered from the scalp, skull and cerebral tissue. Therefore, by subtraction the two detectors measure the oxygen saturation of the underlying cerebral tissue. From previous studies it is estimated that the depth of the signal is at least between 15 and 20 mm, enough to reach the cortical grey and white matter of the infants.[18,19]

For this study, we placed the optical sensor on the left frontoparietal region of the infant's head and held it in place using elastic bandaging. Fifteen minutes were allowed for stabilization of the measurement. For each RBC transfusion, we measured the r_cSO_2 three times for 1 hour at a time. The first measurement was made immediately before (t = 0), the second 1 hour after the completion of the RBC transfusion (t = 1) and the third 24 hours thereafter (t = 24). Simultaneously, we measured $tcSaO_2$ by pulse oximetry. We calculated FTOE using the equation FTOE = $(tcSaO_2 - r_cSO_2)/tcSaO_2$.[11,13,14] The mean values for $tcSaO_2$, r_cSO_2 and FTOE were calculated during the 1-hour period of measurement, and this mean was used as a single value for further analysis. Repeatability of the r_cSO_2 measurements, using the same device as we did, is reported to be stable, with limits of agreement less than 6%.[20] In our hands, repeatability of the r_cSO_2 measurements after refixation of the optode, and allowing 10 min to stabilize r_cSO_2, was similar, with a mean difference of 4.5% (range 1% - 8%) between three consecutive measurements of the same patient (E.A. Verhagen and A.F. Bos, unpublished data). Previous studies have demonstrated that FTOE in preterm infants range from 0.15 to 0.40 during the first weeks after birth.[11,14,20,21]

Clinical variables

In all infants, we collected haemoglobin and haematocrit values before RBC transfusion and 24 hours after transfusion. We prospectively collected data with regard to the perinatal and neonatal characteristics that might influence haemodynamics. These included gestational age, birth weight, patency of the ductus arteriosus and ventilatory status. Simultaneously to r_cSO_2 and $tcSaO_2$ measurements we recorded the infants' heart rate, respiratory rate, blood pressure and blood gas values.

Statistical analysis

SPSS 16.0 software for Windows (SPSS Inc., Chicago, Illinois, USA) was used for most statistical analyses. The mean and median values for r_cSO_2 and FTOE, along with the other variables, were calculated for the 1-hour periods of measurement: t = 0, t = 1 and t = 24. The Spearman rank order correlation test (two-tailed) was used to determine correlations between the haemoglobin concentration before transfusion and NIRS parameters, and between the absolute rise in haemoglobin following transfusion and NIRS parameters.

Next, we categorised the infants into three groups, on the basis of their initial haemoglobin concentration, following the threshold levels of our guidelines (Table 1): group A, haemoglobin level

before transfusion < 6.0 mmol/l (< 97 g/l); group B, haemoglobin level before transfusion 6.1 - 7.0 mmol/l (98 - 113 g/l); group C, haemoglobin level before transfusion 7.1 - 8.0 mmol/l (114 - 129 g/l). In order to detect differences in time, and between the three categories of haemoglobin level before transfusion, we built a multilevel model in which NIRS measurements (level 1) were nested within subjects (level 2), thereby taking dependency between measurements into account. We used this model to test differences between means. To test for differences between an estimated mean and the intercept one uses a *t* test.[22] To test for differences between two estimated means one tests the contrast of the sum of the parameters from which each estimate is derived, using a χ^2 test with 1 degree of freedom. Results were reported including 95% CI. We used MLwiN 2.11 (University of Bristol, Bristol, UK) for these multilevel statistical analyses.

To investigate whether other clinical conditions confounded our results, we tested the correlations between NIRS parameters, blood pressure and partial pressure carbon dioxide (PCO_2) with the Spearman rank correlation test. We used the Mann-Whitney *U* test to test whether patency of the ductus arteriosus and ventilatory status at the time of measurement confounded the NIRS parameters before and after transfusion. We also tested whether patency of the ductus arteriosus and ventilatory status were evenly distributed among the three categories of haemoglobin level before transfusion, using the χ^2-for-trend test. A *P* value less than 0.05 was considered significant.

Results

Thirty-three infants were included in this study. Their gestational age ranged from 25 to 34 weeks (median 27.3 weeks) and their birth weights ranged from 605 to 2080 grams (median 1010 grams). In 14 infants an RBC transfusion was given twice. The study sample therefore consisted of 47 instances of RBC transfusion, which was applied between 1 and 93 days after birth (median 17 days), at a postmenstrual age between 25.9 and 39.0 weeks (median 30.1 weeks). The haemoglobin values before transfusion ranged from 3.7 to 7.9 mmol/l (60 - 128 g/l), with the median 6.9 mmol/l (111 g/l). Haematocrit values ranged from 0.19 to 0.36 (median 0.31). In 11 transfusions, the infants did not require respiratory support, in 18 transfusions, the infants were on continuous positive airway pressure and in 18 transfusions the infants were ventilated. In 17 transfusions, the infants required additional oxygen, but in only three infants was the inspiratory fraction of oxygen more than 0.30.

TABLE 2. Pre and post-transfusion values of haemoglobin (Hb), haematocrit (Ht), $tcSaO_2$, r_cSO_2 and FTOE

	Hb (mmol/l)	Ht	$tcSaO_2$ (%)	r_cSO_2 (%)	FTOE
Before transfusion	6.9 (3.7-7.9)	0.31 (0.19-0.36)	93 (85-99)	60 (28-82)	0.35 (0.07-0.70)
1 hour after transfusion			92 (86-99)	71 (46-94)	0.25 (0.03-0.53)
24 hours after transfusion	8.4 (6.9-10.6)	0.40 (0.32-0.46)	92 (84-100)	71 (45-96)	0.22 (0.07-0.49)

Values are given as median (min - max). FTOE indicates fractional tissue oxygen extraction, r_cSO_2; regional cerebral tissue oxygen saturation, $tcSaO_2$; transcutaneous arterial oxygen saturation.

The course of $tcSaO_2$, r_cSO_2 and FTOE in relationship to haemoglobin

Before transfusion median $tcSaO_2$ was 93% (range 85% - 99%), median r_cSO_2 was 60% (range 28% - 82%) and median FTOE was 0.35 (range 0.07 - 0.70) (Table 2). R_cSO_2 and FTOE correlated strongly with the haemoglobin levels before the transfusion (Spearman's $\rho = 0.414$, $P = 0.004$ and $\rho = -0.462$, $P = 0.001$, respectively; Figure 1). $TcSaO_2$ did not correlate with the haemoglobin levels. R_cSO_2 and FTOE values after the transfusion did also not correlate with haemoglobin levels after the transfusion.

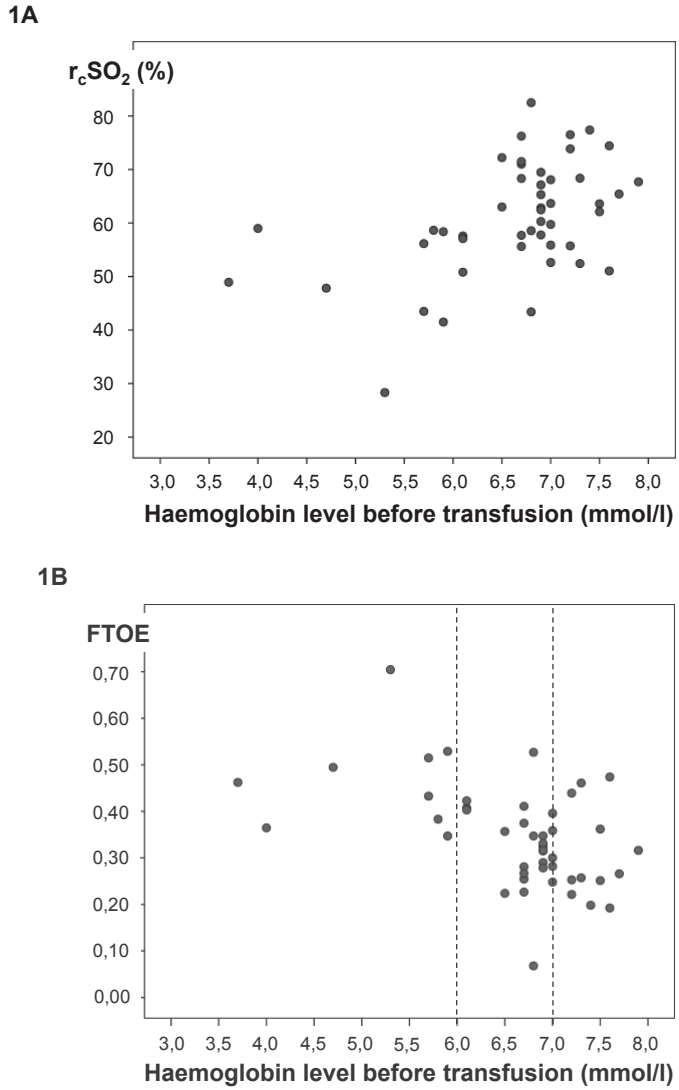

FIGURE 1. The relation of haemoglobin levels before transfusion to regional cerebral tissue oxygen saturation (r_cSO_2) (A) and fractional tissue oxygen extraction (FTOE) (B)

The correlation was highly significant (A, Spearman's $\rho = 0.414$, $P = 0.004$; B, Spearman's $\rho = -0.462$, $P = 0.001$). Note: A haemoglobin level of 3 mmol/l corresponds to 48 g/l, 6 mmol/l to 97 g/l, 7 mmol/l to 113 g/l and 8 mmol/l to 129 g/l.

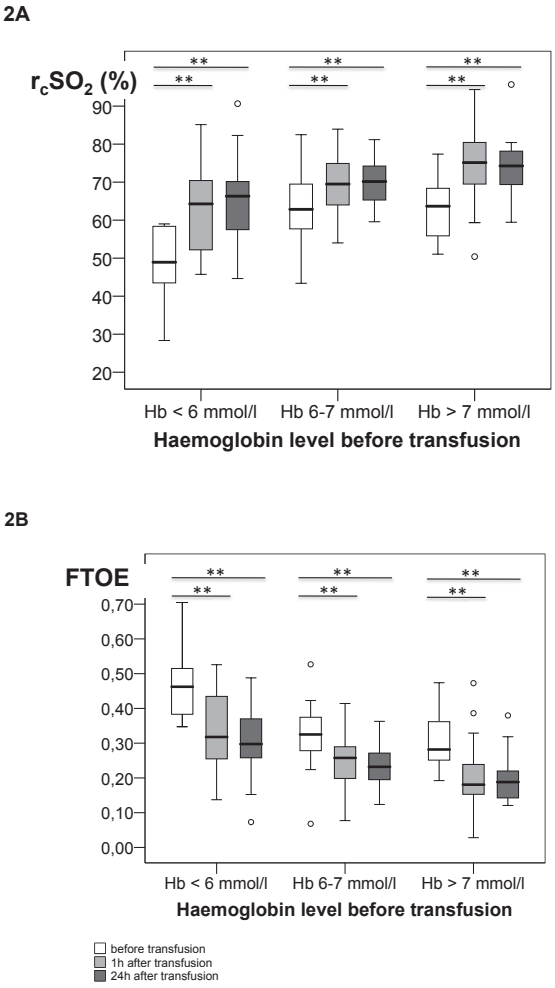

FIGURE 2. The course of regional cerebral tissue oxygen saturation (r_cSO_2) (A) and fractional tissue oxygen extraction (FTOE) (B) (shown as box-and-whisker plots) before (t = 0), 1 hour after transfusion (t = 1) and 24 hours after transfusion (t = 24) in three groups, according to the haemoglobin level before transfusion: (A) haemoglobin less than 6 mmol/l (< 97 g/l), (B) haemoglobin 6 - 7 mmol/l (97 - 113 g/l) and (C) haemoglobin 7 - 8 mmol/l (113 - 129 g/l)
Significant differences were tested by a multilevel model in which r_cSO_2 (A) and FTOE (B) measurements (level 1) were nested within subjects (level 2). Significant differences are marked by asterisks: ** $P < 0.001$.

We categorised the infants receiving RBC transfusions into three groups, following the threshold levels of our guidelines. When taking an arbitrary cut-off point for FTOE at 0.40 (Figure 1B), six of nine infants in group A (67%), five of 21 infants in group B (24%) and three of 17 infants in group C (18%) had higher FTOE values (χ^2-for-trend test 5.5, $P = 0.019$). The courses of r_cSO_2 and FTOE following RBC transfusion in the three groups are shown in Figures 2 and 3. Differences were tested by a multilevel model in which r_cSO_2 and FTOE measurements (level 1) were nested within subjects (level 2). Following

the transfusion, r_cSO_2 increased (Figure 2A) and FTOE decreased (Figure 2B) in each group. These changes were already present at 1 hour after the transfusion (average increase in r_cSO_2 9%, 95% CI 7 to 11, $P < 0.001$; average decrease in FTOE -0.10, 95% CI -0.12 to -0.08, $P < 0.001$). This differed significantly between group A compared with group B, with an average difference in r_cSO_2 of 8.4% (95% CI 2.0 to 14.8) $P = 0.01$, and near-to-significant in FTOE of -0.06 (95% CI -0.13 to 0.00) $P = 0.06$, but not between group A compared with group C (for r_cSO_2, $P = 0.17$; for FTOE, $P = 0.31$) and between group B and group C (for r_cSO_2, $P = 0.15$; for FTOE, $P = 0.31$). There was no significant subsequent change after the next 23 hours (average change 1 - 24 hours in r_cSO_2 -1.1%, 95% CI -3.6 to 1.4, $P = 0.37$, and in FTOE -0.02, 95% CI -0.04 to 0.01, $P = 0.26$) and this did not differ between haemoglobin groups for r_cSO_2 and FTOE, respectively (group A versus B, $P = 0.75$ and $P = 0.70$; group A versus C, $P = 0.53$ and $P = 0.54$; and group B versus C, $P = 0.68$ and $P = 0.76$).

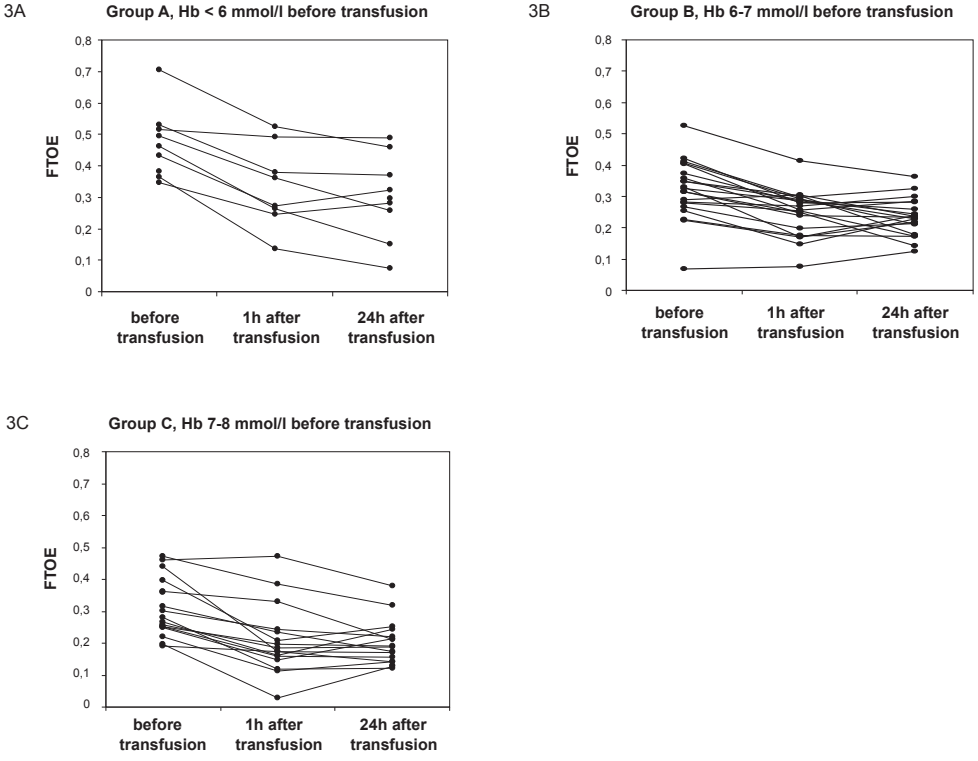

FIGURE 3. The changes of fractional tissue oxygen extraction (FTOE) in individual infants from before transfusion (t = 0), 1 hour after transfusion (t = 1) until 24 hours after transfusion (t = 24) in the three groups created according to the haemoglobin level before transfusion: (A) haemoglobin less 6 than mmol/l (< 97 g/l); (B) haemoglobin 6 - 7 mmol/l (97 - 113 g/l) and (C) haemoglobin 7 - 8 mmol/l (113 - 129 g/l)
In all groups the changes were highly significant ($P \leq 0.001$) between t = 1 and t = 0, and between t = 24 and t = 0, but not between t = 24 and t = 1.

Next, we analysed whether there were any differences between the three groups at each time point (t = 0, t = 1 and t = 24). At t = 0, r_cSO_2 was lower in group A compared with B and C (average difference 12%, 95% CI 8 to 17, $P < 0.001$), whereas there was no significant difference between groups B and C (B on average 2.8% higher (95% CI -1.9 to 7.6) $P = 0.24$). At t = 1, r_cSO_2 was near-to-significantly lower in group A compared with groups B and C (average difference 5.4%, 95% CI -0.1 to 10.9, $P = 0.054$), but not at t = 24, $P = 0.15$. At all three time points, r_cSO_2 values did not differ between groups B and C, $P = 0.24$, $P = 0.70$ and $P = 0.95$, respectively. FTOE values were higher in group A compared with groups B and C at all three time points (average differences: t = 0, 0.13 (95% CI 0.08 to 0.17) $P < 0.001$; t = 1, 0.08 (95% CI 0.02 to 0.13) $P = 0.009$; t = 24, 0.06 (95% CI 0.00 to 0.11) $P = 0.04$). There were no significant differences at any time point between groups B and C ($P = 0.19$, $P = 0.83$ and $P = 0.58$, respectively).

Twenty-four hours after the RBC transfusion, r_cSO_2 had increased from a weighted mean 61% - 72% and FTOE had decreased from a weighted mean of 0.34 - 0.23. At t = 24 FTOE was still above 0.40 in two infants, both were from group A. Both infants were born at 25 weeks, with a birth weight of 730 and 900 grams, respectively. One of them was transfused at postnatal day 30, with haemoglobin 5.7 mmol/l (92 g/l) before transfusion, and haemoglobin 6.9 mmol/l (111 g/l) 24 hours later, the other was transfused at postnatal day 90, with haemoglobin 5.3 mmol/l (85 g/l) before transfusion and haemoglobin 7.8 mmol/l (126 g/l) 24 hours later. In the first infant, there were no clinical factors identified that could account for the higher FTOE, in the second infant a concurrent septicaemia with a coagulase-negative staphylococcus species was diagnosed on the same day as the transfusion.

The change in FTOE following a RBC transfusion correlated strongly with the absolute rise in haemoglobin level. This correlation was already present 1 hours after RBC transfusion (Spearman's $\rho = -0.320$, $P = 0.039$), but was strongest at 24 hours after RBC transfusion (Spearman's $\rho = -0.466$, $P = 0.002$).

The relationship between r_cSO_2 and FTOE and the clinical variables

As several clinical conditions may influence cerebral haemodynamics and oxygenation, we investigated whether these conditions confounded the differences found in r_cSO_2 and FTOE. We checked blood pressure, PCO_2, patency of the ductus arteriosus and ventilatory status during the period of measurement. The clinical characteristics of the groups A, B and C are shown in Table 3. We found no differences between the three groups in any of the clinical variables. All intraventricular haemorrhages were already diagnosed before the occurrence of anaemia. The ductus arteriosus was patent in seven children at the time of transfusion, and closed at the time of the other 40 measurements. R_cSO_2 and FTOE before and following transfusion were not associated with the patency of the ductus arteriosus, or with ventilatory status. Mean blood pressure and PCO_2 did not correlate with the simultaneously measured r_cSO_2 and FTOE. In the absence of significant associations of potential confounders, we refrained from multivariate regression analyses.

TABLE 3. Patient characteristics of the three study groups

	Group A	Group B	Group C
	Hb < 6 mmol/l	Hb 6 - 7 mmol/l	Hb 7 - 8 mmol/l
Number of infants	N=8	N=18	N=14
Number of measurements	N=9	N=21	N=17
Hb before transfusion (mmol/l)	5.7 (3.7-5.9)	6.7 (6.1-6.9)	7.3 (7.0-7.9)
Ht before transfusion	0.26 (0.19-0.28)	0.31 (0.27-0.33)	0.34 (0.31-0.36)
Hb after transfusion (mmol/l)	8.0 (6.9-9.5)	8.2 (7.3-9.9)	8.8 (7.9-10.6)
Ht after transfusion	0.35 (0.32-0.43)	0.39 (0.34-0.46)	0.40 (0.35-0.42)
Gestational age (weeks)	27.0 (25-32)	27.3 (25-34)	26.6 (25-30)
Birth weight (grams)	1010 (730-1400)	990 (605-2080)	1000 (615-1840)
Male/Female	5/4	10/11	14/3
Age (days) at transfusion	27 (1-93)	17 (2-75)	13 (1-41)
Postmenstrual age (weeks)	31.0 (26-39)	31.3 (27-35)	30.0 (26-34)
Intraventricular haemorrhage gr 1-2	n=2	n=1	n=2
Intraventricular haemorrhage gr 3	n=0	n=1	n=1
Conventional ventilation	n=3	n=7	n=8
CPAP / nasal IMV	n=3	n=8	n=7
No respiratory support	n=3	n=6	n=2
FiO_2 > 0.21	n=3	n=8	n=6
Patency of the ductus arteriosus	none	n=4	n=3
PCO_2 (kPa)	5.8 (5.0-7.3)	5.9 (4.1-7.8)	6.0 (4.4-9.0)
Arterial blood pressure (mm Hg)	41 (36-49)	45 (31-66)	41 (34-56)

Data are expressed as median (min - max) or as numbers. There were no differences between groups (Mann-Whitney *U* test and χ^2-for-trend test, when appropriate). CPAP indicates continuous positive airway pressure, FiO_2; fractional inspired oxygen, IMV; intermittent mandatory ventilation.

Discussion

The present study indicated that r_cSO_2 decreased and FTOE increased in preterm infants who were to receive an RBC transfusion according to our rather restrictive transfusion guidelines.[4] Following an RBC transfusion, cerebral oxygenation improved quickly. Further improvement during the following 24 hours did not occur. However, in infants with low haemoglobin levels before transfusion (haemoglobin < 6.0 mmol/l, haemoglobin < 97 g/l), cerebral FTOE was still higher 24 hours after the transfusion, compared with the infants who had a higher haemoglobin level (> 6.0 mmol/l, > 97 g/l) before the transfusion. This was present despite haemoglobin levels that were no different between groups after the transfusion. From our findings we speculate that cerebral oxygenation in preterm infants may be at risk when haemoglobin levels decrease under 6.0 mmol/l (97 g/l).

The increased cerebral oxygen extraction can be explained by a decreased oxygen transport capacity, as a result of the lower haemoglobin level. It is possible for preterm infants to compensate for a low haemoglobin level by increasing cardiac output.[5,23] Along with the higher cardiac output, cerebral blood flow will also increase[24-26], but this increase might be absent or limited in cases of mild anaemia.[27,28] In fact, our data suggest that the increase in cerebral blood flow is insufficient to compensate for the decreased oxygen transport capacity. As the FTOE reflects the balance between oxygen supply and oxygen consumption, this means that oxygen delivery to the brain may be at risk in cases of anaemia. It is interesting to note that changes were detectable 1 hour after transfusion, and did not increase further at 24 hours after the transfusion. This adds support to the hypothesis that increasing the fraction of haemoglobin A and therefore tissue oxygen delivery might be an important factor in the benefits of neonatal blood transfusion.

Our data support the notion that adherence to a too restrictive RBC transfusion policy in preterm infants may be harmful. When compared with studies that have investigated liberal versus restrictive guidelines, our transfusion guidelines are rather restrictive.[2,3] In the randomised study that found significant differences in neurodevelopmental outcome[2], the liberal guidelines in the sickest infants allowed the haematocrit level to be as low as 40% (in our study it was approximately 36%) and in the more stable infants 30% (we had 26%).[4] A few other studies have used NIRS techniques to determine the course of oxygenation parameters following RBC transfusions in preterm infants.[26,27,29-31] Some of those studies were performed several years ago, when haemoglobin levels were not allowed to get as low as in the present study. Despite this, the results of those studies also indicated that oxygen extraction in peripheral and cerebral tissue improved following transfusion.[26,27,29-31] Nevertheless, the studies did not provide a cut-off point for haemoglobin at which cerebral oxygen delivery may be at risk.

Our study may have implications for clinical practice. NIRS can play a role in determining whether cerebral oxygenation is at risk in cases in which the haemoglobin level approaches the level for RBC transfusion according to one's guidelines. Those infants with a high cerebral FTOE might benefit from an earlier RBC transfusion than strictly required according to one's guidelines. This has been studied previously measuring peripheral tissue oxygen extraction of the forearm with NIRS, and taking a cut-off point of 0.47 for giving a blood transfusion.[30] These peripheral fractional oxygen extraction measurements, however, failed to identify many of the infants felt by the clinicians to require blood transfusions.[30] It might be that cerebral FTOE, rather than peripheral fractional oxygen extraction, is a more sensitive predictor of the need for a transfusion. This requires further study.

There are several limitations to our study. Being a single centre study, our results may not be applicable to the general population. Our study group is relatively small, and it may be that clinical variables that we are not aware of also contributed to the variation in r_cSO_2 and FTOE. We did not measure cerebral blood flow. It is difficult to obtain reliable indices of cerebral blood flow, and we refrained from measuring blood flow velocity by Doppler ultrasound. Doppler flow measurements in, for example, the middle cerebral artery could have given an extra dimension to this study, as high values would underscore the presence of haemodynamic significant anaemia, and also indicate the presence of a ductal steal in the case of patent ductus arteriosus.

The values we found for r_cSO_2 and FTOE showed a wide range. This finding is confirmed by various other studies[6,11,14,32] and points to a large interindividual variation. We stress the fact that we did not identify any clinical variables other than haemoglobin related to this variation. A final limitation is that we did not include long term follow-up in the present study. The prevalence of cerebral haemorrhages in our group was low and there were no children with periventricular leukomalacia. However, our study is definitely underpowered to find clinically relevant diverse outcomes in these groups as a result of the haemoglobin level before transfusion.

In conclusion, the present study indicates that cerebral oxygenation in preterm infants may be at risk when haemoglobin levels decrease below 6 mmol/l (97 g/l). Following an RBC transfusion, cerebral oxygenation improved quickly (within 1 hour). Further improvement during the 24 hours following did not occur. However, in infants with low haemoglobin levels before transfusion (haemoglobin < 6.0 mmol/l, haemoglobin < 97 g/l), cerebral FTOE was still higher at 24 hours after the transfusion, compared with the infants who had higher haemoglobin levels (haemoglobin > 6.0 mmol/l, haemoglobin > 97 g/l) before the transfusion. Our findings may have implications for the treatment of anaemia with RBC transfusions.

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Part 2

Maternal risk factors for disturbed cerebral oxygenation

chapter 9

Maternal antihypertensive drugs may influence cerebral oxygen extraction in preterm infants during the first days after birth

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provisionally accepted

Abstract

Objective To determine whether maternal antihypertensive drugs influenced cerebral oxygenation in preterm infants during the first days after birth.

Methods We included 49 preterm infants (median gestational age 30.3 weeks, (range 26.0 - 31.9), birth weight 1250 grams, (560 - 2250)). Regional cerebral oxygen saturation (r_cSO_2) was measured by near-infrared spectroscopy on postnatal days 1, 2, 3, 4, 5. Fractional tissue oxygen extraction (FTOE) was calculated using r_cSO_2 and arterial oxygen saturation (SpO_2) values: (SpO_2 - r_cSO_2)/SpO_2.

Results Nine mothers were treated with labetalol and/or $MgSO_4$ during pregnancy, three mothers with labetalol, $MgSO_4$, and nifedipine, and 19 mothers with nifedipine only. Eighteen infants served as controls. Multivariate linear regression analysis showed that exposure to labetalol and/or $MgSO_4$ during pregnancy decreased FTOE during days 1, 2, and 4 after birth, while nifedipine did not.

Conclusions Treating pregnant women with labetalol and/or $MgSO_4$ may influence cerebral oxygen extraction in their offspring during the first days after birth.

Introduction

Hypertension is a known complication in pregnancy and may result in maternal and neonatal morbidity and mortality.[1,2] The incidence varies in different parts of the world; for the Netherlands it is approximately 16%.[3] Hypertension with a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg, after the 20th week of gestation, is referred to as gestational hypertension (GH).[4] It may occur in isolation or in association with proteinuria (> 300 mg/ 24h) and possibly with multi-organ failure, in which case it is called pre-eclampsia (PE).[4] PE occurs in approximately 1.4% of Dutch pregnancies.[3]

Labetalol and $MgSO_4$ are used to treat GH and PE in pregnant women. Labetalol is an alpha-1 and beta-adrenoreceptor antagonist, yielding a hypotensive effect by causing peripheral vasodilation.[5,6] $MgSO_4$ also causes vasodilation, possibly by inhibiting the increase in intracellular calcium needed for smooth muscle cell contractions.[5,7] Nifedipine is used as a tocolytic agent. Since it is a calcium antagonist, however, it also influences blood pressure.[5] These drugs cross the placenta and may cause fetal and neonatal hypotension.[8] A lower blood pressure in the fetus and newborn infant is potentially harmful because it could affect oxygen supply to cerebral tissue, particularly if autoregulation fails.[9] Labetalol also depresses fetal and neonatal heart rate, and may therefore, possibly, depress cardiac output and cerebral perfusion as well.[5]

Near-infrared spectroscopy (NIRS) is a non-invasive method that may help to clarify the effect of maternal antihypertensive drugs in newborn infants.[10-16] NIRS measures regional cerebral tissue oxygen saturation (r_cSO_2). This measure reflects the oxygen saturation in a mixed vascular bed dominated by venules. R_cSO_2 serves as an indicator of cerebral hypoxic hypoxia.[12] Fractional tissue oxygen extraction (FTOE) is calculated on the basis of the values for r_cSO_2 and arterial oxygen saturation (SpO_2). It reflects the balance between cerebral oxygen supply and cerebral oxygen consumption.[13] FTOE serves as an indicator of cerebral ischemic hypoxia.[14]

Our aim was to determine the influence of maternal antihypertensive drugs on cerebral oxygenation in preterm infants during the first days after birth. Since FTOE serves better as a marker for ischemic hypoxia than r_cSO_2 alone - it being more independent of changes in arterial oxygen saturation - we focused on the effect of maternal antihypertensive drugs on FTOE. We hypothesized that as a result of a lower blood pressure that may subsequently affect cerebral perfusion FTOE during the first days after birth would be higher in infants exposed to maternal antihypertensive drugs during pregnancy.

Methods

Patient population

We performed an observational study. Forty-nine preterm infants admitted to the neonatal intensive care unit of University Medical Center Groningen were included. The selection criterion was a gestational age (GA) of < 32 weeks. Infants with major chromosomal defects, congenital abnormalities or germinal matrix hemorrhages were not included in the study.[11] The review board of University Medical Center Groningen approved the study. Written, informed parental consent was obtained in all cases.

Near-infrared spectroscopy

We used the INVOS 4100-5100 monitor (Somanetics Corporation, Troy, Michigan, USA) in combination with the pediatric SomaSensor to obtain r_cSO_2 values. The SomaSensor was placed on the left frontoparietal side of the infant's head and kept in place by an elastic bandage. A more detailed description of the method was described previously.[11]

R_cSO_2 was measured for a period of two hours (h) within the first 24h after birth and subsequently on days 2, 3, 4, and 5. Simultaneously, arterial oxygen saturation (SpO_2) was measured by pulse oximetry. We calculated FTOE with the equation FTOE = $(SpO_2 - r_cSO_2)/SpO_2$.[14,15]

Clinical variables

Prospectively, details on perinatal and neonatal characteristics that might influence hemodynamics were collected. These included GA, birth weight, small-for-gestational-age, birth asphyxia, early and late-onset sepsis, signs of circulatory failure, ventilatory status, patency of the ductus arteriosus and medication. While measuring r_cSO_2 and SpO_2, we also recorded the infant's heart rate, respiratory rate, blood gas values, glucose, and hemoglobin concentration. In addition, the infant's blood pressure was carefully recorded to determine whether or not neonatal hypotension had occurred.

Data from the medical charts on the occurrence of GH or pre-existing hypertension, PE, and the occurrence of complications such as the hemolysis, elevated liver enzymes, and low platelets (HELLP)-syndrome, as well as maternal use of antihypertensive drugs during pregnancy were collected. We recorded additional pregnancy-related variables such as intrauterine growth restriction, premature rupture of membranes (> 24h), signs of maternal intrauterine infection, and placental characteristics, and maternal medication other than antihypertensive drugs.

Statistical analysis

We used SPSS 18.0 software for Windows (SPSS Inc., Chicago, Illinois, USA) for statistical analyses. R_cSO_2 and SpO_2 values were collected every five seconds. Mean values for r_cSO_2, SpO_2, and FTOE, along with the other variables, were calculated for the two-hour recording periods. Data were tested for normality and showed a normal distribution.

Next, we categorized the infants into four groups according to maternal antihypertensive drugs: exposure to labetalol and/or $MgSO_4$, exposure to labetalol and/or $MgSO_4$ and nifedipine, exposure to nifedipine only, and no exposure to maternal antihypertensive drugs. The latter group served as control group. The association of maternal antihypertensive drugs with FTOE was determined using univariate linear regression analyses, separately for each group by creating dummy variables. Subsequently and where appropriate, the independent samples *t* test, 1-way ANOVA, and the Pearson correlation test (2-tailed) were used to determine which variables were associated with FTOE. The independent samples *t* test, Fisher exact test, χ^2-for-trend test, or the Mann-Whitney test were also used to compare between clinical variables of infants who had been exposed to maternal antihypertensive drugs and infants who had not been exposed to maternal antihypertensive drugs.

Finally, we used backward multiple linear regression analyses to determine which variables, detected by the univariate analyses with $P < .15$, were independently associated with FTOE throughout the analyses. A P value of $< .05$ was considered significant.

Results

Of 49 infants, seven mothers were treated with labetalol and $MgSO_4$, one mother had been treated with labetalol only, and one mother had been treated with $MgSO_4$ only. So, nine infants had been exposed to labetalol and/or $MgSO_4$. Three infants had been exposed to labetalol, $MgSO_4$, and nifedipine. Nineteen infants had been exposed to nifedipine only.

Maternal and neonatal characteristics

Table 1 presents an overview of the maternal and neonatal characteristics. None of the infants died during the study period. Of the nine mothers who had been treated with labetalol and/or $MgSO_4$, eight mothers had PE and one mother had GH. Two mothers continued to use labetalol after discharge from hospital. The others stopped taking labetalol and/or $MgSO_4$ after median 4 days (range 1 - 6 days). Of the three mothers who had been treated with labetalol, $MgSO_4$, and nifedipine, two had GH and one had PE. None of the mothers who had been treated with nifedipine only had developed either GH or PE.

The course of PE and GH was complicated by HELLP-syndrome in six mothers, four of whom had been treated with labetalol and/or $MgSO_4$. Two mothers had not received antihypertensive drugs.

Regarding medication other than hypertensive drugs, four mothers had been treated with antidepressants. Of these four, one had not received antihypertensive drugs, two had received labetalol and/or $MgSO_4$ and nifedipine, and one mother had received nifedipine only. Seven mothers had been treated with antibiotics, six of whom had also been treated with nifedipine. Forty-four mothers had received betamethasone to improve lung maturation of the fetus, six of whom had been treated with labetalol and/or $MgSO_4$, three with labetalol, $MgSO_4$, and nifedipine, and nineteen had been treated with nifedipine only.

Influence of maternal antihypertensive drugs on FTOE

Figure 1 illustrates the course of FTOE during the first five days after birth. Infants who had been exposed to labetalol and/or $MgSO_4$ during pregnancy had the lowest FTOE values, varying between days from median 0.07 to 0.14. For the infants who had been exposed to nifedipine only, FTOE varied between days from median 0.14 to 0.17. FTOE for infants who had been exposed to labetalol, $MgSO_4$, and nifedipine during pregnancy, varied from median 0.06 to 0.17. FTOE was highest in infants who had not been exposed to maternal antihypertensive drugs and varied from median 0.15 to 0.22. For days 1, 2, and 4, linear regression analysis showed that exposure to labetalol and/or $MgSO_4$ was associated with lower FTOE ($P < .05$) (Table 2).

TABLE 1. Neonatal and maternal characteristics

Treatment with	labetalol and/or $MgSO_4$	labetalol and/or $MgSO_4$, nifedipine	nifedipine only	no maternal antihypertensive drugs
Female/Male	4/5	2/1	11/8	6/12
Gestational age, weeks	29.9 (26.0-31.7)	27.9 (26.3-27.9)	30.3 (26.0-31.6)	30.4 (27.0-31.9)
Birth weight, grams	1215 (615-1600)	730 (730-980)	1340 (830-2047)	1263 (560-2250)
Head circumference, centimeters	28.2 (22.8-29.5)	24.5 (23.0-24.5)	27.4 (23.0-30.0)	27.0 (22.8-30.6)
Intrauterine growth restriction	2	1	1	5
Asphyxia	0	1	2	1
Early-onset sepsis	0	0	0	3
Late-onset sepsis	4	2	6	4
Circulatory failure†				
Fluid resuscitation	4	2	9	6
Inotropes,	0	1	0	3
Ventilatory status††				
Mechanical ventilation	4	3	8	9
CPAP	5	0	7	9
Low flow or no support	0	0	4	0
Patent ductus arteriosus	2	2	7	8
Heart rate††	143 (140-155)	141 (135-145)	149 (137-159)	149 (138-165)
Mean arterial blood pressure††	44 (41-46)	33 (31-34)	34 (28-55)	34 (25-40)
Gestational hypertension	1	2	0	2
Pre-eclampsia	8	1	0	2
HELLP syndrome	4	0	0	2
Premature rupture of membranes	1	0	8	2
Placental inflammation				
Fetal side	1	0	4	2
Maternal side	1	0	5	1

Data are expressed as median (range) or as number (percentage) unless otherwise specified. † indicates during the first 48 hours after birth; ††, during the first day after birth; CPAP, continuous positive airway pressure; HELLP-syndrome, hemolysis, elevated liver enzymes, and low platelets syndrome.

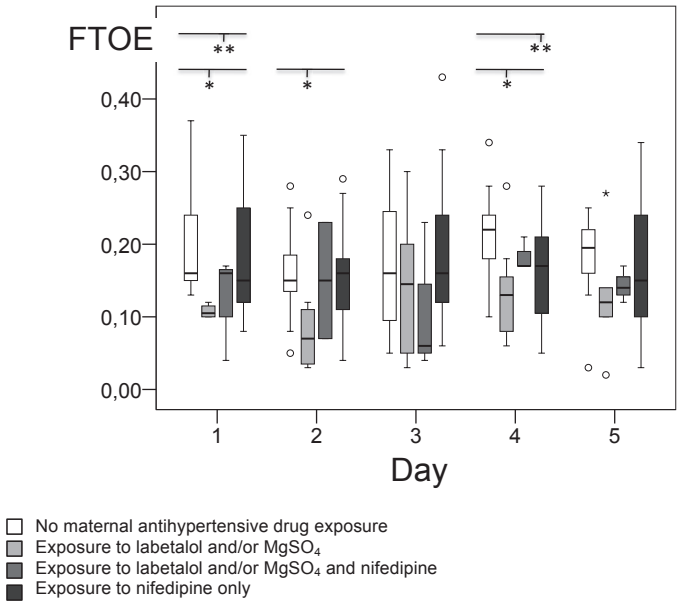

FIGURE 1. The effect of labetalol and/or $MgSO_4$ on FTOE in preterm infants exposed to labetalol and/or $MgSO_4$ versus no exposure, or exposure to labetalol, $MgSO_4$, and nifedipine, or exposure to nifedipine only
Data are shown in box and whisker plots. The boxes represent the individual values between the 25th and 75th centile (interquartile range), the whiskers represent the range of the values, with the exception of outliers. Circles represent outliers, stars extreme outliers. Differences between the groups are marked by * (** ≤ 0.15, * ≤ 0.05, exposure to maternal labetalol and/or $MgSO_4$ exposure versus no exposure, or exposure to labetalol, $MgSO_4$, and nifedipine, or exposure to nifedipine only during pregnancy).

Associations between maternal antihypertensive drugs and perinatal and neonatal variables

The association between exposure to labetalol and/or $MgSO_4$ or nifedipine and lower birth weight nearly reached significance ($P = .076$). Infants who had been exposed to all the antihypertensive drugs had the lowest birth weight (mean 813 grams, SD 120), then came the infants who had been exposed to labetalol and/or $MgSO_4$ (mean 1136 grams, SD 308), followed by infants who had not been exposed to antihypertensive drugs (mean 1349 grams, SD 482). Infants who had been exposed to nifedipine only had a birth weight of mean 1400 grams, SD 344. Neither head circumference nor any of the other variables tested, differed between the groups.

Relationship between FTOE and perinatal and neonatal variables

Blood pressure, P_aCO_2, hemoglobin concentration, heart rate, respiratory rate, ductal patency, and ventilatory status during measuring did not correlate with FTOE, nor did the relationship between FTOE and respiratory rate on day 1 ($\rho = 0.269$, $P = .125$), placental inflammation on the fetal side and FTOE on day 1 ($P = .146$), and FTOE and hemoglobin concentration on day 2 ($\rho = -0.351$, $P = .057$). Two infants developed clinical hypotension even though neither had been exposed to any antihypertensive drugs during pregnancy.

TABLE 2. Linear regression analysis for FTOE and exposure to antihypertensive drugs on days 1, 2, 3, 4, and 5 after birth

Exposure to	B	Std. error	β	*t*	*P* value	95% CI
Day 1						
No exposure	.19	.02		10.05	.000	.15 to .23
Labetalol and/or $MgSO_4$	-.09	.04	-.37	-2.10	.044*	-.17 to -.00
Nifedipine only	-.02	.03	-.13	-0.72	.480	-.08 to .04
Labetalol and/or $MgSO_4$ and nifedipine	-.07	.05	-.27	-1.52	.138	-.16 to .02
Day 2						
No exposure	.16	.02		9.63	.000	.13 to .19
Labetalol and/or $MgSO_4$	-.07	.03	-.40	-2.48	.017*	-.13 to -.01
Nifedipine only	-.00	0.2	-.02	-.09	.929	-.05 to .04
Labetalol and/or $MgSO_4$ and nifedipine	-.01	0.5	-.03	-.17	.870	-.11 to .09
Day 3						
No exposure	.17	.02		7.35	.000	.13 to .22
Labetalol and/or $MgSO_4$	-.03	.05	-.11	-.64	.529	-.12 to .06
Nifedipine only	.01	.03	.07	.40	.689	-.05 to .08
Labetalol and/or $MgSO_4$ and nifedipine	-.06	.06	-.18	-1.07	.291	-.18 to .06
Day 4						
No exposure	.21	.02		12.61	.000	.17 to .25
Labetalol and/or $MgSO_4$	-.08	.03	-.43	-2.58	.014*	-.14 to -.02
Nifedipine only	-.05	.02	-.33	-1.94	.060	-.09 to .00
Labetalol and/or $MgSO_4$ and nifedipine	-.03	.04	-.11	-.67	.508	-.11 to .06
Day 5						
No exposure	.18	.02		8.72	.000	.14 to .22
Labetalol and/or $MgSO_4$	-.05	.04	-.24	-1.36	.183	-.13 to .03
Nifedipine only	-.01	.03	-.09	-.48	.634	-.07 to .04
Labetalol and/or $MgSO_4$ and nifedipine	-.04	.05	-.13	-.73	.467	-.14 to .06

* Indicates $P < .05$. B indicates un-standardized coefficient, Std. error; standard error, β; standardized coefficient.

Multiple linear regression analysis

Multiple linear regression analysis was used to determine whether antihypertensive drugs contributed independently to FTOE. Factors that had shown associations with FTOE at $P < .15$ were entered as predictors (Table 3). These included exposure to labetalol and/or $MgSO_4$, exposure to labetalol, $MgSO_4$, and nifedipine, respiratory rate, and placental inflammation on day 1; exposure to labetalol and/or $MgSO_4$ and hemoglobin concentration on day 2; and exposure to labetalol and/or $MgSO_4$ and exposure to nifedipine only on day 4. Following backward selection, only exposure to labetalol and/or $MgSO_4$ remained significant in the model on day 1 ($P = .006$). On day 2, exposure to labetalol and/or $MgSO_4$ ($P = .053$) and hemoglobin concentration ($P = .073$) remained nearly significantly in the model. On day

4, exposure to labetalol and/or $MgSO_4$ ($P = .016$) and exposure to nifedipine ($P = .074$) remained in the model. The multivariate models explained 40% of the variance on day 1, 25% on day 2, and 16% on day 4.

TABLE 3. Multiple linear regression models for FTOE and exposure to antihypertensive drugs on days 1, 2, and 4 after birth

Exposure to	B	Std. error	β	*t*	*P* value	95% CI
Day 1						
constant	.19	.02		12.8	.000	.16 to .22
Labetalol and/or $MgSO_4$	-.09	.03	-.55	-3.1	.006*	-.16 to -.03
Placental inflammation on the fetal side	-.05	.03	-.31	1.74	.099	-.01 to .10
Day 2						
constant	.29	.07		4.26	.000	.15 to .43
Labetalol and/or $MgSO_4$	-.06	.03	-.34	-2.03	.053	-.13 to .00
Hemoglobin concentration	-.01	.01	-.32	-1.86	.073	-.03 to .00
Day 4						
constant	.21	.02		13.61	.000	.18 to .24
Labetalol and/or $MgSO_4$	-.07	.03	-.40	-2.51	.016*	-.13 to -.01
Nifedipine only	-.04	.02	-.29	-1.83	.074	-.09 to .00

* Indicates $P < .05$. B indicates un-standardized coefficient, Std. error; standard error, β; standardized coefficient.

Discussion

Our study demonstrated that preterm infants who had been exposed to labetalol and/or $MgSO_4$ during pregnancy had lower FTOE on days 1, 2, and 4 after birth than infants not exposed. This finding was not in line with our hypothesis. We expected FTOE to be higher, and not lower, during the first days after birth in infants who had been exposed to maternal antihypertensive drugs. Regarding nifedipine, we found no difference in FTOE between the exposed and non-exposed infants.

We propose several explanations for our findings. Decreased FTOE can be explained either by an increased supply of oxygen or decreased oxygen consumption.[15,16,17] Increased supply of oxygen could be the result of increased cerebral blood flow (CBF). In 31 infants whose mothers had been treated with antihypertensive drugs, Kluckow *et al.* found higher superior vena cava flow during the first 24h after birth.[16] This is in line with our results since superior vena cava flow is a reflection of CBF.[17] They suggested that antihypertensive drugs possibly protect the brain against a low superior vena cava flow shortly after birth due to vasodilation, which off-loads the adapting preterm heart. We speculate that this vasodilation might have led to relatively luxury brain perfusion, explaining the decreased FTOE. Possibly, luxury perfusion declined during the first days after birth as labetalol gradually disappeared.

The effect of labetalol on mothers and fetuses has been widely studied. Labetalol decreases cerebral perfusion pressure in pregnant women.[18] Most studies concerning the fetus, however, determined the uteroplacental blood flow on the maternal side, which is not influenced by labetalol.[6,19,20] Two studies

reported that labetalol does not influence the fetal middle cerebral artery flow.[20,21] Another study reported mild transient hypotension in term neonates which resolved within 24h after birth.[8] Labetalol is an alpha-1 and beta-adrenoreceptor antagonist. It poses a risk to the infants of beta-blockade.[8,22,23] No difference in clinical characteristics due to beta-blockade between groups was found. Since we only measured two hours a day, we could have missed some events. The two infants who had hypotension during measurements, were infants who had not been exposed to antihypertensive drugs during pregnancy. That we did find a difference in FTOE anyway, can be explained by the fact that CBF is poorly predicted by systemic blood pressures.[17]

Previous studies with regards to $MgSO_4$ reported conflicting results. $MgSO_4$ causes vasodilation, possibly by inhibiting the increase in intracellular calcium needed for smooth muscle cell contractions.[5] Two studies found no difference in middle cerebral artery velocities in preterm fetuses after $MgSO_4$ exposure.[7,23] In contrast, in a study involving 19 preterm infants, Rantonen *et al.* found a decrease in cerebral perfusion pressure on the first day after birth.[24] During the first week after birth, however, they found increased cerebral perfusion pressure and blood flow.[24] This latter finding is in support of our findings.

Another explanation for lower FTOE could be decreased oxygen consumption. Brady and Williams reported neurological depression following exposure to $MgSO_4$ during pregnancy.[25] Ayromlooi *et al.*, who studied fetal lambs following $MgSO_4$ exposure, found no change in CBF, but a temporary decrease in cerebral oxygen consumption.[26] These findings support our assumption that decreased oxygen consumption may, at least partly, explain lower FTOE.

No association was found between FTOE and nifedipine. Perhaps this is explained by a different working mechanism of nifedipine and the fact that it was administered with a view to postponing delivery rather than lowering blood pressure. Several studies reported that nifedipine does not affect uteroplacental, fetal, and neonatal circulation.[27-29] Our data confirm the studies on the neonatal circulation.

The question remains whether our findings can be explained by exposure to labetalol and/or $MgSO_4$. Belfort *et al.* reported that mothers with PE have decreased oxygen consumption.[30] To our knowledge no studies have reported on decreased oxygen consumption in the fetus or neonate following PE. It is a known fact that placental blood flow is reduced and uteroplacental resistance is increased in hypertensive pregnancies.[20,28] This poses a risk to the infants of hypoxia, leading to brain sparing. If so, increased CBF may not have been the result of treatment but rather due to brain sparing. It would appear that this presumption is supported by our findings concerning birth weight and head circumference. Infants exposed to all antihypertensive drugs as well as in infants who had been exposed to labetalol and/or $MgSO_4$ tended to have a lower birth weight, while head circumference did not differ between the groups.

Our findings could also be explained by the underlying hypertensive disease of the fetus and newborn infant following maternal hypertension during pregnancy. Rasmussen found a high resistance in the peripheral fetal circulation and a reduced flow in the fetal aorta, suggesting that fetuses might have hypertension themselves during hypertensive pregnancies.[31] The problem was resolved by

administering labetalol. This caused peripheral vasodilation by decreasing the high resistance and yielded increased blood flow, possibly also to the brain. Once the infant is born, the vessels are still dilated yielding luxury perfusion. After a couple of days the infant adapts to the extrauterine environment and neither luxury perfusion nor decreased FTOE are evident any longer.

We recognize several limitations of our study. Although our findings are well explained in terms of increased CBF, it remains a speculative explanation because we did not actually measure CBF. Another limitation is that only a small number of infants who had been exposed to maternal antihypertensive drugs participated in this study. A larger study is required to confirm our results. Since seven out of nine mothers who had been exposed to labetalol and/or $MgSO_{4,}$ had been exposed to both drugs, we could not determine the effect of each drug separately. We stress the fact that it is unlikely that confounders could be held responsible for the differences in FTOE between the groups. No differences between the groups with regard to clinical variables, especially mean arterial blood pressure, heart rate, and SpO_2 were found. On day 2, hemoglobin concentration remained in the model, influencing FTOE, albeit not significantly. Lower FTOE on day 4 might have been the result of markedly higher FTOE values in the control infants compared to the other days, especially during the first three days after birth. We could not explain this higher FTOE even though we analyzed all possible clinical variables.

In summary, treatment of pregnant women with labetalol and/or $MgSO_4$ was associated with reduced cerebral oxygen extraction during the first days after birth. Whether this phenomenon will have consequences for the infant's neurobehavioral outcome has yet to be researched. Our findings might have implications for clinical practice. They provide yet another reason for the necessity of carefully monitoring infants who have been exposed to labetalol and/or $MgSO_4$ during pregnancy. When these infants are exposed to other clinical conditions that influence cerebral hemodynamics, previous exposure to labetalol and/or $MgSO_4$ could increase their vulnerability to cerebral sequelae.

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Chapter 7

Prenatal tobacco exposure influences cerebral oxygenation in preterm infants

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Abstract

Aim Our aim was to determine the influence of prenatal tobacco exposure on regional cerebral tissue oxygen saturation (r_cSO_2) and fractional tissue oxygen extraction (FTOE) in preterm infants. We hypothesised that as a result of vasoconstriction caused by prenatal tobacco exposure r_cSO_2 would be lower and FTOE would be higher during the first days after birth in infants exposed to tobacco during pregnancy.

Methods Sixty preterms were included in this prospective, observational cohort study (median gestational age 29.9 weeks, range 26.0 - 31.8, median birth weight 1248 grams, range 615 - 2250). Fourteen infants had been exposed to tobacco during pregnancy. All mothers smoked more than five cigarettes a day till delivery. We measured r_cSO_2 and transcutaneous arterial oxygen saturation ($tcSaO_2$) in all infants on days 1 - 5, 8, and 15. FTOE was calculated: FTOE = ($tcSaO_2$ - r_cSO_2)/ $tcSaO_2$.

Results In preterm infants exposed to tobacco during pregnancy, r_cSO_2 was lower during days 1, 2, and 8 after birth, median 73% versus 81%, 73% versus 80% and 71% versus 78% respectively. FTOE was higher during days 1 and 8 after birth, median 0.24 versus 0.15 and 0.26 versus 0.19 respectively. On the second day, FTOE tended to be higher, 0.18 versus 0.14.

Conclusions During the first two days and day 8 after birth cerebral oxygen saturation is lower and oxygen extraction higher in preterm infants following prenatal tobacco exposure. Our data suggest that prenatal tobacco exposure may have an effect on cerebral oxygenation of the infant.

Introduction

Tobacco exposure still accounts for significant morbidity and mortality in the foetus and infant. This might be mediated by placental conditions that affect oxygenation of the foetus. It is suggested that smoking compromises foetal placental blood flow due to increased vascular resistance of the placenta from the foetal side.[1] One out of five foetuses exposed to tobacco are possibly in a state of chronic hypoxia.[2] Tobacco exposure during pregnancy increases the risk for preterm birth, low birth weight, smaller head circumference, neurological deficits, sudden infant death syndrome, and adverse neurobehavioral outcome in the long term, including attention deficit disorders and hyperactivity.[3-6]

In the Netherlands, 14% of pregnant women smoke.[7] Tobacco smoke contains more than 4000 chemical compounds.[8] Major foetal neurotoxic components in cigarette smoke are nicotine, carbon monoxide (CO), and lead.[3,4] CO is an important hypoxaemia mediating agent since it has a higher affinity to bind to maternal and foetal haemoglobin compared to oxygen. It forms carboxyhaemoglobin, which may result in chronic cellular hypoxia in the foetus.[3,4] Nicotine affects smooth muscle cells and causes abnormal vascular responses in animals.[9] Recently it was found that nicotine increases foetal cerebrovascular resistance.[10] However, the effects of nicotine on human vascular resistance and foetal development remain controversial.[3]

A non-invasive method that could help to determine the effect of tobacco exposure is near-infrared spectroscopy (NIRS).[11] NIRS measures the regional cerebral tissue oxygen saturation (r_cSO_2).[12-15] This measure reflects the oxygen saturation in a mixed vascular bed dominated by venules. R_cSO_2 serves as an indicator of cerebral hypoxic hypoxia. Fractional tissue oxygen extraction (FTOE) is calculated on the basis of the values for r_cSO_2 and transcutaneous arterial oxygen saturation ($tcSaO_2$)[13-16] and reflects the balance between cerebral oxygen supply and cerebral oxygen consumption. FTOE serves as an indicator of cerebral ischaemic hypoxia.[14,17]

To clarify the effects of prenatal tobacco exposure on newborn infants, especially in the brain, we designed this study. Our aim was to determine the influence of prenatal tobacco exposure on r_cSO_2 and FTOE in preterm infants. We hypothesised that as a result of chronic vascular changes caused by prenatal tobacco exposure r_cSO_2 would be lower and FTOE would be higher during the first days after birth in infants exposed to tobacco during pregnancy.

Methods

Patient population

We performed a prospective, observational, longitudinal study. Initially, we selected 80 preterm infants admitted to the neonatal intensive care unit (NICU) of the University Medical Center Groningen between May 2006 and March 2008. The selection criterion was a gestational age of less than 32 weeks. Infants with major chromosomal or congenital abnormalities were not included in the study group. Later, we excluded 20 infants from further analysis that had developed a germinal matrix haemorrhage or a periventricular haemorrhagic infarction. These conditions might interfere with cerebral oxygenation.[18] Our final study group, therefore, consisted of 60 preterm infants. In this group, we determined whether

the mothers of the infants had smoked or not. The study was approved by the review board of the University Medical Center Groningen and written informed parental consent was obtained in all cases.

Prenatal tobacco exposure

To determine whether infants had been exposed to tobacco prenatally, their mothers were asked whether they had smoked during pregnancy and if so, how many cigarettes they had smoked a day. They were questioned by their gynaecologist or a NICU nurse.

Near-infrared spectroscopy

We used the INVOS 4100 monitor (Somanetics Corporation, Troy, Michigan, USA) in combination with the paediatric SomaSensor to measure r_cSO_2 values. This technology is based on the fact that biological tissue is relatively transparent to near-infrared light (600 - 900 nm wavelength). The optical sensor measures the quantity of reflected light photons as a function of two wavelengths (730 and 805 nm) and determines the spectral absorption of the underlying tissue.[13,19] Due to the fact that oxygenated haemoglobin and deoxygenated haemoglobin have distinct absorption spectra, NIRS can differentiate between the two. The ratio of oxygenated haemoglobin to total haemoglobin reflects the regional oxygen saturation of cerebral tissue. The SomaSensor has two detectors at a distance of 3 and 4 cm from the near-infrared optode. The detector placed 3 cm from the optode receives light scattered predominantly from the scalp and skull. The detector placed at 4 cm receives light scattered from the scalp, skull, and cerebral tissue. Thus, by subtraction, the two detectors measure the oxygen saturation in the underlying cerebral tissue.

R_cSO_2 was measured within the first 24 hours of birth and subsequently on the 2nd, 3rd, 4th, 5th, 8th, and 15th days. On these days, r_cSO_2 was measured over a two-hour period. Infants were in the incubator in supine position while at rest or asleep. Fifteen minutes were allowed for stabilisation of the measurement. The optical sensor was placed to the left frontoparietal side of the infant's head and held in place by elastic bandaging. We marked the location of the sensor to ensure that the sensor was placed in the same position for each measurement. Simultaneously, we measured transcutaneous oxygen saturation ($tcSaO_2$) by pulse oximetry. We calculated FTOE with the equation $FTOE = (tcSaO_2 - r_cSO_2)/tcSaO_2$.[13,14,16] FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen consumption.[14,17] FTOE serves better as a marker for ischaemic hypoxia than r_cSO_2 alone since it is more independent of changes in arterial oxygen saturation.

Clinical variables

Prospectively, we collected details on perinatal and neonatal characteristics that might influence haemodynamics. These included gestational age, birth weight, birth asphyxia, early-onset and late-onset sepsis, signs of circulatory failure, ventilatory status, and medication. Patency of the ductus arteriosus was routinely determined by echocardiography on the 3rd to 5th days after birth if the infants were artificially ventilated, needed continuous positive airway pressure, or had other clinical signs suggesting a patent ductus arteriosus. Maternal and pregnancy-related variables included medication or intoxications, intrauterine growth restriction, premature rupture of membranes (> 24 hours), pre-

eclampsia, and signs of maternal intrauterine infection. The placenta was examined for histological characteristics of inflammation.

An Apgar score of less than 5 at 5 min or resuscitation or both (external heart massage or use of epinephrine or both), and an umbilical cord pH (arterial pH < 7.10) were considered as signs of birth asphyxia. Early-onset sepsis was diagnosed by clinical signs such as increased rate of apnoeas, lethargy, increased capillary refill time and fever or by a positive blood culture or both within the first 48 hours after birth. Late-onset sepsis was diagnosed from 48 hours after birth. Circulatory failure was defined as haemodynamic instability, based on clinical signs as tachycardia, low blood pressure, oliguria, and increased capillary refill time and scored by the need for volume administration or the use of inotropes or both during the first 24 hours after birth. Intrauterine growth restriction was scored if birth weight was below the 10th centile according to the Dutch intrauterine growth standards.[20] Maternal intrauterine infection was based on clinical signs such as foetal tachycardia and maternal fever (< 38 °C), often combined with the mother taking antibiotics.

At the same time as measuring r_cSO_2 and $tcSaO_2$, the infant's heart rate, respiratory rate, blood pressure, blood gas values, and haemoglobin concentration were recorded.

Statistical analysis

We used SPSS 14.0 software for Windows (SPSS Inc., Chicago, Illinois, USA) for the statistical analyses. R_cSO_2 and $tcSaO_2$ values were collected every 5 seconds. The mean and median values for r_cSO_2, $tcSaO_2$, and FTOE, along with the other variables, were calculated for the two-hour recording periods. The median values were analysed by the Mann-Whitney *U* test for non-normal distributions. The Spearman Rank Order Correlation test (two-tailed) was used to determine correlations between the clinical and NIRS parameters during the first two weeks after birth in infants exposed to maternal tobacco smoking during pregnancy and infants that had not been exposed. When appropriate, proportions of categorical data were tested by the Fisher's exact-test or the X^2-for-trend test. The Kruskal-Wallis H test was used to determine a dose-response relationship between the number of cigarettes a day during pregnancy (none, less than ten, more than ten) and NIRS parameters. A *P* value < 0.05 was considered significant.

Results

Out of 60 infants, 14 infants had been exposed to tobacco during pregnancy. Perinatal and neonatal characteristics were similar for infants exposed to tobacco and infants not exposed to tobacco (Table 1). The group of infants exposed to tobacco prenatally consisted of two sets of twins and ten singletons. All 12 mothers who had smoked during pregnancy had smoked more than five cigarettes a day until delivery, while six mothers had smoked more than ten cigarettes a day until delivery. The mothers of the infants that were not exposed to tobacco during pregnancy did not smoke any cigarettes. Five infants died before the 15th day after birth. Of the infants who had been exposed to tobacco prenatally, two infants died: one of asphyxia on the 4th day and the other of combined respiratory and circulatory failure on the 13th day. Three infants that had not been exposed to tobacco prenatally died of combined respiratory and circulatory failure on the 6th day, a massive lung bleeding on the 10th day, or circulatory failure on the 11th day.

TABLE 1. Perinatal and neonatal characteristics

	Tobacco Exposure	No Tobacco Exposure
Number	14	46
Gestational age, weeks	30.4 (28.7-31.1)	29.1 (27.9-30.9)
Birth weight, grams	1288 (1075-1593)	1235 (1049-1563)
Female/Male	10/4*	21/25
Apgar score at 5 minutes	9 (6-9)	8 (7-8)
Umbilical cord pH	7.23 (7.14-7.28)	7.22 (7.14-7.31)
Head circumference, centimeters	26.9 (23.4-29.5)	27.0 (22.8-30.6)
Small-for-gestational age, (%)	4 (29)	4 (9)
Maternal pre-eclampsia, (%)	1 (7)	10 (22)
Premature rupture of membranes, (%)	4 (29)	11 (24)
Intrauterine infection, (%)	0 (-)	5 (11)
Early-onset sepsis, (%)	1 (7)	3 (7)
Late-onset sepsis, (%)	4 (29)	13 (28)
Circulatory failure		
Fluid resuscitation, (%)	6 (43)	18 (39)
Inotropes, (%)	1 (7)	7 (15)

Data are expressed as median (p25 - p75) or as numbers unless otherwise specified. Differences between groups are marked by * ($P < 0.05$ prenatal tobacco exposure versus no prenatal tobacco exposure during pregnancy).

The influence of prenatal tobacco exposure on r_cSO_2, FTOE and $tcSaO_2$

On the first day after birth we found a lower r_cSO_2 in infants that had been exposed to tobacco prenatally (median 73% versus 81%, tobacco versus no tobacco, Mann-Whitney *U* test, $P = 0.005$, Figure 1A). FTOE was higher in infants exposed to tobacco prenatally (median 0.24 versus 0.15, tobacco versus no tobacco, Mann-Whitney *U* test, $P = 0.042$, Figure 1B). $TcSaO_2$ did not differ between the two groups (median 94% versus 96%, tobacco versus no tobacco, Mann-Whitney *U* test, $P = 0.131$, Figure 1C). On the second day after birth, we again found a lower r_cSO_2 in infants exposed to tobacco prenatally (median 73% versus 80%, tobacco versus no tobacco, Mann-Whitney *U* test, $P = 0.022$). We found that FTOE tended to be higher in infants exposed to tobacco prenatally (median 0.18 versus 0.14, tobacco versus no tobacco, Mann-Whitney *U* test, $P = 0.094$). $TcSaO_2$ was lower in infants exposed to tobacco prenatally (median 91% versus 94%, tobacco versus no tobacco, Mann-Whitney *U* test, $P = 0.035$). From the 3rd day onwards we found no differences in r_cSO_2, FTOE, or $tcSaO_2$ between the group exposed and the group that had not been exposed to tobacco prenatally, except on the 8th day (r_cSO_2 median 71% versus 78%, tobacco versus no tobacco, Mann-Whitney *U* test, $P = 0.029$, FTOE median 0.26 versus 0.19, tobacco versus no tobacco, Mann-Whitney *U* test, $P = 0.042$, $tcSaO_2$ median 96% versus 96%, tobacco versus no tobacco, Mann-Whitney *U* test, $P = 0.735$). When we excluded the infants that had died, we found the same results: significant differences on days 1, 2, and 8 for r_cSO_2, FTOE, and $tcSaO_2$.

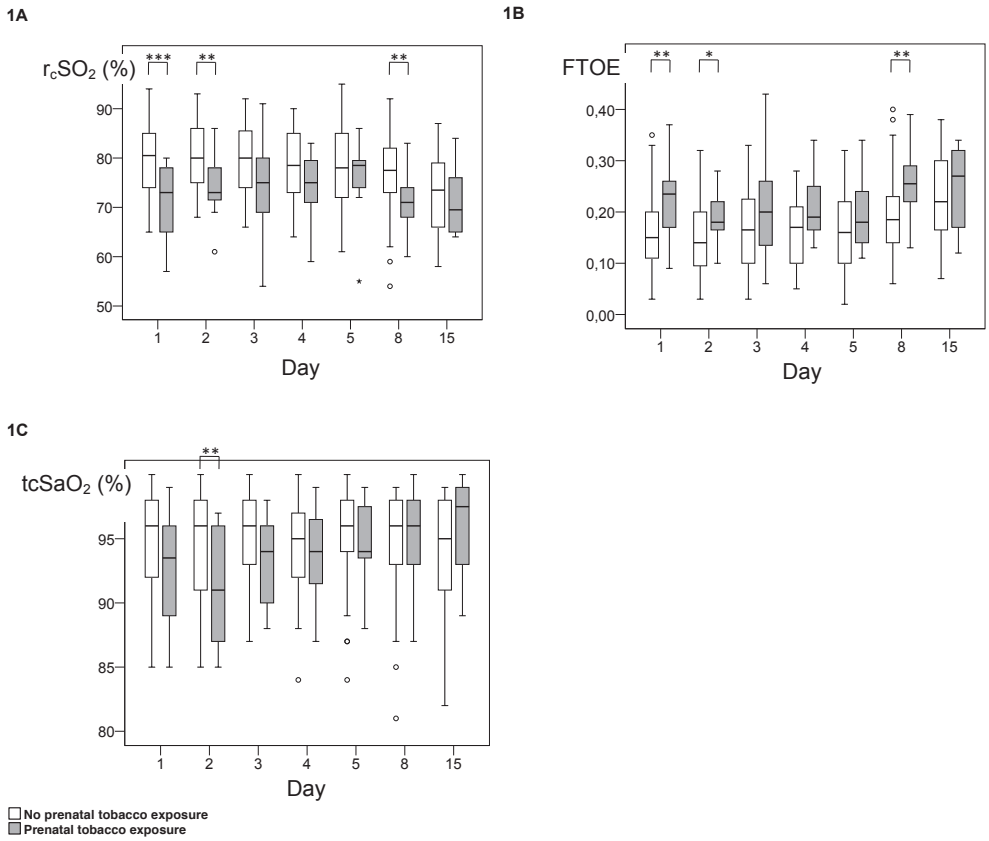

FIGURE 1. Course of r_cSO_2 (A), FTOE (B), and $tcSaO_2$ (C) in preterm infants following prenatal tobacco exposure versus no exposure

Data are shown in box and whisker plots. Dots and stars represent outliers. Differences between the two groups are marked by * (*** ≤ 0.005, ** ≤ 0.05, * ≤ 0.1, prenatal tobacco exposure versus no prenatal tobacco exposure).

The dose-response relationship between the amount of tobacco exposure and r_cSO_2 and FTOE

On the 1st, 2nd, and 8th days we found a dose-response relationship between the number of cigarettes mothers had smoked daily and r_cSO_2 and FTOE (Figure 2). When we divided exposure into three groups, i.e. no exposure, exposure to less than ten cigarettes a day, and exposure to ten or more cigarettes a day, we found the largest effect on the 8th day (Kruskal-Wallis H; $P = 0.019$ for r_cSO_2 and $P = 0.026$ for FTOE). Infants exposed to ten or more cigarettes had the lowest r_cSO_2 (median 69% versus 74% and 78% for exposure to ten or more cigarettes, less than ten cigarettes, and no prenatal exposure to tobacco) and the highest FTOE (median 0.29 versus 0.22 and 0.19 for exposure to ten or more cigarettes, exposure to less than ten cigarettes, and no prenatal exposure to tobacco). We found no dose-response relationship between the extent of exposure to tobacco and $tcSaO_2$.

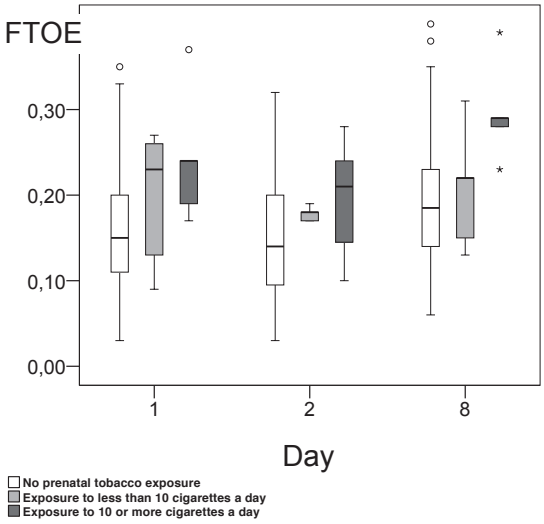

FIGURE 2. Dose-response relationship between the amount of tobacco exposure and FTOE
Data are shown in box and whisker plots. Dots and stars represent outliers.

The relationship between r_cSO_2 and FTOE and perinatal and neonatal variables

In order to investigate whether other variables during the perinatal period had confounded our findings on cerebral haemodynamics, we checked maternal medication and other intoxications, pre-eclampsia, premature rupture of membranes, signs of placental inflammation, and asphyxia. We found no differences between the groups. We also found no difference between the groups with regard to the presence of a patent ductus arteriosus based on clinical signs and confirmed by echocardiography. In addition, we checked blood pressure, $PaCO_2$, haemoglobin concentration, heart rate, respiratory rate, and ventilatory status during the two-hour period of measurement. These variables did not correlate with r_cSO_2 or FTOE measured simultaneously. We found no differences between the two groups on any of these measurements except for day 15 when the infants who were not exposed to tobacco during pregnancy had a higher heart rate compared to the infants who were exposed to tobacco during pregnancy (Table 2).

TABLE 2. Neonatal characteristics

Days	1 - 5		8		15	
	Tobacco exposure – No exposure		Tobacco exposure – No exposure		Tobacco exposure – No exposure	
Number	14	46	14	45	13	43
Haemoglobin concentration, mmol/l	10.5	10.0	7.8	8.4	7.3	8.0
$PaCO_2$, kPa	4.6†	4.4†	5.6	5.8	5.5	6.0
Mean arterial blood pressure, mmHg	36†	34†	33	36	41	40
Heart rate, beats per minute	146‡	150‡	154	153	148	159*
Patent ductus arteriosus	7	17	3	5	3	3
Respiratory rate, breaths per minute	49‡	48‡	43	45	44	47
Ventilatory support						
Mechanical ventilation	7§	25§	3	3	2	3
CPAP	7§	19§	4	17	1	7
Low flow	0§	2§	5	15	3	12
Supplemental oxygen	5§	6§	2	3	1	2

Data are expressed as median or as numbers unless otherwise specified. Differences between groups are marked by * ($P < 0.05$ prenatal tobacco exposure versus no prenatal tobacco exposure). † indicates median lowest value over days 1 – 5, ‡; median value over days 1 – 5, §; number for day 1.

Discussion

Our study demonstrated that preterm infants that had been exposed to tobacco during pregnancy had a lower r_cSO_2 and a higher FTOE on the days 1, 2, and 8 after birth in comparison to infants that had not been exposed to tobacco during pregnancy. This finding was in line with our hypothesis: we expected r_cSO_2 to be lower and FTOE to be higher during the first days after birth in infants that had been exposed to tobacco during pregnancy.

We offer several possible explanations for our findings. As FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen consumption, increased FTOE can be explained either by a lower supply of oxygen or increased oxygen consumption.[14,17,21,22] A lower supply of oxygen could be the result of lower cerebral blood flow (CBF). In foetuses the vascular resistance on the foetal side of the placenta and cerebrovascular resistance are believed to be increased after tobacco exposure.[1,10] This may be mediated by either repetitive vasoconstriction or proliferative vascular changes. In newborn infants the arterial oxygen saturation and tissue oxygen saturation are affected by prenatal exposure to tobacco.[23,24] Furthermore, the microcirculatory function of newborn infants that had been exposed to tobacco prenatally was found to be impaired during the first 48 hours after birth.[25] Previously, increased CBF velocities at 20 to 42 hours after birth were found in newborn infants exposed to tobacco prenatally.[26] As a result of these changes in cerebrovascular resistance, cerebral perfusion may become impaired, resulting in an increase of FTOE. Nicotine may mediate this process. The implications of prenatal exposure to tobacco in preterm infants may be even higher since the half-life of nicotine is longer in newborn infants than in adults (9 to 11 hours versus 1 to 3 hours).[27-29] The effect might also be mediated by cotinine, the primary metabolite of nicotine, which has vasoactive properties on its own.[30] Cotinine has a longer half-life than nicotine and ranges from 15 to 29 hours in newborns have been reported.[27,31,32] Finally, there may be other mediators of the changes in cerebral oxygenation than those we found. Tobacco smoke contains more than 4000 chemical compounds.[8] The effect could also be caused, for example, by nitric oxide or cadmium.

We did find a dose-response relationship between the extent of prenatal exposure to tobacco and r_cSO_2 and FTOE. We speculate that the higher tobacco exposure led to more severe cerebral vasoconstriction and resulted in a lower r_cSO_2 and higher FTOE. In adults who smoke the CBF increases when they stop smoking.[33] This is in line with our results. Apparently, prenatal exposure to tobacco resulted in foetal cerebral vasoconstriction. After birth, tobacco exposure ceased and therefore cerebral vasoconstriction gradually resolved, resulting in an increase of CBF. With increasing CBF, oxygen supply to the brain increased and FTOE decreased as a result. Although our findings are well explained by cerebral vasoconstriction due to tobacco exposure, it remains a speculative explanation. We did not, for example, measure CBF.

Another possible explanation for a higher FTOE was increased oxygen consumption. We did not investigate whether oxygen consumption in the brain increased due to exposure to tobacco. The authors of a study on Rhesus monkeys speculated that exposure to tobacco leads to reactive sprouting of neuronal cells after damage due to tobacco exposure.[34] Theoretically, this may lead to higher oxygen consumption. This is very unlikely since we only found differences in r_cSO_2 and FTOE on days 1, 2, and 8.

Withdrawal effects and subsequently higher oxygen consumption are also unlikely. Other researchers reported on the effects of prenatal tobacco exposure on neurobehaviour during the first 24 to 48 hours after birth.[35] Infants are more excitable, hypertonic, and more difficult to soothe when they had been exposed to tobacco postnatally. Nevertheless, we do not expect that these signs had such an impact on cerebral oxygenation.

On the 8th day after birth we found a lower r_cSO_2 and a higher FTOE. This was unexpected and could be based on coincidence. Another possible explanation could be breastfeeding. Cotinine is transferred from the mother to the infant through breast milk.[31] The policy in our unit is to stimulate all mothers to supply their own milk to their preterm infants. During the first days after birth the amount of breast milk is limited, but intake increases during the course of the first week. At the end of the first week the amount of cotinine the infant receives through breast milk may again be such that it leads to some cerebral vasoconstriction. This is highly speculative, however, and does not explain why we did not find a difference on the 5th and 15th days. As we did not record the amount of breast milk the infants received, further support for this hypothesis is lacking.

We found no differences between the two groups with regard to clinical variables, including inflammatory variables. Haemoglobin levels were also the same in both groups, ruling out the possibility of a lower oxygen supply due to differences in haemoglobin levels. Furthermore, lower arterial oxygen content was not likely because $tcSaO_2$ did not differ between groups, except for day 2. This may explain why there was only a trend for higher FTOE on day 2 since both r_cSO_2 and $tcSaO_2$ were lower. We believe that it was unlikely that confounders could be held responsible for the differences in r_cSO_2 and FTOE between the two groups.

The values we found for r_cSO_2 and FTOE showed a wide range. This finding is confirmed by various other studies[13,15,16] and points to large inter-individual variation. Naulaers *et al*. found cerebral oxygenation values around 70% and FTOE values around 0.30 during the first days after birth.[36] Petrova *et al*. showed a mean r_cSO_2 value of 66% in ten preterm infants older than seven days while receiving mechanical ventilation.[37] Compared to these studies the r_cSO_2 values of the infants in our study were somewhat higher. Sorensen *et al*. found a mean r_cSO_2 of 79% in preterm infants during the first day after birth.[38] This is in line with our study. Naulaers *et al*. and Sorensen *et al*. used a different spectrophotometer to measure cerebral oxygenation (NIRO, Hamamatsu Photonics, Hamamatsu City, Japan). During measurements of cerebral oxygenation in healthy adults, no significant differences between mean values were found comparing both spectrophotometers.[39] We stress the fact that we did find differences of approximately 8% (r_cSO_2) and approximately 0.15 (FTOE) between the group of infants exposed and the group of infants that had not been exposed to tobacco prenatally.

One of the limitations of our study is that possibly mothers underreported their smoking habits. Furthermore, we had no information about the smoking habits in the mothers' households. Nor did we determine nicotine or cotinine levels in hair or urine although a high degree of concordance exists between parents' reports of exposure to tobacco and children's urine cotinine.[40] There were only a small number of infants in this study exposed to tobacco. A larger study should be conducted to confirm our results.

To the best of our knowledge the present study is the first to demonstrate the effect of prenatal tobacco exposure on cerebral oxygen saturation and extraction. Our findings have implications for clinical practice. It provides yet another reason for strongly recommending pregnant women, or women who are trying to fall pregnant, to refrain from smoking. In addition to placental changes, smoking may compromise brain oxygenation of the newly born infant.

Conclusion

Cerebral oxygen extraction was higher in preterm infants during days 1, 2, and 8 after birth following prenatal tobacco exposure. Our data indicated that prenatal tobacco exposure may have an effect on cerebral oxygenation of the infant.

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Part 3

Other techniques to determine the (prognostic) value of NIRS

Chapter 8

The relationship between electrocerebral activity and cerebral fractional tissue oxygen extraction in preterm infants

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Abstract

Impaired cerebral oxygen delivery may cause cerebral damage in preterm infants. At lower levels of cerebral perfusion and oxygen concentration, electrocerebral activity is disturbed. The balance between cerebral oxygen delivery and oxygen use can be measured by near-infrared spectroscopy (NIRS), and electrocerebral activity can be measured by amplitude-integrated EEG (aEEG). Our aim was to determine the relationship between regional cerebral tissue oxygen saturation (r_cSO_2), fractional tissue oxygen extraction (FTOE), and aEEG. We recorded longitudinal digital aEEG and r_cSO_2 prospectively in 46 preterm infants (mean GA 29.5 weeks, SD 1.7) for 2 hours on the 1st to 5th, 8th, and 15th days after birth. We excluded infants with germinal matrix hemorrhage exceeding grade I and recordings of infants receiving inotropes. FTOE was calculated using transcutaneous arterial oxygen saturation ($tcSaO_2$) and r_cSO_2 values: ($tcSaO_2$ - r_cSO_2)/$tcSaO_2$. aEEG was assessed by calculating the mean values of the 5th, 50th, and 95th centiles of the aEEG amplitudes. The aEEG amplitude centiles changed with increasing GA. FTOE and aEEG amplitude centiles increased significantly with postnatal age. More mature electrocerebral activity was accompanied by increased FTOE. FTOE also increased with increasing postnatal age and decreasing Hb levels.

Introduction

Preterm infants are at risk of developing intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL)[1], two conditions that may lead to permanent cerebral damage. Impaired cerebral oxygenation may contribute to the development of both IVH and PVL and may thus contribute to the development of permanent cerebral damage.[2] We defined oxygenation of the brain as oxygen delivery to the brain, which depends on cerebral perfusion and arterial oxygen content. Impaired oxygen delivery may also cause cerebral damage, independently of IVH and PVL.

The balance between cerebral oxygen delivery and oxygen use can be monitored by near-infrared spectroscopy (NIRS).[3] This is a non-invasive method that measures regional cerebral oxygen saturation (r_cSO_2). R_cSO_2 reflects the oxygen saturation in a mixed vascular bed dominated by venules. Fractional tissue oxygen extraction (FTOE) can be calculated from r_cSO_2 and transcutaneous arterial oxygen saturation ($tcSaO_2$).[4] It reflects the balance between oxygen supply and oxygen consumption and may thus indicate cerebral hypoxemia or ischemia.

At lower levels of cerebral perfusion and oxygen delivery, electrocerebral activity is disturbed. Amplitude-integrated EEG (aEEG) is a marker of electrocerebral activity. In term infants, aEEG can be severely abnormal following perinatal asphyxia.[5] In preterm infants, electrocerebral activity is generally discontinuous and changes with gestational and postnatal age.[6,7] With increasing gestational and postnatal age, continuous electrocerebral activity increases.

Little is known about the relationship between electrocerebral activity and cerebral oxygen delivery in relatively healthy preterm infants. Therefore, the aim of our study was to investigate the relationship between r_cSO_2, FTOE, and aEEG. We hypothesized that increased electrocerebral activity will lead to higher oxygen consumption and, as a result, to higher FTOE, as long as it is not accompanied by increased oxygen delivery.

Methods

For this prospective observational study, we initially selected 50 preterm infants that had been admitted to the NICU of the University Medical Center Groningen between May 2006 and July 2007. All preterm infants with a GA of 26 to 32 weeks admitted on their first day after birth were eligible for inclusion. We excluded infants with major chromosomal or congenital abnormalities. After the initial selection, we excluded three infants from further analysis that had developed an IVH exceeding grade I according to Volpe.[8] We also excluded 11 recordings of infants that required inotropes to maintain blood pressure at the time of the aEEG and NIRS recordings. The final study cohort consisted of 46 infants. Written informed consent was obtained from both parents. The study was approved by the review board of the University Medical Center Groningen.

The aEEG and r_cSO_2 were measured simultaneously within the first 24 hours after birth and subsequently on the 2nd, 3rd, 4th, 5th, 8th, and 15th days.

The aEEG measurements

We used a digital cerebral function monitor (CFM) that was not commercially available at the time of the study. It consisted of an amplifier connected to a laptop computer that contained software for digital aEEG processing. In addition to displaying the aEEG pattern, it also displayed the original EEG. The device recorded the aEEG through two neonatal ECG electrodes with a diameter of 15 mm (Neotrode II, Conmed, Utica, NY, USA). The electrodes were placed in P3 and P4 position (international 10 - 20 system). The common electrode was placed conveniently anywhere on the infant's body. We used a digital direct current common average reference amplifier (Porti-X by TMSi, Enschede, the Netherlands) comprising a high input impedance (> 2 GΩ) and a 22-bits sigma-delta Analog to Digital Converter with a resolution of 0.0715 μV per bit. The electrodes were connected to the amplifier by means of shielded cables to prevent electrical noise and alternating current (AC) power interference pick-up. Loss of electrode contact was sensed by the amplifier's input circuitry and signaled to the data acquisition software. Low (< 0.5 Hz) and high frequencies (> 25 Hz) were attenuated by first-order high and low pass filtering. The EEG was stored on a hard disc, and the aEEGs were processed.

The aEEG processor was constructed in software and comprised a signal shaping filter, a semi-logarithmic rectifier, a peak detector, and a smoothing filter. Its characteristics were similar to the CFM constructed and described by Maynard *et al*.[9] and to all commercially available machines. All values were filtered by box-car averagers with a time window of 60 seconds. To obtain additional information, the mean of the aEEG amplitude and the mean peak and trough values were computed and displayed. The mean trough and mean peak values represented the 5th and 95th centiles of the aEEG amplitudes. An example of an aEEG recording, which also displays the aEEG amplitude centiles, is shown in Figure 1.

We assessed the aEEGs by pattern recognition and by calculating the centiles of the aEEG amplitudes.

While the aEEGs were being recorded, the nursing staff noted down any handling of the infant, clinical seizures, and administration of anticonvulsant or sedative drugs.

Pattern recognition

Different background patterns were distinguished according to Hellström-Westas and Rosén.[10] Background patterns were characterized as follows: continuous normal voltage (CNV), discontinuous normal voltage (DNV), burst suppression (BS), continuous low voltage, or flat trace.

The presence or absence of sleep-wake cycling (SWC) and the occurrence of epileptic activity were also noted down. SWC was recognized as cyclical variations in the bandwidth of the aEEG trace indicating cycling of sleep stages.

The aEEG amplitude centiles

To obtain additional quantitative measures, we calculated the mean of the 5th, 50th, and 95th centiles of the aEEG amplitude for the recording period on each day. Artifacts were identified and confirmed with the use of the raw EEG, after which they were excluded from quantitative analysis.

Near-infrared spectroscopy

R_cSO_2 was measured with the INVOS 4100 near-infrared spectrometer (Somanetics Corporation, Troy, Michigan, USA) in combination with the pediatric SomaSensor. This technology is based on the fact that biological tissue is relatively transparent to near-infrared light (600 - 900 nm). The optical sensor measures the quantity of reflected light photons as a function of two wavelengths (730 and 805 nm) and determines the spectral absorption of the underlying tissue.[11,12] NIRS differentiates oxygenated Hb from deoxygenated Hb that has distinct absorption spectra. The ratio of oxygenated Hb to total Hb reflects the regional oxygen saturation of tissue. The SomaSensor has two detectors at a distance of 3 and 4 cm from the near-infrared optode. The detector placed at 3 cm from the optode receives light scattered predominantly from scalp and skull. The detector placed at 4 cm receives light scattered from scalp, skull, and cerebral tissue. Thus, by subtraction, the two detectors measure the oxygen saturation in the underlying cerebral tissue.

R_cSO_2 was measured over a 2-hour period. Fifteen minutes were allowed for the measurement to stabilize. The optical sensor was placed to the left frontoparietal side of the infant's head and held in place by elastic bandaging. Simultaneously, we measured transcutaneous arterial oxygen saturation ($tcSaO_2$) by pulse oximetry. We calculated FTOE with the equation FTOE = ($tcSaO_2$ - r_cSO_2)/$tcSaO_2$.

Statistical analysis

SPSS software for Windows, version 14.0 (SPSS Inc., Chicago, Illinois, USA), was used for all analyses. Because of normal distribution, differences in centiles of the aEEG amplitude between certain types of background patterns were analyzed using *t* test. Results were expressed as mean values ± SD. The Pearson correlation coefficient (two-tailed) was calculated to test the correlation between FTOE, r_cSO_2, and the centiles of the aEEG amplitude. The variables that were tested for their relationship with the aEEG amplitude centiles and FTOE were postnatal age, GA, mean arterial blood pressure, Hb level, and arterial PCO_2 ($PaCO_2$). To test whether clinical data were different between subgroups of our cohort (e.g. infants with and without SWC), we used the Mann-Whitney *U* test for continuous variables and Fisher's exact test for categorical variables. Finally, we performed a multivariate linear regression analysis to find the most significant model explaining FTOE. Variables entered the model at a significance level of $P \leq 0.1$. A P value of < 0.05 was considered statistically significant.

Results

Study group

Data were collected on 46 infants whose GAs ranged from 26 to 31.9 weeks (mean 29.4 ± 1.7). We obtained 238 combined recordings of aEEG and r_cSO_2 (Figure 1). The mean daily recording time was 128 minutes (SD 31). Twenty-nine infants needed artificial ventilation for initial stabilization and all but one received surfactant. During 55 recordings, infants were mechanically ventilated, and nasal continuous positive airway pressure (CPAP) was given during 98 recordings. During the remaining 86 recordings, infants either received low flow via nasal canula or they had no respiratory support. The majority of

infants could be weaned off the ventilator within 5 days. Because we do not routinely sedate infants during artificial ventilation, none of the infants received morphine during the study period. Clinical data of the study population are summarized in Table 1.

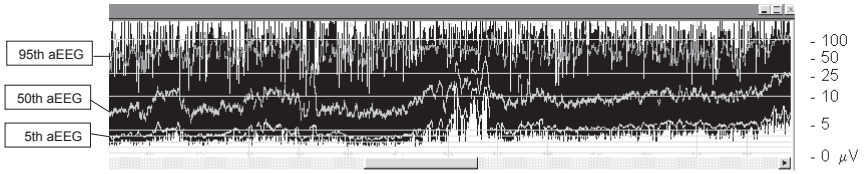

FIGURE 1. Example of a display of the digital recorded aEEG
5th aEEG: 5th centile of the aEEG amplitude; 50th aEEG: 50th centile of the aEEG amplitude; 95th aEEG: 95th centile of the aEEG amplitude.

TABLE 1. Patient characteristics

Gestational age, mean (SD), weeks	29.5 (1.7)
Birth weight, mean (SD), grams	1311 (390)
Antenatal steroids, n (%)	44 (96)
Apgar score at 5 minutes, mean (range)	7.4 (3-10)
Apgar score at 5 minutes ≥ 7, n (%)	35 (76)
MABP, mean (SD), mmHg	37 (6.8)
IVH	
No, n (%)	44 (96)
grade I, n (%)	2 (4)
Initial ventilation	
low flow, n (%)	1 (2)
nasal CPAP/IMV, n (%)	16 (35)
artificial ventilation, n (%)	29 (63)
Surfactant, n (%)	28 (61)
Hb, mean (SD), mmol/l	8.7 (1.6)
$PaCO_2$, mean (SD), kPa	5.5 (0.9)

SD indicates standard deviation, MABP; mean arterial blood pressure, CPAP; continuous positive airway pressure, IMV; intermittent mandatory ventilation, Hb; haemoglobin level, $PaCO_2$; arterial partial pressure of carbon dioxide.

Pattern recognition

During the entire study period, the aEEG traces of the majority of infants showed the discontinuous background patterns BS or DNV (Table 2). From the 5th day after birth, the frequency of continuous patterns increased (χ^2-for-trend, $P = 0.036$). SWC was present in some infants from the first day after birth and increased on the 3rd postnatal day (χ^2-for-trend, $P = 0.003$). Postmenstrual age (GA + postnatal age) was significantly higher when SWC was present (30.3 versus 29.7 weeks, $P = 0.023$). Postnatal age was also significantly higher when SWC was present (6.0 versus 4.6 days, $P = 0.015$).

TABLE 2. Frequencies of aEEG background patterns and sleep wake cycling in relation to postnatal age

	Postnatal day						
aEEG n (%)	1	2	3	4	5	8	15
BS	6 (23)	7 (19)	4 (12)	3 (8)	4 (12)	1 (3)	0
DNV	17 (65)	26 (70)	25 (73)	30 (81)	26 (74)	27 (77)	23 (66)
CNV	3 (12)	4 (11)	5 (15)	4 (11)	5 (14)	7 (20)	12 (34)
SWC (%)	8 (31)	20(54)	26 (76)	26 (70)	24 (69)	23 (66)	27 (77)

SWC already appeared on the first day after birth. No differences were found in GA, birth weight, and Apgar scores between infants with and without SWC on the first day after birth. Infants without SWC were significantly more often treated with surfactant ($P = 0.026$) and more often artificially ventilated ($P = 0.073$).

The different background patterns had significantly different aEEG amplitude centiles (Table 3). In comparison to DNV, BS had a significantly lower mean 5th ($P < 0.001$) and 50th amplitude centile ($P < 0.001$). In comparison to CNV, the mean 5th and 50th centiles of BS were also significantly lower ($P < 0.001$), and the mean 95th centile was higher ($P = 0.011$). In comparison to CNV, DNV had significantly lower mean 5th and 50th amplitude centiles ($P < 0.001$), while the mean 95th amplitude centile was higher ($P = 0.002$).

TABLE 3. aEEG amplitude centiles in relation to background patterns

	Amplitude centiles (mean, SD)		
aEEG (n)	5th	50th	95th
BS (40)	3.9, 0.94	9.1, 1.7	37, 7.8
DNV (174)	6.1, 1.5	12, 2.6	36.5, 10.7
CNV (25)	8.1, 1.6	14.2, 3	32, 7.2

The aEEG amplitude centiles

There was a change of electrocerebral activity with both postnatal age and GA. The 5th amplitude centile correlated positively with both postnatal age ($\rho = 0.19$, $P = 0.004$) and GA ($\rho = 0.56$, $P < 0.001$). We found the opposite effect on the 95th amplitude centile: both postnatal age ($\rho = -0.14$, $P = 0.037$) and GA ($\rho = -0.19$, $P = 0.003$) had negative correlations with the 95th amplitude centiles.

We found no significant changes in the aEEG amplitude centiles during the first 5 days after birth. The 5th amplitude centile increased significantly between the 5th and 15th days (*t* test, $P = 0.001$) and between the 8th and 15th days (*t* test, $P = 0.021$). The changes in the aEEG amplitude centiles are shown in Figure 2.

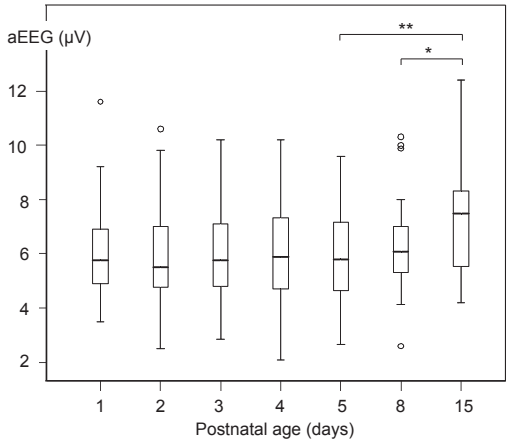

FIGURE 2. The relationship between the 5th aEEG amplitude centile and postnatal age
*$P < 0.05$; **$P < 0.01$.

The relationship between r_cSO_2, FTOE, and postnatal age

FTOE changed with postnatal age. R_cSO_2 decreased ($\rho = -0.27$, $P < 0.001$), whereas FTOE increased with postnatal age ($\rho = 0.32$, $P < 0.001$).

We found no changes in r_cSO_2 and FTOE during the first 5 days after birth. After the 5th day, there were significant changes in r_cSO_2 and FTOE. R_cSO_2 decreased from 79% on the 5th day to 76% on the 8th day and 70% on the 15th day. The differences between the 5th and 15th days and the 8th and 15th days were significant ($P < 0.001$ and $P = 0.007$, respectively). Although r_cSO_2 decreased, there was an increase in FTOE from 0.16 to 0.20 between the 5th and 8th days ($P = 0.03$) and from 0.20 to 0.26 between the 8th and 15th days ($P = 0.013$). The increase between the 5th and 15th days was highly significant ($P < 0.001$). The course of FTOE is shown in Figure 3.

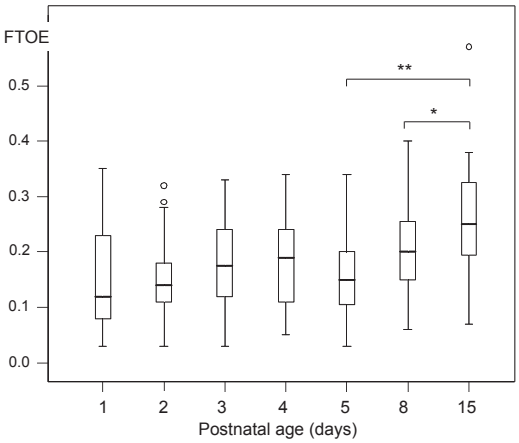

FIGURE 3. The relationship between FTOE and postnatal age
*$P < 0.01$; **$P < 0.001$.

The relationship between aEEG amplitude centiles and FTOE

The 5th and 50th aEEG amplitude centiles correlated positively with FTOE (ρ = 0.26, $P < 0.001$ and ρ = 0.14, $P = 0.035$, respectively). The 95th amplitude centile correlated negatively with FTOE (ρ = -0.13, P = 0.042).

The relationship between FTOE and the clinical variables

Because several clinical conditions may interfere with cerebral oxygen delivery, we investigated whether these conditions confounded the relationships we found for FTOE and aEEG amplitude centiles. We checked blood pressure, persistent ductus ateriosus (PDA), ventilatory support, $PaCO_2$, and Hb levels. There was no relationship between FTOE and blood pressure or $PaCO_2$. We did, however, find a negative correlation between Hb levels and FTOE (ρ = -0.30, P = 0.001). FTOE was influenced by the mode of ventilation. We found that infants on low flow or infants without ventilatory support had higher FTOE compared with infants on nasal CPAP or artificial ventilation (0.22 versus 0.16, $P < 0.001$). The values of $tcSaO_2$ were significantly higher in infants on low flow or infants without ventilatory support compared with infants with CPAP (97% versus 94%, $P < 0.001$) and artificial ventilation (97% versus 91%, $P < 0.001$). There was no difference between infants on nasal CPAP or artificial ventilation. There was a slightly lower FTOE in case of a PDA during recording (0.17 versus 0.19, P = 0.029). The Hb levels were not different between the infants with and without a PDA.

Multivariate linear regression

Because individual variables are likely to be interdependent, we performed a multivariate linear regression analysis to examine the determinants of FTOE. The variables we entered into the model were aEEG amplitude centiles, postnatal age, Hb level, mode of ventilation, and PDA. The 5th aEEG amplitude centile (β = 0.12 [95% CI: 0.003 to 0.20], P = 0.01), 95th aEEG amplitude centile (β = -0.002 [95% CI: -0.003 to 0], P = 0.022), postnatal age (β = 0.005 [95% CI: 0.001 to 0.009], P = 0.009), and Hb level (β = -0.011 [95% CI: -0.021 to -0.001], P = 0.030) remained in the model, explaining 22.5% of the variance.

Discussion

Our study demonstrated a clear relationship between electrocerebral activity and FTOE. We found increased FTOE with changing electrocerebral activity. Increase of the 5th aEEG amplitude centile, decrease of the 95th aEEG amplitude centile, and, consequently, a narrower bandwidth of the aEEG were associated with higher FTOE. This higher FTOE may indicate higher cerebral oxygen consumption.

Electrocerebral activity changed with increasing gestational and postnatal age. The 5th aEEG amplitude centile increased while the 95th amplitude centile decrease concurrently. We consider this narrower bandwidth of the aEEG to be a more mature background pattern. We observed the same maturational effects on aEEG with increasing postnatal age. From the 5th day onward, a larger proportion of aEEGs showed continuous normal voltage. The aEEG amplitude centiles also changed significantly after the 5th postnatal day. Several previous publications reported maturational effects of

both postnatal and GA on electrocerebral activity.[7,13,14] Mostly, these studies recorded aEEG at weekly intervals. Our study indicated that this change in electrocerebral activity generally took place in the second week after birth.

From the 5th postnatal day onward, the maturation of electrocerebral activity occurred simultaneously with an increase of FTOE, an observation we reported previously.[15] It has been reported that increased cerebral oxygen consumption in case of increased metabolism is met by an increase of cerebral blood flow[16], the so-called neurovascular coupling. In that case, FTOE is expected to remain stable. Similar as Yoxall and Weindling[16], we found no increase of FTOE during the first week after birth. Instead, we found an increase of FTOE during the second week after birth, which was independent of Hb levels and more mature electrocerebral activity. We speculate that this higher FTOE is at least partly the result of increased oxygen consumption, due to increased metabolism. It is an established fact that metabolism in preterm infants nearly doubles after the first week after birth.[17] Theoretically, the increase of FTOE could also be the result of impaired oxygen delivery due to decreased cerebral blood flow. In case of lower cerebral blood flow, however, we would expect a decrease in electrocerebral activity. Moreover, none of the infants were treated with inotropes, and their blood pressure was within the normal ranges. We found no relationship between FTOE and blood pressure. This suggests that within normal ranges of blood pressure, cerebral autoregulation is intact.

Independent of electrocerebral activity and postnatal age, we found an increase of FTOE with decreasing Hb concentration. If the Hb concentration decreases, the absolute amount of oxygen transported is decreasing. Within ranges of constant oxygen demand, this may lead to a higher extraction of oxygen.[18,19] These results are in line with the study of Roche-Labarbe *et al*.[20] They found an increase in cerebral oxygen consumption during the first 6 weeks after birth, which was related to a decrease of Hb over the same period. The infants in our study cohort were relatively healthy preterm infants. If an infant's clinical condition worsens, oxygen supply can become critical. Under such circumstances, maintaining the Hb level can become crucial in preserving oxygen supply to the brain, and thus preventing brain damage.

FTOE was influenced by the mode of ventilatory support. Infants that were artificially ventilated or treated with nasal CPAP during aEEG recordings had lower FTOE than infants that only required low flow or no support at all. This was, however, not independent of other factors such as aEEG amplitude centiles and postnatal age. Oxygen delivery to the brain was not impaired in infants without artificial ventilation or nasal CPAP, $tcSaO_2$ was even higher in infants on low flow via nasal canula. Therefore, in hemodynamically stable infants, cerebral oxygen delivery is not influenced by the severity of RDS and the mode of ventilation itself. This is in line with a previous study that compared cerebral oxygen delivery and oxygen extraction in preterm infants with and without RDS.[12] If, however, respiratory failure is accompanied by circulatory insufficiency, this may lead to impaired cardiac output, and, consequently, to lower cerebral perfusion. Lower cerebral perfusion may lead to higher FTOE and to a change of electrocerebral activity.

A negative effect on electrocerebral activity was reported following the so-called "InSurE" procedure for the treatment of RDS.[21] This negative effect was attributed to the administration of opioids before

intubation. With this opoid-induced change in electrocerebral activity, cerebral oxygen delivery and FTOE were constant, suggesting a decreased oxygen demand in case of decreased electrocerebral activity. Because none of the infants in our study received opioids, changes in electrocerebral activity in this study reflect differences in postnatal and GA.

We checked for other potentially confounding factors such as CO_2 level and PDA. Hypocarbia causes cerebral vasoconstriction that results in decreased cerebral blood flow. Decreased cerebral blood flow may result in increased FTOE. A recent study reported a negative correlation between transcutaneous PCO_2 and FTOE.[22] By contrast, we found no correlation between FTOE and $PaCO_2$ level. One of the differences was that we did take blood samples, either arterial or capillary, to measure $PaCO_2$ level. The $PaCO_2$ levels were within the normal ranges. It may well be possible that at lower CO_2 levels cerebral blood flow diminishes and that FTOE increases as a result. Wardle *et al*.[23] found an increase in FTOE with a decrease of $PaCO_2$ levels. They found an effect of $PaCO_2$ levels within infants. We did not perform repeated and combined measurements of $PaCO_2$ levels with FTOE. Therefore, it is possible that we have missed temporal changes of FTOE in relation to $PaCO_2$ levels. We did find a lower FTOE in case of a PDA during recording. Because several factors were likely to be interdependent, we performed a multivariate analysis. A PDA did not contribute to FTOE independently. Lower FTOE seems to be explained by a lower gestational and postnatal age in infants with a PDA. Infants with a lower GA had less mature electrocerebral activity, which was associated with lower FTOE. The difference in FTOE could not fully be explained by a difference in Hb levels.

In conclusion, this study demonstrated a tight relationship between electrocerebral activity and oxygen consumption. As electrocerebral activity matured, oxygen consumption increased. Other factors influencing oxygen consumption were postnatal age and Hb level. This combination of FTOE and electrocerebral activity may be a useful biomarker of brain function in high-risk infants. The combination of high FTOE and low electrocerebral activity may well reflect impairment of cerebral oxygen delivery or perfusion and may be an indication for clinicians to focus on preserving oxygen supply to the brain to limit brain damage. More research is needed to study the implications of this combined monitoring approach for treating infants during the neonatal period.

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chapter 6

Cerebral oxygenation is associated with neurodevelopmental outcome of preterm-born children at age two to three

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Abstract

Objective Disturbed cerebral oxygenation may pose preterm infants with the risk of neurological deficits. Our aim was to determine whether regional cerebral tissue oxygen saturation (r_cSO_2) and fractional tissue oxygen extraction (FTOE), measured by near-infrared spectroscopy, were associated with neurodevelopmental outcome of preterm-born infants.

Patients and methods We included 83 preterm infants (gestational age < 32 weeks), measured r_cSO_2 on days 1, 2, 3, 4, 5, 8, and 15 after birth, and calculated FTOE = (SpO_2 - r_cSO_2)/SpO_2. Additionally, we determined the area under the curve (AUC) of r_cSO_2 and FTOE. Cognitive, motor, neurological, and behavioral outcomes were determined at 2 to 3 years. Multiple linear regression analyses were used to determine whether r_cSO_2 and FTOE contributed to outcome using un-standardized coefficients (B) to determine effect sizes.

Results We included 67 infants for follow-up. Lower quartile (P_{25-50}) and highest quartile (P_{75-100}) of the r_cSO_2 values on day 1 were associated with poorer cognitive outcome (B: -5.77, *P* = .044 and B: -6.64, *P* = .008, respectively). Lower AUC of r_cSO_2 was associated with poorer cognitive outcome (B: 0.43, *P* = .014). Lower quartile (P_{25-50}) AUC of r_cSO_2 was associated with poorer fine motor outcome (B: -1.35, *P* = .004). The amount of time r_cSO_2 < 50% on day 1 was negatively associated with gross motor outcome (B: -0.16, *P* = .002). Highest quartile of the FTOE values on day 1 was associated with poorer total motor outcome (B: -8.01, *P* = .041).

Conclusions: Cerebral oxygen saturation during the first two weeks after birth is associated with neurodevelopmental outcome of preterm-born infants at 2 to 3 years. Both high and low r_cSO_2 on day 1 were negatively associated with neurodevelopmental outcome.

Introduction

Preterm infants are at risk of neurodevelopmental sequelae.[1-4] There are strong indications that cerebral ischemia, hypoxia, and fluctuations in cerebral perfusion are involved in the development of cerebral pathology.[5,6] Unfortunately, it is difficult to predict whether and to what degree the individual infant will develop neurodevelopmental sequelae.[7] Prognostic tools are, therefore, urgently needed for the early postnatal phase.

A promising, non-invasive, bedside tool is near-infrared spectroscopy (NIRS). It measures regional cerebral tissue oxygen saturation (r_cSO_2).[8-10] This measure reflects oxygen saturation in a mixed vascular bed dominated by venules. R_cSO_2 serves as an indicator of cerebral hypoxic hypoxia.[9] Fractional tissue oxygen extraction (FTOE) is calculated on the basis of r_cSO_2 and on the values of arterial oxygen saturation (SpO_2). FTOE reflects the balance between cerebral oxygen supply and consumption.[11,12] FTOE serves as an indicator of cerebral ischemic hypoxia.[13]

Increasingly, NIRS is used in routine clinical practice and is proving a useful tool for monitoring trends in individual cerebral oxygenation.[14] Furthermore, it was shown to have prognostic value for predicting outcome in term-born neonates following asphyxia.[11,15] As yet it is unclear whether NIRS also has prognostic value for outcome of preterm-born infants. Animal studies indicated that cerebral oxygenation < 40% - 50% could be dangerous for the newborn brain and possibly causes damage.[16,17]

The aim of this prospective, longitudinal, cohort study was to determine whether r_cSO_2 and FTOE were associated with neurodevelopmental outcome of preterm-born children at 2 to 3 years of age. We hypothesized that preterm-born children with adverse outcomes would have had lower r_cSO_2 and higher FTOE.

Patients and Methods

Patient population

We performed a prospective, longitudinal, and observational cohort study between May 2006 and March 2008. We included 83 preterm infants, with a gestational age (GA) < 32 weeks, who had been admitted to the neonatal intensive care unit of University Medical Center Groningen, the Netherlands. Infants with major chromosomal or congenital abnormalities were not included. Our center's ethics review board approved the study and written, informed parental consent was obtained in all cases.

Near-infrared spectroscopy

We used the INVOS 4100-5100 monitor (Somanetics Corporation, Troy, Michigan, USA) in combination with pediatric SomaSensors to measure r_cSO_2. The SomaSensor was placed on the left frontoparietal side of the infant's head and kept in place by an elastic bandage. A more detailed description of the method was published previously.[18]

We measured r_cSO_2 within the first 24 hours after birth and subsequently on days 2 to 5, 8, and 15 during a two-hour period. Simultaneously, we measured SpO_2 by pulse oximetry. We calculated FTOE as $(SpO_2 - r_cSO_2)/SpO_2$.[11,13]

Neurodevelopmental outcome

Neurodevelopmental outcome was determined at 2 to 3 years of age. Cognitive and motor development were assessed with Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III).[19] Children with a cognitive composite score < 85 were classified as mildly abnormal, < 70 as abnormal (mean = 100, SD = 15). Children with a fine, gross, or total motor scaled score < 7 were classified as mildly abnormal, ≤ 3 as abnormal (mean = 10, SD = 3). Neurological outcome was assessed with an age-specific neurological examination. Children were classified as normal, mildly abnormal, or abnormal. Behavioral outcome was assessed with the Child Behavior Checklist (CBCL), which evaluates behavior and emotions and is filled out by parents.[20] Children were classified with normal, mildly abnormal, or abnormal behavioral outcome in accordance with the manual.

Clinical variables

Details on perinatal and neonatal characteristics with probable or known associations with disturbed neurodevelopmental outcome, including parental socioeconomic status (SES), were collected. We recorded GA[4], birth weight[4], gender[4], birth asphyxia[11,15], small-for-GA[21], head circumference[22], sepsis[23,24], signs of circulatory failure, circulatory support, respiratory support[25], cerebral lesions[1,26], intestinal pathologies[27], bronchopulmonary dysplasia[28], and retinopathy of prematurity. Furthermore, clinical variables with probable or known associations with cerebral oxygenation were measured, i.e. heart rate, blood pressure, blood gas values[29,30], hemoglobin[31] and glucose concentration[32].

Data analysis and statistical testing

We used SPSS 18.0 software for Windows (SPSS Inc., Chicago, Illinois, USA) for statistical analyses. Mean values for r_cSO_2, SpO_2 and FTOE were calculated per day. NIRS data were tested for normality and showed a normal distribution. We determined the course during the first two weeks after birth. Daily, we also determined in which infants and for what length of time (number of minutes during the two-hour period) r_cSO_2 was < 50%.

BSID-III data were tested for normality and showed normal distributions. Neurological and behavioral outcome measures showed non-normal distributions. If children were too tired and/or uncooperative we excluded their scores.

We determined whether r_cSO_2 and FTOE on day 1 correlated with outcome measures at 2 to 3 years using the Pearson correlation test (2-tailed), or Spearman's rank correlation test (2-tailed), where appropriate. Furthermore, we categorized r_cSO_2 and FTOE values on day 1 into quartiles to perform linear regression analysis on BSID-III outcome measures. We took the third quartile as reference group since it contained the mean value. Behavioral and neurological outcomes were classified as normal or abnormal (mildly abnormal and abnormal behavioral or neurological outcomes taken together) to perform logistic regression analysis on r_cSO_2 and FTOE values in quartiles on day 1.

We compared outcome measures of infants exposed to r_cSO_2 < 50% on day 1 and over the whole study period with infants not exposed to r_cSO_2 < 50%. We used the independent samples *t* test or Mann-Whitney test, where appropriate. We also checked whether the amount of time of r_cSO_2 < 50% correlated

with outcome measures using the Pearson or Spearman's correlation tests.

Because of the serial measurement design of this study, we chose to summarize the information from the series of measurements in each infant by calculating an average daily area under the curve (AUC) of r_cSO_2 and FTOE. The AUC is a measure regarding exposure to low or high cerebral oxygenation levels. It is the product of time difference and the average of two consecutive measurements. We calculated AUC by adding AUCs between each pair of consecutive measurements. Next, we divided AUC by the number of days to obtain a weighted daily average level over the entire study period. We determined the correlation between AUC of r_cSO_2 and FTOE, and outcome measures with the Pearson correlation test. Furthermore, we categorized the AUC of r_cSO_2 and FTOE into quartiles to perform linear regression analyses with BSID-III outcome measures and logistic regression analyses with neurological and behavioral outcomes. Once again the third quartile acted as reference group.

The independent samples *t* test, 1-way ANOVA, Mann-Whitney, or Kruskal-Wallis tests were used to determine the relationship between r_cSO_2, FTOE, or outcome measures with risk factors.

Finally, we used backward multiple linear regression analyses to determine which variables regarding cerebral oxygen saturation were independently associated with cognitive, motor, neurological, or behavioral outcome at 2 to 3 years of age with and without adjusting for gender and parental SES. Potential confounders, detected by univariate analyses with $P < .10$, were also entered into the model. A P value $< .05$ was considered significant.

Results

Eleven of the 83 infants died during the neonatal period. One child died at two years of age after a respiratory syncytial virus infection. Three children were excluded later due to chromosomal abnormalities. One child was lost to follow-up. The final study population consisted of 67 preterm infants. Table 1 presents the neonatal characteristics. Figure 1A and B show the course of r_cSO_2 and FTOE during the first two postnatal weeks.

Neurodevelopmental outcome

Children were median 30 months of age corrected for prematurity (range 24 - 45 months) at follow-up. The mean cognitive composite score was 99 (SD 8). One child scored mildly abnormal. The mean fine motor score was 11 (SD 2). All children scored normal. The mean gross motor score was 9 (SD 3). Four children scored abnormal and three mildly abnormal. The mean total motor composite score was 99 (SD 11). One child scored mildly abnormal.

Nine children had mildly abnormal neurological outcome and three had abnormal neurological outcomes. These were the same children who also had mildly abnormal or abnormal gross or total motor outcomes. Two children had normal neurological outcomes, but scored mildly abnormal on gross motor outcome of the BSID-III. Sixty-five (97%) parents filled out the CBCL. One child scored mildly abnormal and four scored abnormal total behavior.

TABLE 1. Neonatal characteristics

Female/Male	34/33
Gestational age, weeks	30.0 (25.4-31.9)
Birth weight, gram	1305 (615-2250)
Small for gestational age (P < 10)	7 (10%)
Head circumference, centimeters	27.5 (22.8-30.6)
Apgar score at 5 minutes	8 (3-10)
Early-onset sepsis	4 (6%)
Late-onset sepsis	22 (33%)
Circulatory failure	
Fluid resuscitation	25 (37%)
Inotrope treatment	9 (13%)
Respiratory support	
Mechanical ventilation	35 (52%)
Continuous positive airway pressure	28 (42%)
Low flow or no support	3 (4%)
Cerebral lesions	
Germinal matrix hemorrhage-intraventricular hemorrhage	15 (22%)
Transient periventricular echodensities	33 (49%)
Periventricular leukomalacia	-
Intestinal pathologies	
Necrotizing enterocolitis / spontaneous intestinal perforation	8 (12%)
Patent ductus arteriosus	21 (31%)
O_2-dependency at 36 weeks' postmenstrual age	5 (7%)
Retinopathy of prematurity (all grades)	7 (10%)
Low socioeconomic status	11 (16%)

Data are expressed as median (range) or as numbers (percentage) unless otherwise specified. Socioeconomic status was based on highest education level of either one of the parents.

Relationship between r_cSO_2 and FTOE on day 1, and neurodevelopmental outcome

BSID-III outcome measures did not correlate with r_cSO_2 and FTOE on day 1 after birth. After categorizing r_cSO_2 and FTOE values on day 1 into quartiles, linear regression analyses revealed that infants with r_cSO_2 in the lowest and those in the highest quartiles on day 1 had poorer cognitive outcomes ($P = .025$ and $P = .043$, respectively) (Table 2, 3). Infants with r_cSO_2 values in the lowest quartile on day 1 also tended to have poorer fine motor skills ($P = .062$) and poorer total motor skills ($P = .092$). Infants with FTOE values in the highest quartile on day 1 tended to have poorer gross motor skills ($P = .056$) as well as poorer total motor skills ($P = .067$).

Neurological or behavioral outcome was not related to r_cSO_2 and FTOE.

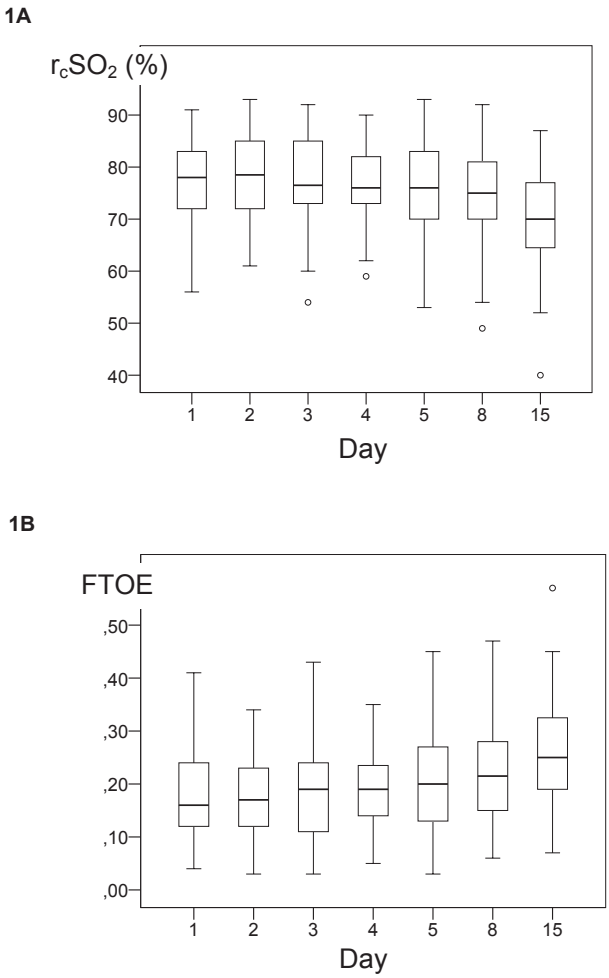

FIGURE 1. The course of r_cSO_2 (A) and FTOE (B) in preterm infants during the first two weeks after birth

TABLE 2. Values for r_cSO_2 and FTOE values on day 1 categorized in quartiles

	r_cSO_2		FTOE	
Quartiles	Minimum	Maximum	Minimum	Maximum
First	56	71	0.04	0.11
Second	72	78	0.12	0.15
Third	79	83	0.16	0.23
Fourth	84	92	0.24	0.41

TABLE 3. Linear regression analyses on outcome measures and r_cSO_2 or FTOE values categorized in quartiles on the first day after birth

	B	Std. error	β	*t*	*P* value	95% CI
Cognition						
r_cSO_2 1st quartile	-7.13	3.08	-0.36	-2.31	.025*	-13.32 to -0.93
r_cSO_2 2nd quartile	-3.17	2.72	-0.19	-1.16	.251	-8.64 to 2.31
r_cSO_2 4th quartile	-5.18	2.492	-0.34	-2.08	.043*	-10.19 to -0.17
Fine motor skills						
r_cSO_2 1st quartile	-1.35	0.70	-0.31	-1.92	.062†	-2.77 to 0.07
r_cSO_2 2nd quartile	-3.70E-16	0.66	0.00	0.00	1.00	-1.32 to 1.32
r_cSO_2 4th quartile	-0.66	0.58	-0.19	-1.15	.258	-1.83 to 0.50
Total motor skills						
r_cSO_2 1st quartile	-8.48	4.92	-0.28	-1.72	.092†	-18.39 to 1.44
r_cSO_2 2nd quartile	-1.23	4.39	-0.05	-0.28	.780	-10.08 to 7.61
r_cSO_2 4th quartile	-3.71	3.86	-0.16	-0.96	.342	-11.50 to 4.08
Gross motor skills						
FTOE 1st quartile	-1.53	1.05	-0.28	-1.46	.153	-3.65 to 0.59
FTOE 2nd quartile	-0.23	1.11	-0.04	-0.21	.836	-2.46 to 2.00
FTOE 4th quartile	-2.30	1.17	-0.36	-1.96	.056*	-4.66 to 0.06
Total motor skills						
FTOE 1st quartile	-6.65	4.30	-0.30	-1.55	.129	-15.32 to 2.01
FTOE 2nd quartile	-0.22	4.30	-0.01	-0.05	.96	-9.49 to 9.05
FTOE 4th quartile	-8.99	4.79	-0.34	-1.88	.067†	-18.65 to 0.67

Third quartile was used as reference group. † indicates $P < .1$, *; $P < .05$, r_cSO_2; regional cerebral tissue oxygen saturation, FTOE; fractional tissue oxygen extraction, B; un-standardized coefficient, Std. error; standard error, β; standardized coefficient, CI; confidence interval.

Relationship between AUC of r_cSO_2 and FTOE, and neurodevelopmental outcome

Median AUC of r_cSO_2 was 69 (range 45 to 79). Median AUC of FTOE was 0.18 (range 0.09 to 0.34). We found a trend for positive correlation between AUC of r_cSO_2 and cognitive outcome ($\rho = .230$, $P = .064$). There was a positive correlation between AUC of FTOE and fine motor skill outcomes ($\rho = .363$, $P = .004$). If we categorized AUC of r_cSO_2 and FTOE values into quartiles, linear regression analyses revealed that infants with AUC of r_cSO_2 in the second quartile had poorer fine motor outcomes ($P = .046$). Infants with AUC of FTOE in the second quartile had better cognitive outcomes ($P = .047$). Infants with AUC of FTOE in the fourth quartile also tended to have better cognitive outcomes ($P = .084$). Infants with AUC of FTOE

in the first, second, and fourth quartiles had better fine motor outcomes ($P = .043$, $P = .030$ and $P = .003$, respectively).

Neurological and behavioral outcomes were not related to AUC of r_cSO_2 and FTOE.

Relationship between r_cSO_2 < 50%, and neurodevelopmental outcome

Twenty-nine infants had some period of r_cSO_2 < 50%. On day 1 there were seven infants (median 1 minute, range 1 to 50). Total time of r_cSO_2 < 50% during all measurements was median 3 minutes, range 1 to 74.

The amount of time r_cSO_2 < 50% on day 1 and during the entire study period correlated negatively with total motor skills ($\rho = -.280$, $P = .030$, and $\rho = -.290$, $P = .024$, respectively) and gross motor skills ($\rho = -.391$, $P = .002$, and $\rho = -.290$, $P = .022$, respectively).

Multiple linear regression analyses

We used multiple linear regression analyses to determine whether r_cSO_2 and FTOE contributed to outcome independently (Table 4). We repeated the multiple linear regression analyses and entered factors, including SES and gender, that had shown associations with outcomes at $P < .10$.

For cognitive outcome, we included r_cSO_2 values in quartiles on day 1, AUC of r_cSO_2, AUC quartiles of FTOE, and respiratory support on day 1. Following backward multivariate linear analysis second quartile ($P_{25 - 50}$), ($P = .044$) and fourth quartile ($P_{75 - 100}$), ($P = .008$) of r_cSO_2 values on day 1, AUC of r_cSO_2 ($P = .014$), respiratory support on day 1 ($P = .017$), and SES ($P = .003$) remained significant in the model, explaining 57% of the variance.

For fine motor outcome we included r_cSO_2 values in quartiles on day 1, AUC quartiles of r_cSO_2, AUC of FTOE, germinal matrix hemorrhage-intraventricular hemorrhage (GMH-IVH), and respiratory support on day 1. Second quartile ($P_{25 - 50}$) of AUC of r_cSO_2 ($P = .004$) as well as GMH-IVH ($P < .001$) remained significant in the model, explaining 64% of the variance.

For gross motor outcome we included FTOE values in quartiles on day 1, amount of time r_cSO_2 < 50% on day 1, and GMH-IVH. The amount of time r_cSO_2 < 50% on day 1 ($P = .002$) remained significant in the model, explaining 46% of the variance.

For total motor outcome we included r_cSO_2 and FTOE values in quartiles on day 1, amount of time r_cSO_2 < 50% on day 1, GMH-IVH, and respiratory support on day 1. The fourth quartile ($P_{75 - 100}$) of FTOE values on day 1 ($P = .041$) and GMH-IVH ($P = .005$) remained significant in the model, explaining 54% of the variance.

TABLE 4. Final model of multiple regression analyses on outcome measures.

	B	Std. error	β	*t*	*P* value	95% CI
Cognitive outcome						
r_cSO_2 2nd quartile	-5.77	2.77	-.33	-2.08	.044*	-11.36 to -0.18
r_cSO_2 4th quartile	-6.64	2.37	-.43	-2.80	.008*	-11.41 to -1.86
AUC r_cSO_2	0.43	0.17	.36	2.55	.014*	0.09 to 0.76
Respiratory support on day 1	-3.82	1.54	-.32	-2.48	.017*	-6.94 to -0.71
SES	4.79	1.51	0.47	3.17	.003*	1.74 to 7.84
Fine motor outcome						
Gender	0.69	0.37	0.24	1.86	.070	-0.06 to 1.43
AUC r_cSO_2 2nd quartile	-1.35	0.44	-.40	-3.11	.004*	-2.23 to -0.47
GMH-IVH	-1.67	0.41	-.52	-4.06	.000*	-2.51 to -0.84
Gross motor outcome						
Amount of minutes $r_cSO_2 < 50\%$	-0.16	0.05	-.46	-3.28	.002*	-0.26 to -0.06
Total motor outcome						
FTOE 4th quartile	-8.01	3.78	-.30	-2.12	.041*	-15.67 to -0.35
GMH-IVH	-9.84	3.30	-.41	-2.98	.005*	-16.51 to -3.16
Respiratory support on day 1	-5.13	2.73	-.26	-1.88	.068	-10.65 to 0.39

* indicates $p < .05$, B; un-standardized coefficient, Std. error; standard error, β; standardized coefficient, CI; confidence interval, r_cSO_2; regional cerebral tissue oxygen saturation, AUC; area under the curve over the whole study period, SES; socio-economic status, GMH-IVH; germinal matrix hemorrhage-intraventricular hemorrhage, FTOE; fractional tissue oxygen extraction.

Discussion

Our study demonstrated that cerebral oxygenation during the first two weeks after birth was associated with neurodevelopmental outcome of preterm-born children at 2 to 3 years of age. The largest effects were found between the lower and highest quartiles of the r_cSO_2 values on day 1 and poorer cognitive outcome, and between the highest quartile of FTOE values on day 1 and poorer total motor outcome. There were no associations of NIRS parameters with neurological or behavioral outcomes, nor after adjusting for possible confounders. This finding was only partially in line with our hypothesis. We expected r_cSO_2 to be lower and FTOE to be higher in infants with adverse outcomes.

It is intriguing that we found both the second and fourth quartiles of r_cSO_2 values on day 1 to be associated with cognitive outcomes. This strongly suggested that both lower and higher values of r_cSO_2 had a negative influence on cognitive outcome. A study with 36 two-year-old preterm-born infants assessed with BSID, Second Edition (BSID-II), reported a negative correlation between mental

developmental index and r_cSO_2 on day 1, and a negative correlation between the psychomotor developmental index and r_cSO_2 on days 1 to 3.[33] Our study confirmed these findings on cognitive outcome. Sorensen *et al*. found higher cerebral oxygen saturation levels in preterm infants on day 1 in comparison to term infants and suggested that preterm infants might be hyperoxygenated.[34] Higher r_cSO_2 values on day 1 possibly exposed infants to oxidative stress. Furthermore, hyperoxia may contribute to neuronal injury by causing a reduction in cerebral blood flow.[5] Both could contribute to hypoxic-ischemic encephalopathy and subsequently, to an adverse neurodevelopmental outcome.[35]
It seemed that infants with r_cSO_2 values in the second quartile on day 1 also acquired some cerebral injury resulting in poorer cognitive outcomes even though their r_cSO_2 values were not considered 'dangerous'. These infants had r_cSO_2 values between 72% and 78%. Several other studies found r_cSO_2 values of approximately 70% on day 1.[36,37] Sorensen *et al*. found mean r_cSO_2 values of 79% in preterm infants on the first day after birth.[34] This is in line with our study despite all the above mentioned studies using different NIRS devices. No consensus has yet been reached on normal values for preterm infants because of the variability in cerebral oxygenation values between NIRS devices, sensors, and variability after sensor replacement.[10,38-43] The present study led us to conclude that r_cSO_2 values between 79% and 83% on day 1 were associated with the best cognitive outcome. Nevertheless, a large, multi-center trial to determine normal cerebral oxygenation values in preterm infants is urgently called for.

The first quartile of r_cSO_2 values did not remain in the final model, possibly because respiratory support did. This suggested that respiratory support on day 1 was more important for cognitive outcome. Perhaps the infants with r_cSO_2 values in the first quartile and on respiratory support were the most ill infants, even though we did not find any of the other parameters concerning illness severity to be associated with cognitive outcome in our study.

We also found that infants with low AUC of r_cSO_2, indicating low r_cSO_2 values over longer periods of time, had poorer cognitive outcomes. No association was found with $r_cSO_2 < 50\%$ or the length of time of $r_cSO_2 < 50\%$ and cognitive outcome. Perhaps it did not matter how long the r_cSO_2 values were < 50%, just having low values already posed the infants with the risk of poorer cognitive outcomes. We did find that the length of time of $r_cSO_2 < 50\%$ on day 1 was most predictive for poorer gross motor outcome. Animal studies indicated that a cerebral oxygenation < 50% could be dangerous for the newborn brain and possibly cause damage, especially if it lasts for > 30 minutes.[16,17] These studies, however, described cellular brain damage and not cognitive or motor outcomes. In our study population the median time of r_cSO_2 values < 50% on day 1 was only 1 minute. Since we only measured for two hours per day it is possibly that r_cSO_2 was < 50% for a longer period. Or perhaps the critical r_cSO_2 value causing cerebral damage in the preterm brain is above 50%.

Concerning FTOE, only the fourth quartile of FTOE values on day 1 was associated with poorer total motor outcome. The effect size was rather large (-8.01). The fourth quartile of FTOE values consisted of values between 0.24 and 0.41. Increased FTOE indicated either decreased cerebral oxygen supply or increased oxygen consumption. One study described poorer motor outcome in term infants with signs of ischemic damage on MRI (increased lactate, reflecting neuronal damage, and accumulation of products of anaerobic metabolism).[44] Our data also suggested that ischemic hypoxia in particular, is a risk factor for poorer total motor outcome.

We recognize several limitations. One being the small number of infants in this study. Outcome measures for BSID-III were rather high for preterm infants. It has been reported that the outcome measures on the BSID-III yield higher values compared to the BSID-II[45]. Nevertheless, we still found associations between neonatal cerebral oxygenation measurements and outcome in a heterogeneous group of preterm infants assessed at 2 to 3 years of age. Whether these associations will also be found in a group of preterm infants assessed at school age, has yet to be researched.

Conclusion

Our study demonstrated that neurodevelopmental outcome at 2 to 3 years of age was associated with cerebral oxygen saturation in preterm infants during the first two weeks after birth. Both high and low r_cSO_2 on day 1 and persistent low r_cSO_2 during the first two weeks after birth were associated with poorer cognitive outcome. The amount of time $r_cSO_2 < 50\%$ on day 1 was negatively associated with gross motor outcome. Highest quartile of FTOE values on day 1 was associated with poorer total motor outcome.

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chapter

10

General discussion and future perspectives

Elise A. Verhagen

The primary aim of this thesis was to determine the added value of monitoring cerebral oxygenation with near-infrared spectroscopy (NIRS) as part of clinical practice in neonatal intensive care. The secondary aim was to determine the course of cerebral oxygen saturation and extraction in newborn infants at risk of disturbances in cerebral oxygenation during the first weeks after birth. We determined cerebral oxygenation by means of NIRS, a technique that measures regional cerebral tissue oxygen saturation (r_cSO_2) and that allows us to calculate fractional tissue oxygen extraction (FTOE) by combining the r_cSO_2 and arterial oxygen saturation (SpO_2) values with the formula FTOE = (SpO_2 - r_cSO_2)/SpO_2. We categorized the variables under investigation into neonatal and maternal risk factors for disturbances in cerebral oxygenation, as well as other diagnostic techniques to determine the prognostic value of NIRS.

Regarding the neonatal risk factors we determined the presence or absence of cerebrovascular autoregulation by using NIRS (*Chapter 2*), and investigated the association of transient periventricular echodensities (TPE) (*Chapter 3*), germinal matrix hemorrhages-intraventricular hemorrhages (GMH-IVH) (*Chapter 4*), and anemia and red blood cell transfusions (*Chapter 5*). Regarding maternal risk factors we investigated the influence of treatment with antihypertensive drugs (*Chapter 6*) and tobacco smoking during pregnancy (*Chapter 7*). The other diagnostic techniques that we used for reasons of comparison or to determine the prognostic value of NIRS were the amplitude-integrated electro encephalogram (aEEG) (*Chapter 8*) and an extensive follow-up examination of neurodevelopmental outcome at two to three years of age of preterm-born infants in whom we had determined cerebral oxygenation during the first two weeks after birth (*Chapter 9*).

This thesis provides evidence that potentially NIRS is a useful clinical tool that reflects cerebral perfusion and helps to identify pathologies. It may therefore contribute towards predicting and possibly preventing adverse outcomes through timely intervention. Such a clinical tool is urgently needed in neonatal practice because the incidence of neurodevelopmental sequelae due to brain damage has not declined despite improved neonatal care.

Cerebral perfusion

Changes in cerebral perfusion expose the preterm infant to the risk of brain damage, especially in case of impaired cerebrovascular autoregulation.[1] In *Chapter 2* we determined the presence or absence of cerebrovascular autoregulation in preterm infants by determining the relationship between mean arterial blood pressure (MABP) and FTOE. Based on the statistically significant negative correlation between MABP and FTOE during 24 hours, cerebrovascular autoregulation appeared to be lacking in 40% of the preterm infants we studied. We were unable to predict absence of cerebrovascular autoregulation in terms of clinical variables. The added value of NIRS had previously been established for determining cerebrovascular autoregulation by using the r_cSO_2 values.[2] In our opinion FTOE is also an invaluable parameter for determining the presence of cerebrovascular autoregulation because the dependency on arterial oxygen saturation is eliminated.

In addition to calculating correlations between MABP and FTOE per infant we made scatter plots of MABP and FTOE and asked three neonatologists, who were unaware of the correlations, to determine the presence or absence of cerebrovascular autoregulation by eye-balling the plots (unpublished data).

The agreement between the three observers and their agreement with the correlations resulted in a kappa value of 0.41, which is reasonable. Thus, besides the calculated correlations between MABP and FTOE, eye-balling is also a reasonably reliable tool for gaining insight into the presence or absence of cerebrovascular autoregulation. Plotting MABP and FTOE (or r_cSO_2) allows bedside visualization of the course of MABP and FTOE and their association in individual infants. Lemmers *et al*. also produced plots of MABP, FTOE, and r_cSO_2 on which the presence and absence of cerebrovascular autoregulation was clearly visualized.[3]

Other methods, such as magnetic resonance imaging, position emission topography scans, single photon emission computed tomography, and Xenon-enhanced computed tomography, can also be used to study cerebral hemodynamics. These techniques, however, are not available at the bedside, may be expensive and time-consuming and usually do not allow for continuous monitoring.[4] Although Doppler ultrasound is available at the bedside and non-invasive, it is unsuitable for prolonged monitoring and only reflects cerebral blood flow velocity in a single artery.[5,6] NIRS is non-invasive, available at the bedside, and it measures oxygen saturation continuously. This combination makes it a useful tool in the process of deciding whether cerebral perfusion is at risk and how it may be improved.

The decision-making process could be improved even further by combining NIRS with measuring electrocerebral activity by amplitude-integrated electro encephalogram (aEEG). In *Chapter 8* we demonstrated a clear relationship between electrocerebral activity and FTOE. We found increased FTOE while the amplitudes of the aEEG narrowed, indicating a more mature background pattern.

FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen consumption.[7,8] We speculate that this higher FTOE indicates higher cerebral oxygen consumption since it seems unlikely that the increase in FTOE is the result of a decrease in cerebral blood flow (CBF). If this were so, we would have expected decreased electrocerebral activity. But this was not the case. The combination of FTOE and electrocerebral activity may be a useful biomarker of brain function in preterm infants at risk of disturbances in cerebral oxygenation. Earlier, aEEG and NIRS measurements were combined in asphyxiated term-born infants and proved to be invaluable in assessing the effect of cooling treatment and pharmacological interventions.[9-11] Thus, NIRS measurements may provide indications for neonatologists to focus on preserving cerebral oxygen supply to protect the brain.

Cerebral oxygenation

This thesis provides insight into the course of cerebral oxygenation in preterm infants exposed to various risk factors related to disturbed cerebral oxygenation during the first two weeks after birth. In *Chapters 3, 4, 6*, and *7* we compared cerebral oxygenation of preterm infants exposed to risk factors with that of non-exposed preterm infants. Table 1 provides an overview of the median r_cSO_2 and FTOE values in preterm infants during the first two weeks after birth with either TPE, GMH-IVH, tobacco exposure during pregnancy, or antihypertensive drug exposure during pregnancy. It also provides the course of r_cSO_2 and FTOE in infants in whom none of these risk factors were present. Overall, and in every study group, we found decreasing r_cSO_2 and increasing FTOE during the first two weeks after birth. In Table 1 we marked the highest (in bold type) and lowest (underlined) r_cSO_2 values and the lowest (in bold type) and highest (underlined) FTOE values.

TABLE 1. The course of cerebral oxygen saturation (r_cSO_2) and extraction (FTOE) during the first two weeks after birth in preterm infants with TPE, GMH-IVH, exposure to tobacco, and antihypertensive drugs during pregnancy

Pathology	r_cSO_2							FTOE						
	day 1	day 2	day 3	day 4	day 5	day 8	day 15	day 1	day 2	day 3	day 4	day 5	day 8	day 15
None (n=20)	82 (74-84)	81 (77-85)	**80** (73-85)	80 (75-86)	78 (72-86)	75 (71-80)	**75** (69-79)	0.16 (0.13-0.25)	0.15 (0.11-0.20)	0.19 (0.15-0.24)	0.17 (0.10-0.21)	0.17 (0.10-0.23)	0.20 (0.16-0.25)	0.20 (0.17-0.24)
TPE (n=25)	79 (73-80)	77 (73-81)	77 (73-86)	76 (72-79)	77 (71-81)	**78** (71-83)	66 (62-74)	0.16 (0.10-0.26)	0.17 (0.12-0.21)	0.14 (0.09-0.23)	0.19 (0.16-0.24)	0.17 (0.12-0.26)	0.17 (0.12-0.25)	0.30 (0.26-0.34)
GMH-IVH (n=17)	72 (66-80)	72 (67-78)	71 (67-75)	70 (65-75)	64 (62-68)	70 (68-75)	62 (61-74)	0.22 (0.13-0.25)	0.25 (0.15-0.28)	0.23 (0.20-0.30)	0.26 (0.21-0.28)	0.30 (0.29-0.36)	0.27 (0.15-0.31)	0.32 (0.24-0.37)
Tobacco exposure (n=14)	73 (64-78)	73 (71-78)	75 (68-80)	75 (70-80)	79 (74-80)	71 (67-74)	70 (65-77)	0.24 (0.16-0.26)	0.18 (0.16-0.22)	0.20 (0.14-0.25)	0.19 (0.16-0.24)	0.18 (0.15-0.23)	0.26 (0.22-0.28)	0.27 (0.17-0.32)
Antihypertensive drug exposure (n=9)	**84** (79-85)	**86** (74-90)	79 (72-90)	**83** (76-86)	**86** (76-87)			**0.11** (0.10-0.12)	**0.07** (0.04-0.11)	**0.14** (0.06-0.20)	**0.13** (0.08-0.16)	**0.12** (0.10-0.14)		

Numbers indicate median (25th – 75th percentile). TPE indicates transient periventricular echodensities, GMH-IVH; germinal matrix hemorrhages-intraventricular hemorrhages. The numbers in bold type indicate highest r_cSO_2 or lowest FTOE per day. The underlined numbers indicate lowest r_cSO_2 and highest FTOE per day, respectively.

Regional cerebral tissue oxygen saturation

R_cSO_2 serves as an indicator of hypoxic hypoxia because it depends mostly on arterial oxygen saturation.[12] If arterial oxygen saturation is low, r_cSO_2 will also be low. We measured r_cSO_2 during the first two weeks after birth to cover the complete transitional phase after birth. The infants we studied were either preterm or very preterm; most had a gestational age of less than 32 weeks.

Several previous studies that described the course of cerebral oxygen saturation are limited to the first hours or one to three days after birth[3,13-16], while several other previous studies covered the first weeks after birth up to six months.[17-22] Two of the latter studies actually described cerebral oxygenation in term infants.[20,21] Our studies confirmed the findings of other longitudinal studies lasting longer than the first week after birth: in preterm infants cerebral oxygen saturation decreases during the first weeks after birth.

We propose several explanations for this phenomenon. First, a decrease in cerebral oxygen saturation could be explained by a decrease in arterial oxygen saturation.[23] This has not been reported earlier; neither in the studies in this thesis, nor in other studies covering cerebral oxygen saturation during the first weeks after birth. Even though, in individual patients, a decrease in arterial oxygen saturation can indeed result in a decrease in cerebral oxygen saturation, the overall decrease in cerebral oxygen saturation during the first two weeks after birth cannot be explained in terms of a decrease in arterial oxygen saturation.

Second, a decrease in cerebral oxygen saturation could indicate a decrease in hemoglobin concentration.[24] In *Chapter 5* we described a positive correlation between hemoglobin concentration and r_cSO_2, and a negative correlation between hemoglobin concentration and FTOE in infants requiring red blood cell transfusions. These infants were at risk of anemic hypoxia and do, therefore, not represent the 'normal' physiological changes in hemoglobin concentration. It is known that hemoglobin concentration decreases during the first weeks after birth because fetal hemoglobin production stops which causes fetal hemoglobin levels to decline.[25] This is followed by an increase in adult hemoglobin, a process that takes several weeks to months. It seems unlikely, therefore, that the decrease in r_cSO_2 during the first two weeks after birth is solely the result of a decrease in fetal hemoglobin concentration. Nevertheless, some influence cannot be ignored.

The question that arises is whether fetal hemoglobin concentration influences the near-infrared light absorption differently or whether adult hemoglobin absorbs near-infrared light better. To the best of our knowledge the definitive answer to this question is lacking but, since most studies focus on the first days or weeks after birth, it probably will not change the results tremendously.

Fractional tissue oxygen extraction

As described above, r_cSO_2 depends largely on changes in arterial oxygen saturation and therefore FTOE is a better indicator of low CBF, which may result in ischemic hypoxia.[7] In case of lower CBF, oxygen extraction needs to increase to maintain the metabolic needs of cerebral tissue. As stated earlier, during the first two weeks after birth we found increased FTOE while the amplitudes of the aEEG narrowed (*Chapter 8*). It seems unlikely that the increase in FTOE is the result of a decrease in CBF, in which case we

would have expected decreased electrocerebral activity. Actually, Roche-Labarbe *et al.* found that CBF increases during the first weeks after birth.[17]

A final explanation can, therefore, be sought by focusing on the cerebral metabolic rate. Previously, the cerebral metabolic rate was found to increase more than two-fold during the first one to two weeks after birth.[26] Roche-Labarbe *et al.* found a strong correlation between cerebral metabolic rate and postnatal age.[17] It seems likely that the increase in CBF does not keep up with the increase of cerebral metabolic rate. If so, oxygen extraction increases even more to meet the metabolic demands of the preterm brain. Thus, we explain the course of r_cSO_2 and FTOE in preterm infants during the first two weeks after birth, in terms of an increased cerebral metabolic rate that results in lower cerebral oxygen saturation and higher cerebral oxygen extraction.

Cerebral pathologies or risk factors

We studied the course of r_cSO_2 and FTOE in preterm infants exposed to a variety of risk factors. All these risk factors influenced the course of cerebral oxygenation. Some exerted this influence for a limited period only, e.g. immediately after birth. Others did so for the entire two-week period under study. In *Chapter 5* we demonstrated that cerebral oxygenation in preterm infants may be at risk when hemoglobin falls below 6 mmol/l (97 g/l). TPE and exposure to tobacco during pregnancy resulted in a relatively short period of lower r_cSO_2 and higher FTOE in comparison to infants without these risk factors (*Chapters 3* and *7*). These effects were subtle, which could explain the lack of an independent effect on neurodevelopmental outcome of these risk factors (*Chapter 9*). On the other hand, both risk factors did lead to disturbed cerebral oxygen saturation and extraction in the infants studied.

The lowest r_cSO_2 and highest FTOE were found in infants with GMH-IVH (*Chapter 4*). This was present from the first day after birth onwards. It is known that infants who develop GMH-IVH are at risk of a disturbed neurodevelopmental outcome even with GMH-IVH grades I or II.[27] We also found disturbed motor outcome in infants with GMH-IVH (*Chapter 9*).

We speculate that the presence of several risk factors possibly have a cumulative effect on disturbing cerebral oxygenation and subsequent neurodevelopmental outcome. This suggests that it is very important to protect the vulnerable preterm brain, especially during the first days after birth.

Predicting outcome

In *Chapter 9* we found that cerebral oxygenation on the first day after birth was associated with the neurodevelopmental outcome of preterm infants at two to three years of age. Both relatively low and high r_cSO_2 values on day 1 were associated with poorer cognitive outcome. A study with 36 two-year old, preterm-born infants assessed with the Bayley Scales of Infant Development, Second Edition (BSID-II), reported a negative correlation between the mental developmental index and r_cSO_2 on day 1 and a negative correlation between the psychomotor developmental index and r_cSO_2 on days 1 to 3 after birth.[28] Our study confirms these findings on cognitive outcome. High FTOE values on day 1 were associated with poorer total motor outcome. One study described poorer motor outcome in term infants with signs of ischemic damage on MRI (increased lactate, reflecting neuronal damage and accumulation of products of anaerobic metabolism).[29]

The presence of low r_cSO_2 values suggests the presence of cerebral hypoxic hypoxia. FTOE serves as an indicator of cerebral ischemic hypoxia.[12] In both types of hypoxia there is less oxygen available to tissue. This results in less ATP production and changes in the membrane potential that, in turn, results in the production of edema.[30] On the one hand, this in itself causes neuronal damage.[31] On the other hand, it is also possible that the actual cerebral damage evolved after the supply of cerebral oxygen was restored.[30] It is known that persistent hypoxia may lead to an adjustment of the oxygen sensing set point in cells. Cells may then sense hyperoxia under otherwise normal oxygen concentrations.[30] Hyperoxia may contribute to neuronal injury by causing a reduction in CBF.[31] Sorensen *et al.* proposed that the preterm brain might actually be hyperoxygenated.[16] Hyperoxia causes oxidative stress because of a cascade of production of free radicals and toxic reactive oxygen species.[31] Due to their immature cardiovascular system the risk for preterm infants of oxidative stress is increased. It is difficult to explain why hypoxic hypoxia on day 1 is specifically associated with cognitive outcome while ischemic hypoxia on day 1 is specifically associated with motor outcome. This observation seems to suggest different damaging pathways.

We stress the fact that the group of preterm infants we studied represented a regular, i.e. heterogeneous neonatal intensive care unit population. And it is in this group that we demonstrated the influence of neonatal cerebral oxygen saturation and extraction, as measured by NIRS, on neurodevelopmental outcome at two to three years of age. NIRS, therefore, may contribute towards predicting neurodevelopmental outcome of preterm-born infants at two to three years of age.

Useful clinical tool

Reliability, accuracy, and ease of use are requirements that determine whether a tool has added value in clinical practice In *Chapter 1* we described some of these requirements. Spatially resolved spectroscopy (SRS) was introduced in the late 1990s which improved the usefulness of NIRS.[32,33] It provides absolute values within certain margins. In addition, SRS is less sensitive to movement and easier to use because the optical detector and receiver are combined in one sensor.

In our opinion, this thesis adds to the current understanding of NIRS as a potentially useful clinical tool that reflects cerebral perfusion, helps to identify the pathophysiology of several perinatal and neonatal morbidities, and in so doing may contribute to the prediction and possibly prevention of adverse outcomes. We therefore succeeded in establishing the added value of NIRS in neonatal clinical practice. NIRS, in combination with other physiological and cerebral parameters for monitoring the young brain, seems a promising tool worthy of being optimized further. Others have also shown that NIRS is useful as a screening tool[34], and that it can be readily used to monitor treatment[35], both of which are most relevant from a clinical point of view.

Limitations of this thesis

One of the main criticisms of NIRS is the repeatability of the cerebral oxygen saturation values. These are reported to vary between 5% and 8%.[36-38] We have endeavored to eliminate this by optimizing the research setting. During all the studies described in this thesis the infants were monitored while at rest

or asleep in the incubator and lying in supine position. Fifteen minutes were allowed for stabilization of the measurement. The optical sensor was placed on the left frontoparietal side of the infant's head and held in place by elastic bandaging (Figure 1). We marked the location of the sensor to ensure that the sensor was placed in the same position for each measurement. Unpublished data from our group show a mean difference of 4.5% (range 1% to 8%) between three consecutive r_cSO_2 measurements in the same patient. Nevertheless, we found a relationship between cerebral oxygenation measurements in the neonatal phase and neurodevelopmental outcome at two to three years of age.

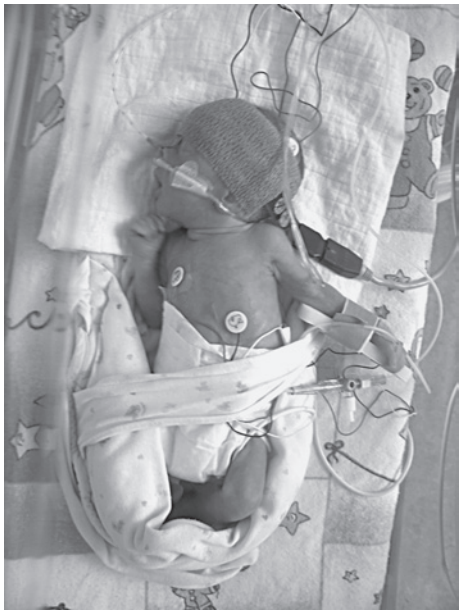

FIGURE 1. Clinical setting of the NIRS measurements
Photo by E.A. Verhagen

No consensus has yet been reached on normal values for preterm infants due to the variability in cerebral oxygenation values between NIRS devices, sensors, and the variability after sensor replacement.[36-42] The results presented in *Chapter 9* led us to conclude that r_cSO_2 values between 79% and 83% on day 1 after birth were associated with best cognitive outcome. All studies presented in this thesis were conducted in the University Medical Center Groningen. Whether our results can be generalized to other centers needs to be established. A large, multi-center trial to determine normal cerebral oxygenation values in preterm infants is therefore urgently required.

Future perspectives

As a consequence of their immature respiratory and circulatory system preterm infants frequently require oxygen therapy. Optimal oxygen exposure, with as little damage as possible due to free radicals for example, remains a matter of debate.[30,43] Since NIRS provides an indication of the oxygen supply

to and consumption by cerebral tissue, the very same tissue where the damage evolves that results in adverse neurodevelopmental outcome, we think that NIRS monitoring may have a key role in determining optimal oxygen exposure. We demonstrated that both relatively low and relatively high cerebral oxygen saturation values are less optimal for neurodevelopmental outcome. Animal studies indicated that cerebral oxygen saturation levels of less than 40% to 50% are dangerous to the newborn brain and possibly cause damage, especially if they last more than 30 minutes.[44,45] Larger trials are needed to determine what values are damaging to the preterm human brain.

Besides cerebral monitoring by means of NIRS, this technique has the potential of being valuable in monitoring systemic oxygenation. Conventional diagnostic possibilities are insufficient to detect low systemic blood flow in preterm infants. There are indications that while cerebral perfusion is still preserved, possibly due to cerebrovascular autoregulation, systemic perfusion might already be disturbed in infants with circulatory failure.[46] Furthermore, systemic and cerebral oxygenation measurements can be used together to determine the splanchnic-cerebral oxygenation ratio (SCOR) as a marker of overall tissue oxygen sufficiency.[47,48] The precision of NIRS depends on tissue homogeneity.[49] Therefore, studies are needed that determine the most valuable and suitable location for systemic NIRS measurements.

The main aim of modern neonatology is the prevention of major neurodevelopmental problems due to brain damage.[50] Whenever possible early intervention is mandatory. NIRS promises to be the technique with which to attain this aim. It is the definitive, non-invasive, clinical bedside tool currently available for determining the presence or absence of cerebrovascular autoregulation. NIRS can, therefore, play an essential tool in the decision-making process involved in improving cerebral circulation. A large-scale, multi-center trial to test this hypothesis is urgently required.

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chapter

11

A main aim of present-day neonatology is the prevention of major neurodevelopmental problems due to brain damage. Potentially, perinatal and neonatal risk factors that cause disturbed cerebral oxygenation may harm the integrity and development of the young preterm and ill term-born brain. Cerebral oxygenation depends on parameters such as oxygen delivery, and oxygen demand and consumption. If oxygen delivery is insufficient the brain is exposed to hypoxia. This can be categorized into three types: hypoxic hypoxia, anemic hypoxia, and ischemic hypoxia. Hypoxic hypoxia results from low arterial oxygen saturation. This causes a decrease in oxygen delivery unless cerebral blood flow (CBF) increases. Anemic hypoxia results from low hemoglobin (Hb) concentration or insufficient availability or capacity of red blood cells. Due to frequent blood sampling in combination with their immature hematopoietic system, preterm infants are at risk of having low Hb concentrations. Ischemic hypoxia is the result of lower CBF. CBF is determined by the infant's cardiac output and cerebrovascular resistance. Within limits, cerebral vessels adapt to changes in blood pressure in order to maintain a constant CBF in case of intact cerebrovascular autoregulation.

Notwithstanding the fact that the clinical status of the infant is monitored by measuring arterial oxygen saturation, blood pressure, heart rate, and respiratory parameters, none of these parameters satisfactorily reflect actual cerebral perfusion or cerebral functioning. Therefore, a non-invasive, bedside tool that reflects cerebral perfusion, that helps to identify pathologies, and in so doing may help to predict or possibly prevent adverse outcomes, is urgently required.

Cerebral perfusion can be assessed indirectly by measuring cerebral tissue oxygenation by means of near-infrared spectroscopy (NIRS). This thesis focuses mainly on the use of NIRS, a non-invasive, bedside technique first introduced in 1977 by Jöbsis to monitor cerebral oxygenation and hemodynamics. In 1985, Brazy *et al.* were the first to use NIRS in neonatal clinical practice. NIRS is based on the fact that biological tissue is relatively transparent to near-infrared light, i.e. wavelengths between 700 to 1000 nm. The chromophores oxygenated Hb and deoxygenated Hb will partly absorb one part of this near-infrared light. A second part of the light will be scattered and a third part will be reflected. The quantity of reflected light photons of two wavelengths in the near-infrared region determines the spectral absorption of the underlying tissue. NIRS differentiates oxygenated Hb from deoxygenated Hb, each of which has a distinct absorption spectrum. The ratio of oxygenated Hb to total Hb reflects the regional oxygen saturation of tissue. About 70% to 80% comes from venous blood, 5% from the capillary compartment, and 20% to 25% comes from arterial blood. In this way, NIRS measures regional cerebral tissue oxygen saturation (r_cSO_2) and allows calculation of fractional tissue oxygen extraction (FTOE) by combining the r_cSO_2 and arterial oxygen saturation (SpO_2) values in the formula $FTOE = (SpO_2 - r_cSO_2)/SpO_2$. R_cSO_2 serves as an indicator of hypoxic hypoxia because it depends mostly on arterial oxygen saturation. FTOE reflects the balance between cerebral oxygen supply and cerebral oxygen consumption. It, therefore, serves as an indicator of cerebral ischemic hypoxia. All the studies reported on in this thesis were conducted with the INVOS 4100-5100 (Somanetics Corporation, Troy, Michigan, USA) in combination with pediatric SomaSensors.

The primary aim of this thesis was to determine the clinical value of monitoring cerebral oxygenation by means of NIRS. The r_cSO_2 and FTOE values of preterm infants during the first weeks after birth are

largely unknown. Our second aim was to determine the course of cerebral oxygen saturation and extraction during the first weeks after birth in newborn infants at risk of disturbed cerebral oxygenation. We measured r_cSO_2 during the first two weeks after birth to cover the transitional phase immediately after birth and beyond. All infants studied were preterm-born infants.

In the first part of the thesis we focused on neonatal risk factors for disturbed cerebral oxygenation and the second part focused on maternal risk factors for disturbed cerebral oxygenation. Changes in cerebral perfusion pose the preterm infant with the risk of brain damage, especially in case of impaired cerebrovascular autoregulation. In *Chapter 2* we explored clinical parameters that might predict absence of cerebrovascular autoregulation. We did so by determining the relationship between mean arterial blood pressure (MABP) and FTOE in twenty-five preterm infants for 24 hours during the first 72 hours after birth. We were unable to predict absence of cerebrovascular autoregulation in terms of clinical variables. Nevertheles, by using NIRS, we found a statistically significant negative correlation between MABP and FTOE in 40% of the preterm infants, which suggests absence of cerebrovascular autoregulation. In *Chapters 3, 4, 6,* and *7*, we compared cerebral oxygenation of preterm infants exposed to risk factors with the cerebral oxygenation of non-exposed preterm infants. In *Chapter 3* we reported that r_cSO_2 was lower and FTOE higher in preterm infants with persistent transient periventricular echodensities (TPE) after two weeks. This suggests that persistent TPEs were associated with an increased demand for cerebral oxygen from the first week after birth. Cerebral oxygenation may be involved in the recovery of perinatal white matter damage. In *Chapter 4* we found that r_cSO_2 was lower and FTOE higher in preterm infants with germinal matrix hemorrhages-intraventricular hemorrhages (GMH-IVH) during the first two weeks after birth. This suggests that, following GMH-IVH, cerebral perfusion decreased persistently for two weeks after birth or decreased cerebral perfusion increases the risk of developing GMH-IVH. In *Chapter 6* we addressed the influence of antihypertensive drugs on FTOE in preterm infants whose mothers were treated with antihypertensive drugs during pregnancy. We found that treating pregnant women with labetalol and/or $MgSO_4$ was associated with reduced FTOE during the first days after birth. Regarding nifedipine, we found no difference in FTOE between the exposed and non-exposed infants. In *Chapter 7* we described that exposure to tobacco during pregnancy influenced r_cSO_2 and FTOE in preterm infants during the first two days after birth. R_cSO_2 was lower and FTOE higher. This suggests that maternal smoking may compromise brain oxygenation of the newly born infant.

Overall, and in each single study, we found decreasing r_cSO_2 and increasing FTOE during the first two weeks after birth. We proposed several explanations for the decrease in cerebral oxygen saturation and increase in cerebral oxygen extraction during the first two weeks after birth. It seems unlikely, however, that the decrease in cerebral oxygen saturation can be explained in terms of a decrease in arterial oxygen saturation. Nevertheless, a decrease in cerebral oxygen saturation could indicate a decrease in the concentration of Hb. In *Chapter 5* we studied infants who had possibly been exposed to anemic hypoxia. We reported a positive correlation between Hb concentration and r_cSO_2 and a negative correlation between Hb concentration and FTOE in infants requiring red blood cell transfusions. It is known that Hb concentration decreases during the first weeks after birth due to the fact that the production of fetal Hb stops and that fetal Hb levels decline as a consequence. This is followed by an increase in adult Hb,

even though this process takes several weeks to months. It seems unlikely, therefore, that the decrease in r_cSO_2 during the first two weeks after birth is solely the result of a decrease in the concentration of fetal Hb. Some influence, however, cannot be ignored. It seems unlikely that the increase in cerebral oxygen extraction is the result of a decrease in CBF in which case we would have expected a decrease in electrocerebral activity. We actually found increased FTOE while the amplitudes of the amplitude-integrated electro encephalogram (aEEG) narrowed during the first two weeks after birth as reported in *Chapter 8*. A final explanation could, therefore, be sought by focusing on the cerebral metabolic rate. Cerebral metabolic rate increases more than two-fold during the first one to two weeks after birth. CBF also increases, but it seems likely that this increase does not keep up with the increase of cerebral metabolic rate. If so, oxygen extraction increases even more to meet the metabolic demands of the preterm brain. We explained the course of r_cSO_2 and FTOE in preterm infants during the first two weeks after birth by an increased cerebral metabolic rate resulting in lower cerebral oxygen saturation and higher cerebral oxygen extraction.

We speculate that the presence of several risk factors possibly have a cumulative effect on disturbing the cerebral oxygenation and subsequent neurodevelopmental outcome. This suggests that it is very important to protect the vulnerable preterm brain, especially during the first days after birth.

In Part 3 we described two other techniques that helped to determine the prognostic value of NIRS. In *Chapter 8* we studied the relationship between r_cSO_2 and FTOE, and the aEEG. We found increased FTOE while the amplitudes of the aEEG narrowed, indicating a more mature background pattern. The combination of FTOE and electrocerebral activity may be a useful biomarker for neonatologists of brain function in preterm infants at risk of disturbances in cerebral oxygenation. In *Chapter 9* we assessed the predictive value of NIRS for neurodevelopmental outcome on the basis of an extensive follow-up examination. In a heterogeneous group of preterm infants we were able to demonstrate an influence of neonatal cerebral oxygen saturation and extraction measurements by NIRS on neurodevelopmental outcome at two to three years of age. NIRS, therefore, may contribute towards predicting neurodevelopmental outcome of preterm-born infants in this age-range. Both the relatively low and high r_cSO_2 values on day 1 were associated with poorer cognitive outcome. The high FTOE values on day 1 were associated with poorer total motor outcome. The presence of low r_cSO_2 values suggested the presence of cerebral hypoxic hypoxia. FTOE serves as an indicator of cerebral ischemic hypoxia. In both types of hypoxia there is less oxygen available to tissue. Persistent hypoxia may lead to an adjustment of the oxygen sensing set point in cells. Cells may then sense hyperoxia under otherwise normal oxygen concentrations. Hyperoxia may contribute to neuronal injury by causing a reduction in CBF and a cascade of production of free radicals and toxic reactive oxygen species. Because of their immature cardiovascular system preterm infants are especially sensitive to oxidative stress. It is difficult to explain why hypoxic hypoxia on day 1 was specifically associated with cognitive outcome and why ischemic hypoxia on day 1 was specifically associated with motor outcome. Possibly different damaging pathways are involved.

Since a main aim of present-day neonatology is the prevention of major neurodevelopmental problems due to brain damage, early intervention is mandatory, whenever possible. NIRS is instrumental

in achieving this target and this underlines the added value of this technique to neonatology. NIRS seems to be the definitive, non-invasive, bedside tool able to determine the presence or absence of cerebrovascular autoregulation. This allows a role for NIRS in the decision-making process involved in improving cerebral circulation. A large-scale, multi-center trial to test this hypothesis is urgently required. We believe that this thesis adds to the current understanding that potentially NIRS is a useful clinical tool that reflects cerebral perfusion, helps to identify the pathophysiology of several perinatal and neonatal morbidities, and in so doing may contribute to the prediction of and possibly help prevent adverse outcomes. In combination with other cerebral parameters, it seems a promising tool towards further optimizing the monitoring of the young brain. We believe that NIRS monitoring may be instrumental in determining optimal oxygen exposure.

Besides cerebral monitoring by means of NIRS, potentially it is also a valuable tool for monitoring systemic oxygenation. There are indications that while cerebral perfusion is still preserved, possibly due to cerebrovascular autoregulation, systemic perfusion might already be disturbed in infants with circulatory failure. The precision of NIRS depends on tissue homogeneity. Therefore, additional studies are required to determine the most suitable location for systemic NIRS measurements.

chapter

11

Een van de belangrijkste doelen van de moderne neonatologie is preventie van ontwikkelingsproblemen door cerebrale schade. Perinatale en neonatale risicofactoren die van invloed zijn op de cerebrale zuurstofvoorziening, kunnen de bouw en ontwikkeling van hersenen van preterme en zieke a terme pasgeborenen beschadigen. De cerebrale zuurstofvoorziening wordt bepaald door de cerebrale zuurstoftoevoer, zuurstofbehoefte en het zuurstofverbruik. Een gestoorde cerebrale zuurstofvoorziening stelt de hersenen bloot aan hypoxie. Hypoxie kan worden onderverdeeld in drie subtypes: hypoxische hypoxie, anemische hypoxie, ischemische hypoxie. Hypoxische hypoxie ontstaat door een lage arteriële zuurstofsaturatie met onvoldoende zuurstoftoevoer naar de hersenen als gevolg, tenzij de bloedstroom naar de hersenen toeneemt. Anemische hypoxie ontstaat door een laag hemoglobine (Hb) gehalte of onvoldoende aanbod of capaciteit van erytrocyten. Preterme pasgeborenen hebben een verhoogd risico op een laag Hb gehalte omdat er vaak bloed moet worden afgenomen terwijl de erytropoëse nog niet volledig ontwikkeld is. Ischemische hypoxie ontstaat in het geval van onvoldoende bloedstroom naar de hersenen. De bloedstroom naar de hersenen wordt bepaald door de 'cardiac output' en de cerebrovasculaire weerstand. Cerebrale vaten passen zich aan aan veranderingen in bloeddruk om zo een constante bloedstroom naar de hersenen te waarborgen. Dit wordt de cerebrovasculaire autoregulatie genoemd.

Ondanks intensieve metingen van arteriële zuurstofsaturatie, bloeddruk, hartfrequentie en ademhaling om de klinische situatie van een preterme of ziek a terme pasgeborene goed in de gaten te houden, geven geen van deze parameters een goede weergave van cerebrale perfusie of het functioneren van de hersenen. Daarom is er grote behoefte aan een niet-invasieve 'bedside' techniek, om een indruk te krijgen van de cerebrale perfusie, om pathofysiolologische processen in kaart te brengen en mogelijk daarmee ontwikkelingsproblemen te voorspellen of te voorkomen.

De zuurstofvoorziening van de hersenen kan met behulp van het niet-invasieve nabij-infrarood licht spectroscopie (NIRS) worden gemeten. Dit geeft indirect een indruk van de cerebrale perfusie. Jöbsis introduceerde NIRS om de cerebrale zuurstofvoorziening en hemodynamiek te meten in 1977. Het werd voor het eerst toegepast bij pasgeborenen in 1985 door Brazy *et al*. NIRS is gebaseerd op het feit dat biologisch weefsel relatief transparant is voor nabij-infrarood licht (golflengte van 700 tot 1000 nanometer). Zuurstofgebonden Hb (HbO_2) en zuurstofvrij Hb (gedeoxygeneerd Hb, HbD) zijn chromoforen die een deel van dit nabij-infrarood licht absorberen. Een ander deel wordt verspreid door het weefsel en een derde deel wordt gereflecteerd. De hoeveelheid lichtfotonen die gereflecteerd wordt op 2 golflengtes binnen het bijna-infrarode licht domein, geeft een beeld van de absorptie van het onderliggende weefsel. NIRS maakt onderscheid tussen HbO_2 en HbD die elk een verschillend absorptiespectrum hebben. De ratio tussen HbO_2 en totaal Hb bepaalt het regionale weefsel zuurstofgehalte. Deze waarde bestaat voor 70% tot 80% uit het zuurstofgehalte in het veneuze compartiment, voor 5% uit het capillaire compartiment en voor 20% tot 25% uit het zuurstofgehalte in het arteriële compartiment.

NIRS meet de regionale cerebrale weefsel zuurstofsaturatie (r_cSO_2). De fractionele weefsel zuurstofextractie (FTOE) wordt berekend met r_cSO_2 en arteriële zuurstofsaturatie (SpO_2) waarden: $FTOE = (SpO_2 - r_cSO_2)/SpO_2$. R_cSO_2 is een indicator van hypoxische hypoxie omdat deze maat voornamelijk

afhankelijk is van de arteriële zuurstofsaturatie. FTOE is een maat voor de balans tussen de cerebrale zuurstoftoevoer en het zuurstofverbruik waardoor het een indicatie geeft van de aanwezigheid van ischemische hypoxie. Alle studies in dit proefschrift werden uitgevoerd met de INVOS 4100-5100 (Somanetics Corporation, Troy, Michigan, USA) en de SomaSensoren voor kinderen.

Het primaire doel van dit proefschrift was het vaststellen van de klinische waarde van het meten van de cerebrale zuurstofvoorziening met behulp van NIRS. Normaalwaarden van r_cSO_2 en FTOE gedurende de eerste weken na de geboorte zijn onbekend. Het tweede doel van dit proefschrift was daarom het vaststellen van het beloop van de cerebrale zuurstofsaturatie en -extractie van pasgeborenen met een verhoogd risico op een gestoorde cerebrale zuurstofvoorziening gedurende de eerste weken na de geboorte. R_cSO_2 werd gedurende de eerste twee weken na de geboorte gemeten. Alle pasgeborenen waren preterm geboren.

Allereerst richt dit proefschrift zich op risicofactoren op een gestoorde cerebrale zuurstofvoorziening, in het eerste deel op neonatale en in het tweede deel op maternale risicofactoren.

Veranderingen in de cerebrale perfusie verhogen het risico op hersenschade bij preterme pasgeborene, juist bij een gestoorde cerebrovasculaire autoregulatie. In *Chapter 2* onderzochten we klinische parameters die de afwezigheid van cerebrovasculaire autoregulatie zouden kunnen voorspellen. Hiervoor bepaalden wij de relatie tussen de gemiddelde arteriële bloeddruk en FTOE gedurende 24 uur in de eerste 72 uur na de geboorte bij 25 preterme pasgeborenen. Geen van de klinische parameters voorspelden de afwezigheid van cerebrovasculaire autoregulatie. Desalniettemin vonden we een statistisch significante negatieve correlatie tussen de gemiddelde arteriële bloeddruk en FTOE, zoals gemeten met NIRS, bij 40% van de preterme pasgeborenen. Dit suggereert dat de cerebrovasculaire autoregulatie afwezig is. In *Chapters 3, 4, 6* en *7* hebben we de cerebrale zuurstofvoorziening van aan risicofactoren blootgestelde preterme pasgeborenen vergeleken met die van preterme pasgeborenen die niet waren blootgesteld aan de risicofactoren. In *Chapter 3* hebben we beschreven dat twee weken na de geboorte de r_cSO_2 lager en FTOE hoger was bij preterme pasgeborenen met tijdelijke periventriculaire echodense gebieden. Dit suggereert dat in deze gebieden mogelijk een toegenomen zuurstofbehoefte bestaat na de eerste week na de geboorte. De cerebrale zuurstofvoorziening is mogelijk betrokken bij het herstel van perinatale witte stof schade. In *Chapter 4* vonden we dat de r_cSO_2 lager en FTOE hoger was gedurende de eerste twee weken na de geboorte bij preterme pasgeborenen met een germinale laag bloeding. Dit kan doordat de cerebrale perfusie gedurende de eerste weken na de geboorte verminderd is als gevolg van een germinale laag bloeding. Maar het kan ook zijn dat de verminderde perfusie een risico vormt voor het ontwikkelen van een germinale laag bloeding. In *Chapter 6* beschreven we de invloed van het gebruik van antihypertensiva tijdens de zwangerschap op de FTOE bij preterme pasgeborenen. We vonden dat de behandeling van zwangere vrouwen met labetalol en/of $MgSO_4$ gepaard ging met een verminderde FTOE tijdens de eerste dagen na de geboorte. Wij vonden geen verschil in FTOE wanneer moeders tijdens de zwangerschap waren behandeld met nifedipine in vergelijking met kinderen die niet waren blootgesteld aan maternale antihypertensiva. In *Chapter 7* beschreven we dat het roken van de moeder tijdens de zwangerschap van invloed is op de r_cSO_2 en FTOE gedurende de eerste twee dagen na de geboorte bij preterme pasgeborenen. R_cSO_2 was lager en

FTOE was hoger. Het lijkt er dus op dat roken tijdens de zwangerschap de cerebrale zuurstofvoorziening van pasgeborenen kan bedreigen.

In elke afzonderlijke studie vonden we in het algemeen een dalende r_cSO_2 en stijgende FTOE gedurende de eerste twee weken na de geboorte. Er zijn verschillende mogelijke verklaringen voor de gevonden afname in cerebrale zuurstofsaturatie en toename in cerebrale zuurstofextractie. Het lijkt onwaarschijnlijk dat de dalende cerebrale zuurstofsaturatie kan worden verklaard door een dalende arteriële zuurstofsaturatie. Een dalende cerebrale zuurstofsaturatie kan wel het gevolg zijn van een dalende Hb concentratie. In *Chapter 5* onderzochten we pasgeborenen die mogelijk werden blootgesteld aan anemische hypoxie. We beschreven een positieve correlatie tussen Hb concentratie en r_cSO_2 en een negatieve correlatie tussen Hb concentratie en FTOE bij pasgeborenen die een erytrocytentransfusie nodig hadden. De Hb concentratie neemt af gedurende de eerste weken na de geboorte omdat de productie van foetaal Hb stopt waardoor het foetaal Hb gehalte daalt. Dit wordt gevolgd door een toename van adult Hb. Dit proces duurt enkele weken tot maanden. Het lijkt daarom onwaarschijnlijk dat de afname in r_cSO_2 tijdens de eerste twee weken na de geboorte uitsluitend wordt veroorzaakt door een daling van de Hb concentratie. Echter, enige invloed is niet uit te sluiten. Het lijkt onwaarschijnlijk dat de toename in cerebrale zuurstofextractie wordt veroorzaakt door een daling in de cerebrale perfusie. In dat geval zouden we een meer discontinue patroon van electrocerebrale activiteit (EEG) hebben verwacht. Wij vonden juist een meer matuur, minder discontinue patroon op het amplitude geïntegreerde electro-encefalogram (aEEG), geassocieerd met een toename in FTOE gedurende de eerste twee weken na de geboorte, zoals beschreven in *Chapter 8*. Een laatste verklaring kan daarom gevonden worden in het cerebrale metabolisme, dus het cerebrale zuurstofverbruik. Dat neemt meer dan twee keer toe gedurende de eerste twee weken na de geboorte. De cerebrale perfusie neemt ook toe, maar het lijkt er op dat deze toename niet gelijk op gaat met de toename van het cerebrale metabolisme. De zuurstofextractie neemt dan meer toe om aan de cerebrale metabole en zuurstofbehoeften te voldoen. Wij verklaren het beloop van r_cSO_2 en FTOE gedurende de eerste twee weken na de geboorte bij preterme pasgeborenen daarom met een toename van het cerebrale metabolisme, wat dan resulteert in een lagere cerebrale zuurstofsaturatie en een hogere cerebrale zuurstofextractie.

Wij speculeren dat de aanwezigheid van verschillende risico factoren mogelijk een cumulatief effect kan hebben op het verstoren van de cerebrale zuurstofvoorziening en daarmee ook de neurologische ontwikkeling. Dit suggereert dat het erg belangrijk is om de kwetsbare preterme hersenen te beschermen, met name gedurende de eerste dagen na de geboorte.

In het derde deel van dit proefschrift beschreven wij andere bruikbare technieken om de prognostische waarde van NIRS te bepalen. In *Chapter 8* onderzochten wij de relatie tussen r_cSO_2, FTOE en het aEEG. FTOE nam toe terwijl de amplitudes van het aEEG kleiner werden. Dit is een indicatie van een meer matuur achtergrondpatroon. De combinatiemeting van FTOE en de electrocerebrale activiteit kan bruikbaar zijn voor neonatologen om de hersenfunctie van preterme pasgeborenen met een verhoogd risico op een gestoorde cerebrale zuurstofvoorziening in de gaten te houden. In *Chapter 9* onderzochten we de voorspellende waarde van NIRS voor de latere neurologische ontwikkeling op basis

van een uitgebreid follow-up onderzoek. In een heterogene groep preterme pasgeborenen vonden wij dat de cerebrale zuurstofsaturatie en zuurstofextractie in de neonatale periode, zoals gemeten met NIRS, van invloed was op de neurologische ontwikkeling op 2 tot 3 jarige leeftijd. NIRS kan daarom mogelijk een voorspellende waarde hebben voor de neurologische ontwikkeling van preterme pasgeborenen in deze leeftijdsgroep. Zowel relatief lage als hoge r_cSO_2 waarden op dag 1 waren geassocieerd met een slechtere cognitieve ontwikkeling. Hoge FTOE waarden op dag 1 waren geassocieerd met een slechtere motorische ontwikkeling. De lage r_cSO_2 waarden suggereren dat er mogelijk sprake was van cerebrale hypoxische hypoxie. De FTOE is een maat voor cerebrale ischemische hypoxie. In beide gevallen van hypoxie is er onvoldoende zuurstof beschikbaar voor weefsel. Aanhoudende hypoxie kan leiden tot een aanpassing van het setpoint in cellen om zuurstof op te merken. Cellen kunnen dan hyperoxie opmerken onder feitelijk normale zuurstof concentraties. Hyperoxie kan bijdragen aan neurologische schade door het veroorzaken van een verminderde cerebrale perfusie en een cascade van vrijkomen van vrije radicalen en toxische reactieve zuurstof species. Preterme pasgeborenen zijn daarbij extra gevoelig voor oxidatieve stress. Het is moeilijk om een verklaring te vinden waarom hypoxische hypoxie op dag 1 specifiek was geassocieerd met de cognitieve ontwikkeling en waarom ischemische hypoxie op dag 1 specifiek was geassocieerd met de motorische ontwikkeling. Mogelijk zijn er verschillende pathofysiologische processen betrokken.

Eén van de belangrijkste doelen in de huidige neonatologische praktijk is het voorkomen van neurologische ontwikkelingsproblemen door cerebrale schade. NIRS is een instrument dat kan bijdragen aan dit doel door het monitoren van het zuurstofgehalte in hersenweefsel. Hierbij wordt een verstoorde cerebrale zuurstofvoorziening in een vroege fase duidelijk, wat mogelijkheden biedt tot vroege interventie. Dit is een toegevoegde waarde van deze techniek aan de neonatologische praktijk. NIRS is zeer geschikt als een niet-invasieve, bedside beschikbare techniek om de aan- of afwezigheid van cerebrovasculaire autoregulatie vast te stellen. Hiermee is er een rol voor NIRS in het besluitvormingsproces rondom het verbeteren van de cerebrale circulatie. Een grootschalig, multi-center onderzoek is dringend nodig om deze hypothese te testen.

Dit proefschrift draagt bij aan de huidige opvatting dat NIRS mogelijk een bruikbaar klinisch instrument is om cerebrale perfusie te meten, de pathofysiologie van verschillende perinatale en neonatale stoornissen vast te stellen en daarmee een bijdrage kan leveren aan het voorspellen van en mogelijk voorkomen van ontwikkelingsstoornissen. In combinatie met andere cerebrale parameters lijkt NIRS een veelbelovende techniek om bewaking van jonge hersenen verder te optimaliseren. NIRS kan helpen om de optimale blootstelling aan zuurstof te bepalen.

Naast cerebrale metingen met NIRS, is NIRS mogelijk ook een waardevol instrument om de systemische zuurstofvoorziening te meten. Er zijn aanwijzingen dat de systemische perfusie al gestoord is bij pasgeborenen met circulatoire insufficiëntie op het moment dat de cerebrale perfusie nog wordt gehandhaafd, mogelijk door de cerebrovasculaire autoregulatie. De precisie van NIRS is afhankelijk van de homogeniteit van het onderliggende weefsel. Daarom zijn er studies nodig om de meest geschikte plaats voor systemische NIRS metingen te bepalen.

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aEEG – amplitude-integrated electro encephalogram
AUC – area under the curve
BPD – bronchopulmonary dysplasia
BS – burst suppression
BSID-II – Bayley Scales of Infant and Toddler Development, Second Edition
BSID-III – Bayley Scales of Infant and Toddler Development, Third Edition
BW – birth weight
CAR – cerebrovascular autoregulation
CI – confidence interval
CBCL – Child Behavior Check List
CBF – cerebral blood flow
CFM – cerebral function monitor
cm – centimeter
CRIB-score – clinical risk index for babies
CNV – continuous normal voltage
CO – carbon monoxide
COV – coefficient of variation
CPAP – continuous positive airway pressure
DNV – discontinuous normal voltage
FiO_2 – fraction inspired oxygen
FTOE – fractional tissue oxygen extraction
GA – gestational age
GH – gestational hypertension
GMH-IVH – germinal matrix hemorrhage-intraventricular hemorrhage
Hb – hemoglobin
HELLP-syndrome – hemolysis, elevated liver enzymes, and low platelets syndrome
Ht – hematocrit
IMV – intermittend mandatory ventilation
INVOS – In Vivo Optical Spectroscopy
IVH – intraventricular hemorrhage
MABP – mean arterial blood pressure
MAP – mean airway pressure
MHz – megahertz
$MgSO_4$ – magnesium sulfate
mm – millimeter
NICU – neonatal intensive care unit
NIRS – near-infrared spectroscopy
NEC – necrotizing enterocolitis
nm – nanometer

$PaCO_2$ – arterial partial pressure of carbon dioxide
PCO_2 – partial pressure of carbon dioxide
PDA – patent ductus arteriosus
PE – preeclampsia
PHVD – post hemorrhagic ventricular dilatation
PNA – postnatal age
PVHI – periventricular hemorrhagic infarction
PVL – periventricular leukomalacia
RBC – red blood cell
RDS – respiratory distress syndrome
r_cSO_2 – regional cerebral tissue oxygen saturation
ROP – retinopathy of permaturity
SAGM – saline, adenine, glucose and mannitol
SCOR – splanchnic-cerebral oxygenation ratio
SD – standard deviation
SIP – spontaneous intestinal perforation
SpO_2 – arterial oxygen saturation
SWC – sleep-wake cycling
$tcSaO_2$ – transcutaneous arterial oxygen saturation
TPE – transient periventricular echodensities
UMCG – University Medical Center Groningen

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Veel personen zijn direct of zijdelings betrokken geweest bij mijn onderzoek. Ik wil graag iedereen hiervoor bedanken en een aantal mensen bij naam noemen.

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Elise (Elisabeth Anna) Verhagen was born on 30 March 1984 in Gouda, the Netherlands. She grew up there and in Breda and Harderwijk. She graduated from Christelijk College Nassau-Veluwe, a secondary school in Harderwijk in 2002. Subsequently, she completed a Senior Year at Gloucester High School in Gloucester, Virginia, United States of America. In 2003, she started studying Psychology at University of Groningen and obtained the first year degree (*propedeuse*), but switched to Medicine at the same university the following year. During her second year at medical school, at the Department of Neonatology of the Beatrix Children's Hospital and University Medical Center Groningen, she started her research on near-infrared spectroscopy under the supervision of Professor Arend F. Bos. This resulted in her applying for the challenging MD-PhD trajectory for which she was accepted in 2008. During the course of this trajectory she combined doing clinical rotations at the University Medical Center Groningen in Groningen and Isala Klinieken in Zwolle with doing clinical research at the Department of Neonatology of the Beatrix Children's Hospital and University Medical Center Groningen in Groningen.

The results of her research are presented in this thesis. Besides, she has presented her research at several international conferences. She completed her final clinical rotations at the Department of Pediatrics and the Department of Clinical Genetics of Leiden University Medical Center and graduated from medical school in August 2012. Currently, she is participating in the two-year PhD Curriculum TULIPS (Training Upcoming Leaders in Pediatrics and Science) and she is a council member of the section on Circulation, Oxygen Transport and Haematology of the European Society for Paediatric Research. It is her wish and ambition to become an academic pediatrician. In December 2012 she will start working as a junior doctor at the department of Pediatrics at the Reinier de Graaf Gasthuis in Delft. She is married to Pieterjan Rozenberg and they live in The Hague.

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