Colophon

Monitoring the oxygenation of the preterm brain:
What is there to gain?

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Monitoring the oxygenation of the preterm brain

What is there to gain?

MONITOREN VAN DE OXYGENATIE VAN HET PREMATURE BREIN: WAT BRENGT HET ONS?

(met een samenvatting in het Nederlands)

Proefschrift
ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op vrijdag 18 maart 2016 des middags te 2.30 uur

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CONTENTS


Chapter 1

GENERAL INTRODUCTION

PRETERM BIRTH

Premature birth before 32 weeks of gestational age (GA) has an incidence of 10.6-17.1 per 1000 births in Europe.[1] Despite advances in neonatal intensive care, premature birth is still associated with significant morbidity and death.[2] These premature infants are particularly at risk of developing brain injury due to inflammation, and disturbances in cerebral oxygenation and blood flow. The two main types of brain injury in preterm neonates are white matter injury (WMI) and peri-intraventricular haemorrhage (PIVH). Although the pathogenesis of these two types of brain injury is distinct and is certainly multifactorial, it is well recognized that haemodynamic instability plays an important role in the occurrence of preterm brain injury.[3,4] For WMI, hypoxia/ischaemia and inflammation are the key players in the development of neuronal injury. Interestingly also hyperoxia has been reported to be harmful due to limited anti-oxidative capacity of the preterm brain.[5] In case of PIVH, the germinal matrix is extremely vascular and consists of thin-walled capillaries.[4] These capillaries are fragile and a priming episode of hypoxia/ischaemia followed by reperfusion may cause these vessels to rupture.[6] In an effort trying to avoid cerebral injury, infants admitted to a neonatal intensive care unit (NICU) are closely monitored to ensure an adequate and stable supply of oxygen and other nutrients to the vital organs of the body, in particular to the (developing) brain.

MONITORING INFANT WELLBEING

Traditionally, the arterial oxygen saturation (SaO_2), heart-rate (HR), and the blood pressure are used to monitor infant wellbeing, aiming for stable perfusion and oxygenation to the vital organs (e.g. avoiding hypo-perfusion, hypoxia, and large fluctuations). Indeed these three parameters are surrogates of components (i.e. blood oxygen content and cardiac output [CO]) that are vital to ensure adequate supply of oxygen and nutrients to the vital organs. Where SaO_2 and HR are more established in terms of how to interpret them and especially when to intervene, the blood pressure in preterm infants remains the topic of ongoing debate.[7]

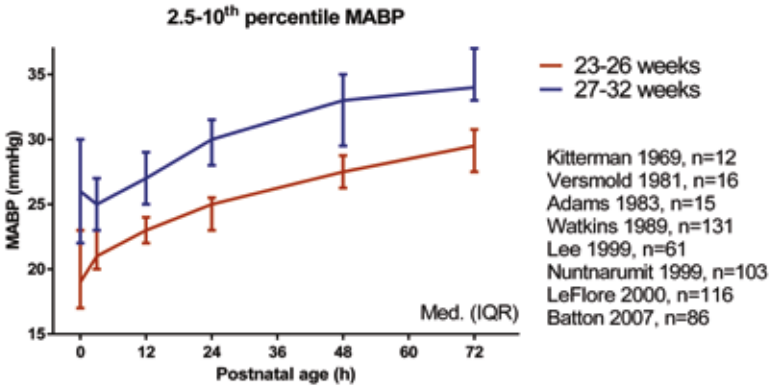
Low blood pressure in preterm infants is a frequently occurring and well recognized clinical problem.[8] Although adequate blood pressure is certainly important, it is unclear how and when to intervene in case of low blood pressure.[9] Treatment thresholds used in daily clinical practice include: 1) a mean arterial blood pressure (MABP in mmHg) below the GA in weeks, 2) a MABP <30mmHg, and a MABP below the 5th-10th percentile according to age specific reference curves.[8,10,11] The documented association between hypotension (using various definitions) and brain damage / neurodevelopmental outcome has served as the main reason for aggressive intervention with volume

expansion and inotropes such as dopamine, dobutamine, and epinephrine.[11–14] A related reason is the lower threshold for cerebral autoregulation, which is reported to be around 28-30mmHg (i.e. close to the 50th percentile of MABP in very preterm infants).[15–17] Cerebral autoregulation is the capacity of the brain to maintain stable perfusion at varying perfusion pressures (i.e. the MABP) by adjusting the vascular resistance in the feeding vessels. Impaired autoregulation results from vessels that reached either their maximum dilation at low perfusion pressures, or their maximum constriction at very high perfusion pressures.

The use of a fixed MABP threshold (e.g. 30 mmHg) has clear limitations, as lower MABP is related to lower GA, and GA in turn is related to the occurrence of brain injury.[8,18,19] A fixed threshold, without correcting for GA dependent increases in MABP, would therefore automatically lead to an association between low blood pressure and brain injury. The use of percentiles also has limitations, specifically because reference values reported in literature are quite variable, which can be seen (**figure 1.1**) from the large inter-quartile range (IQR) of the 2.5-10th percentile values of the most commonly cited MABP reference values.[8,11,20–25]

Likewise, the infants' GA as a treatment threshold has similar limitations as it is basically a translation from the lower percentiles thresholds to a rule of thumb. Recent studies exploring the relation between low blood pressure (by any definition) and either short- or long-term outcome have failed to demonstrate a significant association between presumed hypotension and outcome.[26–28]

Considering the underlying physiology it is not surprising that a unequivocal MABP threshold for treatment remains elusive. As mentioned above, MABP is merely a surrogate for CO as it is the product of systemic vascular resistance (SVR) and CO (i.e. MABP = SVR x CO). Therefore, low CO can be accompanied with a high or normal MABP in case of high SVR.[29] The opposite (i.e. high CO, low MABP) can also be true, for instance during sepsis when vessels dilate as part of the inflammatory response.



↑ **Figure 1.1**
2.5-10th percentile values for MABP, obtained from eight commonly cited papers regarding MABP reference values.

Unfortunately, SVR can only be calculated, and the measurement of CO requires considerable skill and cannot be measured continuously. Moreover, the MABP is a measure of the macro-circulation and does not provide information on circulatory status of individual organs. Because of these limitation, other measures have been proposed as adjuncts to diagnose compromised circulatory function, and thereby compromised delivery of oxygen and nutrients to vital parts of the body.[30]

ADJUNCTS TO BLOOD PRESSURE TO ASSESS CIRCULATORY COMPROMISE IN NEONATES

Ideally, a bedside monitoring parameter should be continuous, non-invasive, and representative of the oxygenation and perfusion of the end-organs, in particular the developing brain. Both HR and SaO_2 have the advantage to be continuous and non-invasive, but they only make up a single link in the chain necessary to maintain adequate oxygen and nutrient supply to the vital organs. The HR represents electrical activity of the heart and can only be used as a trend monitor of CO, when assuming that stroke volume remains stable. Moreover, there is a great variation in normal HR and more importantly it is influenced by factors not related to CO (e.g. stress). Indeed, no strong relation was found between HR and systemic blood flow.[31] The SaO_2 can only be used to assure adequate oxygenation of blood in the feeding arteries, but does not take the absolute blood flow into account and therefore does not provide information on the actual amount of oxygen (in µmol/L) being carried in the blood.[32]

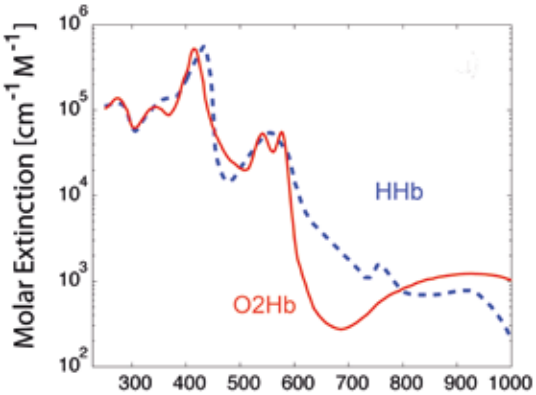
A commonly used parameter in adult intensive care is urine output, which can also be measured continuously. However, this requires placing a catheter, and the interpretation is more complex in neonates because the first void may only occur after the first day of life.[33] Moreover, preterm neonates often lack the ability to concentrate urine and therefore adequate output does not necessarily assures adequate perfusion of the kidneys, especially given the possibility of kidney damage after hypoxic/ischaemic episodes.[34,35] The capillary refill provides yet another measurement, but it is not continuous, subjective, and only has a good negative predictive value (i.e. 93 [CI 89-98] at 3h postnatal age) when comparing to upper body blood flow as assessed by superior vena cava (SVC) flow.[36] The SVC flow was proposed as an echocardiographic measure to overcome the limitations of left ventricular output measurements, as the latter does not necessarily represent systemic perfusion in the presence of shunts such as a haemodynamically significant patent ductus arteriosus (*hs*PDA).[37] Is has been shown that SVC flow <40ml/kg/min does not always coincides with low MABP, the other way around low MABP also does not necessarily imply low upper body flow.[36] The SVC flow is most promising as it represents a direct measure of CO. However, it does require considerable skill to perform, lacks repeatability, is intermittent, and still

only provides a measure of the macro circulation.[38] As stated above, the central issue in any patient admitted to the NICU is preservation of adequate oxygen and nutrient supply to the (immature) brain and other organs systems. A non-invasive, easy to interpret, and continuous measure that provides precise information on end-organ oxygenation and perfusion should be available at every NICU bed. In theory, Near-InfraRed Spectroscopy (NIRS) fulfils these prerequisites.

NEAR-INFRARED SPECTROSCOPY TO MONITOR CIRCULATORY COMPROMISE

NIRS was originally proposed by Jöbsis to non-invasively monitor the cerebral oxygenation.[39] It exploits the relative translucency of biological tissue to light at near-infrared wavelengths (i.e. 700-1000nm) and the fact that oxygenated (O_2Hb) and deoxygenated (HHb) haemoglobin have very distinct absorption spectrums at NIR wavelengths (**figure 1.2**).[40]

The original implementation of NIRS allowed for the measurement of absolute changes in concentration of oxygenated and deoxygenated haemoglobin over time. However, these O_2Hb and HHb measures are in reference to an arbitrary baseline and are very sensitive to probe movements, impeding its usefulness in daily practice on the NICU.[41] Amongst other implementations, a multi distance technique referred to as spatially resolved spectroscopy (SRS) introduced a cost-efficient NIRS implementation that does provide an absolute measure.[42] The use of multiple emitter-detector distances enables the expression of the relative concentration of O_2Hb as a ratio of the relative concentration of total Hb (i.e. O_2Hb + HHb), resulting in an absolute measure. This absolute measure is expressed as a saturation (in %) and often referred to as the regional tissue oxygen saturation ($rStO_2$), tissue oxygenation index



← **Figure 1.2**
Molar extinction spectra for oxygenated and deoxygenated haemoglobin at various at wavelengths visible to the human eye.

(TOI) or tissue saturation index (TSI), depending on the manufacturer. In this thesis we will refer to oxygen saturation estimated by NIRS as the $rStO_2$, with the 't' varying according to tissue being measured. The strength of NIRS lies in the fact that the $rStO_2$ represents the oxygen saturation in a mixed arterial-capillary-venous vascular bed in an approximate 75%/5%/20% distribution, for the brain.[43] Therefore, it captures information on actual oxygen delivery and utilisation in a single measure. However, this also makes interpretation ambiguous, as fluctuation in in $rStO_2$ can result from changes in oxygen supply (e.g. blood flow and blood oxygen content), a change in tissue metabolism, or changes to the venous outflow (e.g. venous pooling).

The main application of NIRS in neonates is to monitor the regional cerebral oxygen saturation ($rScO_2$). Brazy was the first to apply NIRS in neonates back in 1985.[44,45] Measures of cerebral oxygenation obtained by NIRS have also been shown to correlate with cerebral blood flow and therefore the $rScO_2$ can also be used as a surrogate of cerebral perfusion.[46,47] Since its introduction numerous groups have reported on just as many clinical conditions and diseases, including but certainly not limited to: respiratory distress syndrome, hypoxic-ischaemic encephalopathy, *hs*PDA, and hypotension.[48–53] For example, both Bonestroo et al. and Garner et al. demonstrated that $rScO_2$ in infants with presumed hypotension was no different from $rScO_2$ in neonates without hypotension.[48,54] Thereby suggesting that we might be treating hypotension too aggressive and supporting that the MABP might not be the best parameter to guide circulatory support in neonates. Despite the considerable time that NIRS has been around, and it being a very promising technique as demonstrated by numerous studies, NIRS is still not as broadly used as SaO_2, HR, and MABP monitoring.

The slow acceptance of NIRS on the NICU can be attributed to at least three factors: 1) heterogeneity in terms of 'reference values' acquired in different populations (e.g. GA, postnatal age, morbidity), 2) the lack of clear thresholds of $rScO_2$ that identify infants 'at risk', and 3) the multitude of devices available on the market with just as many sensors, all yielding slightly different results.

On average, neonatal and paediatric sensors measure 10% higher than the adult sensors when using the same device.[55] Although the true cause of this difference remains elusive, it is likely that this originates from the way NIRS devices and sensors have been calibrated in neonates. No true gold-standard exists that is comparable to the NIRS $rScO_2$ in a mixed arterial-capillary-venous compartment. In adults, NIRS has been validated against jugular saturations, which are strictly venous and depend strongly on patient and catheter positioning.[56–60] As placing jugular catheters in neonates is not possible, animal validation models, as well as patients on cardio-pulmonary-bypass have been used to validate NIRS.[61,62] These approaches are clearly not comparable to the application of NIRS in 'normal' preterm infants. Besides the limitations regarding validation in neonates, NIRS is restricted by the penetration depth of NIR light in tissue,

limiting reading to the ~2cm directly below the sensor.[63] The $rScO_2$ in relatively stable infants has been reported to be comparable between the left and right fronto-parietal regions, and also between left and right occipital regions.[64,65] Nevertheless, this is only a comparison between a selected number of regions and the $rScO_2$ might not be as comparable between regions in more severely ill infants. A method that facilitates measuring cerebral oxygenation in a mixed (arterial, capillary, and venous) vascular bed, comparable to NIRS, could be of great value to validate NIRS in the neonatal population. In addition, full brain coverage would enable comparison of the more superficial NIRS estimates to oxygen saturation levels in deeper brain structures.

OUTLINE OF THE THESIS

The main aim of this thesis is to study cerebral oxygenation and haemodynamics and their relation with blood pressure in infants who were born very preterm. The **first part** of this thesis focusses on monitoring infant circulatory well-being on the NICU by means of near-infrared technology. In these clinical studies INVOS 4100 and 5100c devices were used in combination with a SAFB-SM SomaSensor (both Covidien, Mansfield, MA) to monitor the regional cerebral oxygenation. In the **second part**, the development and application of MRI sequences that visualize cerebral haemodynamics, the cerebral oxygenation in particular, are studied in conjunction with cerebral NIRS measurements.

PART I: BEDSIDE MONITORING

In **chapter 2**, the patterns of $rScO_2$ and MABP are studied directly before and after the diagnosis of a PIVH on cranial ultrasound.

In **chapter 3**, $rScO_2$ and MABP are studied as predictors of neurodevelopmental outcome in infants who were treated for presumed hypotension.

In **chapter 4**, an interim-analysis of the short-term results of the infants that were included so far in the 'Treatment of Hypotension of Prematurity' trial is presented, with a particular focus on a relatively new circulatory measure: the regional renal oxygen saturation.

In **chapter 5**, the perfusion-index, a measure of systemic perfusion derived from standard pulse-oximetry probes, and its association with MABP, $rScO_2$ and various other clinical parameters is studied.

In **chapter 6**, normative data for $rScO_2$ in preterm infants during the first three days after birth are presented.

In **chapter 7**, different $rScO_2$ thresholds during the first three days after birth are studied in association with long-term outcome.

PART II: BEDSIDE TO BENCH

Chapter 8 provides an overview of the MR techniques that are available to quantify cerebral haemodynamics in neonates.

Chapter 9 describes an approach that requires a hypercapnia-MR experiment to obtain quantitative parameters of cerebral oxygen metabolism, and compares these with parameters that were obtained simultaneously by NIRS

In **chapter 10** a novel MR-sequence to obtain cerebral oxygen saturation without needing a hypercapnia experiment is described, and its agreement with NIRS measurements is investigated.

Chapter 11 describes an improvement of the MR-sequence presented in chapter 10, now including full-brain coverage and a pulse to suppress signal arising from cerebrospinal fluid. Results of this sequence are compared to oxygenation parameters that were obtained simultaneously by means of NIRS

Finally, in **chapter 12** the findings of the previous chapters are summarized, discussing possible implications, and providing suggestions for further research.

Chapter 13 summarizes the results of the studies in Dutch.

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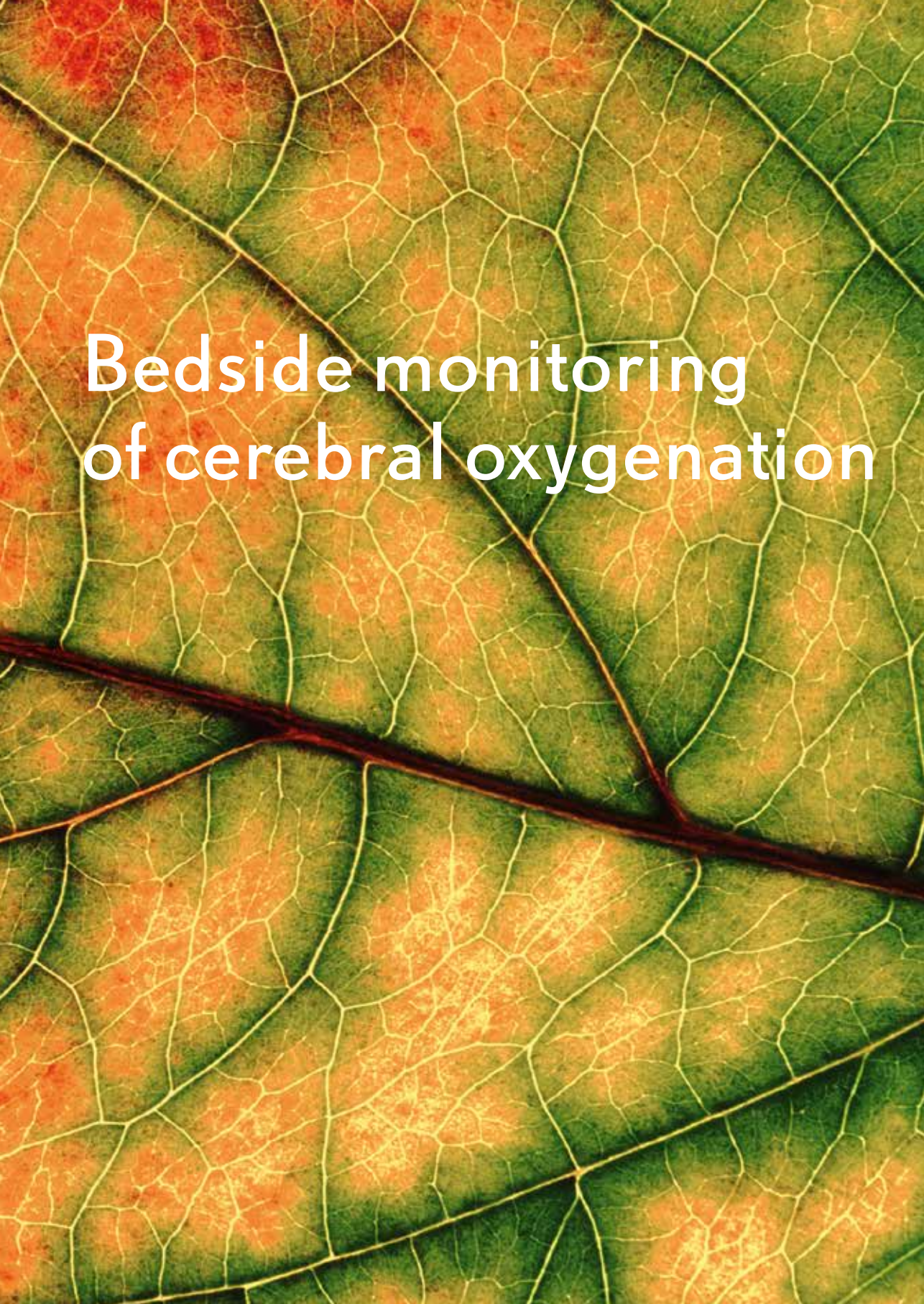
Part 1



Bedside monitoring of cerebral oxygenation


Chapter 2

CEREBRAL OXYGENATION, EXTRACTION, AND AUTOREGULATION IN VERY PRETERM INFANTS WHO DEVELOP PERI-INTRAVENTRICULAR HAEMORRHAGE

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ABSTRACT

OBJECTIVE To test the hypothesis that near-infrared spectroscopy (NIRS)-determined patterns of regional cerebral oxygen saturation ($rScO_2$), cerebral fractional tissue oxygen extraction (cFTOE), and autoregulatory ability can identify neonates at risk for developing peri-intraventricular haemorrhage (PIVH).

STUDY DESIGN This case-control study is a sub-analysis of 30 neonates who developed a PIVH >12 hours after admission, nested in a lager prospective observational cohort study comprising 650 preterm neonates born at <32 weeks' gestational age. PIVH was diagnosed by cranial ultrasound, performed at least once daily. Mean arterial blood pressure (MABP), $rScO_2$, cFTOE, and MABP–$rScO_2$ correlation were monitored from birth until 72 hours of age.

RESULTS Infants with PIVH received more inotropic drugs before being diagnosed with PIVH. Significantly more infants with severe PIVH needed treatment for patent ductus arteriosus. The MABP–$rScO_2$ correlation was >0.5 significantly more often before mild/moderate PIVH and after severe PIVH compared with controls. $rScO_2$ was higher and cFTOE lower in infants before severe PIVH.

CONCLUSION NIRS-monitored $rScO_2$ and cFTOE suggest cerebral hyperperfusion in infants with severe PIVH. Moreover, MABP–$rScO_2$ correlation indicates more blood pressure-passive brain perfusion in infants with PIVH. Continuous assessment of patterns of cerebral oxygenation and arterial blood pressure may identify those preterm infants at risk for severe PIVH and prompt consideration of preventive measures.

INTRODUCTION

Peri-intraventricular haemorrhages (PIVH) that become evident within the first hours after birth in the extremely preterm infant are mostly related to inflammatory factors and the intrapartum period.[1,2] In contrast PIVHs diagnosed more than 12 hours after birth ("late" PIVH), have been suggested to be related to haemodynamic factors, including loss of cerebral autoregulation and/or a fluctuating pattern of cerebral perfusion.[3–6]

PIVH is an important factor with respect to adverse neurodevelopmental outcome, and efforts to reduce its incidence are manifold.[7] Recently it has been shown that prophylactic treatment of the extremely preterm infant with indomethacin decreased the incidence of severe PIVHs.[8] Because "late" PIVHs provide us with a therapeutic window, early recognition of changes in cerebral haemodynamics and cerebral oxygenation prior to PIVH may help us to reduce its occurrence.

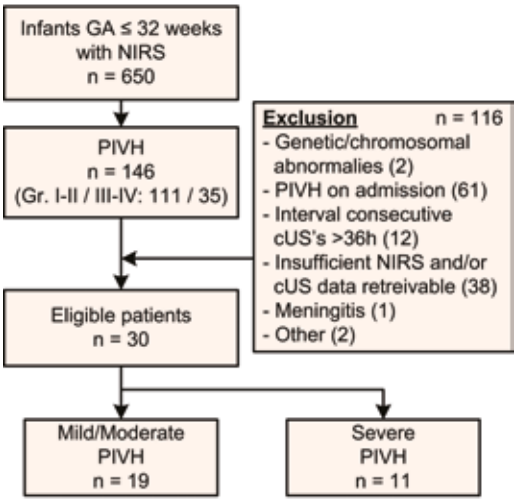
Near-infrared spectroscopy (NIRS) can be used to assess regional cerebral oxygenation saturation ($rScO_2$), fractional tissue oxygen extraction (cFTOE), and autoregulatory ability of the cerebral vascular bed.[9–11] Moreover, it is non-invasive and can be applied for extended periods of time without disturbing the often unstable patient.[10,12]

We hypothesized that the pattern of cerebral oxygenation and assessment of the autoregulatory ability of the cerebral vascular bed, as determined with NIRS, may be used to identify infants at risk of developing "late" PIVHs.

PATIENTS AND METHODS

STUDY POPULATION

The present study presents a sub-analysis of a prospective observational study in which aims to monitor all infants admitted to the NICU of the Wilhelmina Children's Hospital, Utrecht the Netherlands with a gestational age (GA) of less than 32 completed weeks . The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht. Parental informed consent was obtained in all cases. From these infants, continuous data on $rScO_2$ and cFTOE using NIRS were prospectively collected during the first 72 hours after birht.[10,13,14] These data were stored in an electronic database along with clinical, physiological, and cranial ultrasound (cUS) data. From this cohort of 650 infants, 146 infants developed a PIVH. A total of 116 infants were excluded from analysis for reasons listed in **figure 2.1.** Consequently, 30 infants with a postnatal PIVH were eligible for analysis. From the 504 infants without PIVH, 60 infants were matched to the 30 infants with PIVH and served as the control group. Matching criteria were GA, birth weight, gender, and year of birth (ensuring equal treatment strategies).



← **Figure 2.1**
Flow diagram depicting the numbers and reasons for inclusion and exclusion respectively.

CLINICAL DATA

Obstetrical, intrapartum and neonatal data were collected from the database and hospital records. In all infants the arterial oxygen saturation (SaO_2) was measured continuously by pulse oximetry. The mean arterial blood pressure (MABP) was measured by means of an indwelling arterial catheter (umbilical, tibial or radial artery) during the 72 hour study period. These parameters were monitored simultaneously with NIRS-monitored $rScO_2$ and stored with a 1 Hz sample rate on a personal computer for offline analysis (software: Poly 5, Inspector Research Systems, Amsterdam, The Netherlands). Arterial blood gas samples were taken at regular intervals (at least every 4 hours). The cUS's were performed through the anterior fontanel as soon as possible after admission and repeated daily or more frequently if necessary (PST-65AT combined with a Toshiba Aplio MX system, Toshiba Medical Systems Corporation, Otawara-shi, Tochigi-ken, Japan). PIVH was graded according to the classification of Papile: grade I/II being mild-moderate PIVH; grade III/IV severe PIVH.[15] The presence or absence of a haemodynamically significant persistent ductus arteriosus (*hs*PDA) was investigated at least daily and based on clinical indices and confirmed by echocardiography (left atrial and/or left ventricular dilatation; internal ductal diameter >1.4 mm/kg; left pulmonary artery end diastolic flow > 0.2 m/sec). Respiratory distress syndrome was graded in no, moderate (clinically as well as on x-ray) and severe (needing exogenous surfactant therapy). Blood pressure support was scored according to Krediet: 0) no support; 1): volume expansion and/or

dopamine ≤5 µg/kg/min; 2): dopamine 5-10 µg/kg/min; 3): dopamine >10 µg/kg/min or dopamine + dobutamine ≤10 µg/kg/min; 4): dopamine + dobutamine >10 µg/kg/min; 5): additional adrenaline and/or corticosteroids.[16]

NEAR-INFRARED SPECTROSCOPY

The $rScO_2$ was used as an estimator for changes in regional cerebral oxygenation.[13] This parameter provides us with absolute values, is less sensitive for movement artefacts and enables comparisons over time.[10,17]

A 2-wavelength (730 and 810 nm) near-infrared spectrometer (INVOS 4100-5100c, Covidien, Mansfield, MA) was used. A transducer (small adult SomaSensor® SAFB-SM) containing a light emitting diode and two distant sensors (30 and 40 mm) was placed on the fronto-parietal side of the infant's head and attached firmly with an elastic bandage to prevent displacement.[10] The $rScO_2$ was calculated from the differential signals obtained from these two sensors, expressed as the venous-weighted percentage of oxygenated haemoglobin (oxygenated haemoglobin/total haemoglobin [oxygenated haemoglobin + deoxygenated haemoglobin]).[10,18] To investigate the balance between oxygen delivery and oxygen consumption, cFTOE was calculated $(SaO_2\text{-}rScO_2)/SaO_2$: an increase reflecting increased oxygen extraction by brain tissue, a decrease suggests less utilization or increased delivery of oxygen.[19]

The correlation between MABP and $rScO_2$ was used as an estimator of cerebral autoregulation.[9,20,21] MABP-$rScO_2$ correlation coefficients were determined every minute over 10 averaged 1-minute periods. A MABP-$rScO_2$ correlation coefficient r >0.50 was considered a lack of cerebral autoregulation.[9,22]

STUDY DESIGN

In each infant two periods were defined, one before and one after detection of PIVH. The period before PIVH covered the 24-36h (up to 36h when interval between subsequent cUS's was >24h) before the first cUS with PIVH. The period after PIVH covered the 24 hours after detection of PIVH. The defined periods of controls were matched for postnatal age to exclude physiological evolution of the studied variables (i.e. $rScO_2$ and MABP) from influencing the results. For evaluation of $rScO_2$, a mean $rScO_2$ was calculated for both periods. Average cFTOE and % of time with a MABP-$rScO_2$ correlation >0.5, average heart rate (HR), and average SaO_2 were also calculated using the entire periods. Artefacts in SaO_2, MABP, HR and $rScO_2$ (caused by i.e. movement or blood sampling) were manually removed before calculating the results. For MABP-$rScO_2$ correlations time spans with a SaO_2 <85% were not included in the analysis. All calculations were performed by using in house developed software (SignalBase, University Medical Center Utrecht, The Netherlands).

STATISTICS

Clinical data are summarized as mean values ± SD, as median and range, or as counts and percentages where appropriate. Results are presented for the PIVH group as a whole with subsequently the results of the mild-moderate (grade I/II) and severe (grade III/IV) PIVH subgroups. The students' *t*-test, Mann-Whitney U test or $\chi 2$ test were used where appropriate. Patterns of pCO_2 were analysed by using a mixed model approach for repeated measures, taking into account the individual patient (random intercept), allowing for a random slope over time and including group (case or control) as a factor. Data were analysed using R 2.15.0 for Windows (The R Foundation of Statistical Computing, Vienna, Austria). Receiver operator characteristics (ROC) analysis and calculation of an optimal cut-off value was performed by using MedCalc 12.3.0.0 (MedCalc Software, Mariakerke, Belgium). A p-value of <0.05 was considered statistically significant.

RESULTS

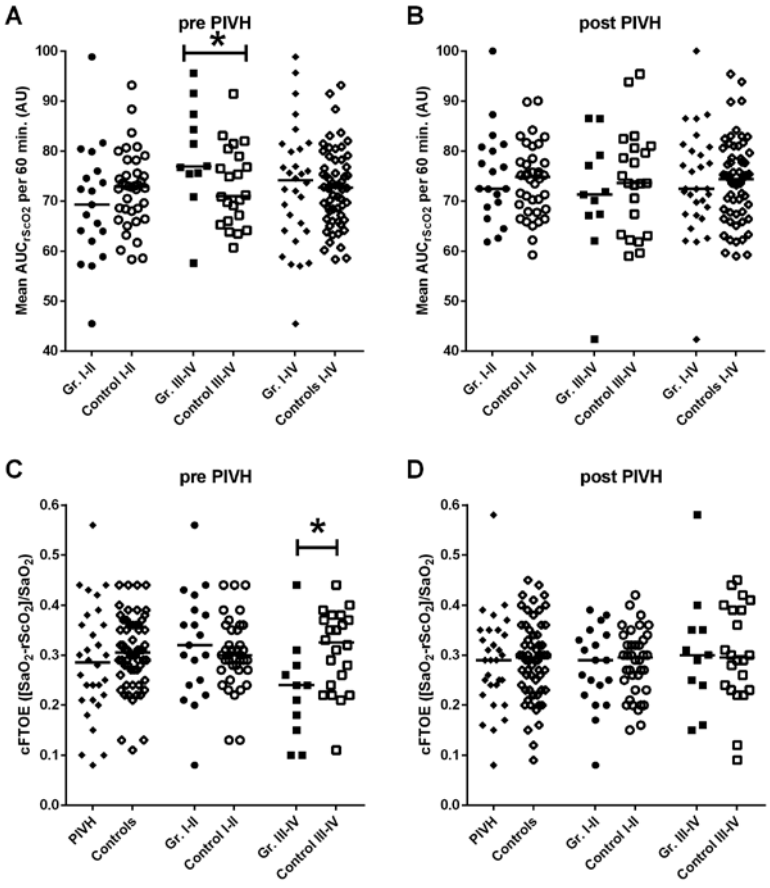
STUDY POPULATION

The clinical characteristics for the mild-moderate and severe PIVH groups are shown separately in **table 2.1.** Analysing all PIVH cases combined, only the inotropes score was significantly higher (median 3 [range 0-5] vs. 0 [0-5], $p < 0.001$) as compared to controls. The mean time between the most recent cUS without and first cUS with PIVH was 21 hours (range 3-36 hours) for the 30 cases.

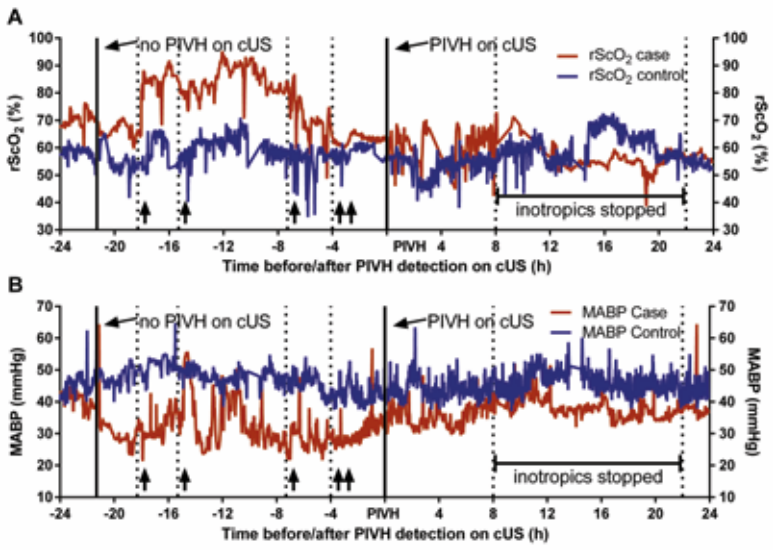
Five infants had grade I, 14 had grade II, 6 had grade III and 5 had grade IV PIVH. Seven infants (37%) with mild-moderate and 10 infants (91%) with severe PIVH had bilateral PIVHs. One infant progressed from an initial grade II to a grade III PIVH within 8 hours, this case was analysed as part of the severe PIVH group. A total of 6 infants died during the neonatal period (all in the PIVH group): 4 died of neurological sequelae following grade III-IV PIVH, 1 infant succumbed of CMV infection combined with the effects of gr. II PIVH, and 1 infant died of necrotizing enterocolitis.

PATTERNS OF $RSCO_2$ AND CFTOE

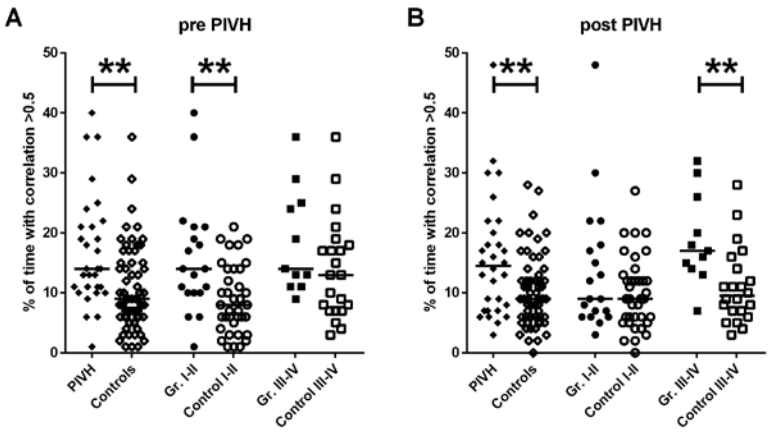
Median duration of available data during selected periods was 24 hours (range 6-31) before and 24 hours (range 3-24) after detection of PIVH in cases, in controls the medians were 23 (range 3-31) and 24 hours (range 12-24) respectively. The number of neonates that demonstrated a $rScO_2$ <50% for more than 30 minutes was not significantly different between cases and controls (23% vs. 18%, $p = 0.781$). Results on $rScO_2$ and cFTOE are presented in **figure 2.2. Figure 2.3** shows representative $rScO_2$ and MABP tracings, in relation with changes in administered inotropes, of an infant with grade III PIVH and a matched control. Data on $rScO_2$ variance is presented in **table 2.2.** Results of ROC analysis are presented in **table 2.3.**



← **Figure 2.2**
Scatter plot of mean $rScO_2$ and cFTOE in cases (filled symbols) and controls (clear symbols) the day before (**A** and **C**) and after (**B** and **D**) detection of PIVH on cUS. Horizontal bars represent group medians * $p \leq 0.05$



← **Figure 2.3**
Graphs of (**A**) $rScO_2$ and (**B**) MABP (without post-processing, thus including artefacts) of an infant with grade III PIVH and *one* (for sake of clarity) corresponding control 24 hours before and after detection of PIVH. Dashed vertical lines and corresponding arrows represent changes in the given amount of inotropes.



→ **Figure 2.4**
Scatter plot of % of time with a MABP-rScO$_2$ correlation > 0.5 in cases (filled symbols) and controls (clear symbols) the day before (**A**) and after (**B**) detection of PIVH on cUS. Horizontal bars represent group medians. ** p ≤ 0.01

MABP-RSCO$_2$ CORRELATION

The percentage of time with a MABP-rScO$_2$ correlation > 0.5 for all cases combined was significantly higher both the day before (median 14% vs. 9%, p <0.01) *and* after detection of PIVH (15% vs. 9%, p <0.01), results are shown in **figure 2.4.** Cumulative time spans with SaO$_2$ <85%, excluded from analysis, were always less than 0.5% of the studied period. Results of ROC analysis are presented in **table 2.3.**

PHYSIOLOGICAL DATA

Data on mean MABP, MABP variance, mean SaO$_2$ and mean HR for cases and controls before and after detection of PIVH on cUS are presented in **table 2.2.** There was no significant difference in the pCO$_2$ intercept nor was there a significant change in pCO$_2$ over time before detection of PIVH between cases and controls (whole group and subgroups). Although not significant, the intercept of pCO$_2$ in the grade III-IV PIVH group was on average 3 mmHg higher as compared to the controls (48 vs. 45 mmHg, p = 0.24).

DISCUSSION

Higher rScO$_2$ and lower cFTOE values suggest an increased perfusion prior to development of a severe PIVH.[23] These higher rScO$_2$'s were not caused by higher SaO$_2$'s (**table 2.2**). This is substantiated by cFTOE values moving in the opposite direction. Moreover, Naulaers et al showed that cerebral oxygenation during the first 3 days after birth in very preterm infants had no relation with SaO$_2$ as long as SaO$_2$ was stable.[14] Our results on MABP-rScO$_2$ correlation suggest that (prolonged) pressure-passive cerebral perfusion may have played an additional role in the occurrence of PIVHs.

Lack of significance in the severe PIVH group can be explained by a lower number of cases and by variation/outliers in the control group.

These findings confirm the results of earlier studies which showed that cerebral hyperperfusion, a fluctuating perfusion pattern, and lack of cerebral autoregulation are associated with the development of major PIVHs.[5,6] We further postulate that the combination of impaired cerebral auto regulation and suboptimal blood pressures that necessitate inotropic medication may induce swings in brain perfusion that put infants at risk of developing (severe) PIVHs.

Our results are in contrast with results of others who demonstrated hypoperfusion to be related to PIVH.[4,24] A recent study demonstrated decreased $rScO_2$ and increased cFTOE during the first 2 weeks of life in 17 preterm infants with grade I-IV PIVH.[25] These opposing results can possibly be explained by differences in study design: i.e. a higher interval between subsequent cUS examinations and the discontinuous nature of performed measurements in these studies. We acknowledge that hypoperfusion indeed plays a role in the pathogenesis of PIVH as showed by Pryds et al.[24] However, our results suggest an additional etiological role for hyperperfusion in the development of severe PIVHs.

A possible contributing factor to the development of severe PIVH may be high cerebral tissue oxygenation induced by hyperperfusion, facilitated by decreased auto regulation of cerebral blood flow in these very preterm neonates. Sorensen et al recently reported that the brains of (stable) very preterm infants seem to be hyperoxygenated as compared to term infants during the first days after birth and that this may pose an increased risk of oxygen toxicity.[23] Hyperoxia is increasingly recognized as a pathogenic factor linked to morbidity in preterm neonates who have an inadequate antioxidant capacity.[26,27]

Neonates with PIVHs more often had a *hs*PDA within 72 hours after birth than their counterparts without PIVH. The presence of an early *hs*PDA is known to be associated with PIVHs.[3] It can, however, not explain the increased $rScO_2$ (and decreased cFTOE), as preterm infants with a *hs*PDA have lower $rScO_2$'s caused by a 'ductal steal'.[28] We speculate that the presence of a PIVH itself induces an inflammatory response whereby associated mediators cause the duct to stay open, in analogy with re-opening/delayed closing of the duct in case of sepsis.[29]

The current study used MABP-$rScO_2$ correlation as an estimator for cerebral autoregulation. Others have used different averages and epoch durations for the assessment of cerebral autoregulation. Moreover, additional methods including gain and coherence analysis can be used to assess cerebral autoregulation.[9,11,20,21] The analysis of cerebral autoregulation using multiple different NIRS based methods was beyond the scope of this study.

There are several limitations to the current study that need to be addressed. First, the

time of development of the PIVHs was determined with a margin of approximately 24 hours, as cUS was performed daily. Although far from exact, this is more accurate than most centres which only perform a cUS once during the first 72 hours of admission. The second limitation is that infants with grade III-IV PIVHs differed from controls in several baseline characteristics. These variables are however known to be associated with PIVH and differences between groups are therefore to be expected.[2,3,6,30] Finally, a larger patient group could have demonstrated even more differences between groups.

The $rScO_2$ and cFTOE currently lack the necessary precision to be used as robust quantitative markers of cerebral oxygenation, oxygen extraction, and as a surrogate for cerebral perfusion.[10,13] Intra- and interpatient variability are too large for this. Absolute cut-off values generated in the current study cannot be considered clinically useful at this time, predictive values are too low and confidence intervals too wide for this purpose. However, NIRS has the great advantage that it is non-invasive and can be used for prolonged periods without putting excess strain on the patient, which facilitates (semi quantitative) trend monitoring in individual patients. Deviations from baseline larger than the limits of agreement (normally considered to be up to ± 7-10%) can provide important clinical information.[10,13] Upon identification of these baseline deviations one can (re)consider changes in care. An example is the very careful titration of inotropes in the management of hypotension in preterm neonates. Future steps to be taken to facilitate broad implementation of NIRS in neonatal intensive care include the generation of reference values in a large group of (premature) neonates.

In conclusion, NIRS-monitored cerebral oxygenation and oxygen extraction suggest cerebral hyperperfusion just prior to development of severe PIVH. Moreover, correlations between mean arterial blood pressure and cerebral oxygenation may indicate (prolonged) blood pressure-passive brain perfusion in neonates with PIVH. Current calculated absolute cut-off values and associated predictive values cannot be considered clinically applicable at this time. However, we do suggest that monitoring of the *trend* of these variables may identify those preterm infants at risk in an early stage and timely preventive actions may be considered upon identification of changes beyond the limits of agreement to reduce the incidence of severe PIVH and improve developmental outcome.

	Mild-moderate PIVH (Gr. I-II)			Severe PIVH (Gr. III-IV)		
	Case	Control	p-value	Case	Control	p-value
n= (M/F)	19 (11/8)	38 (22/16)	ns	11 (7/4)	22 (14/8)	ns
GA (wks) Median [range]	27^{+5} [24^{+6}-30^{+6}]	27^{+4} [25^{+1}-31^{+0}]	ns	27^{+6} [26^{+5}-30^{+0}]	28^{+1} [27^{+0}-30^{+2}]	ns
Birth Weight (g) Median [range]	1160 [685-1770]	1067 [750-1830]	ns	1020 [750-1680]	1100 [700-1550]	ns
Apgar score 5' Median [range]	8 [4-10]	8 [6-10]	ns	6 [4-8]	8 [5-10]	<0.001
RDS, n [%][a]			ns			<0.01
- No	1 [5%]	10 [26.3%]		0 [0%]	15 [68.2%]	
- Moderate	10 [52%]	19 [50.0%]		6 [54.5%]	4 [18.2%]	
- Severe	8 [43%]	9 [23.7%]		5 [45.5%]	3 [13.6%]	
Ventilation, n [%][a]			0.06			<0.01
- None/ Low Flow	0 [0%]	2 [5%]		0 [0%]	1 [4%]	
- CPAP	2 [10%]	9 [24%]		0 [0%]	12 [55%]	
- InSurE + CPAP	1 [5%]	4 [10%]		0 [0%]	1 [4%]	
- SIMV	8 [43%]	19 [50%]		8 [73%]	6 [28%]	
- HFOV	8 [43%]	4 [11%]		3 [27%]	2 [9%]	
***hs*PDA, n [%]**			ns			<0.05
- No	8 [43%]	25 [66%]		5 [46%]	18 [82%]	
- <72h	9 [47%]	10 [26%]		6 [54%]	3 [14%]	
- >72h	2 [10%]	3 [8%]		0 [0%]	1 [4%]	
PPROM, n [%]	4 [21%]	12 [32%]	ns	1 [9%]	8 [36%]	ns
PE/HELLP, n [%]	1 [5%]	4 [11%]	ns	1 [9%]	4 [19%]	ns
Sepsis <48h, n [%]	1 [5%]	2 [5%]	ns	0 [0%]	1 [4%]	ns
Inotropes score[a] Median [range]	1 [0-5]	0 [0-5]	0.01	3 [0-5]	0 [0-2]	<0.001
PA at detection of PIVH (h) Median [range]	33 [13-162]	n/a	n/a	39 [21-66]	n/a	n/a
PA at start pre PIVH period (h) Median [range]	9 [1-143]	11 [1-141]	ns	15 [1-50]	15 [1-42]	ns

CPAP: Continuous positive airway pressure, HELLP: Haemolysis Elevated Liver enzymes and Low Platelets, InSurE: Intubation-Surfactant-Extubation, ns: not significant, PA: postnatal age, *hs*PDA: haemodynamic significant patent ductus arteriosus, PE: pre-eclampsia, PIVH: peri-intraventricular haemorrhage, PPROM: preterm premature rupture of membranes, RDS: respiratory distress syndrome, SIMV: Synchronised intermittent mandatory ventilation, HFOV: High-frequency oscillatory ventilation.
[a] Before detection of PIVH on cUS (or similar time point in controls).

↑ **Table 2.1**
Clinical characteristics of the control, the mild/ moderate PIVH and severe PIVH groups.

	Mild-moderate PIVH (Gr. I-II)		Severe PIVH (Gr. III-IV)	
	Case	Control	Case	Control
n= (M/F)	19 (11/8)	38 (22/16)	11 (7/4)	22 (14/8)
Mean MABP (mmHg)[a]				
- Pre PIVH	35 [29-44]	36 [30-54]	34 [31-47]	38 [30-48]
- Post PIVH	37 [30-47]	40 [27-48]	37 [30-47]	38 [32-48]
MABP variance (σ^2)[a,b]				
- Pre PIVH	16 [7-34]	11 [4-41]	20 [4-36]	15 [6-32]
- Post PIVH	13 [5-47]	11 [4-24]	14 [2-42]	14 [3-24]
SaO_2 (%)[a]				
- Pre PIVH	93 [91-98]	95 [91-99]	93 [92-97]	95 [92-99]
- Post PIVH	94 [92-99]	94 [92-99]	94 [92-95]	96 [89-99]
Mean HR (bpm)[a]				
- Pre PIVH	145 [125-195]	148 [126-167]	154 [141-173]	150 [126-172]
- Post PIVH	146 [130-181]	150 [130-172]	156 [138-176]	153 [124-176]
$rScO_2$ variance (σ^2)[a,b]				
- Pre PIVH	30 [15-75]	32 [4-105]	34 [26-100]	30 [6-99]
- Post PIVH	47 [11-283]	32 [13-102]	38 [23-139]	25 [12-64]

[a] median [range], [b] $\sigma^2 = \text{sum}(X-\mu)^2/N$

← **Table 2.2**
Vital parameters of the control, the mild/ moderate PIVH and severe PIVH groups before and after the detection of PIVH

	Cut-off	Sens (95% CI)	Spec. (95% CI)	PPV (95% CI)	NPV	(95% CI)	AUC (95% CI)
$rScO_2$ (%)[a]	69	47% (28-66%)	70% (57-81%)	44% (15-77%)	72%	(47-90%)	0.53 (0.42-0.63)
- Gr. I-II	60	42% (20-67%)	84% (69-94%)	57% (29-82%)	74%	(59-87%)	0.60 (0.46-0.72)
- Gr. III-IV	69	82% (48-98%)	64% (41-83%)	53% (28-77%)	87%	(61-99%)	**0.72 (0.54-0.86)**
cFTOE[a]	0.26	47% (28-66%)	75% (62-85%)	48% (29-67%)	74%	(61-84%)	0.57 (0.46-0.67)
- Gr. I-II	0.31	53% (29-76%)	66% (49-80%)	43% (23-66%)	74%	(56-87%)	0.55 (0.42-0.69)
- Gr. III-IV	0.28	82% (48-98%)	64% (41-83%)	53% (28-77%)	87%	(61-99%)	**0.75 (0.57-0.89)**
MABP-$rScO_2$ corr.[a,b]	9	87% (69-96%)	53% (39-66%)	48% (34-62%)	89%	(74-97%)	**0.71 (0.60-0.80)**
- Gr. I-II	9	84% (60-97%)	60% (42-75%)	51% (32-69%)	88%	(69-98%)	**0.74 (0.60-0.84)**
- Gr. III-IV	8	100% (71-100%)	36% (17-59%)	44% (24-66%)	100%	(63-100%)	0.67 (0.48-0.82)

cFTOE: cerebral fractional tissue oxygen extraction; CI: confidence interval; NPV: negative predicting value; PPV: positive predicting value; $rScO_2$: regional cerebral saturation; sens.: sensitivity; spec.: specificity; AUC: area under the curve

[a] Grade I-II and grade III-IV PIVH cases combined (cases n=30; controls n=60). Significant results of ROC analysis are displayed in **bold**.

[a,b] Cut-off displayed in % of time with a MABP-$rScO_2$ correlation > 0.5

↑ **Table 2.3**
Results of receiver operator characteristics (ROC) analysis using variables during the period before detection of PIVH (pre PIVH)

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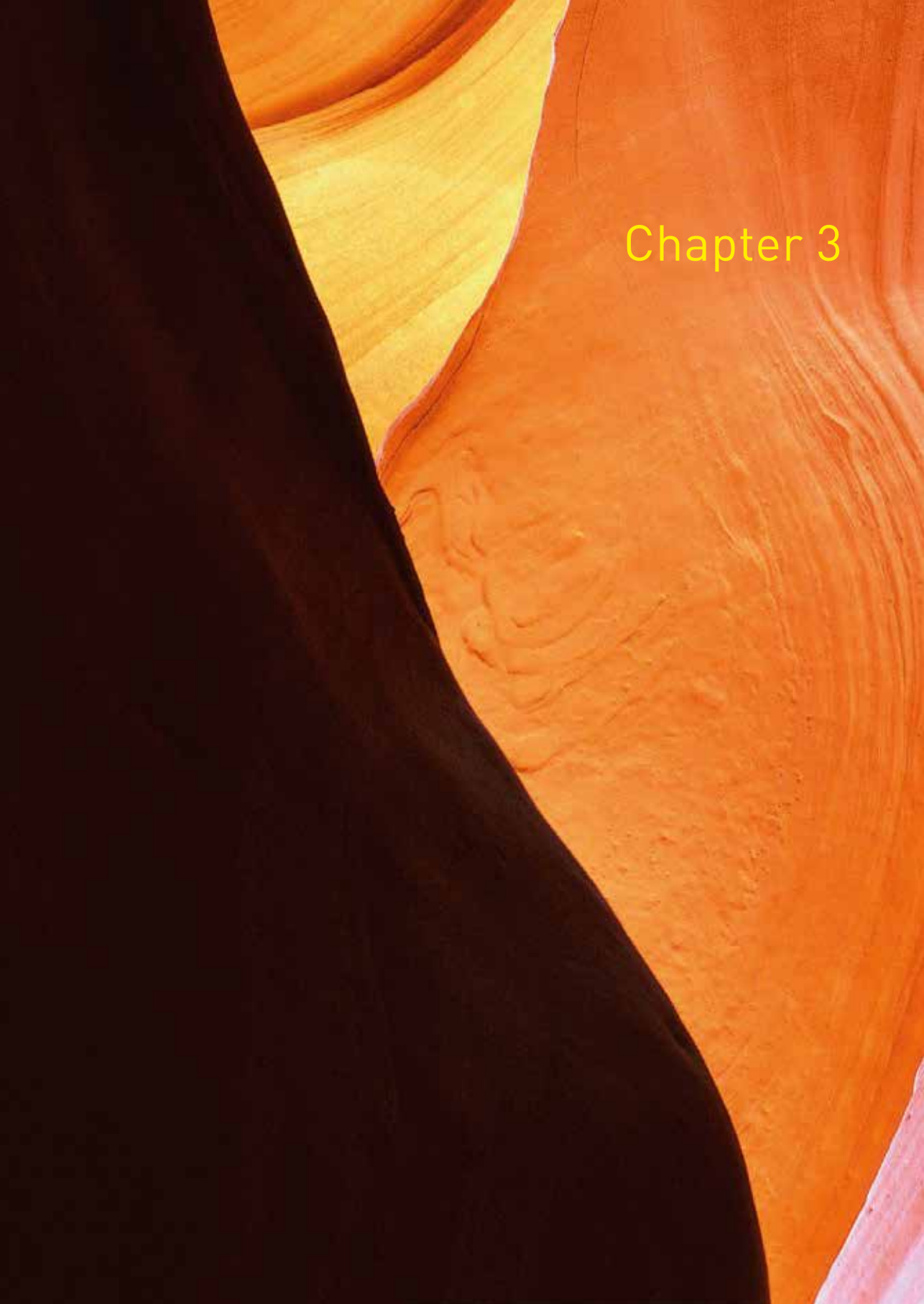

Chapter 3

HYPOTENSION IN PRETERM NEONATES: LOW BLOOD PRESSURE ALONE DOES NOT AFFECT NEURODEVELOPMENTAL OUTCOME

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ABSTRACT

BACKGROUND Hypotension in preterm neonates is treated rather vigorously because of the previously reported associations with adverse (short term) outcomes.

AIM To compare neurodevelopmental outcome (NDO), mean arterial blood pressure (MABP), and regional cerebral oxygen saturation ($rScO_2$) between preterm neonates treated for hypotension and controls.

STUDY DESIGN Sixty-six preterm neonates with a gestational age (GA) ≤32 weeks, without a patent ductus arteriosus, treated for hypotension (dopamine ≥5µg/kg/min) were included. Neonates were matched to controls for GA, birth weight, sex, and year of birth. The $rScO_2$ was determined by Near-InfraRed Spectroscopy. Monitoring of MABP, $rScO_2$ and arterial saturation was started at admission and continued for at least 72 hours. NDO was assessed at 15 and 24 months corrected age (CA) by using the Griffiths Mental Development Scales or the Bayley Scales of Infant and Toddler Development.

RESULTS Cases spent more time with a MABP <GA (median 9% vs. 0%, $p < 0.001$), and time with a MABP/$rScO_2$ correlation >0.5 (27% vs. 17%, $p < 0.001$). Time spent with a $rScO_2$ <50%, and NDO at 15 and 24 months CA were not significantly different between cases and controls. The 26 neonates with a $rScO_2$ <50% for >10% of time had a lower NDO at 15 months (med. 99 vs. 104, p=0.02).

CONCLUSION A MABP < GA in weeks was neither associated with lower $rScO_2$, nor with lower NDO scores. However, irrespective of MABP, low $rScO_2$ was associated with lower NDO scores. This suggests that perfusion/oxygenation parameters could be of additional value in neonatal intensive care.

INTRODUCTION

The incidence of hypotension in preterm neonates is inversely related to gestational age (GA) and birth weight.[1,2] Several definitions are being used as treatment thresholds, including: (1) a mean arterial blood pressure (MABP) in mmHg below the GA in weeks, (2) a MABP <30 mmHg, and a MABP below the 5-10th percentile according to age and birth weight specific reference values.[2–4]

The rationale for application of these treatment thresholds in daily practice is based on two observations. The first is the reported *association* between adverse (short term) outcome and hypotension.[4–6] The second is the suggestion that the lowest threshold for intact cerebral autoregulation is a MABP of 28-30 mmHg.[7–9]

Several studies did not find an association between low blood pressure and either short or long term outcome.[10,11] More specifically, Dempsey et al. reported that neonates with hypotension but without signs of impaired tissue perfusion did not differ from neonates without hypotension in terms of short term outcome.[12] This suggests that monitoring of cerebral haemodynamics, including regional cerebral oxygenation ($rScO_2$), can be an important tool in blood pressure management. A non-invasive method that can be used for assessing cerebral haemodynamics is Near-InfraRed Spectroscopy (NIRS). The advantage of NIRS is that it can be used for prolonged periods of time, even in very preterm neonates.[13] Recent results of our own group and others demonstrated that dopamine significantly increased the MABP. However, the $rScO_2$ values directly pre and post initiation of treatment were in the same range as in the matched controls.[14,15] This raises the question whether we are treating hypotension too vigorously. In the present study, the question is addressed whether early neurodevelopmental outcome (NDO) in neonates treated for systemic arterial hypotension is different from NDO in their counterparts without hypotension.

PATIENTS AND METHODS

PATIENT POPULATION

The current case-control study is nested in a prospective observational cohort study conducted at the level 3 Neonatal Intensive Care Unit of the Wilhelmina Children's Hospital Utrecht, The Netherlands. The aim of this cohort study is to monitor clinical, physiological, and NIRS data during the first 72 hours after birht in all neonates admitted with a GA ≤32 weeks. Cranial ultrasound was performed daily, and Peri-/Intraventricular haemorrhage (PIVH) was graded according to Papile.[16] The presence or absence of a haemodynamically significant patent ductus arteriosus (*hs*PDA) was examined at least daily based on clinical indices and confirmed by echocardiography (i.e. left atrial and/or left ventricular dilatation, internal ductal diameter >1.4 mm/

kg, left pulmonary artery end diastolic flow >0.2 m/s). The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht. Parental informed consent was obtained in all cases.

The present study presents a retrospective review of prospectively collected data between January 2005 and December 2010. Neonates were included when they developed systemic arterial hypotension that required treatment with dopamine ≥5 µg/kg/min in addition to volume expansion in order to maintain a MABP (mmHg) above the GA in weeks. Exclusion criteria were: major congenital malformations, a *hs*PDA, and severe anaemia. Neonates were matched with controls from the same cohort, who did not receive any blood pressure support. Matching criteria were GA, birth weight, sex, and year of birth. Year of birth was chosen to ensure equal treatment strategies, and equal methods of NDO assessment. Part of this population has been described elsewhere.[14]

CLINICAL DATA

Obstetric, intra-partum, and neonatal data were collected from the patient database and hospital records. The $rScO_2$ was measured by using a 2-wavelength (i.e. 730, and 810 nm) NIRS monitor (INVOS 4100-5100c; Covidien, Mansfield, MA, USA) in combination with a small adult sensor (SAFB-SM, Covidien, Mansfield, MA, USA). The $rScO_2$, arterial oxygen saturation (SaO_2), MABP, and heart rate were measured continuously and stored at a sample rate of 1 Hz for off-line analysis.

In addition, data on the socioeconomic status (SES) of the family was obtained from the Central Bureau of Statistics (CBS, The Hague, The Netherlands). This SES combines information on the parents' highest educational degree, total household income, and profession into a Z-score.

NEURODEVELOPMENTAL OUTCOME

Assessment of NDO was performed by certified investigators at 15 and 24 months corrected age (CA). At 15 months CA the assessment was performed by using the Griffiths Mental Development Scales (GMDS). The assessment at 24 months CA was performed using the GMDS, the Bayley Scales of Infant Development, Second Edition (BSID-II-NL), or the Bayley Scales of Infant and Toddler Development, Third Edition (BSITD-III). The BSID-II-NL or BSITD-III was used in neonates with a GA <30 weeks, or a birth weight <1000 grams. In all other cases the GMDS was used.

The GMDS calculates a total developmental quotient (DQ) based on 5 subscales: locomotion, personal-social, hearing-speech, eye-hand coordination, and performance.[17] From the BSID-II-NL and BSITD-III, only the Mental and Motor Scales were used. To facilitate reliable comparison of NDO at 24 months, Z-scores were calculated by using standard deviations of 12 and 15 for the GMDS and the

Bayley Scales, respectively.[17–20] Only the cognitive scores were used for calculating the Z-scores. The NDO at 24 months was also analysed after correcting the BSITD-III scores.[21,22] In addition to comparing means, NDO scores were classified as delayed (< -1SD) or normal (≥ -1 SD).

STUDY DESIGN

For comparison of physiological parameters between cases and controls, a total of five periods were defined. Three periods were based on postnatal age (PA): 0-24, 24-48, and 48-72 hours PA. The two remaining periods were related to the period of hypotension: one period encompassed the entire period of hypotension (between start and complete cessation of anti-hypotensive medication), and the other period consisted of the 24 hours after discontinuation of the anti-hypotensive medication. In controls, these two periods were selected by using the same duration and PA as in their corresponding case.[2,23]

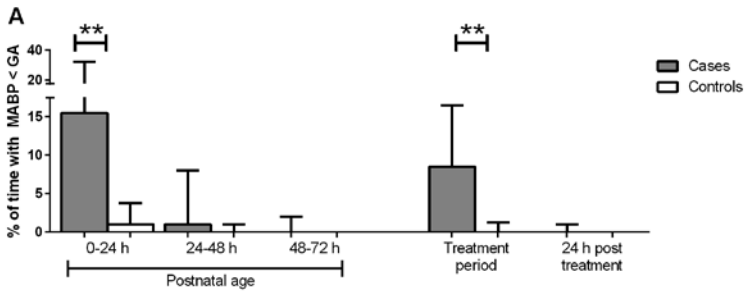
The correlation between the MABP and $rScO_2$ was used as a surrogate marker for cerebral auto-regulation.[24,25] This correlation was determined using 1 minute averages and 15 minute sliding epochs. A correlation coefficient >0.5 was considered to represent a decreased cerebral autoregulatory capacity.[14,24,25] The cerebral fractional tissue oxygen extraction (cFTOE) was calculated ($[SaO_2\text{-}rScO_2]/SaO_2$) to assess the balance between oxygen delivery and oxygen consumption. A $rScO_2$ <50% was defined as low cerebral oxygenation.[13,26–28]

Before calculation, artefacts (e.g. movement, arterial line sampling) were removed manually. All calculations and post processing of the physiological data were performed by using in-house developed software (SignalBase, University Medical Center Utrecht, The Netherlands). To correct for differences in available data between subjects, results are presented as a percentage of time (of a specified period) above or below a pre-defined threshold. In addition, the MABP was adjusted for GA (i.e. MABP – GA).

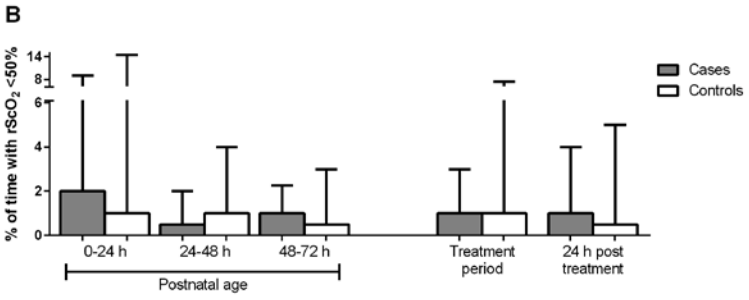
STATISTICAL ANALYSIS

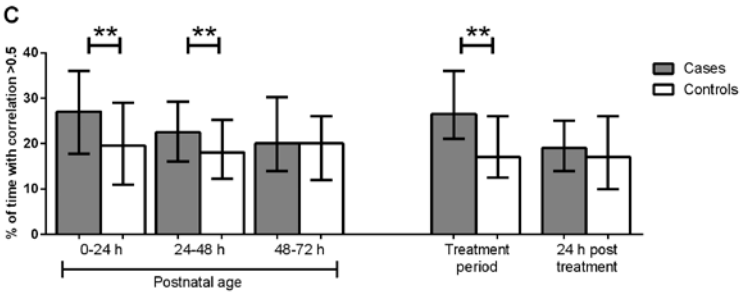
Clinical data are summarized as mean values ± SD, medians and interquartile range (IQR) or numbers and percentages where appropriate. Data were analysed using R 3.0.0 for Windows (The R Foundation for Statistical Computing, Vienna, Austria). Groups were compared by using the paired samples student's *t* test for parametric data or the Wilcoxon signed-rank test for non-parametric data. Categorical data was compared using McNemar's test for case-control studies. With a combined sample size of n = 132 and an alpha of 0.05, the power to detect 1 SD difference in NDO between groups in a 2-tailed test is 0.99. The power for detecting a 0.5 SD difference is 0.81.

To assess whether a $rScO_2$ <50% was associated with NDO at 15 months CA, general

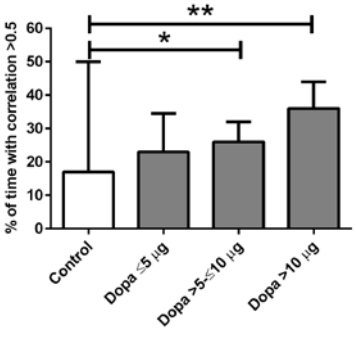


← **Figure 3.1**
Cumulative time shown as a percentage of the total period with A) a MABP < GA in weeks, B) a $rScO_2$ <50%, and C) a MABP/$rScO_2$ correlation coefficient >0.5. 'Treatment': defined as the entire period of anti-hypotensive treatment. Medians are shown; error bars represent the interquartile range. ** P ≤ 0.001





→ **Figure 3.2**
The MABP/$rScO_2$ correlation, as a surrogate for cerebrovascular autoregulation, in relation with the administered dopamine dosage.



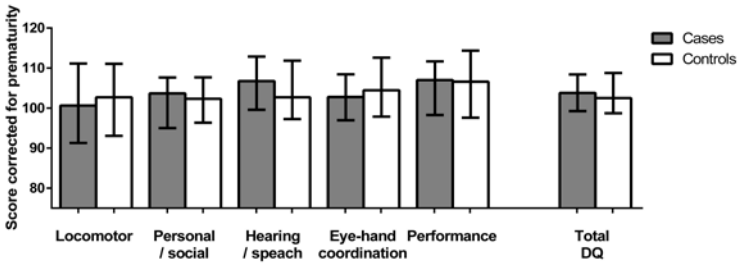
linear modelling was applied with each matched pair as a random factor. Multiple cut-offs were explored for the time with a $rScO_2$ <50% (i.e. 1-20%). To correct for the possible influence of severe PIVH (i.e. grade III/IV) and severe respiratory disease (i.e. needing hydrocortisone yes/no) these factors were also put into the model. Finally, case (yes/no) was put into the model to asses if the association of $rScO_2$ with NDO was different between cases and controls. A p-value of <0.05 was considered statistically significant.

RESULTS

Out of the 549 neonates who were monitored between 2005 and 2010, 137 neonates received inotropes in addition to fluids as a treatment for systemic arterial hypotension (i.e. MABP <GA). Of these 137 neonates, 67 were excluded because of having a *hs*PDA, 3 for having a congenital malformation, and 1 because of severe anaemia. Therefore, 66 cases and 66 controls were included in this study. Clinical data are presented in **table 3.1.** Median duration of blood pressure support was 43 hours (IQR 27-62 hours). Blood pressure support was started at a median PA of 3 hours (IQR 1.3-5.0 hours). **Figure 3.1** panel A, B and C show the percentages of time with a MABP <GA, a $rScO_2$ <50%, and a MABP/$rScO_2$ correlation >0.5, respectively. The mean MABP was significantly lower in cases (all periods, p < 0.001). No significant differences were observed between cases and controls for any of the $rScO_2$, cFTOE, and SaO_2 parameters.
The time with a MABP/$rScO_2$ correlation above 0.5 during the treatment period was higher in neonates treated with higher doses of dopamine (**figure 3.2**). No relation was found between the MABP/$rScO_2$ correlation and any parameter quantifying the severity of hypotension (e.g. mean MABP, minimum MABP, % of time with MABP < GA, or MABP adjusted for GA [MABP-GA]).

NEURODEVELOPMENTAL OUTCOME

At 18 months the median CA was 15.7 months (IQR [15.2-16.8]). The NDO was comparable between cases and controls (**figure 3.3**). Two cases had a total DQ < -1SD. On the locomotor subscale, 9 cases, and 9 controls had scores < - 1SD.



← **Figure 3.3**
Neurodevelopmental outcome, assessed at 15 months CA by using the GMDS. Scores are shown per subscale and as a global developmental quotient (DQ). LM: locomotion, PS: personal social, HS: hearing and speech, EH: eye-hand co-ordination, P: performance. Medians and interquartile ranges are shown.

The mean Z-score of the NDO at 24 months CA was not significantly different between cases and controls (0.07 for controls vs. 0.23 for cases, p = 0.235). One case and 1 control had a Z-score < -1. Differences at 24 months CA remained not significant when using adjusted BSITD-III scores.[21,22]

None of the MABP parameters (e.g. mean MABP, time with MABP <GA) were associated with NDO at 15 months CA. For time with a $rScO_2$ <50%, the cut-off value with maximum discriminative power regarding NDO at 15 months CA was 10%, equal to about 4.5 cumulative hours. On average, the 26 neonates, 13 cases and 13 controls, with a $rScO_2$ <50% for more than 10% of time had a 5 point lower total DQ (intercept 104, coefficient -5, p =0.02). This observation was not significantly different between cases and controls (i.e. no interaction, p = 0.33).

DISCUSSION

In this observational study, neonates treated for hypotension spent more time with a MABP (mmHg) below their GA in weeks than controls, in spite of treatment with fluids and inotropes. No differences were found between cases and controls regarding the cerebral oxygenation and NDO. Interestingly, a $rScO_2$ <50%, for longer than 10% of time, was found to be associated with a lower NDO score. This observation was made irrespective of whether the infant was treated for arterial hypotension or not.

Others have suggested that current definitions of hypotension might not be the appropriate treatment goal.[10–12,29] A small prospective study by Pellicer et al. reported no significant differences in short and long term outcome between infants without and infants with hypotension who responded to carefully titrated therapy.[11] Another study concluded that there was no relation between short term outcome (cerebral lesions on cranial ultrasound) and current definitions of hypotension in preterm neonates.[10] Results of a retrospective study suggest that (untreated) permissive hypotension, defined as a MABP (mmHg) below the GA in weeks without signs of hypoperfusion, was not associated with adverse short term outcome when compared to normotensive counterparts.[12] More recently, a large prospective study including 945 infants of which 78% received either volume expansion or additional vasopressor support, found none of the indicators of hypotension during the first 24 hours to be related with cognitive delay at 24 months of age.[29] However, their study did not include $rScO_2$ or any other perfusion parameter as is the case in the present study.

The contrast between the association of hypotension with adverse (short term) outcome on one hand and reports that fail to demonstrate a relationship with adverse outcome on the other hand can be attributed to several factors: 1) the morbidity inherent to preterm birth, 2) the applied statistical approaches, and 3) the complex interaction between cardiac output, systemic vascular resistance (SVR), and MABP.

The MABP depends on cardiac output and SVR, therefore a MABP within the reference range can be accompanied by a low cardiac output in the presence of a high SVR.[30] As SVR can only be calculated and cardiac output cannot be easily measured continuously, other tools such as NIRS are necessary for continuous monitoring of (tissue) oxygenation and perfusion.[13,30]

For NIRS it has been established that the $rScO_2$ can be used as a surrogate marker for CBF.[13,24,25] The $rScO_2$ did not differ between cases and controls, thereby suggesting comparable cerebral perfusion, which might explain why NDO was comparable between these two groups. More interestingly, $rScO_2$ values <50% seem to be potentially dangerous for the brain, which is in line with previously published research relating $rScO_2$ to brain damage and (short term) outcome.[26–28] Although further research is needed to firmly establish reference values and absolute cut-off values in preterm neonates, the next step would be to intervene when the $rScO_2$ is below a certain cut-off. The $rScO_2$ is measured in a mixed arterial and venous compartment and therefore reflects the balance between oxygen supply (i.e. CBF and oxygenation) and oxygen demand. As the oxygen demand is rather stable in preterm infants, possible interventions should be sought in optimizing the oxygen supply. Parameters that should be evaluated include, but are not limited to, the presence of a *hs*PDA, haemoglobin level, ventilator settings, and also blood pressure.[31]

As for cerebral autoregulation, low MABP has been associated with decreased cerebral autoregulation.[7–9,32] Interestingly, we did not find a relation between any of the parameters quantifying low MABP, and cerebral autoregulatory ability. Instead, an association was found between higher dosages of dopamine and decreased cerebral autoregulatory ability (i.e. a MABP/$rScO_2$ correlation >0.5). This suggests that the dopamine itself accentuates the relationship between the MABP and $rScO_2$. Previously hypotensive neonates have been shown to remain pressure passive after dopamine infusion, even in the high pressure range.[7] Moreover, experimental work suggests vasopressor-inotropes to have direct cerebral actions that might in part explain our observation.[33]

A possible limitation for the generalizability of the results is the exclusion of preterm neonates with a *hs*PDA, who are often younger. This design choice was made to enable reliable comparison of the effect of blood pressure on NDO, independent of the existence of a *hs*PDA and its associated treatments (e.g. indomethacin, surgery). Another limitation is the use of three different methods of NDO assessment at 24 months CA.[22] To increase the reliability of the NDO comparison at 24 months CA, Z-scores were calculated, and the year of birth was included as a matching criterion. Nevertheless, the focus of the current study lies on the NDO at 15 months CA, which has been assessed by using a single method (i.e. the GMDS).

To more firmly establish the lack (or existence) of a relationship between hypotension and short and long term outcome, future studies should use prospective designs and include perfusion parameters in their study setup.[34]

CONCLUSION

In spite of treatment, preterm born neonates treated for arterial hypotension with fluids and inotropes spent more time with a MABP below their GA than controls. The $rScO_2$ was comparable between cases and controls, thereby suggesting comparable cerebral perfusion and possibly explaining the absence of a difference in NDO. Irrespective of whether neonates were hypotensive or not, a $rScO_2$ <50% was found to be associated with a lower NDO score. This suggests that clinical care for preterm neonates could benefit from the inclusion of NIRS in (hypotension) treatment protocols.

↓ **Table 3.1**
Baseline characteristics

	Case	Control	p value
n= (male/female)	31/35	31/35	ns
Gestational age, weeks^{+days} (IQR)	29^{+2} (25^{+6}-31^{+4})	29^{+3} (25^{+5}-31^{+4})	ns
Birth weight, grams (SD)	1195 (354)	1196 (310)	ns
Apgar score at 5 min (IQR)	9 (7-9)	9 (8-9)	ns
Pre-eclampsia, n (%)	18 (27%)	23 (35%)	ns
Antenatal CSS, n (%)	54 (82%)	59 (89%)	ns
eCS, n (%)	36 (55%)	36 (55%)	ns
pPROM, n (%)	9 (14%)	17 (26%)	ns
SGA <p10, n (%)	8 (12%)	7 (11%)	ns
Exogeneous surfactant, n (%)	44 (67%)	20 (30%)	<0.001
Ventilation, n (%)			
CPAP/BPAP	24 (36%)	35 (53%)	ns
SIMV	30 (46%)	25 (38%)	ns
HFOV	11 (17%)	1 (1%)	0.002
Postnatal CCS (hydrocortisone), n (%)	10 (15%)	2 (3%)	0.021
PIVH grade, n (%)			
Grade I or II	14 (21%)	15 (23%)	ns
Grade III or IV	6 (9%)	2 (3%)	ns
Sepsis <48h, n (%)	2 (3%)	3 (5%)	ns
Sepsis ≥48h, n (%)	15 (23%)	21 (32%)	ns
Hypoglycaemia <2.6 mmol/L, n (%)	10 (15%)	6 (9%)	ns
Hyperbilirubinemia, n (%)	59 (89%)	58 (88%)	ns
Retinopathy of prematurity, n (%)	2 (3%)	2 (3%)	ns
NEC treated with surgery, n (%)	3 (5%)	0 (0%)	ns
SES Z-score (SD)	-0.05 (0.99)	0.28 (1.15)	ns
CRIB-II score	7 (6-9)	7 (5-9)	ns
Died	0 (0%)	0 (0%)	ns

BPAP: bi-level positive airway pressure; CCS: corticosteroids; CPAP: continuous positive airway pressure; CRIB-II: clinical risk index for babies version II; eCS: emergency caesarean section; HFOV: high frequency oscillation ventilation; NEC: necrotizing enterocolitis; ns: not significant; PIVH: peri-intraventricular haemorrhage; pPROM: preterm premature rupture of membranes; SES: socioeconomic status; SGA: small for gestational age; SIMV: synchronized intermittent mechanical ventilation
Medians with interquartile ranges are shown unless specified otherwise.

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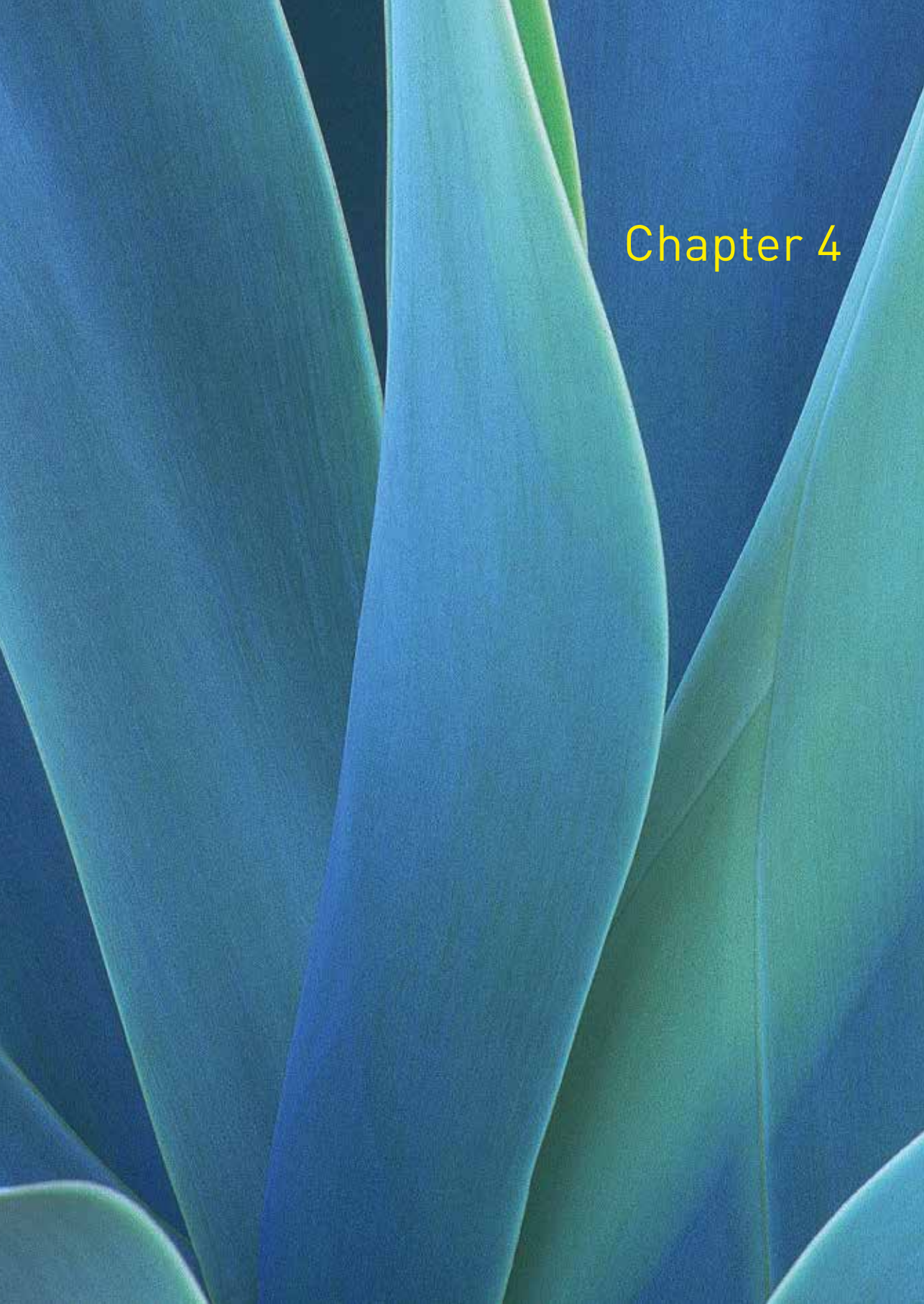

Chapter 4

TREATMENT OF HYPOTENSION OF PREMATURITY (TOHOP) TRIAL: INTERIM ANALYSIS OF SHORT-TERM RESULTS

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Recruitment ongoing

ABSTRACT

BACKGROUND Using the mean arterial blood pressure (MABP) as indication for circulatory compromise in preterm infants, without taking tissue perfusion inconsideration, may lead to unnecessary exposure to potentially harmful vasoactive medication.

AIM To provide an interim analysis of the short-term results of the Treatment of Hypotension of Prematurity (TOHOP) trial.

STUDY DESIGN Infants with a GA <30 weeks, admitted to the neonatal intensive care unit of the Wilhelmina Children's Hospital, who developed hypotension within the first 72h after birth were eligible for randomisation. Infants were randomised to either A) "standard" treatment according to institutional guidelines (i.e. when MABP is below the gestational age [GA] in weeks), or B) "delayed" treatment when the MABP was 5 mmHg below the GA or when there was evidence of compromised tissue perfusion. The MABP, regional cerebral oxygenation ($rScO_2$), regional renal oxygenation ($rSrO_2$), cerebral white- and grey-matter injury, and the number of adverse events (deaths, peri- and intraventricular haemorrhages, retinopathy of prematurity, necrotizing enterocolitis) were compared between the two treatment groups.

RESULTS A total of 69 infants are included thus far. Infants in the delayed treatment group received less circulatory support. There was neither a significant difference between groups, nor a trend showing that that the two treatment groups were different in terms of the MABP, $rScO_2$, $rSrO_2$, number of adverse events, and white- and grey-matter injury scores. The $rSrO_2$ was significantly lower in infants who developed a haemodynamic significant patent ductus arteriosus (*hs*PDA), but the $rScO_2$ was not significantly different between infants with and without a *hs*PDA.

CONCLUSION We did not find any short-term evidence that perfusion guided treatment of low blood pressure is inferior to treatment according to gestational age specific MABP thresholds. A coincidental find suggest that regional renal oxygen saturation might be of added value in the early identification and management of *hs*PDA.

INTRODUCTION

For many years now, hypotension in preterm infants has been subject to a lot of controversy. Although hypotension is a common entity in neonatal intensive care, there is no real consensus on how, but especially when to treat low blood pressure in the (preterm) infant.[1,2] The most commonly used definitions in daily clinical care are: 1) a mean arterial blood pressure (MABP) in mmHg below the gestational age (GA) in weeks, 2) a MABP is less than 30 mmHg, or 3) a MABP below the 5-10th percentile according to reference ranges obtained from literature.[2–4]

These references came into use because of reported *associations* with adverse outcome, and the suggested loss of cerebral-autoregulation below a MABP of 30mmHg.[5,6] On the other hand, there are just as many studies that report MABP to be a poor representative of (tissue) perfusion, and that there is no association between low blood pressure (by any definition) and either short or long term outcome.[7–11] This is not surprising as blood pressure is the product of cardiac output and systemic vascular resistance.[12] and is thereby by no means a direct measure for tissue perfusion

Altogether this suggest that MABP thresholds might not be an appropriate treatment goal to ensure infant circulatory well-being. Therefore, the "Treatment of Hypotension of Prematurity" (TOHOP) randomised controlled trial was set up as a non-inferiority trial. The primary hypothesis is that treatment of low blood pressure according to perfusion parameters is as safe as initiation of treatment when MABP is less than GA in weeks, which is the most commonly used treatment guideline, also in the Wilhelmina Children's Hospital, Netherlands.

METHODS

PATIENTS

The medical ethical board of the University Medical Center Utrecht approved the current study. Written informed parental consent was obtained in all cases. This trial is registered under NL33865.041.10 (www.ccmo.nl) and NCT01434251 (clinicaltrials.gov). All infants with a GA <30 weeks admitted to the NICU of the Wilhelmina Children's Hospital, Netherlands, who developed idiopathic hypotension (i.e. MABP in mmHg < GA in weeks), and in whom written parental informed consent was obtained were eligible for randomization. Exclusion criteria were: indirect clinical or direct laboratory evidence of poor organ perfusion (see below), clinically and/or microbiologically proven sepsis, major congenital abnormalities, postnatal age (PA) >72h at development of hypotension, or the absence of an arterial line for continuous monitoring of the MABP.

STUDY PROTOCOL: RANDOMIZATION AND INTERVENTIONS

Upon eligibility, web based randomisation (t=0) was performed according to a computer generation allocation sequence of 1:1 with block sizes of 4 and 6 in random order. The allocation was stratified for GA (i.e. <27 weeks or ≥27 weeks) and birthweight (i.e. <p10 or ≥p10 for GA). Singleton infants were randomised individually and twins were allocated to the same treatment group.

Infants were randomised to either 1) Standard treatment: start of treatment when MABP in mmHg < the GA in weeks, or 2) Delayed treatment : start of treatment when MABP drops 5 or more mmHg below the GA in weeks, *or* when there was laboratory or clinical evidence of impaired tissue perfusion. Impaired tissue perfusion was defined as: regional cerebral oxygenation ($rScO_2$) <50% for 30 min., two consecutive lactate measurements >6mmol/L, or urine production <0.6ml/kg/h for a 6-hour period.[13,14]. Treatment itself was to the discretion of the physician on duty, but usually encompassed the following treatment scheme: 1) saline bolus at 20ml/kg, 2) dopamine, 3) dobutamine, 4) epinephrine, and 5) corticosteroids.

Patients in both groups were monitored up to 72h PA for: MABP, heart-rate (HR), arterial oxygen saturation (SaO_2), $rScO_2$, regional renal oxygenation ($rSrO_2$), capillary refill, arterial blood gasses, haemoglobin, and lactate. The capillary-refill and laboratory assessments were performed at regular intervals: 4-hour intervals for t=0-24h, and 8-hour intervals for t=24-72h. Monitoring of $rScO_2$ is routine clinical care at the NICU of the Wilhelmina Children's Hospital.[15] The $rSrO_2$ was included as a observational parameter, but not used in deciding whether or not to start anti-hypotensive treatment. The end-point of the monitoring was at 72h PA when the infant had a MABP >GA for at least 6h, without the need of BP support. In case of still receiving BP support at 72h PA, monitoring was continued for as long as needed until MABP was >GA for 6h.

On top of monitoring during the first 3 days after birth, the study protocol specifies a MRI to be performed at term-equivalent age, and a neurodevelopmental outcome assessment at 24 months corrected age by means of the Bayley Scales of Infant and Toddler Development, 3rd Edition.[16] The latter will not be discussed in this preliminary report due to the limited number of infants that reached 24 months corrected age at this moment.

CLINICAL DATA

Obstetrical, intrapartum and neonatal data were collected from the hospital records. The presence of a haemodynamically significant patent ductus arteriosus (*hs*PDA) was defined as a PDA confirmed to be haemodynamically significant on cardiac ultrasound and either treated with indomethacin or surgically closed. The BW z-score was based on recently published Dutch reference curves.[17] Based on in the infants' zip-code, data on the socioeconomic status (SES) of the family was obtained from the

Central Bureau of Statistics (CBS, The Hague, Netherlands). The SES is expressed as a Z-score that combines information on the parents' highest educational degree, total household income, and profession.

MONITORING PARAMETERS

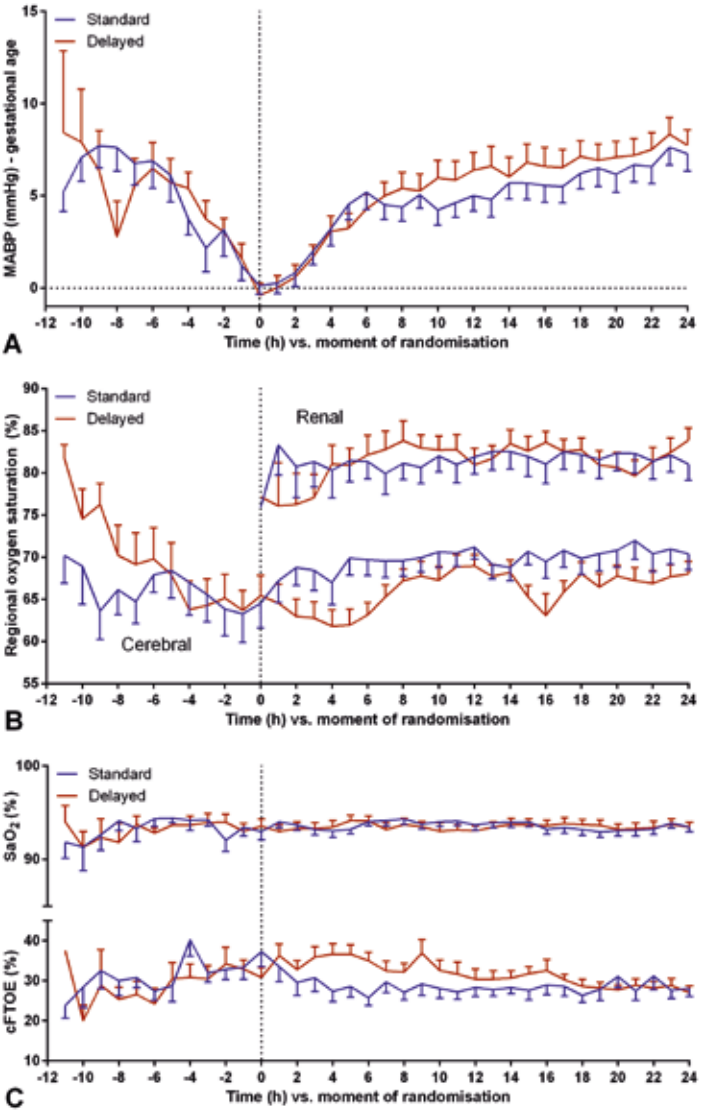
Monitoring of standard physiological parameters was performed by using a patient monitor (IntelliVue MP70, Philips Healthcare, Best, Netherlands): SaO_2 by pulse-oximetry probe, HR by using gel-electrodes, and arterial blood pressure by means of an indwelling catheter (i.e. umbilical, radial or tibial artery). This catheter was also used to obtain blood for the laboratory assessments (Abbott i-STAT System, Abbott Laboratories, Princeton NJ). The $rScO_2$ was monitored by using a two wavelength (i.e. 730 and 810 nm), two distance (i.e. 30 and 40 mm) NIRS monitor (INVOS 4100 or 5100c), Covidien, Mansfield, MA) in combination with a small adult sensor (SomaSensor SAFB-SM, Covidien, Mansfield, MA). An elastic bandage was used for sensor fixation. Until June 2012 data was recorded with Poly 5 (Inspector Research Systems, Amsterdam, Netherlands) at a sample rate of 1 Hz using an INVOS 4100 monitor. Thereafter, in-house developed software (BedBase, University Medical Center Utrecht, Netherlands) was used to record data from the patient monitor and an INVOS 5100c NIRS monitor at a sample rate of 0.4 Hz. In both cases the cerebral fractional tissue oxygen extraction (cFTOE; $[SaO_2\text{-}rScO_2]/SaO_2$) was calculated online. In all cases, the $rSrO_2$ was monitored using an additional 5100c NIRS monitor in combination with a neonatal sensor (OxyAlert CNN NIRsensor, Covidien, Mansfield, MA). This sensor was placed on the right flank at the level of the kidney, in parallel to the spine. The sensor was fixated using double-adhesive Mepitel One (Mölnlycke Health Care, Gothenburg, Sweden). The time of the renal NIRS devices was synchronized with the monitor setup (i.e. Poly 5 or BedBase).

IMAGING: CRANIAL ULTRASOUND AND MR IMAGING

Cranial ultrasounds (PST-65AT combined with a Toshiba Aplio MX system, Toshiba Medical Systems Corporation, Otawara-shi, Tochigi-ken, Japan) were performed daily during the first week of life to assess for the presence of peri- and intra-ventricular haemorrhages (PIVH), which were graded according to the classification of Papile et al.[18]

Infants underwent MR-imaging at term-equivalent age. All MR-imaging was performed on a 3.0 Tesla system (Achieva, Philips Healthcare, Best, Netherlands) with a quadrature body coil for transmission and an 8-element phased-array SENSE head coil as a signal receiver. Infants were sedated by chloralhydrate (50-60mg/kg, oral). A vacuum cushion was used to provide comfort and to minimize motion during imaging. MiniMuffs (Natus Europe, Münich, Germany) and closed headphones

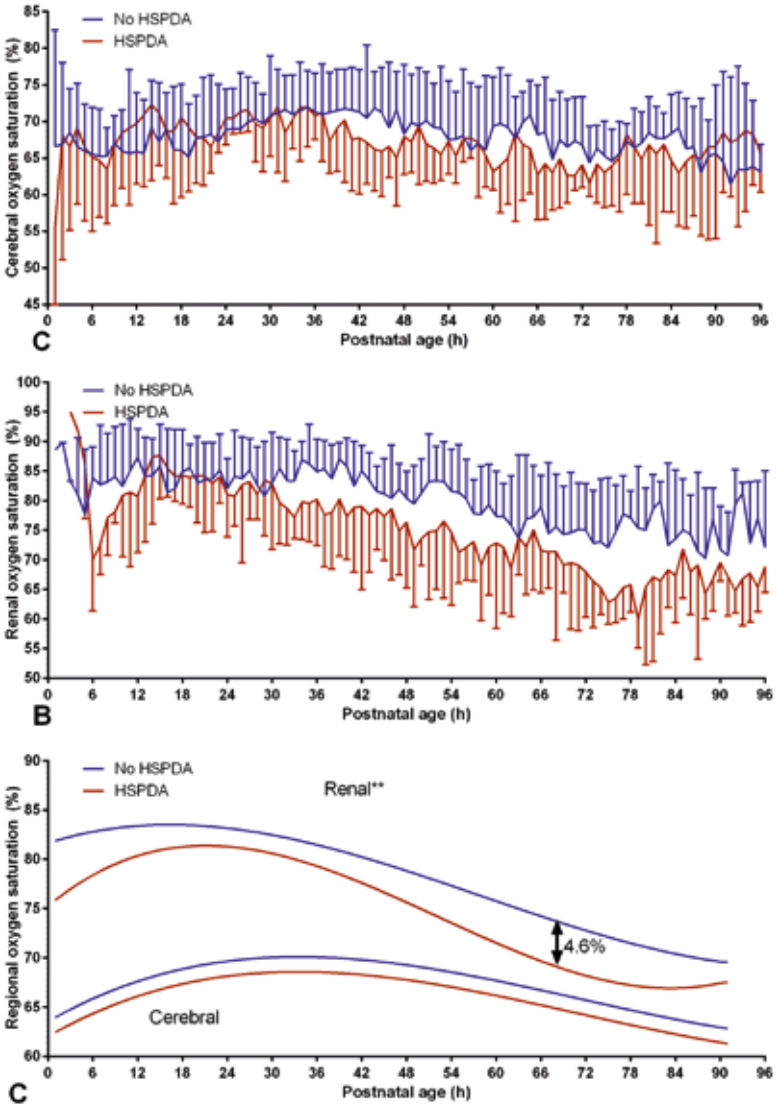
(Philips Healthcare, Best, The Netherlands) were used for noise insulation. Heart rate and arterial oxygen saturation were monitored by pulse-oximetry (Nonin Medical, Plymouth, MN, USA) and respiratory rate by an abdominal transducer (Philips Healthcare, Best, Netherlands). A neonatologist was always present throughout the examination. The MR imaging protocol included sagittal T_1-weighted, coronal T_1- and T_2-weighted, and axial susceptibility weighted (SWI) sequences. Grey- and white-matter abnormalities were scored according to the Woodward score.[19]



→ **Figure 4.1**
Course of A) the MABP, B) the regional cerebral and renal oxygenation, and C) SaO_2 and cFTOE over time for the standard and delayed treatment group in reference to the moment of randomisation.

DATA PROCESSING

All monitoring parameters were analysed with the off-line version of the BedBase software (SignalBase, University Medical Center Utrecht, Netherlands). Before analysis, artefacts were removed manually. For the $rScO_2$, artefacts were defined as: changes in a particular parameter that could not be physiologically explained (e.g. a 30% step change between two subsequent data points), or changes that were accompanied by severe distortion the other parameters suggesting infant movement or handling. Thereafter, 1h-periods were selected during the first 72h after birth and



← **Figure 4.2**
Course over time of A) the regional cerebral and B) renal oxygenation. Panel C) shows the results of the mixed model. ** p<0.01

counted in reference to a patient's birth date and time (i.e. postnatal age, PA). Periods with short drops in SaO_2 (i.e. <85%) were not included in the analysis. In case of SaO_2 drops where additional O_2 was given to assist recovery, the duration of the associated increase in SaO_2 and $rScO_2$ over baseline conditions was also excluded from analysis.[20] Before statistical analysis, 1h-periods containing less than 10-min of data were rejected

STATISTICAL ANALYSIS

All analyses were based on the intention-to-treat principle and conducted blinded for allocation. All statistical analyses were performed in R for Windows 64-bit, version 3.1.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Unless specified otherwise, data are presented as mean±SD for parametric data, median with interquartile range (IQR) for non-parametric data, and counts (%) for categorical data. A p-value <0.05 was considered statistically significant.

RESULTS

Table 4.1 lists the baseline characteristics for the infants that were included thus far. Except for birth-weight, baseline characteristics were comparable between the two groups. Infants in the delayed treatment group received significantly less inotropes.

In two infants (twins) in the delayed treatment group, the study protocol was ended before reaching 72h PA. These infants turned septic and developed severe pulmonary hypotension, necessitating higher MABP's, which was incompatible with the delayed treatment threshold. Both these infants developed a severe IVH a couple of days later.

Five infants died because of various reasons: three in the standard treatment group because of chronic lung disease/pulmonary complications (n=2) or surgical ligation of *hs*PDA that was complicated by a severe IVH (n=1), and two in de delayed treatment group because of severe pulmonary disease.

Figure 4.1 demonstrates the course of MABP, $rScO_2$, $rSrO_2$, SaO_2, and cFTOE over the first 24h following the moment of randomisation (t=0). No statistically significant differences could be demonstrated in any of these variables between the standard and delayed treatment group. Also time spend with an $rScO_2$ <50% during the first 24h after randomisation was not significantly different between the two groups.

A total of 57 infants underwent a MRI examination at term-equivalent age. Parents of one infant did not provide consent for the MRI, and the remainder of the infants who did not underwent an MRI either died or did not reach term-equivalent age yet. The white- and grey-matter injury scores were not significantly different between the two groups, neither was the number and extent of cerebellar haemorrhages (**table 4.2**).

In **figure 4.2**, the effect of a *hs*PDA on $rScO_2$ (panel A) and $rSrO_2$ (panel B) is demonstrated. Infants with a *hs*PDA were found to have significantly lower $rSrO_2$, this

was not significant for $rScO_2$ (**figure 4.2** panel C). A polynomial model (i.e. model with PA, PA^2, and PA^3) provided the best fit (i.e. lowest residuals) to both $rScO_2$ and $rSrO_2$ data (**table 4.3**). For the $rSrO_2$, *hs*PDA had a main effect and interactions with all three representatives of time (PA, PA^2, and PA^3).

DISCUSSION

This work reports on the short-term outcome of the infants included in the TOHOP-trial thus far. This trial was set up as a non-inferiority trial to assess whether treatment according to perfusion parameters was just as safe as treatment according to the current institutional guideline (i.e. MABP <GA). The strength of the current study lies in the fact that it is a interventional trial, which directly compares a broadly used treatment guideline to a perfusion based approach, as opposed to less direct approaches such as observational and case-control studies.[8,10]

The overall trend in the adverse outcomes (i.e. ROP, NEC, IVH, died) does not raise any safety concerns regarding 'delayed treatment'. In all cases, death seems to be unrelated to the hypotensive episode (e.g. pulmonary problems or considerable separation in time between hypotensive episode and the sentinel event leading to death). Although one could argue that the incidence of severe IVH in the delayed treatment group is worrying, the association with the hypotensive episode is unlikely as 1 of these IVHs was present before randomisation, and 3 developed PIVHs considerable time after the hypotensive episode. In the standard treatment group, the 3 infants who developed severe IVH all developed them after randomisation and <4 days of age. In addition, both white- and grey-matter injury, and the number and extent of the cerebellar haemorrhages that were evaluated based on MRI's acquired at term-equivalent did not show any worrying trends. This adds to the confidence that perfusion based treatment, given the used perfusion parameters in the current study, is safe. A retrospective study already suggested that a MABP below the GA does not necessarily needs to be treated, as long as the infants did not show any signs of poor perfusion.[21]

Despite receiving less blood pressure support, the course of MABP in infants in the delayed treatment group was on par with, or maybe even slightly higher than, that of their counterparts in the standard treatment group (**figure 4.1**, panel A). This raises the question, whether we are treating a number or an individual infant? Especially when combining this with results on $rScO_2$ and $rSrO_2$. Both measures of regional oxygen saturation recorded differences between treatment groups that were always < 5%, which is less then 1SD according to reference values, well below the known limits-of-agreement, and lower than the variation encountered after resitting a sensor.[22–24]

A similar argument can be made for the derived cFTOE, which seems to be slightly higher in the delayed treatment group, but considering the scale this difference can

hardly be deemed clinically relevant. This in line with findings of others showing $rScO_2$ to be unrelated to anti-hypotensive treatment.[7,25]

A remarkable observation is that infants in the delayed treatment group had a higher birth-weight than their counterparts in the standard treatment group, despite randomisation. This is even more intriguing because low birth weight (i.e. <p10) was included as a randomisation stratum. Being small-for-gestation is known to be associated with $rScO_2$.[26] The numbers of infants <p10 was comparable between groups and could therefore not be an explanation for the minimal difference in $rScO_2$ between groups.

A coincidental finding was the marked difference in $rSrO_2$ from 48h PA onwards between infants with and without a *hs*PDA. For the $rScO_2$ this has been shown before, also when using comparable analysis.[22,27] Interestingly, the $rScO_2$ did not (yet) demonstrate a difference between infants who develop a *hs*PDA <96h and infants who did not, where the $rSrO_2$ did. This might be explained by the fact that the cerebral circulation benefits from (limited) auto regulatory capability, where the systemic circulation does not.[6] It must be said that the $rSrO_2$ was measured with a neonatal sensor, which on average has an off-set of ~10% compared to the adult sensor when used on the neonatal brain.[28] The exact off-set in the kidney is not known, but when assuming 10% this would still mean that the $rSrO_2$ is higher than the $rScO_2$. Nevertheless, the off-set of the sensor would not influence the recorded difference between infants with and without a *hs*PDA. Altogether, this suggests that the $rSrO_2$ might be of added value in the management of *hs*PDA. Especially if one considers that the individual time at diagnosis of a *hs*PDA was not taken into account in the current analysis, but simply dichotomised at 96h.

As discussed above, this study is limited by the number of included patients thus far, which limits statistical power. Nevertheless, the trend of the reported variables suggests that "delayed treatment" is safe and does not raise concerns at this time. A second limitation at this point is the lack of long-term follow-up data, as the largest part of the participants did not yet reach 24 months corrected age for neurodevelopmental outcome assessment. This is inevitable and will be solved by the passing of time.

CONCLUSION

In conclusion, we did not find any short-term evidence that perfusion guided treatment of low blood pressure is inferior to treatment according to an infants' gestational age. Furthermore, a coincidental find suggest that regional renal oxygen saturation might be of added value in the management of *hs*PDA. We will continue recruiting infants in the TOHOP-trial and are greatly anticipating the long-term follow up data. On a separate track we will explore the relation between $rSrO_2$ and *hs*PDA.

↓ **Table 4.1**
Clinical characteristics

	Standard treatment	**Delayed treatment**	
Baseline characteristics	N=34	N=35	p-value
Gestational age (weeks, mean[SD])	26.6 (1.7)	26.7 (1.7)	ns
Birth weight (grams, mean [SD])	853 (200)	980 (245)	0.022
Birth weight z-score (mean [SD])	-0.3 (0.7)	0.2 (0.8)	0.013
Male gender (n, %)	17 (50%)	23 (66%)	ns
Antenatal corticosteroids (n, %)	23 (68%)	25 (71%)	ns
Apgar score 1 min. (med. [IQR])	5 (4 - 7)	5 (3 - 7)	ns
Apgar score 5 min. (med. [IQR])	8 (7 - 8)	7 (7 - 9)	ns
CRIB-II score (med. [IQR])	12 (10 - 14)	12 (9 - 13)	ns
Lactate at admission (mmol/L, mean[SD])	4.7 (2.2)	4.3 (2.5)	ns
Spontaneous breathing (n, %)			
Day 1	10 (29%)	11 (31%)	ns
Day 2	11 (32%)	11 (31%)	ns
Day 3	10 (29%)	12 (34%)	ns
Days of SIMV/HFOV (med. [IQR])	6 (2 - 14)	8 (1 - 17)	ns
Days of additional O_2 (med. [IQR])	32 (10 - 57)	25 (5 - 54)	ns
Patent ductus arteriosus (n, %)	20 (59%)	18 (51%)	ns
Indomethacin	20 (59%)	17 (50%)	ns
Surgical clip	6 (18%)	8 (23%)	ns
Socioeconomic status z-score (mean [SD])	0.1 (1.1)	0.1 (1.3)	ns
Blood pressure support (n, %)			0.011
Fluids only	12 (35%)	9 (26%)	
Dopamine 5µg	5 (15%)	2 (6%)	
Dopamine 5-10µg	10 (29%)	4 (11%)	
Dopa >10 µg or Dopa + Dobu	1 (3%)	1 (3%)	
Dopa + Dopa >10 µg	1 (3%)	0 (0%)	
Epinephrine or CCS	0 (0%)	1 (3%)	
Adverse events			
IVH (n, %)	12 (35%)	17	ns
Grade I/II	9 (27%)	9 (26)	
Grade III/IV	3 (9%)	8 (23)	
Culture proven sepsis (n, %)	11 (32%)	10 (29%)	ns
Necrotizing enterocolitis (n, %)			ns
Suspicion / conservative	1 (3%)	3 (9%)	
Surgical intervention	3 (9%)	3 (9%)	
Retinopathy of Prematurity (n, %)	6 (18%)	7 (20%)	ns
Conservative treatment	4 (12%)	4 (11%)	
Laser	2 (6%)	3 (9%)	
Died (n, %)	3 (9%)	2 (6%)	ns

CRIB: clinical risk index for babies

↓ **Table 4.2**
MRI scores

	Standard treatment	**Delayed treatment**	
	N=28	N=30	p-value
White matter injury			
White matter signal abnormality			ns
Normal	11 (39.3%)	12 (40.0%)	
Focal abnormalities	14 (50%)	16 (53.3%)	
Multiple regions	3 (10.7%)	2 (6.7%)	
PVWM loss			ns
Normal	15 (53.6%)	21 (70.0%)	
Mild reduction	11 (39.3%)	7 (23.3%)	
Marked reduction	2 (7.1%)	2 (6.7%)	
Cystic abnormalities			ns
Normal	26 (92.9%)	27 (90.0%)	
Single focal cyst <2mm	0 (0%)	0 (0%)	
Multiple or single >2mm	2 (7.1%)	3 (10.0%)	
Ventricular dilatation			ns
Normal	11 (39.3%)	16 (53.3%)	
Moderate	14 (50.0%)	12 (40.0%)	
Global enlargement	3 (10.7%)	2 (6.7%)	
Thinning of corpus callosum			ns
Normal	6 (21.4%)	7 (24.1%)	
Focal thinning	11 (39.3%)	11 (37.9%)	
Global thinning	11 (39.3%)	11 (37.9%)	
Total WMI class			ns
No abnormality	6 (22.2%)	9 (31.0%)	
Mild	17 (63.0%)	17 (58.6%)	
Moderate	3 (11.1%)	1 (3.4%)	
Severe	1 (3.7%)	2 (6.9%)	
Grey matter injury			
Grey matter signal abnormality	0 (0%)	1 (3.3%)	ns
Quality of gyral maturation			ns
Normal	7 (25.0%)	5 (16.7%)	
2-4 weeks delay	20 (71.4%)	24 (80.0%)	
>4 weeks delay	1 (3.6%)	1 (3.3%)	
Subarachnoidal space			ns
Barely visible	6 (21.4%)	8 (26.7%)	
Mildly enlarged	9 (32.1%)	11 (36.7%)	
Substantially enlarged	13 (46.4%)	11 (36.7%)	
Total grey matter class			ns
Normal	17 (60.7%)	20 (66.7%)	
Abnormal	11 (39.3%)	10 (33.3%)	
Cerebellum			
Cerebellar haemorrhages			ns
<6 punctate lesions	6 (21.4%)	5 (16.7%)	
≥6 punctate lesions	0 (0%)	1 (3.3%)	
Large unilateral lesion	1 (3.6%)	0 (0%)	
Vermis involvement	0 (0%)	1 (3.3%)	

↓ **Table 4.3**
Results of the mixed-model with *hs*PDA in relation to $rSrO_2$ and $rScO_2$

Variable	Coefficient (95% CI)	p-value
Regional renal oxygen saturation		
Main effects		
Intercept	81.63 (78.40;84.87)	<0.001
PA(h)	0.243 (0.065;0.422)	0.008
PA^{2a}	-0.0087 (-0.013; -0.0046)	<0.001
PA^{3a}	0.00005 (0.00003; 0.00008)	<0.001
*hs*PDA	-6.40 (-11.70; -1.12)	0.017
Interactions		
PA:*hs*PDA[a]	0.392 (0.112; 0.672)	0.006
PA^2:*hs*PDA[a]	-0.0101 (-0.0164 ; -0.0038)	0.002
PA^3:*hs*PDA[a]	0.00007 (0.00002;0.00011)	0.002
Regional cerebral oxygen saturation		
Main effects		
Intercept	63.52 (60.71; 66.33)	<0.001
PA	0.437 (0.227; 0.646)	<0.001
PA^{2a}	-0.0085 (-0.0136;-0.0035)	0.001
PA^{3a}	0.00004 (0.00001;0.00008)	0.012
*hs*PDA	-1.51 (-4.09; 1.07)	0.246

*hs*PDA: haemodynamically significant patent ductus arteriosus, PA(h): postnatal age in hours [a]PA to the power of 2, and 3 respectively, necessary to obtain a polynominal model.

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Chapter 5

PERFUSION INDEX (PI) IN PRETERM INFANTS DURING THE FIRST 3 DAYS OF LIFE: REFERENCE VALUES AND RELATION WITH CLINICAL VARIABLES

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ABSTRACT

BACKGROUND The perfusion index (PI) derived from pulse oximetry readings represents the ratio of pulsatile (arterial blood) and non-pulsatile contributors to infrared light absorption. The PI has been shown to correlate with cardiac performance. In theory the PI is readily available on every pulse oximeter, therefore no additional sensors or infant handling are required. Currently, reference values are lacking in (preterm) neonates and its association with common clinical conditions is unclear.

OBJECTIVES To establish reference values for the PI in premature infants and at the same time determine the influence of common clinical conditions.

METHODS The PI was prospectively monitored on the lower limb for 72h in 311 neonates born with a gestational age <32 weeks between January 2011 and December 2013. Longitudinal mixed-effects modelling was used. Linear, quadratic and cubic models were explored. Main effects and interactions were investigated.

RESULTS A squared model (0-24h) followed by a linear model (24-72h) provided the best fit of the data. The PI was lowest around 12-18h after birth and showed a steady increase thereafter. PI was positively related with: female gender, gestational age, and pulse pressure. Negative associations were found with: SIMV/HFOV ventilation, dopamine administration, mean arterial blood pressure, and arterial oxygen saturation. Although more complex, the general association with a patent ductus arteriosus was positive.

CONCLUSION The PI varied according to several clinical conditions. The association with common clinical factors suggests that the PI might be used for monitoring neonatal haemodynamics and possibly as an additional guidance for interventions.

INTRODUCTION

Accumulating evidence suggests that monitoring arterial blood pressure might not be sufficient to ensure adequate tissue perfusion in (preterm) neonates admitted to the neonatal intensive care unit (NICU).[1–3] Early recognition of suboptimal organ perfusion is of utmost importance to avoid adverse long term motor and cognitive outcome in these often unstable infants. Although approaches like repeated cardiac ultrasound have proven to be valuable in evaluating neonatal haemodynamics, considerable skill is required to perform and interpret these examinations.[4] Moreover, the nature of the examination still prohibits monitoring and conflicts with the "minimal handling" concept advocated in this patient group. In contrast, Near-InfraRed Spectroscopy (NIRS) does fulfil the minimal handling prerequisite and allows for monitoring for prolonged periods of time, but provides regional information that depends on sensor placement (e.g. brain).[5]

Recently, monitoring of the Perfusion Index (PI) has been suggested as an alternative way of monitoring the general haemodynamic condition of the preterm infant.[6,7] The PI is derived from the plethysmographic signal of pulse oximeters and represents the ratio of the amount of light absorbed by pulsatile (i.e. arterial pulse) and non-pulsatile absorbers (e.g. non-pulsatile blood, tissue, etc.).[8] The PI has been shown to correlate with peripheral perfusion, cardiac output, and stroke volume.[9–11] Moreover, in neonates the PI has been shown to correlate with superior vena cava flow, and that it is associated with volume responsiveness.[7,12] Although further studies are necessary to evaluate its usefulness as an indicator of haemodynamics, the combination with other monitoring tools like aEEG and NIRS seems attractive.

At present little is known about reference values and impact of common clinical variables on the PI in preterm neonates.[8] Therefore, the aim of the current study was to obtain longitudinal data during the first 3 days of life in a large population of preterm neonates admitted to the NICU.

PATIENTS AND METHODS

This observational cohort study is part of a larger prospective observational cohort study which aims to monitor physiological parameters during the first 72h of life in all neonates with a gestational age (GA) < 32 weeks that are admitted to the level 3 NICU of the Wilhelmina Children's Hospital/University Medical Center Utrecht, The Netherlands. The study has been approved by the institution review board and parental informed consent was obtained in all cases. Physiological parameters that were recorded continuously include: arterial blood pressure (ABP), arterial oxygen saturation (SaO_2), heart rate (HR), and the regional cerebral oxygen saturation ($rScO_2$) as determined by NIRS.

The SaO_2 and PI were monitored by using a pulse oximetry probe (Nelcor™, Covidien, Mansfield, MA, USA) placed on one of the lower limbs, preferably the limb without peripheral arterial or venous catheters. The ABP was monitored using an indwelling catheter and HR using gel electrodes. All parameters except the $rScO_2$ were registered by using an Intellivue MP70 patient monitor (Philips Healthcare, Best, The Netherlands). The $rScO_2$ was monitored by using a spectrophotometer combined with a small adult probe (Invos 5100c and SAFB-SM, Covidien, Mansfield, MA, USA). Subsequently, the cerebral Fractional Tissue Oxygen Extraction (cFTOE) was calculated $[SaO_2\text{-}rScO_2]/SaO_2$.[13] Data recording commenced as soon as possible after birth by using BedBase (University Medical Center Utrecht, Utrecht, The Netherlands) connected to the patient monitor using a serial connection. Data was stored at a sample rate of 0.4 Hz.

A cranial ultrasound was performed as soon as possible after birth and thereafter at least daily using a neonatal 3-6 MHz sector transducer (PST-65AT combined with a Toshiba Aplio MX system, Toshiba Medical Systems Corporation, Otawara-shi, Tochigi-ken, Japan) to evaluate the presence of peri-intraventricular haemorrhages (PIVH). PIVHs were graded according to Papile et al.[14] In case of clinical signs of a haemodynamically significant persistent ductus arteriosus (*hs*PDA), the presence or absence of an *hs*PDA was investigated by echocardiography.[15]

Information on relevant clinical parameters was collected from the patient records. In addition, the exact start and stop times of fluid boluses and inotrope administration were documented. Birth weight was converted to a z-score using gender and primi/multiparae specific birth weight curves for the Dutch population.[16]

DATA ANALYSIS

For data processing, SignalBase v. 7.8.1 (University Medical Center Utrecht, Utrecht, The Netherlands) was used. The first 72h after birth were divided in 12 epochs of 6h each (e.g. 0-6h, 6-12h, etc.) Data was discarded when the available data was less than 1 hour (i.e. 1/6 of a 6h epoch). After manual removal of artefacts, median values for each variable were calculated per epoch.

Results were summarized using standard descriptive statistics: counts and percentages for categorical variables, means with standard deviations for parametric data, or medians with interquartile range for non-parametric data.

Mixed-effects modelling was performed by using R 3.0.0 for Windows with the *nlme* package (The R Foundation of Statistical Computing, www.r-project.org). This approach allows for a unequal number of observations per patient. Linear, quadratic, and cubic functions of time after birth were explored to obtain the best fit to the data. To allow for easier interpretation of the model, time after birth was put into the model as hours by converting the epoch number (e.g. 1st, 2nd , etc.) to actual hours after birth

by using the middle of each epoch: 0-6h epoch became 3h, 6-12h became 9h, etc. The PI was used as the dependent variable and the individual patient was always included into the model as a random factor. Fixed effects and interactions were explored for the following variables: gender, the occurrence of a *hs*PDA <84h after birth, the use of a fluid bolus per epoch, the use of dopamine per epoch, the use of dobutamine per epoch, MABP in mmHg, pulse pressure in mmHg, GA in weeks, $rScO_2$ (%), cFTOE (ratio between 0 and 1), SaO_2 in %, HR (bpm), birth weight z-score, the presence of a PIVH, and the use of mechanical ventilation SIMV/HFOV. To yield a value for the intercept that is more readily interpretable, a baseline was used for GA (24 weeks), MABP (20 mmHg), and SaO_2 (85%). Statistical significance was assumed in all tests at $\alpha = 0.05$

RESULTS

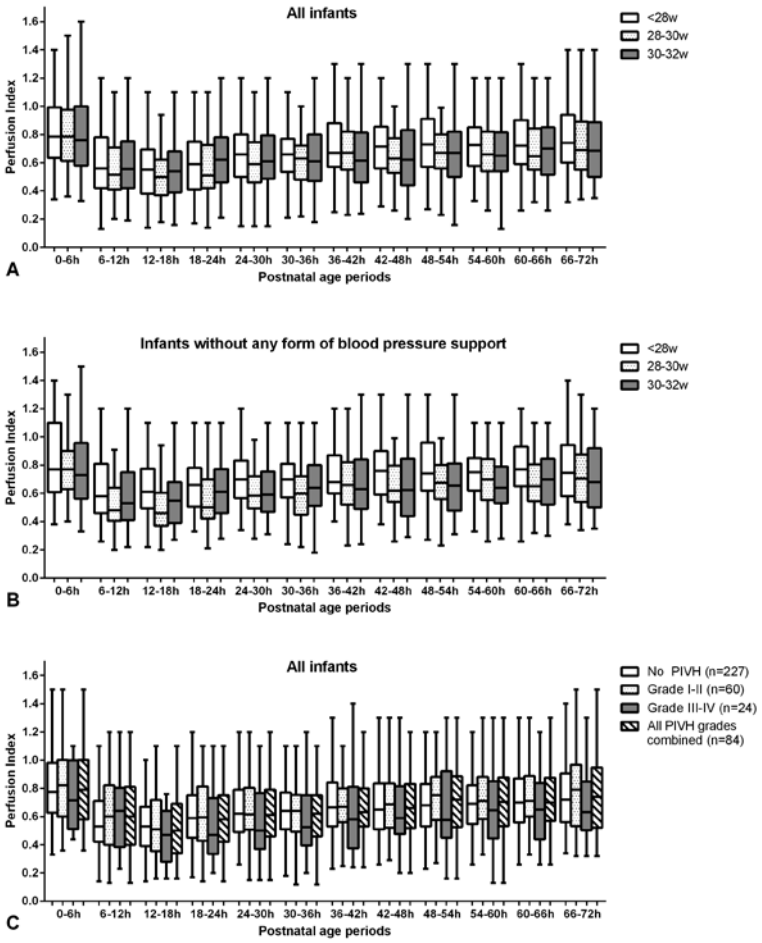
Between January 2011 and December 2013, recording of the PI was performed in 342 neonates. Equipment failure in the early measurements caused data loss in 26 neonates. An additional five neonates were excluded: 1 died within 72h after birth, and 4 had severe cardiac malformations. In total, data of 311 neonates was available for analysis.

Baseline characteristics of the patient population are listed in **table 5.1. Figure 5.1A** shows the raw data per epoch divided according to GA group. A clear minimum is observed 12-18h after birth and a steady increase thereafter. A total of 3342 6h-epochs were available for analysis, with a median of 287 (range 216-303) patients per epoch.

A squared model for 0-24h combined with a linear model for 24-72h provided the best fit to the data. **Table 5.2** provides a list of variables, with corresponding coefficients, that were significantly associated with the PI. In both parts of the model, dopamine, MABP, SaO_2 and artificial ventilation were negatively associated with the PI. The opposite is true for female gender, GA, and pulse pressure. The main effect of having a *hs*PDA switches from positive to negative between the squared and linear model (i.e. 0.171 vs. – 0.057). When taking into account the fact that time is modelled in hours after birth, and that the coefficient of the "time*hsPDA" interaction is positive in the linear model (i.e. 0.0016), the effect of a *hs*PDA switches from negative to positive again after approximately 37 hours (-0.057 ≈ 37*0.0016).

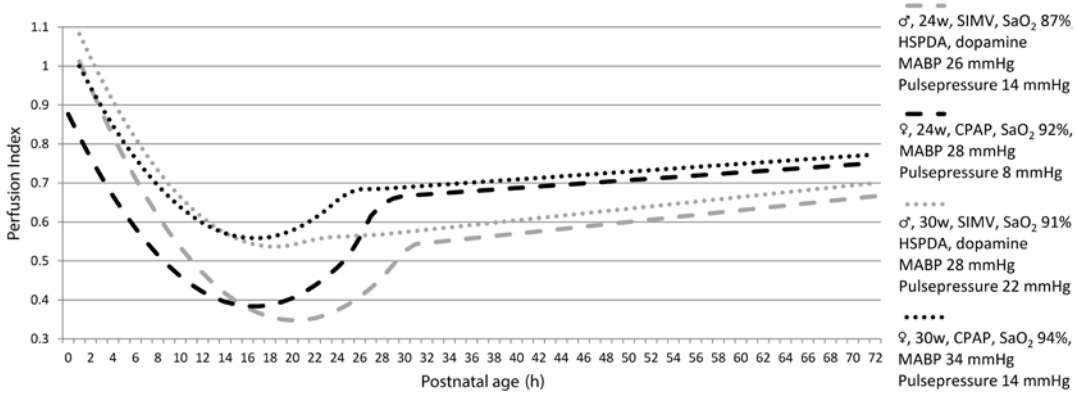
When added to the full model, the association between the PI and cFTOE or $rScO_2$ was significant during 24-72h after birth ($rScO_2$[%] coefficient 0.0033, $p < 0.001$; cFTOE[ratio between 0 and 1] coefficient -0.3092, $p < 0.001$). The $rScO_2$ and cFTOE were not included in the final model to facilitate a more straightforward interpretation, as NIRS is not readily available in every NICU. The effect of dobutamine on top of dopamine was not significantly (0-24h, $p = 0.34$; 24-72h, $p = 0.27$) associated with the PI.

→ **Figure 5.1**
Raw data displayed as median and IQR per 6h epoch, categorized according to A) gestational age group including all infants, B) according to gestational age group only for infants who did not receive any intervention for blood pressure support, and C) according to grade of PIVH including all infants.



Neither was the presence of a PIVH. **Figure 5.1B** shows the raw data for infants who did not receive any intervention for blood pressure support, and **figure 5.1C** shows the raw data per epoch divided according to the grade of PIVH.[14] The effect of a fluid bolus was significant for 0-24h when using a simplified model with time (i.e. PA and PA^2), GA and fluid bolus in the model (coeff. 0.090, p = 0.01).

Model estimates (**table 5.2**) were used to demonstrate the course of the PI in four infants: two with a GA of 24 weeks, and two with a GA of 30 weeks (**figure 5.2**). The difference between the graphs of infants with the same GA is the illness severity (e.g. *hs*PDA, SIMV, dopamine). The two equations to calculate the PI during the first 24h and 24-72 using the model estimates are provided in the appendix.



↑ **Figure 5.2**
Four examples of the model fit. Two infants with a gestational age of 24 weeks (dashed lines): a complicated case (grey) and a relatively uncomplicated case (black). Dotted lines show two infants with a gestational age of 30 weeks: again a complicated (grey) and a less complicated case (black).

DISCUSSION

The PI has the potential to become an additional monitoring tool for neonates admitted to the NICU. One of the main advantages is that monitoring of the PI is readily available to basically anyone who uses pulse oximetry. It has been shown to be associated with measures of cardiac performance and peripheral perfusion both in adults and the neonatal population.[7,9–11]

To our knowledge this is the first paper to report longitudinal reference values of the PI during the first 72h of life in a large cohort (n=311) of preterm infants. Several interesting observations can be made. The first is the lowest PI being recorded between 12-18 hours of life visible both in the raw data and the model fit. Interestingly, this initial decline and subsequent rise are also seen when using other measurements of perfusion, which probably reflects transitional (patho)physiology.[4,17,18] The first day after birth is often characterized by haemodynamic disturbance as transition takes place from a circulation with high right ventricular afterload to a circulation with high left ventricular afterload. After birth, systemic vascular resistance (SVR) increases and the immature myocardium is not able to cope with the relatively sudden increase in left ventricular afterload which consequently results in lower cardiac output.

Reviewing data from **table 5.2**, the PI increases with GA (e.g. the difference between 24w and 28w of gestation is 4 x 0.027 = 0.108), which is in line with the maturation of the

cardiovascular system with every week of gestation.[19] Although the higher PI in females 0-24h might be a reflection of the well-known fact that girls do better in general, one would have expected a (slight) difference in baseline characteristics, which we did not observe. It might be that the differences are more subtle and we did not pick them up in current dataset.

Earlier studies showed conflicting results concerning the effect of a *hs*PDA on the PI.[20,21] In the current study, there was a positive association with the PI during the first 24h and from 37h onwards (when taking into account the *hs*PDA*time interaction) as also has been found by others.[20] One might expect a decrease in (peripheral) perfusion due to the ductal steal phenomenon and thereby a decrease in PI. However, a *hs*PDA is often accompanied by a hyper dynamic circulation as demonstrated by an increased left ventricular output and a widened pulse pressure.[15,22] Combining the increased pulse amplitude with a decrease of the static component (i.e. decrease in overall perfusion), a *hs*PDA can result in a higher PI. The independent association between the PI and pulse pressure probably reflects the associations that both parameters have with cardiac performance.[10]

The negative association of dopamine might be due to its (peripheral) vasoconstrictive action that outweighs the effect of positive inotropy and chronotropy at moderate dosages.[23] In addition, the increase in blood pressure results in an afterload increase and could consequently decrease stroke volume.[24,25] The association with dobutamine was not significant, probably due to the limited number of neonates receiving dobutamine in addition to dopamine in this population (n=5). For fluid boluses a positive relation might be expected.[12] This effect is demonstrated when using a simplified model (i.e. coeff. 0.09, p = 0.01). The lack of significance in the full model is most likely caused by using 6h epochs whilst the effect of a fluid bolus is more short lasting.[3] Also, fluid boluses might have been given to infants with presumed hypotension that were actually not hypovolemic.[26] The lack of significance >24h is most likely a statistical phenomenon as only 8 infants received fluid boluses 24-48h. Interestingly the MABP was negatively associated with the PI. This might be explained by how both parameters are related to SVR. The SVR has a positive correlation with MABP, whereas SVR has a negative correlation with the amplitude of the peripheral arterial pulse and therefor also with the PI.[27,28]

The negative association with SIMV/HFOV is most likely explained by a higher illness severity in this population (median CRIBII 7 [IQR 5-9] vs. 11 [8-14]). Illness severity has been reported to be negatively related to the PI.[6,9] Infants who were receiving SIMV/HFOV had, on average, 2% lower SaO_2. Thereby, the difference in SaO_2 cannot explain the negative effect of SIMV/HFOV, as lower SaO_2 would only slightly increase the PI (i.e. during 0-24h, 2% lower SaO_2 equals -2*-0.028 = 0.056 higher PI, **table 5.2**). Interestingly, the negative relation between the PI and the SaO_2 is independent from

receiving artificial ventilation. We speculate that this can be explained by the effect that oxygen tension has on vascular tone and thereby on the PI.

Finally, the $rScO_2$ and cFTOE were related to the PI 24-72h. Although it could not be determined from this data set, we speculate that the lack of a relationship <24h might be due to the effect of cerebral auto regulation. The benefit of cerebral auto regulation, and thereby also the contrast with circulations that do not auto regulate, is probably greatest during situations of haemodynamic disturbance (i.e. transition during 0-24h after birth).

A possible limitation of this study could be the limb that was used for the measurements, as reported by Kinoshita et al.[29] In our hospital neonates receive their first pulse oximetry probe in the delivery room on one of the upper limbs. However, the probe was switched to one of the lower limbs upon arrival on the NICU. The bias would therefore be minimal, and in the worst case limited to the first 6h. Moreover, even when taking into account a potential upper/lower limb bias during the first 6h, the course of the PI would remain the same. However, we do want to stress that one always needs to pay attention to the limb of measurement when interpreting PI values.[29] The same goes for using different devices and sensors, which calculate the PI slightly different and also have a different precision.[8,30]

In summary, we modelled the PI during the first 72h of life in a large group of premature infants. We consider this the first step in elucidating the value of the PI as a monitoring tool on the NICU. Results show that several common clinical parameters are associated with the PI. These should be taken into account when interpreting this 'new' parameter. Moreover, it provides possible directions for future research into the use of the perfusion index as a haemodynamic monitoring tool.

APPENDIX

The PI during the first 24h can be calculated using the coefficients from **table 5.1**. In this equation, **t** is the time after birth in hours and **{I}** the interaction term:

PI = *1.080 – (0.157+**{I}**)(**t**)+ 0.0018(**t**²) + 0.110(**female gender**[y/n]) + 0.027(**GA**[w]-24) + 0.171(**hsPDA[**y/n]) – 0.084(**dopamine**[y/n]) – 0.021(**MABP**[mmHg]-20) + 0.014(**pulse pressure**[mmHg]) – 0.028(**SaO**$_2$[%]-85) – 0.098(**SIMV/HFOV**[y/n]).*

{I} = *-0.008(**hsPDA**[y/n]) – 0.004(**female gender**[y/n]) + 0.0005(**MABP**[mmHg]) + 0.0009(**SaO**$_2$[%]).*

↓ **Table 5.1**
Baseline characteristics

Male / Female, n	162 / 149		
Gestational age, wks (SD)	28.8 (2.15)		
Birth Weight, g (SD)	1127 (319)		
Apgar score 1 minute, med (IQR)	6 (4-8)		
Apgar score 5 minutes, med (IQR)	8 (7-9)		
CRIB II score, med (IQR)	8 (6-12)		
BW-z score	0.18 (0.25)		
BW-z score < - 1, n (%)	32 (10.3%)		
***hs*PDA**	88 (28.3%)		
*hs*PDA <84h, n (%)	59 (18.9%)		
*hs*PDA >84h, n (%)	29 (9.3%)		
Treated with Indomethacin, n (%)	81 (26.0%)		
Treated with Indomethacin and clip	23 (7.4%)		
Blood pressure support	Day 1	Day 2	Day 3
None, n (%)	210 (67.5%)	256 (82.3%)	274 (88.0%)
Fluid bolus, n (%)	51 (16.4%)	8 (2.6%)	2 (0.6%)
Dopamine <5, n (%)	27 (8.7%)	22 (7.1%)	19 (6.1%)
Dopamine 5-10, n (%)	14 (4.5%)	17 (5.5%)	5 (1.6%)
Dopa >10 or Dopa + Dobu, n (%)	4 (1.3%)	5 (1.6%)	5 (1.6%)
Dopa + Dobu >10, n (%)	1 (0.3%)	2 (0.6%)	3 (1.0%)
Additional medication, n (%)	2 (0.6%)	3 (1.0%)	5 (1.6%)
Predominant form of resp. support <72h	Day 1	Day 2	Day 3
Lowflow/CPAP/BIPAP, n (%)	170 (55%)	177 (57%)	188 (60%)
SIMV/ HFOV, n (%)	141 (45%)	134 (43%)	123 (40%)
IVH	Visible on first cUS	During admission	Combined
Grade I, n (%)	16 (5.1%)	14 (4.5%)	30 (9.7%)
Grade II, n (%)	13 (4.2%)	17 (5.5%)	30 (9.7%)
Grade III, n (%)	5 (1.6%)	8 (2.6%)	13 (4.2%)
Grade IV, n (%)	3 (1.0%)	8 (2.6%)	11 (3.6%)
Hospital mortality, n (%)	13 (4.2%)		

How a certain variable should be entered is noted as *[xx]*, with *xx* depicting the units of the variable. For example gestational age, for a baby of 29.4 weeks of gestation, *0.027(**GA**[w]-24)* would become 0.027*(29.4-24) = 0.027*5.4 = 0.1458.
In case of a variable that is either present or not *[y/n]*, this translates to either a 1 (yes) or a 0 (no). For example gender, *0.110(**female gender**[y/n])* would become 0.110*1 = 0.110 for a female and 0.110*0 = 0 for a male infant.

For the model of the PI during 24-72h after birth the single interaction term "0.001(***hs*PDA**[y/n])" was integrated in the equation:

PI = *0.724 + (0.002+0.001(**hsPDA**[y/n]))(**t**) + 0.011(**GA**[w]) – 0.057(**hsPDA**[y/n]) – 0.154(**dopamine**[y/n]) + 0.013(**MABP**[mmHg]-20) + 0.006(**pulse pressure**[mmHg]) – 0.009(**SaO_2**[%]-85) – 0.051(**SIMV/HFOV**[y/n]).*

↓ **Table 5.2**
List of coefficients for both the squared and linear parts of the perfusion index model.

	0-24h squared model			24-72h linear model		
	Coeff.	S.E.	%[e]	Coeff.	S.E.	%[e]
Intercept	1.080‡	0.0809	-	0.724‡	0.0460	-
PA (h)	-0.157‡	0.0352	-	0.002‡	0.0003	-
PA*PA[a]	0.0018‡	0.00018	-	NA	NA	-
Female gender	0.110†	0.0353	10%	NS	NS	-
Gestational age (w)[b]	0.027	0.0074	3%	0.011*	0.0059	2%
***hs*PDA (<84h age, y/n)**	0.171**	0.0491	15%	-0.057	0.0436	-10%
Dopamine (y/n)	-0.084†	0.0312	-9%	-0.154‡	0.0218	-21%
MABP (mmHg)[c]	-0.021‡	0.0034	-2%	-0.013‡	0.0014	-2%
Pulse pressure (mmHg)	0.014‡	0.0024	1%	0.006‡	0.0015	1%
SaO_2 (%)[d]	-0.028‡	0.0056	-2%	-0.009‡	0.0023	-1%
SIMV/HFOV (y/n)	-0.098†	0.029	-9%	-0.051*	0.0245	-7%
Interactions						
Time*_hs_PDA (y/n)	-0.008**	0.0025	-	0.002*	0.0006	-
Time*Female gender	-0.004*	0.0019	-	ns	ns	-
Time*MABP (mmHg)	0.0005**	0.0002	-	ns	ns	-
Time*SaO_2 (%)	0.0009*	0.0004	-	ns	ns	-

*hs*PDA: haemodynamically significant patent ductus arteriosus; NS: not significant; PA: postnatal age; SIMV: synchronized intermittent mandatory ventilation; HFOV: high frequency oscillatory ventilation
[a] Term to create a squared model for the first 24h, [b] Baseline set at 24 weeks by subtracting 24 from actual value, [c] Baseline set at 20mmHg by subtracting 20 from actual value, [d] Baseline set at 85% by subtracting 85 from actual value, [e] Change in % with respect to intercept caused by a 1 step/unit change. P-values: * <0.05; **<0.01; †<0.001; ‡<0.0001

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Chapter 6

REFERENCE VALUES OF REGIONAL CEREBRAL OXYGEN SATURATION DURING THE FIRST 3 DAYS OF LIFE IN PRETERM NEONATES

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ABSTRACT

BACKGROUND Currently, reliable reference values of regional cerebral oxygen saturation ($rSc0_2$) for different gestational age (GA) groups are lacking, which impedes the implementation of Near-InfraRed Spectroscopy (NIRS) alongside monitoring arterial oxygen saturation ($Sa0_2$) and blood pressure in neonatal intensive care. The aim of the current study was to provide reference values for $rSc0_2$ and cerebral fractional oxygen extraction (cFTOE, [$Sa0_2$ – $rSc0_2$] / $Sa0_2$) for small adult and neonatal NIRS sensors.

METHODS 999 infants born preterm (GA<32w) were monitored with NIRS during the first 72 hours of life. Mixed-modelling was used to generate reference curves grouped per 2 weeks of GA. In addition, the influence of a haemodynamically significant patent ductus arteriosus , gender, and birth weight were explored.

RESULTS Average $rSc0_2$ was approximately 65% at admission, increased with GA (1% per week), and followed a parabolic curve in relation to postnatal age with a peak at ~36h. The cFTOE showed similar but inverse effects. On average, the neonatal sensor measured 10% higher than the adult sensor.

CONCLUSION $rSc0_2$ and cFTOE reference curves are provided for the first 72h of life in preterm infants, which might support the broader implementation of NIRS in neonatal intensive care.

INTRODUCTION

Despite advances in neonatal intensive care that have led to a decline in morbidity, preterm birth is still associated with neurological sequelae.[1] Brain injury in preterm infants is often caused by disturbances in cerebral blood flow (CBF) and oxygenation.[2–4] Evidence is accumulating that monitoring blood pressure alone is not enough to ensure adequate (cerebral) perfusion and oxygenation.[5,6]

Near-InfraRed Spectroscopy (NIRS) is a technique that can be used to monitor regional cerebral oxygen saturation ($rScO_2$), being both a measure of cerebral oxygenation as well as a surrogate of CBF. NIRS monitoring can be applied for prolonged periods of time, even in the most vulnerable infants.[7] It uses multiple wavelengths of NIR light, and relies on the distinct absorption spectra of oxygenated (O_2Hb) and deoxygenated (HHb) haemoglobin to calculate relative concentrations of O_2Hb and HHb, which are then used to calculate the $rScO_2$ (O_2Hb / [O_2Hb + HHb]). Where pulse-oximetry only measures the oxygen saturation in arterial blood (SaO_2), NIRS makes no distinction between different (cerebral) blood volume compartments, therefore the $rScO_2$ represents the oxygen saturation in a mixed arterial-capillary-venous compartment in an approximate 20:5:75 distribution.[8]

NIRS is increasingly being used as a trend monitor of cerebral oxygen supply in neonates admitted to the Neonatal Intensive Care Unit (NICU). Readily interpretable reference values could provide another way of using NIRS in neonates by identifying high-risk neonates. In other words, to identify neonates whose $rScO_2$ resides at the outskirts (high or low) of what is considered 'normal'. Furthermore, reliable reference values could benefit NIRS research by suggesting thresholds that should be explored in relation to interventions and (neurodevelopmental) outcome. However, current literature is quite heterogeneous, with different age groups, small sample sizes, different onsets and durations of measurement, and the use of different devices and sensors.[9–15] Therefore, the aim of the current study was to construct gestational age (GA) specific reference curves during the first 72h of life for $rScO_2$ and its derived cerebral fractional tissue oxygen extraction (cFTOE [SaO_2 – $rScO_2$] / SaO_2) in a large group of neonates who were measured with a small adult NIRS sensor (SomaSensor SAFB-SM using INVOS 4100 or 5100c monitors, Covidien, Mansfield, MA).[16] The second aim was to provide a conversion model to convert $rScO_2$ values obtained by a neonatal sensor (OxyAlert CNN cerebral NIRsensor, Covidien, Mansfield, MA) to the adult sensor equivalent.

METHODS

PATIENTS

This study is part of an ongoing prospective observational cohort study which aims to record physiological parameters during the first 72h of life in all infants born with a GA <32 weeks who are admitted to the NICU of the Wilhelmina Children's Hospital, Utrecht, The Netherlands. The medical ethical board of the University Medical Center Utrecht approved the current study. Informed parental consent was obtained in all cases. Data collection was attempted in 1059 infants admitted between January 2005 and September 2013.

DATA COLLECTION

Obstetrical, intrapartum and neonatal data were collected from the hospital records. Peri- and intra-ventricular haemorrhages (PIVH) were graded according to the classification of Papile et al.[17] The presence of a *hs*PDA was defined as a PDA confirmed to be haemodynamically significant on cardiac ultrasound and either treated with indomethacin or surgically closed.[2]

Standard physiological parameters were monitored by using a patient monitor (Intellivue MP70, Philips Healthcare, Best, The Netherlands): SaO_2 using a pulse-oximetry probe, arterial blood pressure by means of an indwelling catheter (e.g umbilical, radial or tibial artery), and heart rate by using gel electrodes. In general, the pulse-oximetry probe was placed on one of the lower limbs. In case of a *hs*PDA the probe was placed on the right hand (i.e. pre-ductal). $rScO_2$ was monitored by using a two wavelength (i.e. 730 and 810 nm) NIRS monitor (INVOS 4100 or 5100c), Covidien, Mansfield, MA) in combination with a small adult sensor (SomaSensor SAFB-SM, Covidien, Mansfield, MA). An elastic bandage was used for sensor fixation. Until June 2012 data was recorded with Poly 5 (Inspector Research Systems, Amsterdam, The Netherlands) at a sample rate of 1 Hz using an INVOS 4100 monitor. Thereafter, in-house developed software (BedBase, University Medical Center Utrecht, The Netherlands) was used to record data from the patient monitor and an INVOS 5100c NIRS monitor at a sample rate of 0.4 Hz.

DATA PROCESSING

Data was analysed with the off-line version of the BedBase software (SignalBase, University Medical Center Utrecht, The Netherlands). Before analysis, artefacts were removed manually. Artefacts were defined as: changes in $rScO_2$ that could not be physiologically explained (e.g. a 30% step change between two subsequent data points), or changes that were accompanied by severe distortion in the other parameters suggesting infant movement or handling. Thereafter, 1h-periods were

selected during the first 72h of life and counted in reference to a patient's birth date and time (i.e. PA). Periods with short drops in SaO_2 (i.e. <85%) were not included in the analysis. In case of SaO_2 drops where additional O_2 was given to assist recovery, the duration of the associated increase in SaO_2 and $rScO_2$ over baseline conditions was also excluded from analysis.[18]

STATISTICAL ANALYSIS

Before statistical analysis, 1h-periods containing less than 10-min of data were rejected. Mean values of the 1h-periods were used for analysis. A mixed-model approach was performed using R for Windows 64-bit, version 3.1.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria) with the *nlme* package. This approach can handle missing data, and obsoletes correcting for multiple comparisons.

The decision was made, *a priori,* to investigate 4 variables: GA (in weeks), birth weight (BW) z-score, gender, and the presence of a *hs*PDA. The BW z-score was based on recently published Dutch reference curves and explored as a continuous variable, and dichotomized at -1SD and -2SD.[19]

The time of diagnosis of a *hs*PDA was expressed as PA at time of the cardiac ultrasound. This PA at diagnosis was dichotomized at different cut-offs, ranging from 60h-132h with 12h increments.

Linear, squared and polynomial models of time (i.e. PA) were explored to find the best fit to the data. Either the $rScO_2$ or cFTOE was selected as the dependent variable, with the individual subject as a random factor. Both main effects and interactions with PA were explored. It was decided *a priori* that interactions with PA would be included into the final model when the interaction was statistically significant and caused at least a 10% coefficient change with respect to the coefficient for PA in the model with only main effects.

The final model was used to create $rScO_2$ and cFTOE reference curves by generating predictions based on a new set of predictors (i.e. PA, GA, *hs*PDA, gender and BW). This new set was generated as follows: PA ranging 1-72h with 0.2h increments (e.g. 1.0h, 1.2h, 1.4h etc.), GA ranging 24-32 with 1 week increments, *hs*PDA yes/no, female gender yes/no, and BW <-1SD yes/no. The standard errors of these predictions were calculated and used to create percentile plots based on a normal distribution.

To assess the robustness of the results, analysis was also performed by using Generalized Additive Models for Location, Scale and Shape (GAMLSS) in R with the *GAMLSS* package.[20]

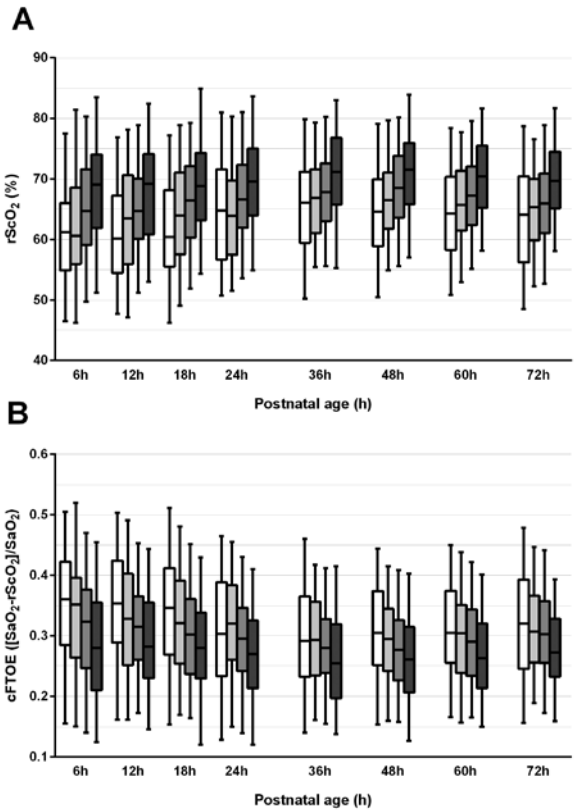
Unless specified otherwise, data are presented as mean with standard deviation (SD) for parametric data, median with interquartile range (IQR) for non-parametric data, and counts (%) for categorical data. A p-value <0.05 was considered statistically significant.

CONVERSION MODEL FOR A NEONATAL NIRS SENSOR

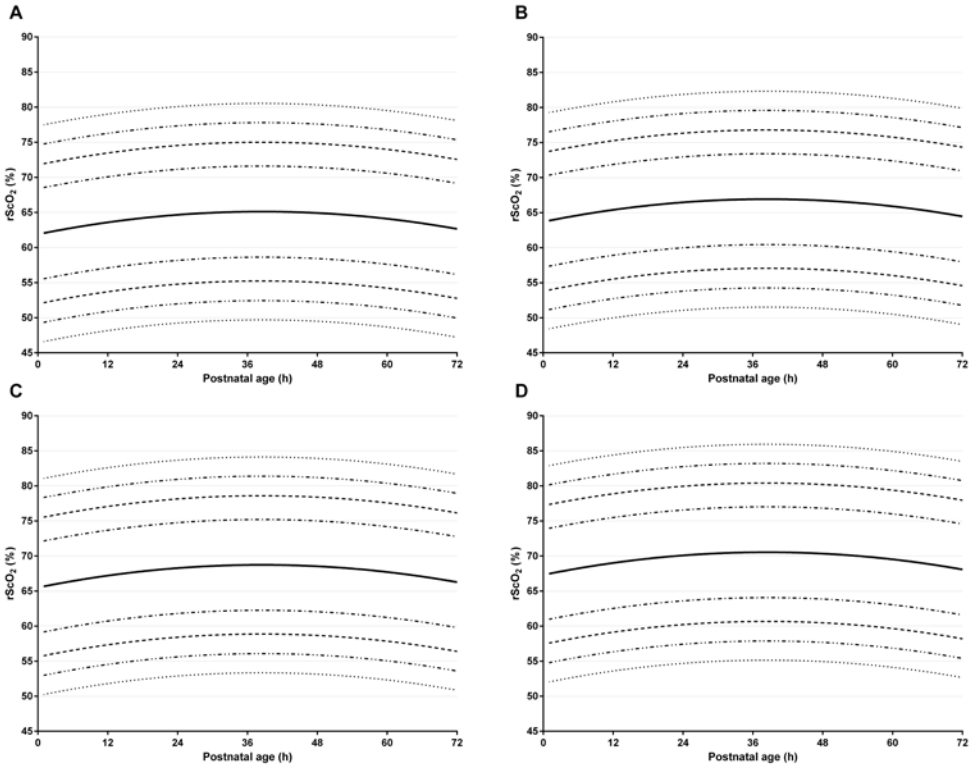
In a subset of infants (n=16, GA 30±3 wk.), $rScO_2$ was recorded bilateral by using the neonatal (OxyAlert CNN cerebral NIRsensor, Covidien, Mansfield, MA) and small adult sensor (SAFB-SM, as described above), as reported previously.[14] Both sensors use a single LED light source, two distant detectors (30 and 40mm), and two wavelengths (i.e. 730nm and 810nm). After at least one hour of stable recordings (e.g. free of care, feedings, and interventions), the sensors were switched to the contralateral side and data collection continued for another hour. Data analysis was performed in MATLAB (vR2011b, The Mathworks Inc., Natick, MA) and artefacts were removed manually. A linear model, polynomial models, and a piecewise linear model were examined to find the best fit (i.e. lowest residuals and highest R^2) in order to convert data obtained by the neonatal sensor to small adult sensor equivalent values and vice versa.

RESULTS

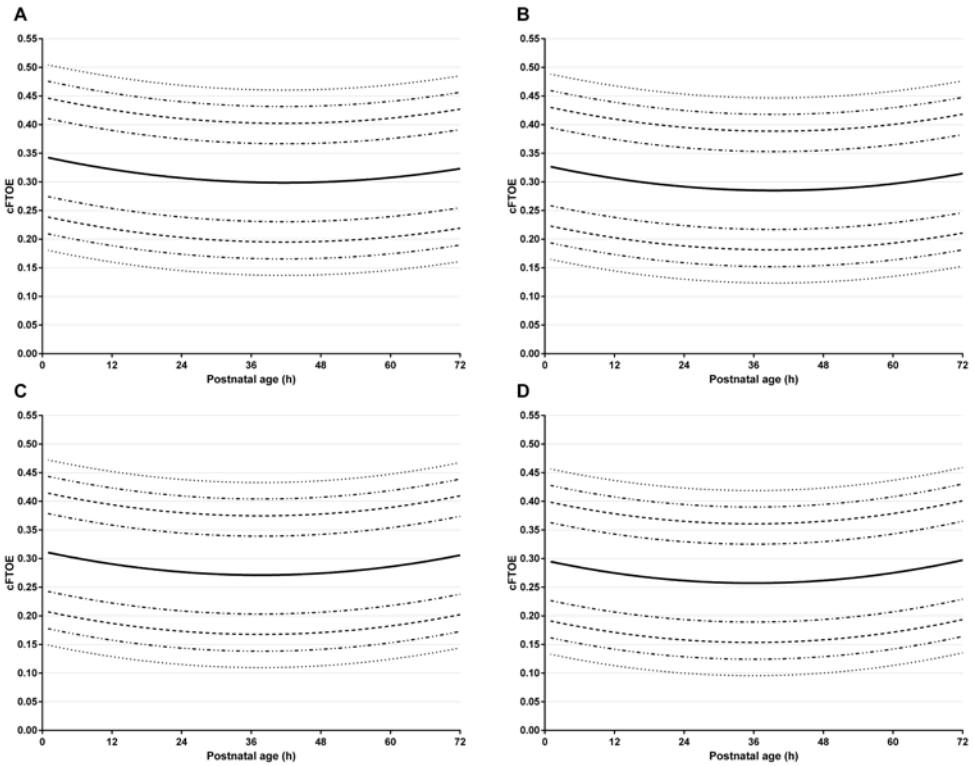
Out of the 1059 participating infants, 41 were excluded because of technical problems during data collection (e.g. data corruption, missing cable connections, or electrical interference). An additional 19 infants were excluded either for having cardiac



→ **Figure 6.1**
Boxplots of the raw data are displayed for four GA groups: □ 24-25w, ▫ 26-27w, ▪ 28-29w, and ■ 30-31w for A) regional cerebral oxygen saturation ($rScO_2$) and B) cerebral fractional tissue oxygen extraction (cFTOE). Data are displayed in 6h periods for 0-24h after birth and in 12h periods for 24-72h after birth.



← **Figure 6.2**
$rScO_2$ reference value curves for neonates of A) 24-25 weeks GA, B) 26-27 weeks GA, C) 28-29 weeks GA, and D) 30-31 weeks GA. The line patterns depict different percentiles: ⋯ p2.3 and p97.7, — ·· — p5 and p95, — — — p10 and p90, — · — p20 and p80, and —— p50.

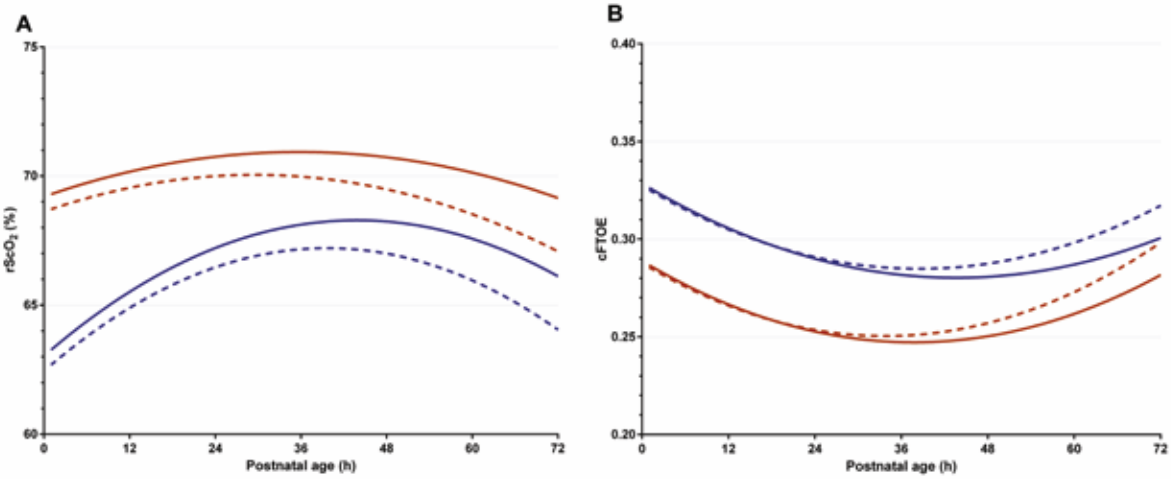


← **Figure 6.3**
cFTOE reference value curves for neonates of A) 24-25 weeks GA, B) 26-27 weeks GA, C) 28-29 weeks GA, and D) 30-31 weeks GA. The line patterns depict different percentiles: ⋯ p2.3 and p97.7, — ·· — p5 and p95, — — — p10 and p90, — · — p20 and p80, and —— p50.

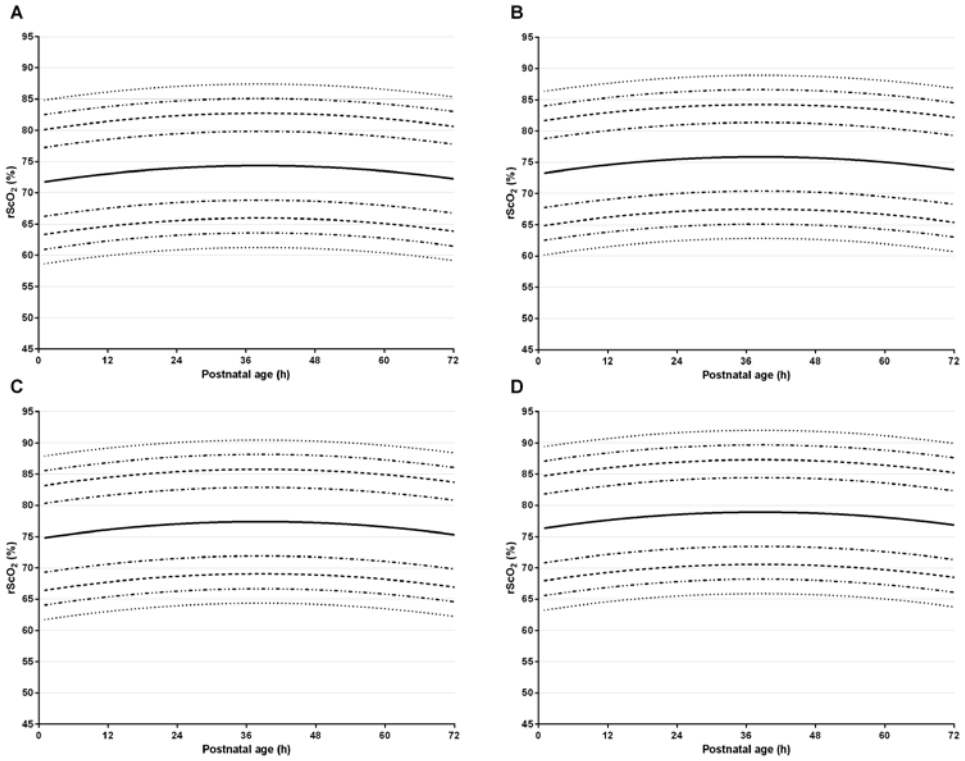
malformations (n=8), chromosomal or severe genetic abnormalities (n=6), or severe congenital malformations (n=5). **Table 6.1** summarizes the clinical characteristics of the study population.
A total of 59135 1h-periods (med. 873, IQR 817-905 per 1h-period) were available for analysis. Although 1h-periods were used for modelling, **figure 6.1** displays the raw $rScO_2$ and cFTOE data in 6h-averages for the first 24h and 12h-averages for the 48h thereafter to yield an easily interpretable figure.

Figures 6.2 and 6.3 display the main results: the $rScO_2$ and cFTOE reference curves for 4 different GA groups. Depending on GA and postnatal age (PA), the mean $rScO_2$ during the first 72h of life ranges from 62-71%, with a positive association between $rScO_2$ and GA. A single SD in $rScO_2$ is approximately 7%, which provides a ±2SD bandwidth of approximately 30%. Likewise, mean cFTOE ranges from 0.25-0.34 during the first 72h of life, a single SD is 0.08 which provides a ±2SD bandwidth of 0.32.

Table 6.2 lists the coefficients of the $rScO_2$ and cFTOE models. For example, mean $rScO_2$ increases 0.9% per week of GA. In the models, PA is also represented by the square of PA (PA-sq) to model the parabolic relationship that $rScO_2$ and cFTOE have with PA. The PA, PA-sq, and interactions with PA and PA-sq determine the location of



↑ **Figure 6.4**
Graphic representation of the interactions of *hs*PDA and SGA with the representatives of postnatal age in the model for A) rScO2 and B) cFTOE. Red line: SGA, blue line AGA, dashed *hs*PDA, solid without *hs*PDA



← **Figure 6.5**
$rScO_2$ reference value curves obtained with the neonatal sensor for neonates of A) 24-25 weeks GA, B) 26-27 weeks GA, C) 28-29 weeks GA, and D) 30-31 weeks GA. The line patterns depict different percentiles: ‥ p2.3 and p97.7, — ‥ p5 and p95, — — — p10 and p90, — · — p20 and p80, and —— p50. Note: the neonatal probe (CNN) $rScO_2$ values were obtained by using a conversion from the adult (SAFB-SM) probe values: $rScO_{2\text{-neo}}$ = 0.8481 $rScO_{2\text{-adult}}$ + 19.11.

the vertex (i.e. maximum for $rScO_2$ and minimum for cFTOE) and the curvature of the parabola. **Figure 6.4** is a graphical representation of the main effects and interactions of being born with a birth weight <-1SD (i.e. small for gestational age, SGA) and having a haemodynamically significant patent ductus arteriosus (*hs*PDA) as reported in **table 6.2**. Infants who developed a *hs*PDA <84h of life had a lower $rScO_2$ and demonstrated a sharper decline after approximately 24h. Infants born SGA started off with a higher $rScO_2$ and had slightly lower values at 72h compared to those at 1h PA, whereas infants with a birthweight appropriate for gestational age (AGA) had higher values at 72h than at 1h PA. This difference between infants born SGA and those born AGA diminishes over time (i.e. SGA and AGA lines in **figure 6.4** converge), but is still present at 72h PA. No significant differences were found between data obtained before or after June 2012 (i.e. Poly 5 software + INVOS 4100 vs. BedBase software + INVOS 5100c).

CONVERSION DIAGRAM

A strict linear model provided the best fit to convert data obtained by the SAFB-SM (adult) sensor to the CNN (neonatal) sensor: $rScO_{2\text{-neo}} = 0.8481 * rScO_{2\text{-adult}} + 19.11$, $R^2 = 0.65$. **Figure 6.5** is a conversion of **figure 6.2** by using this equation.

DISCUSSION

This is the first study to report reference values of $rScO_2$ and cFTOE obtained by using NIRS during the first 72h of life in a large cohort of preterm neonates born at a GA <32 weeks.

Four factors should be taken into account when comparing the work reported here to work of others: i) GA, ii) PA, iii) sample size, and iv) the sensor and device that were used (see discussion below). Values found in literature agree quite well with the values reported here (**table 6.3**, mean difference -0.9%). The differences are likely explained by the characteristics of the reported populations (e.g. GA, PA, specific morbidity), duration of measurements, and small sample sizes.[9–11,21–24] It seems likely that the $rScO_2$ will either stabilize or may even increase again after 72h.[12,13,15,25] Note that Hoften et al. collected data with a paediatric sensor, and Pocivalnic et al. and Pichler et al. with a neonatal sensor.[15,24,25]

It is noteworthy how close the -2SD bands (i.e. p2.3) are to the $rScO_2$ threshold (i.e. 33-44%) reported to be associated with functional impairment of the brain.[26,27] A lower CBF, either regional or global, in infants with a lower GA is the most plausible explanation for the positive association between GA and $rScO_2$. Roche-Labarbe et al., while using a frequency domain NIRS system, also demonstrated lower levels of cerebral oxygenation during the first 7 weeks of life in infants with a GA <31 weeks compared to infants with a GA >31 weeks.[28] Furthermore, their data shows that infants with a GA of 24-27 weeks have the lowest blood flow index, supporting lower CBF as an explanation for lower cerebral oxygenation in younger infants. No associations were found between head circumference and $rScO_2$, and SaO_2 and GA. This makes the influence of head circumference (i.e. different curvature of the head influencing NIRS from a technical point of view) or SaO_2 unlikely. Furthermore, a similar (inverse) association was found between GA and cFTOE. An increased metabolic demand in neonates of lower GA seems unlikely as cerebral activity increases with GA.[29]

Female neonates had lower $rScO_2$ as compared to male neonates. This gender difference was also observed by the group of Pichler during transition from foetal to neonatal life (personal communication, data not published). Again, this could not be explained by a difference in SaO_2 or head circumference. Therefore, possible explanations are a higher (regional) CBF, or lower metabolic demand. A *hs*PDA can cause a ductal steal phenomenon with a surplus of pulmonary flow at the cost of systemic perfusion, and thus CBF.[30] Although notably increasing with PA, the effect of an *hs*PDA seems rather limited during the first 3 days of life. The most plausible explanation for this is the fact that most *hs*PDA's become clinically apparent from day 3 onwards. In addition, an objectively present *hs*PDA (i.e. confirmed by cardiac ultrasound) does not necessarily decrease CBF, and thus $rScO_2$, as the magnitude of

systemic steal depends on shunt volume and left ventricular output. Moreover, in the current study the PA at diagnosis was dichotomized (i.e. ≤84h), therefore the exact PA of the individual at diagnosis and start of treatment was not taken into account. In previous publications we took a different approach with case-control designs and the start of indomethacin or surgery as time reference, at median postnatal day 2 and 7, respectively.[30,31]

Higher $rScO_2$ values in infants born SGA demonstrate the brain-sparing effect with a compensatory higher CBF. This has been demonstrated previously with other techniques.[32] The difference in $rScO_2$ between SGA and AGA infants diminishes over time, suggesting that the CBF returns to normal after day 3. Interestingly, unlike in AGA infants, $rScO_2$ values in SGA infants were slightly lower at 72h PA compared to $rScO_2$ values shortly after birth. This suggests down regulation of compensatory mechanisms instead of a relative lack of haemodynamic development in SGA infants as an explanation for values converging towards AGA values. The limited number of severe SGA cases (i.e. birth weight z-score <-2, n=18) prevented statistical significance of the effect that being severely SGA has on $rScO_2$ and cFTOE.

We preferred to present 'overall' reference curves over presenting numerous curves according to different morbidities. Therefore the reference curves are valid for a population of preterm infants admitted to the NICU, with inherent morbidity. The GAMLSS results were similar to the mixed-model approach. We chose to report the mixed-model procedure because results are easier to interpret and there is more extensive expertise in mixed-model procedures in our department.

As mentioned before, substantial differences exist between different NIRS sensors.[14,15] The correlation between values obtained with the adult and neonatal sensor is not perfect (R^2 = 0.65), which is probably caused by the in-vivo nature of our experiment. However, the relation is clearly linear, which has also been demonstrated in-vitro.[33] Although the difference between the neonatal and adult sensor can be as high as 15% (mean 10%), trend monitoring is still possible in a way similar to adult sensors. Moreover, the ±2SD limits provide a bandwidth' of ~30% which makes the max. 15% difference less stressing. However, awareness of a possible offset between sensors is crucial when comparing data between patients, institutions, or devices. For example, a $rScO_2$ of 55% seems low but acceptable when using an adult sensor (**figure 6.2**), but is below the -2SD threshold in all GA groups when using a neonatal sensor (**figure 6.5**). A $rScO_2$ of 55% with the neonatal sensor converts to an adult sensor value of approximately 40%, which is close to or below the thresholds (33-50%) reported to be associated with neuronal damage and adverse neurodevelopmental outcome.[5,26,27] Two of these thresholds were established in piglet studies by using devices that are not commercially available.[26,27] Therefore, these thresholds should be used with caution, especially in preterm neonates. Also higher values pose pitfalls, as most devices have

an upper detection limit of 95%. Any value >85% obtained by an adult sensor would register as 95% (i.e. 85+10%) with a neonatal sensor, losing the ability to monitor the variability in $rScO_2$. This is particularly relevant for the prognostic value of the $rScO_2$, for example in asphyxiated neonates.[34,35]

A possible limitation of this study is the generalizability of the results as participants were admitted to a single level III NICU. However, neonatal intensive care in the Netherlands takes place in 10 NICU's and admissions are purely based on geography. Equipment availability was the only factor to prohibit data collection. As unavailability was supposedly random, this influence should be minimal. Moreover, the number of recording setups substantially increased over the years, now ensuring round-the-clock availability. The second limitation is the restriction to the first 72h of life. This choice was made to encompass the most vulnerable period of life, and to limit strain on nursing staff at the same time. The final limitation is clinical practice in our unit regarding the used SaO_2 thresholds (i.e. 85-92%), which might differ from other institutions. The $rScO_2$ results were not corrected for SaO_2 to avoid overly complicating results and because model coefficients (**table 6.2**) changed less than 5% when correcting to a SaO_2 of 90%. Moreover, the cFTOE curves already provide a form of SaO_2 correction. Altogether, we feel confident that the current results are generalizable to other populations of preterm neonates with a GA <32 during the first 72 hours of life.

Currently, the core application of NIRS on the NICU lies in trend monitoring. For the inexperienced user, we suggest plotting an infants' $rScO_2$ in the appropriate GA specific reference curve. In case of sudden changes in $rScO_2$ ≥7% (i.e. 1SD), we recommend evaluation of clinical parameters (e.g. ventilator settings, haemoglobin levels, presence of a *hs*PDA, medication, perform a cranial ultrasound), but only after ensuring that the measurement setup has not changed (e.g. sensor displacement). Likewise, absolute $rScO_2$ values close to or outside the ±2SD bands, should trigger an evaluation, similar to what has been done during the SafeBoosC trial (Safeguarding the Brains of our smallest Children).[36] For a more detailed discussion on which parameters to evaluate in case of either low or high levels or $rScO_2$ we refer to the treatment guideline published by the 'SafeBoosC' research group.[37]

CONCLUSION AND FUTURE RESEARCH DIRECTIONS

This study provides reference values for $rScO_2$ and cFTOE measured by NIRS during the first 72 hours of life in premature infants. Both $rScO_2$ and cFTOE are influenced by GA, PA, *hs*PDA, gender, and being born SGA. Furthermore, an equation is provided to extend the results to $rScO_2$ values obtained with a neonatal NIRS sensor. These data provide an additional way for applying NIRS on the NICU, on top of trend monitoring. Furthermore, reliable reference data can be useful in future studies. Indices of

cerebral oxygenation have only been suggested to be related to outcome.[5,26] Future research should focus on developing robust indices of cerebral oxygenation that are related to (long-term) outcome and can be used to guide interventions. Our results suggest that GA and PA specific thresholds are worth exploring in this regard. The SafeBoosC research group already reported that NIRS can be used to stabilize the cerebral oxygenation in preterm infants.[36]

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↓ **Table 6.1**
Baseline characteristics of the study population

		Total	24-25 wks	26-27 wks	28-29 wks	30-31 wks
Male / Female, n		539 / 460	57 / 50	128 / 111	171 / 143	183 / 156
Gestational age [weeks], mean (SD)		28.7 (1.96)	25.1 (0.57)	27.0 (0.59)	28.9 (0.57)	30.8 (0.55)
Birth weight [gram], mean (SD)		1150 (330)	770 (110)	930 (180)	1193 (244)	1385 (318)
Birth weight z-score, mean (SD)		0.02 (0.90)	0.40 (0.84)	0.04 (0.83)	0.12 (0.88)	-0.21 (0.91)
Birth weight <-1SD, n (%)		142 (14.2)	5 (4.7)	29 (12.1)	40 (12.7)	68 (20.1)
Apgar score 1 min, median (IQR)		7 (5-8)	5 (3-6)	6 (4-7)	7 (5-8)	7 (6-8)
Apgar score 5 min, median (IQR)		8 (7-9)	7 (6-8)	8 (7-9)	8 (8-9)	9 (8-9)
Head circumference [cm], mean (SD)		26.1 (2.3)	22.8 (1.4)	24.7 (1.6)	26.4 (1.6)	27.8 (1.9)
aCCS full course, n (%)		725 (72.6)	78 (72.9)	172 (72)	236 (75.2)	239 (70.5)
Spontaneous breathing, n (%)		Day 1 Day 2 Day 3	Day 1 Day 2 Day 3	Day 1 Day 2 Day 3	Day 1 Day 2 Day 3	Day 1 Day 2 Day 3
		535 (53.6) 547 (54.8) 573 (57.5)	20 (18.7) 22 (20.6) 26 (24.3)	69 (28.9) 74 (31.0) 84 (35.4)	188 (59.9) 189 (60.2) 194 (61.8)	258 (76.1) 262 (77.3) 269 (79.4)
*hs*PDA, n (%)		294 (29.4)	78 (72.9)	127 (46.9)	75 (23.9)	29 (8.6)
PA at *hs*PDA diagnosis [h], med (IQR)		55 (29-91)	58 (38-113)	59 (28-81)	52 (25-86)	62 (28-82)
PIVH, n (%)	None	713 (71.4)	54 (50.5)	156 (65.3)	231 (73.6)	272 (80.2)
	Grade 1	88 (8.8)	6 (5.6)	18 (7.5)	34 (10.8)	30 (8.8)
	Grade 2	110 (11.0)	28 (26.2)	25 (10.5)	35 (11.1)	22 (6.5)
	Grade 3	59 (5.9)	16 (15.0)	32 (13.4)	10 (3.2)	1 (0.3)
	Grade 4	29 (2.9)	3 (2.8)	8 (3.3)	4 (1.3)	14 (4.1)
Hospital mortality, n (%)		65 (6.5)	23 (21.5)	25 (10.5)	13 (4.1)	4 (1.2)
CRIB II score, median (IQR)		9 (7-11)	14 (13-16)	12 (10-13)	8 (7-9)	6 (5-7)

aCCS: antenatal corticosteroids; CRIB: Clinical Risk Index for Babies; *hs*PDA: haemodynamically significant Patent Ductus Arteriosus; IQR: Inter-quartile range; PA: postnatal age; PIVH: periventricular/intraventricular haemorrhage; SD: standard deviation

↓ Table 6.2
Final model coefficients for the $rScO_2$ and cFTOE

	$rScO_2$			cFTOE	
	Coefficient	95% C.I.		Coefficient[C]	95% C.I.
Intercept	59.415	58.138; 60.693†		36.033	34.581; 37.486†
Main effects					
PA (h)	0.240	0.207; 0.273†		-0.221	-0.255; -0.188†
PA sq[a]	-0.003	-0.003; -0.002†		0.0023	0.002; 0.003†
*hs*PDA ≤ 84h	-0.581	-1.652; 0.490		-0.113	-1.263; 1.036
GA[b]	0.904	0.699; 1.108†		-0.795	-1.039; 0.552†
Female gender	-1.565	-2.308; -0.822†		1.729	0.946; 2.512†
BW ≤ -1SD	6.172	4.371; 7.973†		-3.960	-5.243; -2.677†
Interactions					
PA : BW ≤ -1SD	-0.1431	-0.2325; -0.0536**		n/a	
PA sq : *hs*PDA ≤ 84h	-0.00029	-0.00052; -0.00005*		0.00034	0.00007; 0.00061*
PA sq : BW ≤ -1SD	0.0014	0.0002; 0.0025*		0.00040	0.00008; 0.00071*
PA sq : GA	n/a	n/a		0.00007	0.00001; 0.00013*

BW: birth weight; GA: gestational age; *hs*PDA: haemodynamically significant patent ductus arteriosus; n/a: not applicable; PA: postnatal age
[a] PA sq: postnatal age squared to enable a squared model
[b] GA -24, to make 24 weeks of gestation the reference point to yield a interpretable intercept.
[c] $[SaO_2 – rScO_2] / SaO_2$ multiplied by 100, to obtain coefficients with the same effect size as for the $rScO_2$
*p <0.05, ** p <0.01, † p <0.001

↓ Table 6.3
$rScO_2$ values obtained from literature compared to $rScO_2$ reference values established in the current study

	Literature values					Current study		Difference
Author	Time	Measure	GA (w)	n	$rScO_2$ / TOI (%)	Ref. GA group	$rScO_2$ (%)	Literature - current
0-72h of life								
Naulaers et al. 2002[9]	d1	TOI	28 [25-30]	15	57 [54–66]	28-29 (12h)	67.2	10.2
	d2				66 [62–82]	28-29 (36h)	68.7	2.7
	d3				76 [68–80]	28-29 (60h)	67.7	-8.3
Lemmers et al. 2006[21]	6-12h	$rScO_2$	29.3 (1.7)	20	70 [61-77]	28-29 (12h)	67.2	-2.8
	18-24h				68 [63-75]	28-29 (24h)	68.3	0.3
	36-48h				73 [65-84]	28-29 (48h)	68.5	4.5
	60-72h				71 [64-75]	28-29 (72h)	66.3	-4.7
Sorensen et al 2006[11]	19h (6)	TOI	27.6 [23.9-33]	37	74.6 (8.5)	26-27 (18h)	66.5	-8.1
Moran et al. 2009[23]	d1	TOI	29 [25.3-31.5]	27	68.1 (7.9)	28-29 (12h)	67.2	-0.9
Pichler et al. 2013[24]	<1h	$rScO_2$	34.9 (1.4)	27	80 [62-92]	34-35 (1h)[a]	79.5[ab]	-0.5
Sirc et al. 2013[22]	6h	TOI	25.9 (1.7)	22	65.2 (10)	24-25 (6h)	62.8	-2.4
	12h				63.9 (5.9)	24-25 (12h)	63.6	-0.3
	24h				68.8 (5.7)	24-25 (24h)	64.7	-4.1
	48h				67.2 (7.2)	24-25 (48h)	64.9	-2.3
Hyttel-Sorensen et al. 2013[10]	avg 3days	$rScO_2$	26.3 (-)	10	64.2 (4.5)	26-27 (36h)	66.9	2.7
Average difference								**-0.9**
>7d of life								
Hoften et al. 2010[25]	17d (1-93d)	$rScO_2$	27.3 [25-34]	33	71 [65-96]	26-27 (72h)	74.7[b]	3.7
Petrova et al 2006[12]	>7d	$rScO_2$	[24-32]	20	66 (8.8)	28-29 (72h)	66.3	0.3
Petrova et al 2010[13]	~5 weeks	$rScO_2$	26 (2.4)	10	68.5 (4.6)	26-27 (72h)	66.3	-2.2
Pocivalnic et al 2011[15]	3.9d (4.8)	$rScO_2$	35.2 (3.0)	37	84.1 (6.4)	34-35 (72h)[a]	80.0[ab]	-4.1
		TOI			72.2 (6.0)	34-35 (72h)[a]	71.3[a]	-0.9

[a] Model fit was obtained in subjects ≤32 GA, therefore data >32 weeks GA was extrapolated
[b] Study used a neonatal sensor, for comparison the conversion was used: $rScO_{2\,[neo]} = 0.8481 * rScO_{2\,[adult]} + 19.11$
GA: gestational age; $rScO_2$: regional cerebral oxygen saturation; TOI: tissue oxygenation index

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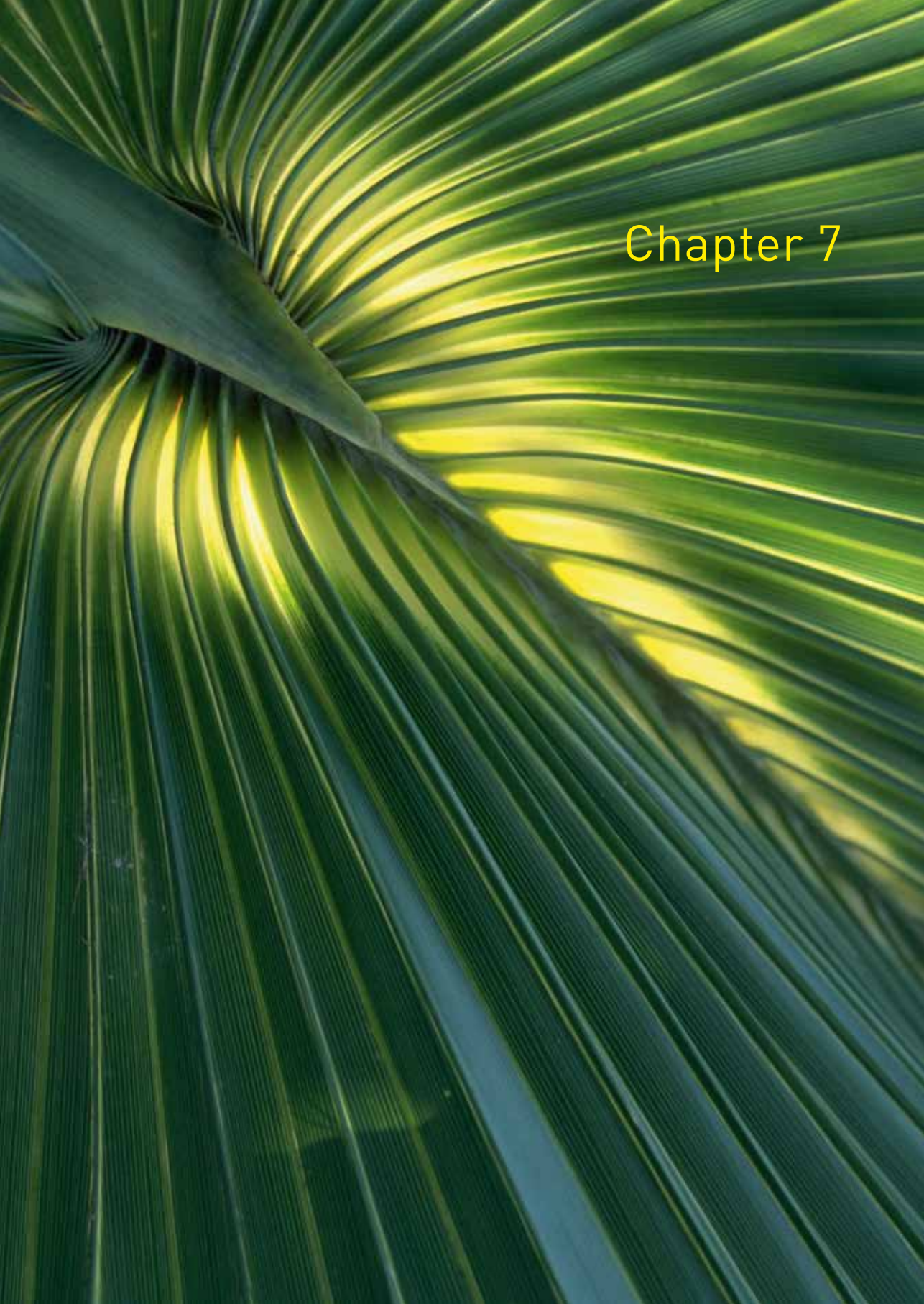
Chapter 7

CEREBRAL OXYGENATION IN INFANTS BORN PREMATURE IS RELATED TO ADVERSE NEURODEVELOPMENTAL OUTCOME AT 15 AND 24 MONTHS CORRECTED AGE

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ABSTRACT

INTRODUCTION Cerebral injury is not uncommon in infants who are born before 32 weeks of gestation and is often related to disturbances in cerebral blood flow and oxygenation. Near-InfraRed spectroscopy (NIRS) can be used to monitor the regional cerebral oxygen saturation ($rScO_2$). The aim was to explore which thresholds of $rScO_2$ have the strongest association with neurodevelopmental outcome.

METHODS The $rScO_2$ was continuously monitored during 0-72 hours after birth. Fixed thresholds (range 30-90%, with 5% increments) and thresholds according to published reference values (range -2SD to +2SD, with 0.5SD increments) were explored in association with outcome. Neurodevelopmental outcome was assessed at 15 and 24 months corrected age using the Griffiths Mental Development Scales and Bayley-III scales, respectively. Composite adverse outcomes were defined as a neurodevelopmental outcome score <-1SD or death.

RESULTS Infants with adverse outcome spent more time with $rScO_2$ below fixed thresholds 45-60%, with <55% being the most discriminative fixed threshold. Overall, time spent with $rScO_2$ below -1.5SD or outside ±1.5SD (i.e. <-1.5 or >+1.5SD combined in one measure), based on reference values, had the strongest independent association with outcome. This could already be demonstrated on the first day after birth.

CONCLUSIONS Low, and to a lesser extent high, $rScO_2$ according to recently published reference values provided the most significant difference between infants with an adverse outcome and infants with a favourable outcome. This suggests we should have a slightly different approach regarding the interpretation of absolute values of $rScO_2$, both clinically and in future trials that use NIRS.

INTRODUCTION

During the last decades, survival of infants born premature has increased dramatically. The incidence of brain injury, however, has remained relatively stable. Common entities are peri-and intraventricular haemorrhage (PIVH), post haemorrhagic ventricular dilatation, white matter injury, and cerebellar lesions. Although their pathophysiology has not been completely resolved, the development of these lesions seems to be at least partially related to disturbances in cerebral blood flow and oxygenation.[1,2]

Traditionally, the wellbeing of infants is assessed by monitoring arterial oxygen saturation (SaO_2), heart rate, and blood pressure. Although these three parameters are of vital importance, neither of them can be used as a direct measure to ensure adequate perfusion and oxygenation of the vital organs, such as the brain.

Near-Infrared Spectroscopy (NIRS) can provide information on end-organ perfusion and oxygenation by providing a direct, continuous, and absolute estimate of the tissue oxygen saturation. Currently NIRS is mostly used as a trend-monitor of the cerebral oxygenation, absolute values are not used often due to the lack of reliable thresholds that are associated with outcome. A recent randomised trial demonstrated that the cerebral oxygenation could be stabilised by combining commercially available NIRS devices with an intervention guideline.[3,4] Although, indices of cerebral oxygenation as obtained by NIRS have been associated both with short and more long-term outcome, the impact of these results are limited because of heterogeneous study designs and relatively small sample sizes.[5–7] To transform NIRS from a predominantly research tool and trend monitor to an established clinical monitoring tool, reliable thresholds of cerebral oxygenation should be available that are associated with outcome parameters. The association between hypoxia and brain injury is most intuitive, but also hyperoxia seems harmful for the preterm brain.[8–10]

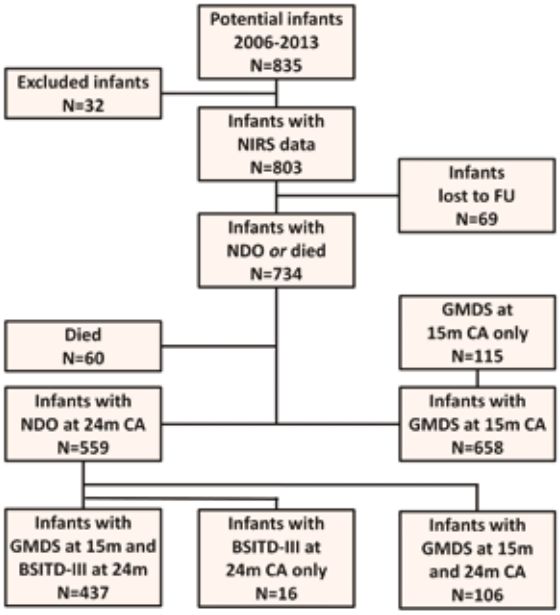
The primary aim of this study was to investigate whether or not absolute thresholds of cerebral oxygenation, quantifying both hypo- and hyperoxia during the first 72h after birth, are associated with an adverse neurodevelopmental outcome (NDO) at 15 and 24 months corrected age (CA). The secondary aim was to assess if infants with an adverse outcome had a different pattern of cerebral oxygenation during these 72h. We hypothesized that both hypo- and hyper oxygenation of the brain are associated with adverse outcome.

METHODS

PATIENTS

This study is part of an ongoing prospective observational cohort study which aims to record physiological parameters during the first 72h after birth in all infants born with a gestational age (GA) <32 weeks who are subsequently admitted to the level three NICU of the Wilhelmina Children's Hospital, Utrecht, The Netherlands. The ethics committee of the University Medical Center Utrecht, which adheres to the "Medical Research Involving Human Subjects Act (WMO)", approved the current study (protocol 14-335/C). Data collection was attempted in 835 infants between April 2006 and April 2013. **Figure 7.1** displays the flowchart of the included patients. A total of 32 patients were excluded because of various reasons: chromosomal abnormalities, congenital abnormalities, or data corruption.

→ **Figure 7.1**
Flow-chart of included Patients



DATA COLLECTION

The methods of data collection is reported in more detail elsewhere.[11] Obstetrical, intrapartum and neonatal data were collected from the hospital records. Diagnosed PIVHs were graded according to the classification of Papile et al.[12] The presence of a haemodynamically significant patent ductus arteriosus (*hs*PDA) was defined as a PDA confirmed to be haemodynamically significant on cardiac ultrasound and either treated with indomethacin or surgically closed.[13] The birth weight (BW) z-score was

based on recently published Dutch reference curves.[14] Based on the infants' zip code data on the socioeconomic status (SES) of the family was obtained from the Central Bureau of Statistics (CBS, The Hague, The Netherlands). The SES is expressed as a z-score that combines information on the parents' highest educational degree, total household income, and profession into a single score.

Standard physiological parameters were monitored by using a patient monitor (IntelliVue MP70, Philips Healthcare, Best, The Netherlands): SaO_2 by pulse-oximetry, arterial blood pressure by means of an indwelling catheter (e.g umbilical, radial or tibial artery), and heart rate by using gel electrodes. $rScO_2$ was monitored by using a two wavelength (i.e. 730 and 810 nm) NIRS monitor (INVOS 4100 or 5100c, Covidien, Mansfield, MA) in combination with a small adult sensor (SomaSensor SAFB-SM, Covidien, Mansfield, MA). While neonatal sensors are more commonly used due to their more elegant form factor, we prefer to use the adult sensor (i.e. algorithm) because it enables monitoring variability in $rScO_2$ at higher levels (i.e. >85%). Results can be converted to neonatal values with relative ease by adding ~10% to the values reported here.[11,15] For example, a threshold of <55% in this study would translate into <65% when using either neonatal or paediatric sensors. An elastic bandage was used for sensor fixation. Until June 2012 data was recorded with Poly 5 (Inspector Research Systems, Amsterdam, The Netherlands) at a sample rate of 1 Hz using an INVOS 4100 monitor. Thereafter, in-house developed software (BedBase, University Medical Center Utrecht, Utrecht, The Netherlands) was used to record data from the patient monitor and an INVOS 5100c NIRS monitor at a sample rate of 0.4 Hz.

DATA PROCESSING

Data was analysed with the off-line version of the BedBase software (SignalBase, University Medical Center Utrecht, The Netherlands). Before analysis, artefacts were removed manually. For the $rScO_2$, artefacts were defined as: changes in a particular parameter that could not be physiologically explained (e.g. a 30% step change between two subsequent data points), or changes that were accompanied by severe distortion in the other parameters suggesting infant movement or handling. Thereafter, 1h-periods were selected during the first 72h after birth and counted in reference to a patient's birth date and time (i.e. postnatal age, [PA]). Periods with short drops in SaO_2 (i.e. <85% for <10s) were not included in the analysis. In case of SaO_2 drops where additional O_2 was given to assist recovery, the duration of the associated increase in SaO_2 and $rScO_2$ over baseline conditions was also excluded from analysis.[16] Before statistical analysis, 1h-periods containing less than 10-min of data were rejected.

NEURODEVELOPMENTAL OUTCOME

Assessment of NDO was performed by certified investigators at 15 (DQ15) and 24 (NDO24) months CA. At 15 months CA the assessment was performed by using the Griffiths Mental Development Scales (GMDS). The GMDS calculates a total developmental quotient (DQ) based on 5 subscales: locomotion, personal-social, hearing-speech, eye-hand coordination, and performance.[17] The assessment at 24 months CA as performed using the GMDS or the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III). At 24 months CA, the neonatal follow-up program of the Wilhelmina Children's Hospital aims to perform the Bayley-III in all neonates with a GA <30 weeks or GA ≥30 weeks with a BW <1000 grams. Otherwise, in infants with a normal BW, the GMDS was used. From the Bayley-III, only the cognitive and motor scales were assessed.

STATISTICAL ANALYSIS

For cognitive outcome at 24m CA, the Bayley-III cognitive composite scores using the Dutch normative values that are available since October 2014 [mean 100, SD 15] and GMDS DQ [mean 100, SD 12], excluding the locomotion subscale, were combined.[17–20] For gross motor outcome at 24m CA, the Bayley-III gross motor score [mean 10, SD 3] and GMDS locomotion subscale [mean 100, SD 16] were combined.[17–20] In all analyses, composite adverse outcomes were defined as a NDO score <-1SD *or* death. The number of deceased infants that were included in the composite adverse outcome depended on the age at death, which was explored using four cut-offs: ≤7 days after birth, ≤14d, ≤28d, and death during hospital stay.

Four approaches were used to study the $rScO_2$ in relation to outcome: 1) The course of the $rScO_2$ over the first 72h after birth was compared between infants with a favourable and an adverse outcome, 2) the $rScO_2$ was studied in relation to multiple fixed thresholds (i.e. range 30-90% with 5% steps), 3) the average $rScO_2$ per 1h-period was classified into quartiles, and 4) according to thresholds based on recently published reference values (i.e. range -2SD to +2SD with 0.5SD steps).[11]

Ad 1. First, PA and the $rScO_2$ were modelled on an individual basis using a squared design.[11] The coefficients (i.e. intercept, PA, and the square of PA), model significance and r-squared value were extracted per individual subject. These coefficients were then compared between infants with/without composite adverse outcome by using an independent two-sample student's *t*-test.

Ad 2. The $rScO_2$ was expressed in relation to fixed thresholds by using the four definitions available in SignalBase (University Medical Center Utrecht, The Netherlands): i) time in sec. below/above a threshold, ii) area under/above a threshold (i.e. [deviation from threshold in %]*[duration in sec.]), iii) time expressed as % below/above a threshold

(i.e. [time in sec.]*100/[total available data in sec. during the selected period]), and iv) area expressed as % below/above a threshold (i.e. [area below/above threshold]*100/[total area below/above the curve during the selected period]). In addition, time and area outside 55-85% was determined.[3,4]

Ad. 3. The average $rScO_2$ per 1h-period was classified into quartiles based on the quartile boundaries per 1h-period. In every individual the number of 1h-periods fulfilling a certain classification was divided by the total number of periods with data available in that individual. For example, 12 hours in the lowest quartile with a total of 66 available periods during 72 hours = 12/66 = 18.1% of time the $rScO_2$ was in the lowest quartile during the first 72h after birth.

Ad 4. The average $rScO_2$ per 1h period was classified as being either above or below SD-thresholds based on GA, PA, and gender.[11] Subsequently, the number of periods was expressed as a percentage of the total number of periods.

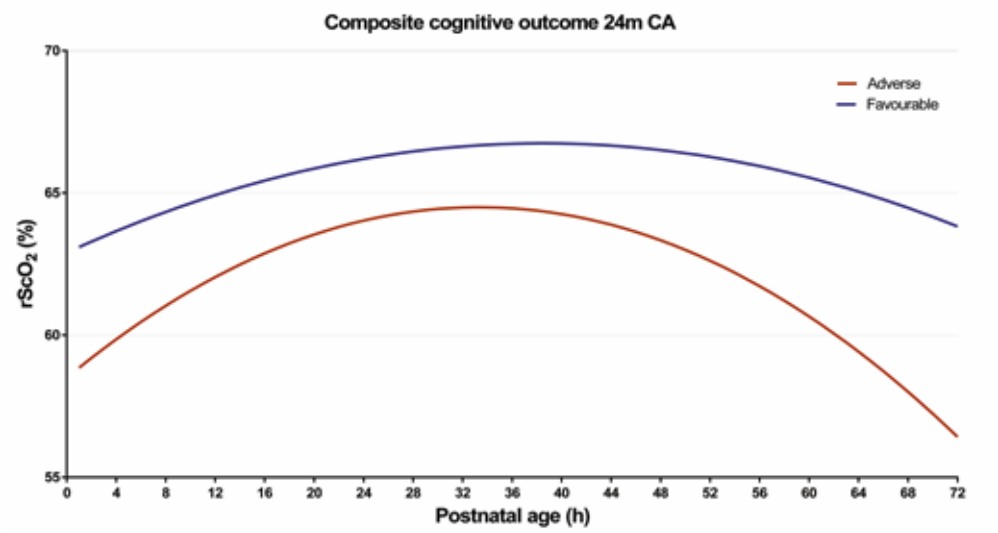
To determine which of the abovementioned thresholds had the strongest association with composite outcome, a stepwise logistic regression analysis was performed. All statistical analyses were performed in R for Windows 64-bit, version 3.1.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Unless specified otherwise, data are presented as mean ± SD for parametric data, median with interquartile range (IQR) for non-parametric data, counts (%) for categorical data, and odds with 95% confidence interval (CI) for logistic regression analysis. A p-value <0.01 was considered statistically significant in case of multiple comparisons. For logistic regression analysis, variables were kept in the model at $p<0.05$.

RESULTS

Data from 734 infants was available for analysis. **Table 7.1** shows the baseline clinical characteristics and follow-up parameters of the population. None of the reported clinical characteristics were significantly different between the group of infants included in this study and the infants who were lost to follow-up.

THE COURSE OF THE $RSCO_2$ OVER THE FIRST 72H OF LIFE

In **figure 7.2**, curves are plotted for infants with and without an adverse composite cognitive outcome at 24m CA. The average coefficients for the different composite outcomes are listed in **table A7.1** (appendix). In all subsequent analysis, a cut-off age at death ≤14d, as compared to ≤7d, ≤28d, and death during hospital stay, yielded the largest differences between favourable and adverse outcome groups.

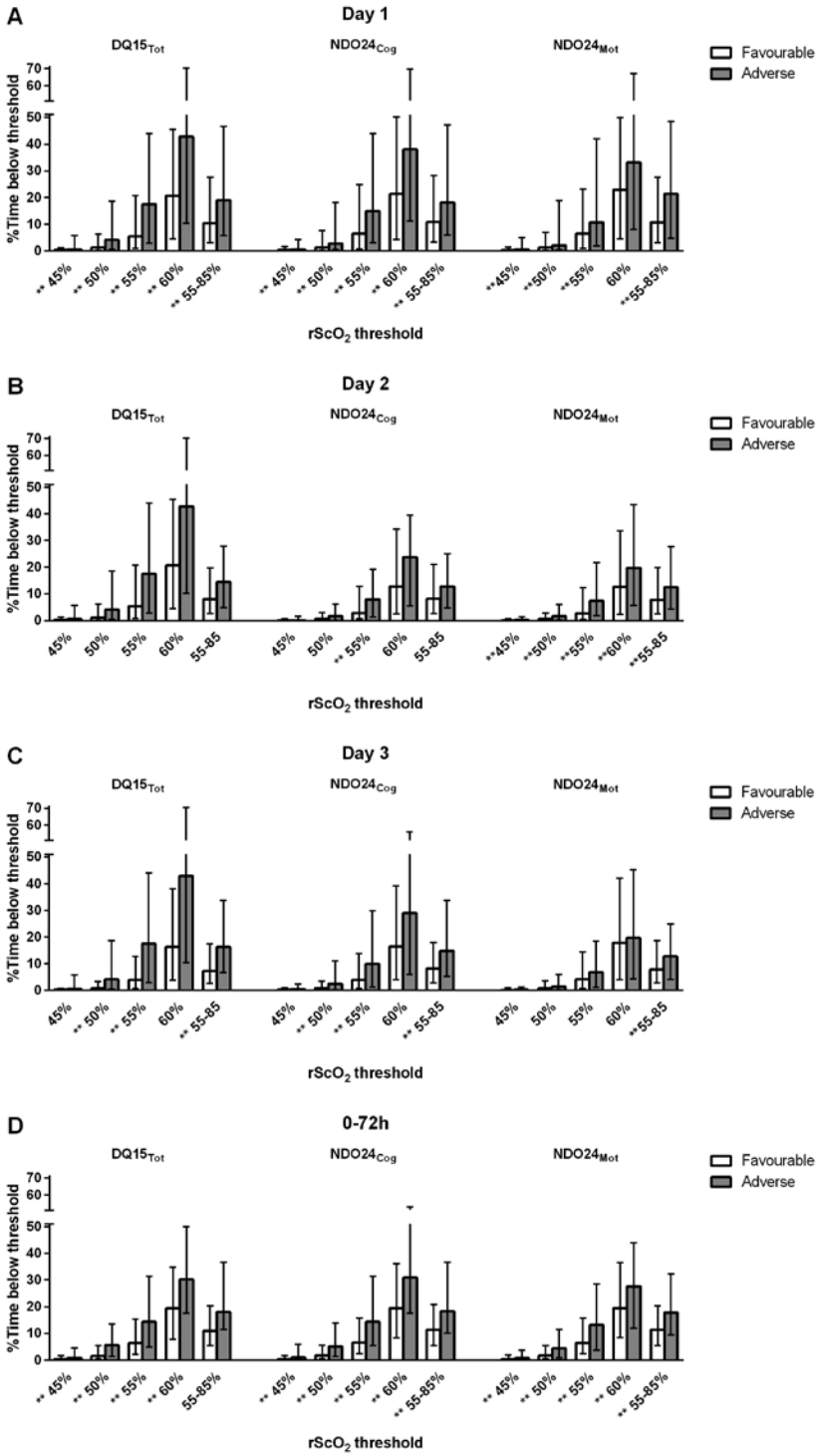


↑ **Figure 7.2**
Course of the $rScO_2$ over the first 3 days of life for infants with and without composite adverse cognitive outcome at 24 months CA.

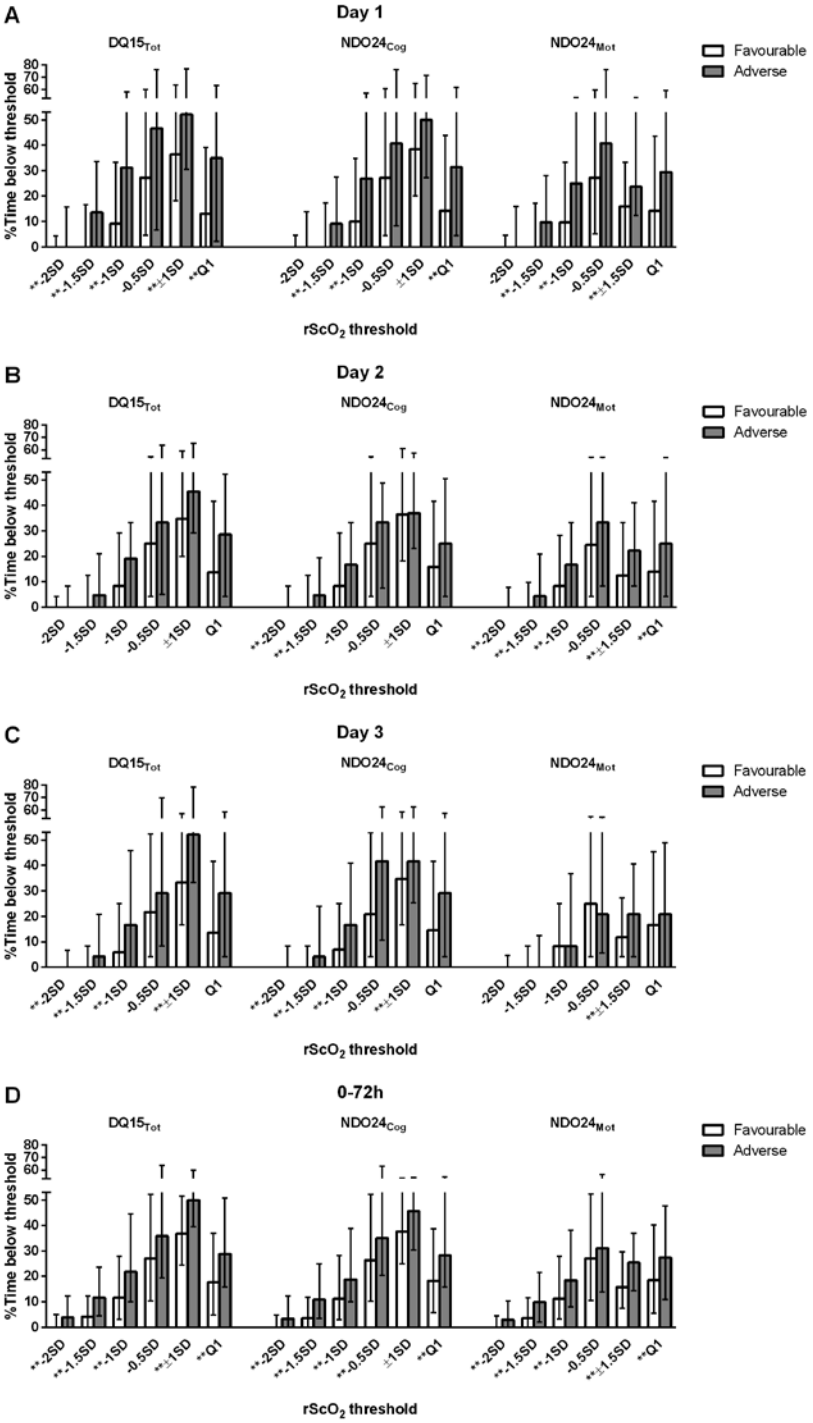
LOW VALUES OF $RSCO_2$: 'HYPOXIA'

For the fixed thresholds quantifying low $rScO_2$, thresholds between 45-60% proved to be significantly different between infants with and without a composite adverse outcome, both at 15m and 24m CA. In **table A7.2** (appendix), results are presented for these fixed thresholds, for all three composite outcomes, and for the four ways of quantifying the thresholds (i.e. time in s, area, time in % and area in %). The percentage of time spent below a threshold had the most significant association with outcome (**figure 7.3**). Logistic regression analysis demonstrated that an $rScO_2$ <55% had the strongest association with outcome, both at 15 and 24m CA (**table 7.2**).

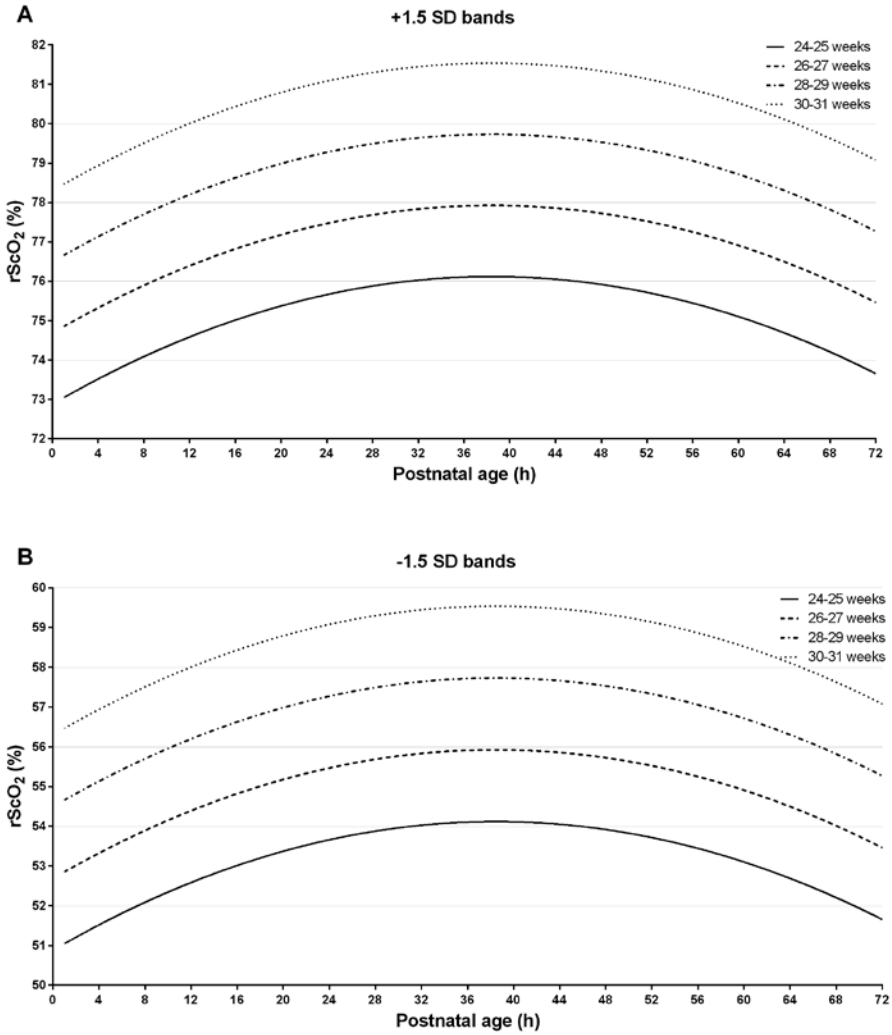
In **table A7.3** results are presented for all three composite outcomes when classifying $rScO_2$ according to published reference values, and when classifying the $rScO_2$ into quartiles. **Figure 7.3** displays the results for time spent below the SD-bands, and time spent in the 1st quartile. Logistic regression analysis demonstrated that time spent with a $rScO_2$ below -1SD to -1.5SD had the strongest association with outcome (**table 7.2**). Time below SD-bands on day 2 did not remain significant in the logistic regression analysis with composite motor outcome at 24m CA.



← **Figure 7.3**
Time in % spent below (i.e. <45%, <50%, <55%, and <60%) or outside (i.e. <55 *or* >85%) fixed thresholds for day 1, 2, 3, and 0-72h after birth. ** p< 0.01



← **Figure 7.4**
Time in % spent below (i.e. <-2SD, <-1.5SD, <-1SD, and <-0.5SD) or outside (e.g. <-1.5SD *or* >1.5SD) thresholds according to reference values, and in the lowest quartile (i.e. Q1) for day 1, 2, 3, and 0-72h after birth. ** p< 0.01



← **Figure 7.5**
The A) +1.5SD and B) -1.5SD band for the $rScO_2$ during the first 3 days after birth for four gestational age groups.

HIGH VALUES OF $RSCO_2$: 'HYPEROXIA'

Although a trend was observed in infants with an adverse outcome spending more time (~2% more) above the various SD-bands, none of the investigated thresholds quantifying high levels of oxygenation (i.e. absolute thresholds 70-90%, 3rd and 4th quartiles, or time spend >0.5 to 2.0SD) reached statistical significance.

TIME SPENT OUTSIDE 'REFERENCE RANGE'

In general, infants with an adverse outcome spent significantly more time outside 55-85% on days 1 and 3 (**figure 7.3**). In the logistic regression analysis, % time below 55% always came out on top of % time outside 55-85%, except for day 2 in relation to outcome at 15m CA (**table 7.2**).

Of the time spent outside reference values, % of time outside ±1SD and outside ±1.5SD had the strongest association with outcome at 15m and 24m CA, respectively (**table 7.2** and **figure 7.4**). **Table A7.3** (appendix) provides medians and IQRs for all investigated SD-bands and composite outcomes.

OVERALL BEST DISCRIMINATING THRESHOLD AND MULTIVARIABLE LOGISTIC REGRESSION

To sort out which thresholds had the strongest association with outcome, a stepwise logistic regression procedure was followed. This was done while always adjusting for GA, as the fixed thresholds and quartiles are not adjusted for GA effects, while the SD-bands are. Overall, either <-1SD or outside ±1SD demonstrated the strongest association with outcome at 15m CA, and <-1.5SD or outside ±1.5SD had the strongest association with outcome at 24m CA (**table 7.2**). **Figure 7.4** shows the ±1.5SD $rScO_2$ bands during the first 3 days after birth for gestational ages between 24-32 weeks, per 2 weeks of gestation.

Multivariable logistic regression was used to assess whether $rScO_2$ contributed to a composite adverse outcome independently, which was performed for the $rScO_2$ on day 1, and for all available $rScO_2$ variables over the first 3 days after birth (**table 7.3**).

DISCUSSION

Cerebral oxygenation measured by NIRS during the first three days after birth is related to NDO. The largest effect sizes were found on day 1 and day 3, with slightly larger effect sizes on day 3. The strength of this study lies in the size of the study population, and the fact that $rScO_2$ was measured continuously over the first 72h.

Values below 50% have previously been reported to be associated with NDO.[5,6] Interestingly, both studies used different sensors (i.e. adult vs. paediatric sensors). Considering the offset, the threshold used by Verhagen et al. would translate into the adult sensor equivalent ~40%, which could explain the absence of a relation between this threshold and cognitive outcome in their dataset, as the incidence of values <40% is very low.[11] This is confirmed by their observation that the duration of $rScO_2$ <50% on day 1 was only 1 minute.[6]

Contra-intuitively, when analysing the fixed thresholds, in virtually all cases the % of time and not the area under the curve had the strongest association with outcome. This suggests, when selecting an appropriate threshold, that the absolute deviation from that threshold is of less importance than the actual duration of the period of low oxygenation and thereby points to an 'all-or-nothing' threshold below which an adverse effect occurs.

Although we could not confirm our hypothesis that hyper oxygenation was independently associated with outcome, time spent outside "reference values" was often more strongly associated with outcome than just time spent below a threshold. This would suggest that hyperoxia does indeed have adverse effects on the preterm brain, as suggested before.[8,21] For sustained global hyperoxia, the mechanism would be oxidative stress in absence of adequate anti-oxidative countermeasures, which is particularly harmful for pre-oligodendrocytes.[2,9,22] In addition, the pathophysiology of PIVH has been linked to fluctuations in cerebral oxygenation and blood flow, including high rScO2.[7,13] Intriguingly, associations with gross motor outcome at 24m CA was most significant for time spent outside ±1.5SD, as opposed to time spent below -1.5SD for cognitive outcome. This suggests that hyperoxia is important as far as motor outcome is concerned. Although speculative, this could be explained by the specific sensitivity to hyperoxia of the cerebellum.[23]

The use of the Bayley-III can be a limitation, as it is known that outcome scores have an upwards bias as compared to version II of the Bayley.[24,25] Using the normative values of the Bayley-III-NL at 24m CA seems to generate different outcomes compared to the original Bayley-III.[19] Moreover, associations with outcome at 15m CA were similar, which strengthens confidence in the reported results. It would be very interesting to perform the analysis in relation to outcome at school age. Unfortunately these data are not yet available for this population. Another potential limitation is the dichotomisation of outcome scores. This was done because we did not want to exclude infants who passed away. Moreover, it allowed the simultaneous analysis of infants with a GA above and below 30 weeks (i.e. 30-32 weeks were assessed with the GMDS at 24m CA).

The question remains whether we can actually improve outcome by intervening postnatally, or that we merely record 'events' that took place before birth. Recent results suggest that the burden of hypoxia and hyperoxia, defined as <55% and >85% based on a cohort of infants from our unit, can be influenced by using NIRS.[3] The paucity of significant results on day 2, on which $rScO_2$ was generally higher, and 'return' of significance on day 3 also suggest this is not a static situation and thus there is still room for improvement. For daily clinical care, we prefer the use the SD-based thresholds (**figure 7.5**), as these are adjusted for the association that GA, PA, and gender have with $rScO_2$. The $rScO_2$ increases with GA and the use of a single fixed threshold would therefore automatically associate $rScO_2$ with outcome if not corrected for GA, as low GA is also associated with adverse outcome.[26,27] This is supported by the observation that SD-based thresholds came out on top in a direct comparison with fixed thresholds, but with GA in the model. Nevertheless, avoiding values <55%, irrespective of GA, can be a good rule of thumb when $rScO_2$ reference charts are not within reach, as the average -1.5SD $rScO_2$ on day 1 and 3 (i.e. at 12h and 60h PA) comes down to 55-56%. Upon identification of low $rScO_2$, a check of the measurement-setup

to rule-out measurement errors should always be the first step. Thereafter, a routine evaluation of clinical parameters (e.g. haemoglobin levels, cardiac ultrasound for a *hs*PDA, ventilator settings, cranial ultrasound) is probably the best approach.[4]

CONCLUSION

To assess whether the $rScO_2$ was related to neuromotor outcome, a sweep was made through multiple fixed thresholds, and multiple thresholds varying according to GA

↓ **Table 7.1**
Clinical characteristics

	Total N=734	**24-25 weeks** N=90
Clinical parameters		
Sex (male/female)	399/335	51/39
Gestational age (weeks, mean [SD])	28.5 [2.0]	25.1 [0.6]
Birth weight (grams, mean [SD])	1130 [331]	771 [112]
Birth weight z-score (mean, [SD])	0.03 [0.93]	0.42 [0.86]
Mechanical ventilation (day 1/2/3, %)	367 (50%) / 374 (51%) / 390 (53%)	74 (82%) / 73 (81%) / 72 (80%)
Apgar score 1 min (med, [IQR])	7 [5-8]	5 [3-6]
Apgar score 5 min (med, [IQR])	8 [7-9]	7 [6-8]
aCCS full course (n, %)	557 (76 %)	69 (76.7%)
Patent ductus arteriosus (n, %)		
During admission	230 (31.1%)	64 (71.1%)
<84h	170 (23.2%)	45 (50.9%)
Culture proven sepsis (n, %)		
<48h	16 (2%)	5 (5.6%)
>48h	177 (24%)	33 (36.7%)
IVH (n, %)	223 (31.2%)	45 (50.0%)
Grade I/II	152 (20.6%)	29 (32.4%)
Grade III/IV	71 (10.6%)	16 (17.8%)
CRIB II score (med, [IQR])	9 [6-11]	8 [6-12]
Follow-up parameters		
15m corrected for prematurity	N=658	N=64
Corrected age (months, med [IQR])	15.8 [15.3-17.4]	15.9 [15.4-16.6]
GMDS DQ (mean, [SD])	102.2 [9.3]	100.2 [11.2]
24m corrected for prematurity	N=559	N=61
Corrected age (months, mean [SD])	24.7 [2.4]	24.3 [0.6]
GMDS	N=106	N=0
GMDS DQ (mean, [SD])	97.0 [9.3]	n/a
GMDS DQ excl. locomotor	97.1 [9.4]	n/a
GMDS DQ locomotor only	96.4 [11.1]	n/a
BSITD-III-NL	N=453	N=61
BSITD-III-NL cognition (mean, [SD])	102.2 [14.0]	99.5 [16.1]
BSITD-III-NL total motor (mean, [SD])	108.4 [12.1]	105.4 [12.7]
BSITD-III-NL gross motor (med, [IQR])	10 [9-12]	10 [8-12]
SES z-score (mean, [SD])	-0.09 [1.06]	-0.17 [1.04]
Died (n, %)	60 (8.2%)	24 (26.7%)
<7d (n, %)	21 (2.9%)	4 (4.4%)
<14d (n, %)	34 (4.6%)	10 (11.1%)
<28d (n, %)	45 (6.1%)	18 (20.0%)

aCCS: antenatal corticosteroids; BSITD: Bailey Scales of Infant and Toddler Development; CRIB: Clinical Risk Index for

and PA. A $rScO_2$ <-1.5SD and outside ±1.5SD based on GA and PA had the strongest association with cognitive and motor outcome at 24 months CA, respectively. Although the strongest associations were found beyond the first day after birth, the $rScO_2$ on day 1 was already independently associated with cognitive and motor outcomes. The $rScO_2$ thresholds reported here are slightly higher than previously reported, suggesting that more infants are at risk than previously thought. The next step is to study whether interventions targeted to maintain $rScO_2$ within the ±1.5SD range could help to improve outcome.

26-27 weeks N=186	**28-29 weeks** N=231	**30-31 weeks** N=227
100/86	124/107	124/103
27.0 [0.6]	28.9 [0.56]	30.7 [0.55]
925 [182]	1194 [241]	1374 [339]
0.01 [0.86]	0.15 [0.89]	-0.23 [0.97]
130 (70%) / 127 (68%) / 119 (64%)	101 (44%) / 101 (44%) / 96 (42%)	62 (27%) / 59 (26%) / 55 (24%)
6 [4-8]	7 [5-8]	7 [6-8]
8 [6-9]	8 [8-9]	9 [8-9]
136 (73.1%)	180 (77.9%)	272 (75.8%)
87 (46.8%)	56 (24.2%)	23 (10.1%)
68 (36.6%)	40 (17.3%)	17 (7.5%)
4 (2.2%)	4 (1.7%)	3 (1.3%)
56 (30.1%)	47 (20.3%)	41 (18.1%)
63 (33.9%)	64 (27.7%)	51 (22.5%)
32 (17.2%)	52 (22.5%)	39 (17.2%)
31 (16.7%)	12 (5.2%)	12 (5.3%)
9 [7-12]	9 [7-11]	8 [6-11]
N=155	N=217	N=222
15.7 [15.2-16.5]	15.6 [15.2-16.6]	16.8 [15.4-18.5]
102.1 [9.0]	103.7 [8.7]	101.7 [9.2]
N=160	N=202	N=136
24.1 [0.5]	24.2 [1.3]	24.9 [2.5]
N=0	N=21	N=85
n/a	99.3 [6.7]	96.8 [8.7]
n/a	99.7 [7.3]	97.0 [8.8]
n/a	99.4 [9.9]	96.1 [10.3]
N=160	N=181	N=51
102.1 [13.4]	104.1 [13.6]	99.6 [13.8]
107.7 [12.6]	110.5 [11.0]	107.3 [12.0]
10 [9-12]	10 [9-12]	10 [8-11]
0.04 [1.10]	-0.08 [1.02]	-0.19 [1.07]
22 (11.8%)	10 (4.3%)	4 (1.8%)
10 (5.4%)	5 (2.2%)	2 (0.9%)
16 (8.6%)	6 (2.6%)	2 (0.9%)
18 (9.7%)	7 (3.0%)	2 (0.9%)

Babies; GMDS: Griffiths Mental Development Scales; SES: socioeconomic status

↓ **Table 7.2**
Results from logistic regression analysis: below, outside, and overall best threshold

	Odds Ratio for composite outcome					
	GMDS total DQ 15m CA		Cognitive outcome at 24m CA		Gross motor outcome at 24m CA	
Time spend below, in %						
Below fixed thresholds (not adjusted for gestational age)						
Day 1	<55%	1.018 [1.009-1.027]	<55%	1.014 [1.004-1.023]	<55%	1.014 [1.005-1.033]
Day 2	ns	ns	ns	ns	<55%	1.015 [1.003-1.027]
Day 3	<55%	1.018 [1.007-1.030]	<55%	1.019 [1.009-1.029]	<55%	1.011 [1.002-1.021]
0-72h	<55%	1.026 [1.013-1.040]	<55%	1.024 [1.011-1.037]	<55%	1.022 [1.010-1.034]
Below SD-based thresholds						
Day 1	<-1SD	1.014 [1.006-1.022]	<-1SD	1.010 [1.003-1.018]	<-1.5SD	1.013 [1.005-1.022]
Day 2	ns	ns	<-1.5SD	1.013 [1.002-1.023]	ns	ns
Day 3	<-1SD	1.014 [1.005-1.023]	<-1.5SD	1.018 [1.008-1.028]	<-1.5SD	1.010 [1.000-1.021]
0-72h	<-1SD	1.017 [1.007-1.028]	<-1.5SD	1.029 [1.013-1.044]	<-1.5SD	1.021 [1.009-1.034]
Time spend outside, in %						
Outside 55-85% (not adjusted for gestational age)						
Day 1	55-85%	1.016 [1.007-1.026]	55-85%	1.013 [1.004-1.022]	55-85%	1.016 [1.008-1.025]
Day 2	55-85%	1.015 [1.002-1.029]	ns	ns	55-85%	1.013 [1.001-1.024]
Day 3	55-85%	1.023 [1.012-1.034]	55-85%	1.020 [1.009-1.030]	55-85%	1.015 [1.005-1.024]
0-72h	55-85%	1.030 [1.016-1.044]	55-85%	1.024 [1.011-1.037]	55-85%	1.025 [1.013-1.038]
Outside SD-based thresholds						
Day 1	±1SD	1.012 [1.004-1.024]	ns	ns	±1.5SD	1.012 [1.005-1.020]
Day 2	ns	ns	ns	ns	±1.5SD	1.010 [1.000-1.018]
Day 3	±1SD	1.020 [1.011-1.030]	±1.5SD	1.012 [1.003-1.021]	±1.5SD	1.012 [1.004-1.020]
0-72h	±1SD	1.022 [1.011-1.034]	ns	ns	±1.5SD	1.020 [1.009-1.030]
Overall best discriminating threshold						
Day 1	<-1SD	1.013 [1.005-1.021]	<-1SD	1.009 [1.002-1.017]	±1.5SD	1.012 [1.005-1.020]
Day 2	55-85	1.015 [1.001-1.029]	<-1.5SD	1.013 [1.002-1.023]	±1.5SD	1.010 [1.000-1.018]
Day 3	±1SD	1.020 [1.011-1.030]	<-1.5SD	1.018 [1.008-1.028]	±1.5SD	1.012 [1.004-1.020]
0-72h	±1SD	1.022 [1.011-1.034]	<-1.5SD	1.029 [1.013-1.044]	±1.5SD	1.020 [1.009-1.030]
In all cases the OR relates to 1% of time of a specified period (i.e. one day, or 72h), ns: not significant in logistic regression analysis						

↓ **Table 7.3**
Final models of multi-variable logistic regression analysis on composite outcomes

$rScO_2$ on day 1					
Total DQ at 15m CA <-1SD *or* death ≤14d					
	B	S.E.	p-value	Odds ratio	95% C.I.
$rScO_2$ time (%) < -1SD d1[a]	0.010	.005	.036	1.010	1.001 - 1.019
Birth weight z-score	-0.404	.169	.017	0.668	0.479 - 0.931
Grade III/IV IVH	1.259	.369	.001	3.523	1.709 - 7.264
Mechanical ventilation day 1	1.225	.334	.000	3.406	1.770 - 6.553
Apgar score 5 min	-0.252	.075	.001	0.777	0.671 - 0.900
Male sex	0.994	.336	.003	2.703	1.398 - 5.226
Cognitive outcome at 24m CA <-1SD *or* death ≤14d					
$rScO_2$ time (%) < -1SD d1[a]	0.008	0.004	0.044	1.008	1.000 - 1.016
Birth weight z-score	-0.334	0.138	0.015	0.716	0.547 - 0.937
Grade III/IV IVH	1.314	0.324	<0.001	3.723	1.974 – 7.023
Mechanical ventilation day 1	0.832	0.267	0.002	2.299	1.361 - 3.883
SES z-score	0.239	0.113	0.034	1.270	1.018 - 1.584
Gross motor outcome at 24m CA <-1SD *or* death ≤14d					
$rScO_2$ time (%) < -1.5SD d1[a]	0.008	0.004	0.05	1.008	1.000 – 1.016
Birth weight z-score	-0.514	0.133	<0.001	0.598	0.461 – 0.776
Grade III/IV IVH	1.674	0.329	<0.001	5.335	2.798 – 10.172
*hs*PDA <84h	0.842	0.252	0.001	2.322	1.418 – 3.803
Gestational age (wks)	-0.186	0.070	0.008	0.830	0.724 – 0.952
$rScO_2$ 0-72h					
Total DQ at 15m CA <-1SD *or* death ≤14d					
	B	S.E.	p-value	Odds ratio	95% C.I.
$rScO_2$ time (%) < -1SD d1[a]	0.009	0.005	0.05	1.009	1.000 - 1.019
$rScO_2$ time (%) outside ±1SD d3[a]	0.022	0.006	<0.001	1.022	1.011 – 1.033
Birth weight z-score	-0.503	0.182	0.006	0.605	0.423 - 0.865
Grade III/IV IVH	1.572	0.402	0.000	4.818	2.192 - 10.590
Mechanical ventilation day 1	1.216	0.364	0.001	3.373	1.653 - 6.882
Apgar score 5 min	-0.176	0.083	0.034	0.839	0.713 - 0.987
Male sex	1.120	0.357	0.002	3.065	1.521 – 6.176
Cognitive outcome at 24m CA <-1SD *or* death ≤14d					
$rScO_2$ time (%) < -1.5SD d3[a]	0.013	0.006	0.025	1.013	1.002 - 1.024
Birth weight z-score	-0.365	0.148	0.014	0.694	0.519 - 0.929
Grade III/IV IVH	1.546	0.344	<0.001	4.693	2.393 – 9.201
*hs*PDA <84h	0.624	0.283	0.028	1.866	1.071 – 3.251
Gestational age (wks)	-0.166	0.078	0.033	0.847	0.727 – 0.987
Gross motor outcome at 24m CA <-1SD *or* death ≤14d					
$rScO_2$ time (%) outside ±1.5SD 0-72h[a]	0.013	0.006	0.027	1.013	1.001 – 1.025
Birth weight z-score	-0.581	0.141	<0.001	0.560	0.424 – 0.738
Grade III/IV IVH	1.546	0.343	<0.001	4.691	2.394 – 9.191
Apgar score 5 min	-0.152	0.073	0.037	0.859	0.745 – 0.991
*hs*PDA <84h	0.822	0.261	0.002	2.276	1.364 – 3.798
Gestational age (wks)	-0.170	0.074	0.021	0.843	0.730 – 0.975

[a]The OR relates to 1% of time of a specified period (e.g. 10% and a OR per % of 1.013 would yield a total OR of 1.13)

APPENDIX

↓ Table A7.1
Difference in model coefficients using squared model between postnatal age and $rScO_2$

	Favourable		Adverse		Favourable		
	Intercept	IQR	Intercept	IQR	PA(h)	IQR	PA(h)
Composite outcome died ≤7d							
$DQ15_{tot}$	63.0*	55.3 - 71.5	58.9*	46.4 - 69.7	0.20*	-0.21 - 0.64	0.42*
$NDO24_{cog}$	62.9**	55.2 - 71.7	58.5**	47.8 - 69.3	0.20*	-0.21 - 0.63	0.36*
$NDO24_{mot}$	62.3	54.2 - 71.3	62.8	48.4 - 71.7	0.22	-0.18 - 0.67	0.30
Composite outcome died ≤14d							
$DQ15_{tot}$	62.9*	53.0 - 71.8	59.0*	47.2 - 69.2	0.27	-0.18 - 0.78	0.31
$NDO24_{cog}$	62.9**	55.2 - 71.7	58.8**	48.2 - 69.2	0.20*	-0.21 - 0.63	0.29*
$NDO24_{mot}$	62.6	54.7 - 70.8	61.6	51.2 – 70.4	0.22	-0.15 - 0.67	0.21

$DQ15_{tot}$: Total developmental quotient at 15 months corrected age; IQR: inter-quartile range; NDO24: Difference between favourable and adverse outcome group: *p<0.05, ** p<0.01

↓ Table A7.2
Median + IQR for $rScO_2$ under fixed thresholds for day 1, 2, 3, and 0-72h

	Day 1													
	GMDS 15m CA total DQ					Cognitive outcome at 24 months					Gross motor outcome at 24 months				
	favourable		Comp. Adverse died ≤14d			Favourable		Comp. Adverse died ≤14d			Favourable		Comp. Adverse died ≤14d		
Threshold	Med	IQR	Med.	IQR	p	Med.	IQR	Med.	IQR	p	Med.	IQR	Med.	IQR	p
45															
Time (s) / h	7.9	0.3 - 41.3	21.4	1.5 - 179.4	0.006	8.8	0.2 - 47.3	18.7	2.2 - 144.1	0.002	8.7	0.3 - 42.8	17.6	1.5 - 167.8	0.003
Area (au) / h	24.6	0.1 - 181	83.9	1.9 - 539.9	ns	30.0	0 - 200.5	71.3	6.1 - 480	0.007	29.7	0.1 - 191.2	86.2	1.9 - 465.3	0.003
%Time	0.3	0 - 1.3	0.6	0 - 5.8	0.003	0.3	0 - 1.5	0.5	0.1 - 4.3	0.001	0.3	0 - 1.4	0.5	0 - 5	0.001
%Area	0.0	0 - 0.5	0.2	0 - 4.3	0.003	0.1	0 - 0.6	0.2	0 - 3.2	ns	0.1	0 - 0.6	0.2	0 - 3.5	0.002
50															
Time (s) / h	36.8	5.8 - 197.2	147.4	14.9 - 650.6	0.003	40.2	5.4 - 237.3	92.2	15.5 - 626.3	0.003	40.4	5.5 - 196.4	66.2	11.5 - 661.6	0.006
Area (au) / h	146.3	14.1 - 694.1	329.2	30.6 - 2061.3	0.007	168.4	13.5 - 752.5	315.6	36.6 - 2032.9	0.004	155.1	14.8 - 704.8	366.0	30.4 - 2571.4	0.002
%Time	1.2	0.2 - 6.3	4.1	0.5 - 18.6	0.003	1.3	0.2 - 7.7	2.7	0.6 - 18.2	0.004	1.3	0.2 - 6.9	2.0	0.4 - 18.9	0.007
%Area	0.4	0 - 4.8	2.2	0.1 - 18.2	0.003	0.5	0 - 6.1	1.1	0.1 - 17.8	0.006	0.5	0 - 5.3	1.1	0.1 - 18.1	0.004
55															
Time (s) / h	172.3	28.6 - 652	604.7	76.1 - 1465.9	<0.001	182.5	27.9 - 801	469.1	78.6 - 1459.1	0.001	189.7	28.6 - 723.7	371.8	61.6 - 1454.1	0.001
Area (au) / h	624.5	105.4 - 2833.9	1710.8	226.5 - 8128.6	0.001	670.0	98.6 - 3207.7	1378.9	287.9 - 7027.6	0.002	704.6	99 - 3002.9	1425.4	219.8 - 8183.3	0.002
%Time	5.4	0.9 - 20.7	17.5	2.9 - 44	<0.001	6.4	0.8 - 24.9	14.9	3 - 44	<0.001	6.4	0.9 - 23.1	10.7	1.9 - 42	0.002
%Area	3.5	0.4 - 19.3	16.7	1.5 - 43.5	<0.001	4.3	0.4 - 23	13.1	1.8 - 43.2	0.001	4.5	0.4 - 21.8	11.1	1 - 42.3	0.003
60															
Time (s) / h	673.4	149.1 - 1492.7	1302.9	282.3 - 2288.9	0.001	706.4	145.3 - 1624.2	1236.5	339.5 - 2088.4	0.001	738.8	149.4 - 1600.6	1135.8	268.3 - 2156	0.010
Area (au) / h	2517.1	526.7 - 8290.2	8297.8	1205.2 - 17125.4	0.001	2733.9	519.5 - 9379.2	6266.8	1345 - 15967.7	0.001	2789.9	535.6 - 9025.4	5805.3	892.6 - 16168.5	0.004
%Time	20.7	4.5 - 45.5	42.8	10.3 - 70.3	0.001	21.3	4.3 - 50.3	38.0	11.1 - 69.7	0.002	22.9	4.5 - 49.9	33.1	7.9 - 67.3	0.011
%Area	20.1	3.2 - 46.6	41.0	6.5 - 70.4	0.001	21.1	3.1 - 51.6	38.0	10 - 69.9	0.002	21.7	3.3 - 50.8	33.0	5.8 - 67.5	0.016

Adverse	Favourable		Adverse	
IQR	PA(h)^2	IQR	PA(h)^2	IQR
-0.04 - 0.98	-0.0024*	-0.0079 - 0.0025	-0.0049*	-0.0142 - 0.0009
-0.03 - 0.9	-0.0026*	-0.0083 - 0.0024	-0.0054*	-0.0128 - 0.0011
-0.2 - 0.89	-0.0029	-0.0085 - 0.0021	-0.0047	-0.0132 - 0.0020
-0.09 - 0.82	-0.0030	-0.0097 - 0.0025	-0.0044	-0.0119 - 0.0018
-0.09 - 0.86	-0.0026*	-0.0083 - 0.0024	-0.0049*	-0.0103 - 0.0013
-0.21 - 0.56	-0.0029	-0.0084 - 0.0017	-0.0021	-0.0070 - 0.0029
neurodevelopmental outcome at 24 months corrected age; PA: postnatal age;				

	Day 2													
	GMDS 15m CA total DQ					Cognitive outcome at 24 months					Motor outcome at 24 months				
	favourable		Comp. Adverse died ≤14d			Favourable		Comp. Adverse died ≤14d			Favourable		Comp. Adverse died ≤14d		
	Med	IQR	Med.	IQR	p	Med.	IQR	Med.	IQR	P	Med.	IQR	Med.	IQR	p
45															
Time (s) / h	4.9	0.3 - 23.8	7.5	0.9 - 45.3	ns	5.5	0.5 - 22.8	8.0	1 - 50.3	ns	4.9	0.4 - 21.3	10.3	2 - 46.1	0.002
Area (au) / h	13.8	0.4 - 101.6	35.8	1.9 - 197.4	ns	15.7	0.5 - 103.7	36.3	1.7 - 200.7	ns	14.0	0.4 - 101.1	34.2	2.9 - 202.2	0.002
%Time	0.2	0 - 0.8	0.2	0 - 1.4	ns	0.2	0 - 0.7	0.2	0 - 1.6	ns	0.2	0 - 0.7	0.3	0.1 - 1.4	0.002
%Area	0.0	0 - 0.2	0.1	0 - 0.5	ns	0.0	0 - 0.2	0.1	0 - 0.8	ns	0.0	0 - 0.2	0.0	0 - 0.5	0.002
50															
Time (s) / h	18.0	4 - 98.9	38.3	7.4 - 241.5	ns	18.7	4.8 - 97	51.8	6.4 - 219.8	ns	17.4	4.1 - 94.4	54.5	11.1 - 207.7	<0.001
Area (au) / h	81.8	10.5 - 389.1	141.0	17.2 - 733.3	ns	84.2	13.5 - 388.1	153.4	25.4 - 807.5	ns	80.4	11.7 - 358.9	154.6	38.9 - 723.2	<0.001
%Time	0.6	0.1 - 3.1	1.3	0.2 - 6.8	ns	0.6	0.1 - 2.9	1.7	0.2 - 6.3	ns	0.6	0.1 - 2.8	1.7	0.4 - 6	<0.001
%Area	0.2	0 - 2	0.5	0 - 5.8	ns	0.2	0 - 2	0.9	0 - 5.1	ns	0.2	0 - 1.7	0.7	0.1 - 4.3	<0.001
55															
Time (s) / h	98.0	17.6 - 415	212.9	41.2 - 680.7	ns	95.2	19.3 - 423.4	253.4	40.9 - 668.6	0.010	89.0	17.8 - 412.6	247.9	48.2 - 728.1	<0.001
Area (au) / h	353.1	67 - 1604.1	649.8	115.1 - 2761.7	ns	354.3	78.5 - 1553.8	892.9	133.4 - 2868	0.010	339.3	71.1 - 1461.1	846.1	214.7 - 3028.6	<0.001
%Time	3.0	0.5 - 12.9	6.9	1.7 - 19.5	ns	2.9	0.6 - 12.9	8.0	1.3 - 19.1	0.008	2.8	0.5 - 12.3	7.4	1.8 - 21.7	<0.001
%Area	1.5	0.2 - 11.5	4.8	0.3 - 18.9	ns	1.5	0.2 - 11.5	6.6	0.5 - 17.1	0.008	1.4	0.2 - 11.3	5.9	0.7 - 19.9	<0.001
60															
Time (s) / h	426.1	80.1 - 1135.6	705.1	188.6 - 1522.5	ns	440.2	85.7 - 1153.6	746.2	186.2 - 1398.6	ns	428.8	82 - 1136.7	678.4	197.2 - 1517	0.004
Area (au) / h	1627.0	305.9 - 5425.9	2876.6	899.3 - 8048.3	ns	1627.0	341.1 - 5567.8	3619.3	834.2 - 7344.4	0.008	1531.6	316.7 - 5300.8	3089.3	1015.6 - 9424.9	<0.001
%Time	12.8	2.4 - 33.7	23.7	5.7 - 43.9	ns	12.8	2.6 - 34.2	23.9	5.5 - 39.5	ns	12.7	2.4 - 33.7	19.8	5.8 - 43.4	0.004
%Area	11.5	1.3 - 33.8	22.1	4.3 - 39.6	ns	11.6	1.4 - 33.8	21.8	4.2 - 39.6	ns	11.5	1.4 - 33.7	20.1	5.1 - 41.6	0.004

↓ Table A7.2 continued

	Day 3													
	GMDS 15m CA total DQ					Cognitive outcome at 24 months					Motor outcome at 24 months			
	favourable		Comp. Adverse died ≤14d			Favourable		Comp. Adverse died ≤14d			Favourable		Comp. Adverse died ≤14d		
	Med	IQR	Med.	IQR	p	Med.	IQR	Med.	IQR	p	Med.	IQR	Med.	IQR	P
45															
Time (s) / h	4.6	0.3 - 24.8	16.6	0.6 - 54.5	ns	5.1	0.4 - 26.7	14.7	0.3 - 70.4	ns	5.1	0.4 - 27	9.6	0.5 - 42.6	ns
Area (au) / h	12.8	0.2 - 97.9	59.3	0.9 - 234.3	ns	15.3	0.5 - 111.2	36.2	0.4 - 232.6	ns	14.7	0.5 - 114.4	36.0	0.6 - 156.9	ns
%Time	0.2	0 - 0.8	0.5	0 - 1.5	ns	0.2	0 - 0.9	0.5	0 - 2.5	ns	0.2	0 - 0.9	0.3	0 - 1.2	ns
%Area	0.0	0 - 0.2	0.1	0 - 0.9	ns	0.0	0 - 0.3	0.1	0 - 0.9	ns	0.0	0 - 0.3	0.1	0 - 0.4	ns
50															
Time (s) / h	20.8	2.8 - 106.9	100.0	11 - 190.2	0.004	24.1	3.6 - 112.1	62.8	11.1 - 354.5	0.003	25.3	3.9 - 112.5	47.0	5.4 - 188.6	ns
Area (au) / h	79.1	8.2 - 410.9	320.0	25.9 - 887.8	0.007	93.2	12.3 - 435.3	258.7	28.3 - 1138.8	ns	92.4	12.6 - 436.6	184.4	15.5 - 720	ns
%Time	0.7	0.1 - 3.3	2.8	0.3 - 5.8	0.005	0.8	0.1 - 3.5	2.5	0.3 - 11	0.002	0.8	0.1 - 3.6	1.4	0.2 - 5.9	ns
%Area	0.2	0 - 1.9	1.3	0 - 4.3	0.007	0.3	0 - 2.1	0.8	0 - 9	0.009	0.3	0 - 2.1	0.8	0 - 4.4	ns
55															
Time (s) / h	119.1	22.7 - 422.6	328.7	41.5 - 748.8	0.003	133.3	23.6 - 456.3	313.5	43.6 - 983.8	0.002	137.2	24.1 - 483	230.8	31.5 - 644.8	ns
Area (au) / h	401.3	73.3 - 1663.9	1178.8	169.8 - 2712.1	0.003	441.8	80 - 1725.6	1244.6	172 - 4787.8	0.002	465.2	80.4 - 1730.3	931.9	104.6 - 2513.2	ns
%Time	3.9	0.7 - 12.8	10.3	1.1 - 21.5	0.006	4.0	0.7 - 13.7	9.9	1.3 - 29.9	0.003	4.1	0.8 - 14.4	6.8	1 - 18.5	ns
%Area	2.2	0.2 - 11.3	8.6	0.4 - 21.2	0.008	2.8	0.2 - 11.7	8.1	0.3 - 30.6	0.003	2.9	0.2 - 12.1	6.1	0.3 - 18.1	ns
60															
Time (s) / h	518.5	114.1 - 1261	918.0	256.3 - 1637.5	0.006	568.3	125 - 1326	946.3	181.1 - 1831.9	ns	578.4	128.1 - 1395.9	658.2	156.8 - 1582.3	ns
Area (au) / h	1934.0	392.6 - 5874.6	4602.8	746.8 - 8751.6	ns	2053.4	439.9 - 6351.2	4166.8	760.4 - 12337.1	ns	2170.9	470.9 - 6682.9	3120.1	512.6 - 7543.4	ns
%Time	16.3	3.6 - 38.1	25.9	7.7 - 46.1	ns	16.4	3.9 - 39.1	28.9	6 - 56.2	ns	17.9	4 - 42	19.6	4.3 - 45.2	ns
%Area	13.9	2.3 - 38.6	24.9	4.3 - 51	ns	14.7	2.5 - 39.4	25.7	4.7 - 55.6	ns	16.3	2.5 - 41	17.8	2.7 - 47	ns

↓ Table A7.3

Median + IQR for $rScO_2$ under thresholds based on reference values for day 1, 2, 3, and 0-72h.

		GMDS 15m CA total DQ			Cognitive outcome at 24 months			Motor outcome at 24 months		
		Below								
	Threshold	Favourable Med. [IQR]	Adverse Med. [IQR]	p	Favourable Med. [IQR]	Adverse Med. [IQR]	p	Favourable Med. (IQR)	Adverse Med. [IQR]	p
d1	<-2SD	0 [0 - 4.35]	0 [0 - 15.79]	0.001	0 [0 - 4.76]	0 [0 - 13.8]	ns	0 [0 - 4.8]	0 [0 - 16]	ns
	<-1.5SD	0 [0 - 16.67]	13.6 [0 - 33.65]	<0.001	0 [0 - 17.39]	9.1 [0 - 27.63]	0.003	0 [0 - 17.2]	9.8 [0 - 28.1]	0.000
	<-1SD	9.1 [0 - 33.33]	31.3 [0 - 58.58]	0.002	10 [0 - 34.78]	27 [0 - 57.14]	0.005	9.8 [0 - 33.3]	25 [0 - 53.8]	0.007
	<-0.5SD	27.3 [4.8 - 60]	46.7 [6.7 - 76.19]	ns	27.3 [4.5 - 60.87]	40.7 [8.3 - 76.19]	ns	27.3 [5.3 - 59.8]	40.7 [0 - 76.2]	ns
	1st quartile	13 [0 - 39.13]	35 [2.2 - 63.31]	0.001	14.3 [0 - 43.75]	31.4 [4.5 - 61.9]	0.006	14.3 [0 - 43.5]	29.5 [0 - 59.6]	ns
	2nd quartile	22.7 [6.3 - 39.13]	19 [5.7 - 35.68]	ns	21.7 [5.9 - 38.89]	22.7 [5.8 - 39.17]	ns	22.2 [5.6 - 39.1]	20.5 [7.1 - 37.1]	ns
	3rd quartile	23.5 [6.7 - 39.13]	14.3 [0 - 33.33]	ns	23.5 [5.6 - 39.13]	15.4 [0 - 35.07]	ns	23.8 [5.9 - 39.1]	11.8 [0 - 33.8]	0.002
	4th quartile	9.5 [0 - 40]	4.8 [0 - 38.49]	ns	9.1 [0 - 41.67]	0 [0 - 37.16]	ns	8.7 [0 - 40.9]	5.4 [0 - 38.3]	ns
d2	<-2SD	0 [0 - 4.17]	0 [0 - 8.33]	ns	0 [0 - 0]	0 [0 - 8.33]	0.009	0 [0 - 0]	0 [0 - 7.7]	0.008
	<-1.5SD	0 [0 - 12.5]	4.8 [0 - 21.05]	ns	0 [0 - 12.5]	4.7 [0 - 19.49]	0.004	0 [0 - 9.8]	4.3 [0 - 20.8]	0.001
	<-1SD	8.3 [0 - 29.17]	19 [0 - 33.33]	ns	8.3 [0 - 29.17]	16.7 [0 - 33.33]	ns	8.3 [0 - 28.2]	16.7 [0 - 33.3]	0.008
	<-0.5SD	25 [4.2 - 54.55]	33.3 [5 - 63.64]	ns	25 [4.2 - 54.55]	33.3 [7.5 - 48.76]	ns	24.4 [4.2 - 54.2]	33.3 [8.3 - 54.2]	ns
	1st quartile	13.6 [0 - 41.67]	28.6 [4.2 - 52.17]	ns	15.8 [0 - 41.67]	25 [4.2 - 50.54]	ns	14 [0 - 41.7]	25 [4.2 - 53.7]	ns
	2nd quartile	25 [8.7 - 41.67]	25 [8.3 - 37.5]	ns	25 [8.3 - 41.67]	25 [8.9 - 37.65]	ns	25 [8.3 - 41.7]	21.7 [10.3 - 36]	ns
	3rd quartile	21.4 [8.3 - 39.13]	18.2 [7.7 - 33.33]	ns	20.8 [8.3 - 37.5]	20.8 [11.8 - 37.5]	ns	21.1 [8.3 - 40]	20.4 [8.3 - 31.8]	ns
	4th quartile	12.5 [0 - 37.5]	12.5 [0 - 41.67]	ns	12.5 [0 - 37.5]	4.9 [0 - 38.63]	ns	9.5 [0 - 34.9]	12.5 [0 - 41.7]	ns
d3	<-2SD	0 [0 - 0]	0 [0 - 6.67]	0.006	0 [0 - 0]	0 [0 - 8.33]	0.001	0 [0 - 0]	0 [0 - 4.8]	ns
	<-1.5SD	0 [0 - 8.33]	4.2 [0 - 20.83]	0.001	0 [0 - 8.33]	4.2 [0 - 23.96]	0.002	0 [0 - 8.3]	0 [0 - 12.5]	ns
	<-1SD	5.9 [0 - 25]	16.7 [0 - 45.83]	0.002	6.9 [0 - 25]	16.7 [0 - 40.97]	0.009	8.3 [0 - 25]	8.3 [0 - 36.8]	ns
	<-0.5SD	21.7 [4.2 - 52.38]	29.2 [8.3 - 69.57]	ns	20.8 [4.2 - 52.83]	41.7 [10.6 - 62.15]	ns	25 [4.2 - 54.5]	20.8 [5.7 - 54.2]	ns
	1st quartile	13.6 [0 - 41.67]	29.2 [4.2 - 58.33]	ns	14.6 [0 - 41.67]	29.2 [4.2 - 57.29]	ns	16.7 [0 - 45.5]	20.8 [0 - 49]	ns
	2nd quartile	22.2 [8.3 - 41.67]	20.8 [8.3 - 33.33]	ns	22.5 [8.6 - 41.67]	20.8 [8.3 - 33.33]	ns	25 [9.1 - 41.7]	20.8 [5.8 - 33.3]	ns
	3rd quartile	23.1 [7.7 - 39.13]	13 [4.2 - 33.33]	ns	21.2 [4.8 - 37.5]	13 [4.2 - 33.33]	ns	20.8 [4.5 - 37.5]	20.8 [4.5 - 38.5]	ns
	4th quartile	12.5 [0 - 38.89]	16.7 [0 - 45.83]	ns	12.5 [0 - 37.5]	12.5 [0 - 31.16]	ns	12.5 [0 - 36.4]	15.5 [0 - 40.6]	ns
72h	<-2SD	0 [0 - 5.04]	3.8 [0 - 12.28]	<0.001	0 [0 - 4.91]	3.3 [0 - 12.28]	<0.001	0 [0 - 4.5]	3 [0 - 10.4]	0.000
	<-1.5SD	4.2 [0 - 12.31]	11.6 [4.5 - 23.53]	<0.001	3.6 [0 - 11.86]	10.9 [3.4 - 25]	<0.001	3.6 [0 - 11.6]	9.9 [2.1 - 21.5]	0.000
	<-1SD	11.7 [3.1 - 27.99]	21.7 [10.1 - 44.62]	<0.001	11.2 [3 - 28.2]	18.8 [10 - 38.81]	<0.001	11.3 [3.2 - 28]	18.4 [8 - 38.2]	0.001
	<-0.5SD	27.1 [10.3 - 52.13]	35.8 [19.4 - 63.77]	ns	26.4 [10.3 - 52.17]	35 [20.5 - 63.08]	0.008	27.1 [10.5 - 52.3]	31 [13.9 - 56.3]	ns
	1st quartile	17.7 [4.9 - 37]	28.8 [15.7 - 50.75]	0.001	18.2 [5.6 - 38.69]	28.3 [15.7 - 54.41]	<0.001	18.5 [5.6 - 40.2]	27.4 [11 - 47.7]	0.004
	2nd quartile	25.8 [14.9 - 35.21]	23.2 [14.9 - 31.43]	ns	25.4 [14.9 - 34.89]	25.4 [14.9 - 33.33]	ns	26.2 [15.2 - 35.3]	23.7 [14.3 - 31.3]	ns
	3rd quartile	25.2 [13.6 - 35.74]	19.7 [10.8 - 27.69]	0.002	24.2 [13 - 34.85]	21 [10.8 - 29.85]	ns	24.5 [13.1 - 35.2]	20 [10.6 - 31.1]	0.009
	4th quartile	16 [3.6 - 35.01]	16.4 [4.3 - 33.33]	ns	16.4 [3.9 - 34.85]	13.3 [1.6 - 32.79]	ns	16 [3.3 - 33.9]	15.7 [3.2 - 34.7]	Ns

	0-72h													
	GMDS 15m CA total DQ					Cognitive outcome at 24 months					Motor outcome at 24 months				
	favourable		Comp. Adverse died ≤14d			Favourable		Comp. Adverse died ≤14d			Favourable		Comp. Adverse died ≤14d		
	Med	IQR	Med.	IQR	p	Med.	IQR	Med.	IQR	p	Med.	IQR	Med.	IQR	p
45															
Time (s) / h	13.1	2.9 - 51.2	27.83	12.5 - 151.9	<0.001	13.8	3.1 - 54.8	31.2	12 - 182.6	<0.001	13.1	2.8 - 52.7	25.1	8.8 - 116.6	<0.001
Area (au) / h	56.3	7.6 - 182.1	124.64	35.9 - 698.5	<0.001	64.5	8.3 - 198	114.3	32.5 - 698.5	<0.001	60.0	8.3 - 203.3	107.5	32.5 - 449.5	<0.001
%Time	0.4	0.1 - 1.6	0.92	0.4 - 4.6	<0.001	0.4	0.1 - 1.7	1.0	0.4 - 5.8	<0.001	0.4	0.1 - 1.8	0.8	0.3 - 3.7	<0.001
%Area	0.1	0 - 0.9	0.59	0.1 - 4	<0.001	0.1	0 - 1	0.4	0.1 - 4.8	<0.001	0.1	0 - 1	0.3	0.1 - 3.2	<0.001
50															
Time (s) / h	49.3	12.5 - 181.3	152.27	46 - 449.1	<0.001	53.7	14.8 - 181.5	144.2	44 - 455.3	<0.001	51.8	14.6 - 178.8	139.3	31.4 - 400.1	<0.001
Area (au) / h	203.2	49.9 - 746.8	495.18	214.9 - 2146.7	<0.001	213.6	56.3 - 756.8	573.1	182.6 - 2217.9	<0.001	211.6	55.8 - 754.1	430.1	178.8 - 1608.1	<0.001
%Time	1.6	0.4 - 5.5	5.55	1.4 - 13.4	<0.001	1.7	0.5 - 5.6	5.1	1.3 - 13.8	<0.001	1.7	0.5 - 5.5	4.4	0.9 - 11.4	<0.001
%Area	0.9	0.1 - 4.7	4.08	0.9 - 12.1	<0.001	1.0	0.2 - 4.8	4.1	0.7 - 12.3	<0.001	1.0	0.2 - 4.7	3.6	0.5 - 10.8	<0.001
55															
Time (s) / h	203.6	67.7 - 512.2	480.88	175.1 - 1103.4	<0.001	204.4	75.8 - 513.9	480.9	172 - 1089	<0.001	203.0	75.9 - 512.8	436.6	135.3 - 981.2	<0.001
Area (au) / h	770.8	235.7 - 2357.2	2190.45	646.9 - 5028.8	<0.001	837.8	255.4 - 2359.4	2342.5	611.3 - 6141.8	<0.001	796.0	258.5 - 2350.2	1804.0	507.2 - 4713.4	<0.001
%Time	6.4	2.2 - 15.4	14.35	4.9 - 31.4	<0.001	6.6	2.3 - 15.7	14.4	5.4 - 31.4	<0.001	6.4	2.3 - 15.7	13.2	3.8 - 28.5	<0.001
%Area	5.3	1.3 - 14.9	13.14	4.8 - 29.3	<0.001	5.5	1.4 - 15.4	13.1	4 - 29	<0.001	5.5	1.5 - 15.4	12.5	3 - 27.5	<0.001
60															
Time (s) / h	639.7	254.2 - 1156.5	964.2	626.6 - 1628.3	0.001	636.1	276.2 - 1190.5	1032.6	603.3 - 1743.2	0.002	640.6	277.6 - 1197.1	939.9	404.7 - 1492.2	0.002
Area (au) / h	2770.8	1017 - 6899.2	6148.9	2742.8 - 12146.7	<0.001	2864.1	1078.1 - 6980.5	6148.9	2718.7 - 13136.6	<0.001	2776.2	1083.4 - 6977.1	5527.0	1937.1 - 10929	<0.001
%Time	19.4	7.8 - 34.9	30.19	17.6 - 50.1	<0.001	19.5	8.3 - 36.2	30.9	17.6 - 53.1	<0.001	19.5	8.5 - 36.6	27.6	11.9 - 43.9	0.002
%Area	18.2	6.4 - 34.4	28.88	15.8 - 50	0.001	18.3	6.7 - 36.2	29.5	15.8 - 52.5	<0.001	18.5	6.8 - 36.4	25.9	11.7 - 44.2	0.002

		GMDS 15m CA total DQ			Cognitive outcome at 24 months			Motor outcome at 24 months		
		Outside								
d1	out ±2SD	0 [0 - 15]	9.1 [0 - 22.6]	0.006	0 [0 - 15.8]	6.3 [0 - 22.3]	ns	0 [0 - 14.3]	10.9 [0 - 30.3]	<0.001
	out ±1.5SD	15.8 [0 - 33.3]	22.7 [13.2 - 51.1]	0.004	17.6 [0 - 33.3]	21.4 [8.2 - 41.4]	ns	16 [0 - 33.3]	23.7 [12.5 - 53.8]	<0.001
	out ±1SD	36.4 [18.2 - 63.6]	52.2 [30.5 - 76.6]	0.003	38.5 [20 - 65]	50 [27.3 - 71.4]	ns	37.5 [19.3 - 64.3]	50 [26.5 - 78]	<0.001
	out ±0.5SD	70 [54.5 - 86.4]	75 [63.4 - 93.3]	ns	72.2 [56.3 - 88.2]	74.5 [60.5 - 93.1]	ns	71.4 [55.6 - 86.9]	76.2 [61.2 - 92.9]	ns
	Q1 and Q4	47.7 [25 - 73.9]	58.3 [39.1 - 75]	0.009	50 [26.1 - 73.3]	54.5 [33.5 - 77.6]	ns	47.6 [25.3 - 72.6]	55 [36.2 - 82.6]	0.007
d2	out ±2SD	0 [0 - 13.6]	4.2 [0 - 16.7]	ns	0 [0 - 14.3]	4.2 [0 - 16.7]	ns	0 [0 - 14.3]	4.2 [0 - 16.7]	ns
	out ±1.5SD	12.5 [0 - 33.3]	23.5 [8.3 - 41.7]	0.007	13.6 [0 - 33.3]	17 [4.5 - 33.7]	ns	12.5 [0 - 33.3]	22.2 [8.3 - 41]	0.001
	out ±1SD	34.8 [20 - 59.1]	45.5 [29.2 - 65]	ns	36.4 [18.2 - 61]	36.9 [23.1 - 57.2]	ns	33.3 [16.7 - 58.3]	45.8 [28.7 - 63.4]	0.001
	out ±0.5SD	69.6 [54.2 - 83.3]	75 [62.5 - 87]	ns	69.6 [54.5 - 83.3]	70.8 [54.9 - 83.3]	ns	69.6 [54.2 - 83.3]	72.7 [58.3 - 83.3]	ns
	Q1 and Q4	45.8 [26.3 - 70.8]	56.5 [37.5 - 72.5]	ns	45.8 [28.9 - 70.8]	50 [33 - 70.8]	ns	45.6 [26.1 - 69.6]	54.9 [34.4 - 73.6]	0.010
d3	out ±2SD	0 [0 - 8.7]	8.3 [0 - 20.8]	0.001	0 [0 - 9.6]	5.7 [0 - 16.7]	ns	0 [0 - 9.1]	5.3 [0 - 17.4]	0.001
	out ±1.5SD	10 [0 - 27.3]	25 [12.5 - 45.8]	<0.001	11.8 [4.2 - 29.2]	20.8 [7.9 - 40.6]	0.002	11.8 [4.2 - 27.3]	20.8 [4.2 - 40.6]	0.004
	out ±1SD	33.3 [16.7 - 57.1]	52.2 [33.3 - 78.3]	<0.001	34.8 [16.7 - 58.3]	41.7 [25.3 - 62.3]	0.009	33.3 [16.7 - 58.3]	50 [21.1 - 65.6]	0.001
	out ±0.5SD	66.7 [50 - 83.3]	75 [62.5 - 91.7]	0.005	66.7 [50 - 83.3]	69.6 [58.3 - 83.3]	ns	66.7 [52.2 - 83.3]	75 [58.3 - 90.4]	0.009
	Q1 and Q4	45.8 [27.3 - 70.8]	54.2 [40 - 82.6]	0.003	45.8 [27.3 - 70.8]	54.2 [37.5 - 75]	ns	45.8 [27.3 - 70.8]	51.1 [33.3 - 78.3]	ns
72h	out ±2SD	5.1 [1.4 - 13.7]	12 [4.5 - 18.3]	<0.001	5.6 [1.4 - 14.3]	9.1 [3 - 17.5]	ns	5.1 [0 - 14.1]	10.1 [3.9 - 18]	<0.001
	out ±1.5SD	16 [7.5 - 29.6]	27.5 [17.4 - 37.5]	<0.001	16.4 [7.7 - 30.8]	25 [13.6 - 34.6]	0.006	15.8 [7.4 - 29.6]	25.4 [14.3 - 37]	<0.001
	out ±1SD	36.8 [24.5 - 51.6]	50 [39.6 - 60]	<0.001	37.7 [25 - 53.5]	45.6 [30.3 - 53.8]	ns	36.1 [24.6 - 52.3]	47.2 [36 - 61.6]	<0.001
	out ±0.5SD	67.2 [56.3 - 78.1]	75 [66.7 - 80.7]	0.001	68.1 [57.7 - 78.9]	71.9 [61.1 - 79]	ns	67.5 [56.7 - 78.5]	73.3 [62.8 - 81.5]	0.002
	Q1 and Q4	45.7 [33.3 - 63.3]	56.7 [44.5 - 67.7]	<0.001	46.2 [33.8 - 63.6]	54.4 [40.3 - 64.6]	ns	45.4 [33.3 - 63.1]	53.8 [41.2 - 66.2]	0.001

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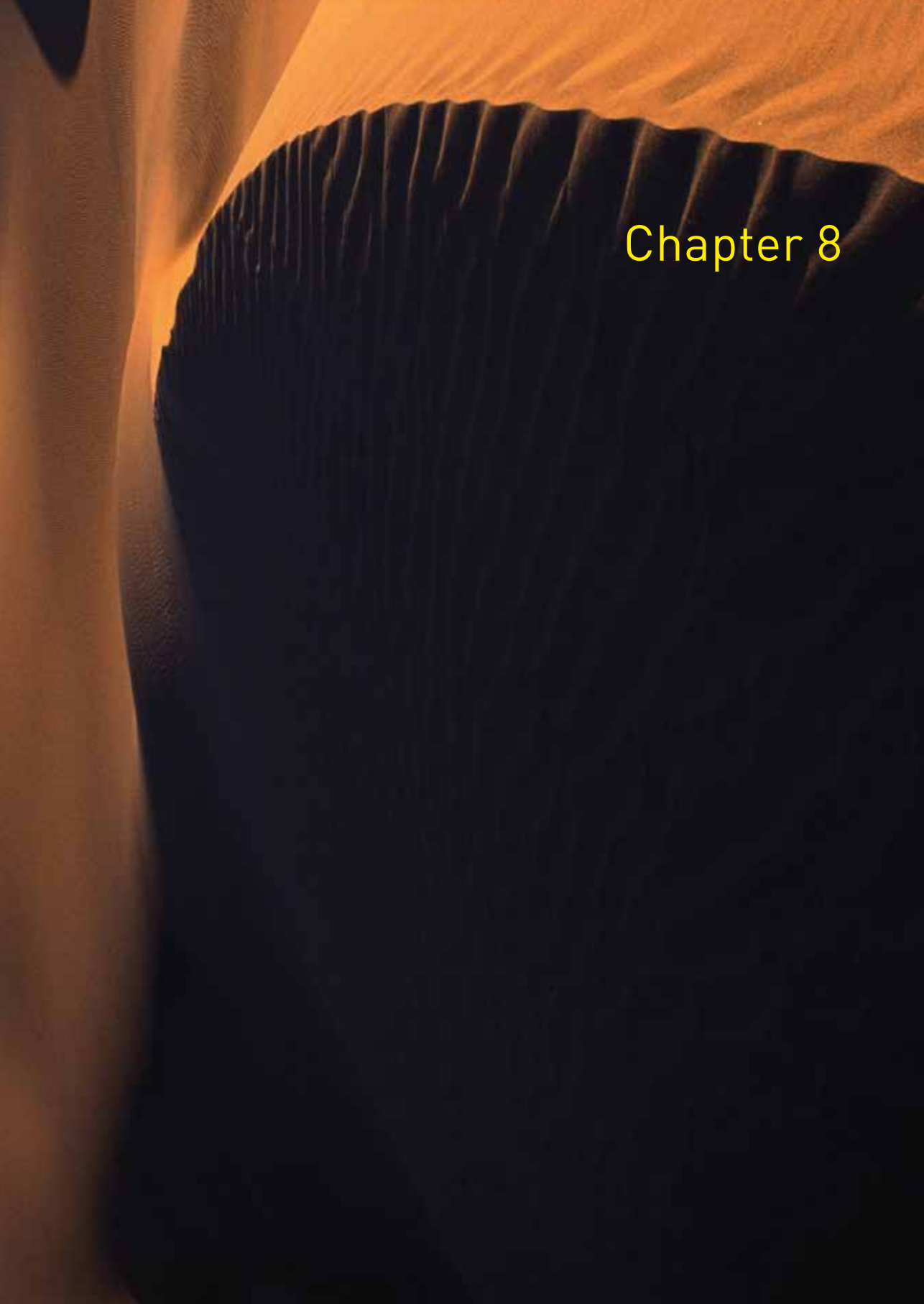
Part 2

Bedside to bench: imaging of cerebral haemodynamics by MRI


Chapter 8

MAGNETIC RESONANCE IMAGING BASED NON-INVASIVE MEASUREMENTS OF BRAIN HAEMODYNAMICS IN NEONATES: A SYSTEMATIC REVIEW

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Submitted

ABSTRACT

Perinatal disturbances of brain haemodynamics can have a detrimental effect on the brain's parenchyma with consequently adverse neurodevelopmental outcome. Non-invasive and reliable tools to evaluate the neonate's brain haemodynamics are scarce. Advances in magnetic resonance imaging have provided new methods to non-invasively assess brain haemodynamics. More recently these methods have made their transition to the neonatal population. The aim of this review is twofold. Firstly, to describe these newly available non-invasive methods to investigate brain haemodynamics in neonates. Secondly, to discuss the results that were obtained with these techniques, identifying both potential clinical applications as well as gaps of knowledge.

INTRODUCTION

The neonatal brain is most vulnerable during perinatal life when neuronal proliferation, neuronal migration, white matter myelinisation, glial cell migration and cortical folding are at their maximum depending on their gestational ages.[1-3] Any disturbance in the delivery of blood or oxygen to the brain tissue can have a deleterious effect leading to hypoxic-ischaemic encephalopathy (HIE), perinatal arterial ischaemic stroke (PAIS), periventricular leukomalacia and other brain injuries, which are known to be related to adverse neurodevelopmental outcome.[4-10] Therefore, a sensitive assessment of the perfusion and oxygenation status of the brain tissue is important to monitor the neonate's brain. As well, it could provide invaluable insight into the effect of neuroprotective agents. Unfortunately, the gold-standards to evaluate brain haemodynamics, oxygen-15 positron emission tomography (PET) and Xenon clearance, are invasive and therefore not feasible in neonates.[11,12] Although non-invasive techniques like Doppler flow measurements and near-infrared spectroscopy (NIRS) have been used as alternatives, these techniques have their own limitations.[13,14] NIRS only provides localized information (i.e. depending on sensor position) and is limited to the most superficial 2cm of the brain.[15] Doppler flow measurements on the other hand only provide flow measurements in cm/s or ml/min and do not actually measure tissue perfusion (e.g. in ml/100g/min).

Advances in Magnetic Resonance Imaging (MRI) have brought forward techniques which allow non-invasive evaluation of brain haemodynamics. More recently, these techniques have made their transition to the neonatal population. This review provides an overview of these non-invasive techniques, summarizes the clinical studies that have been done so far, and ends with suggestions for future research.

CEREBRAL HAEMODYNAMICS

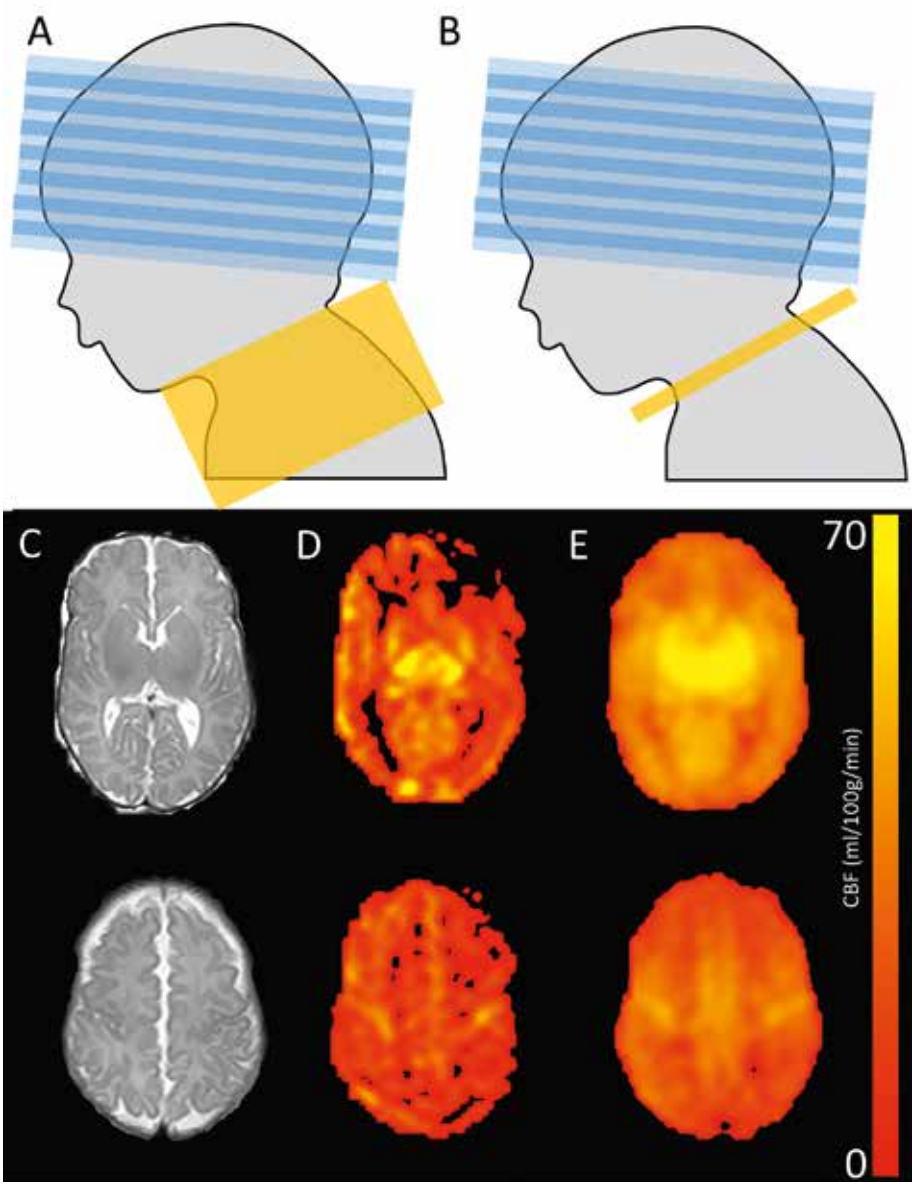
The delivery of oxygen and other nutrients to the brain tissue is essential for the brain tissue to survive. The delivery and consumption of oxygen and nutrients can be defined by four different haemodynamics parameters: the cerebral blood flow (CBF), the oxygen extraction fraction (OEF), the oxygen saturation (SO_2), and the cerebral metabolic rate of oxygen ($CMRO_2$). The different MRI techniques to measure CBF, OEF, SO_2 and $CMRO_2$ will be discussed separately.

CEREBRAL BLOOD FLOW (CBF)

Phase-contrast magnetic resonance angiography (PC-MRA) and arterial spin labelling (ASL) are the two MRI-techniques available to estimate CBF. In PC-MRA two datasets are acquired, one with and one without flow sensitivity. This is accomplished

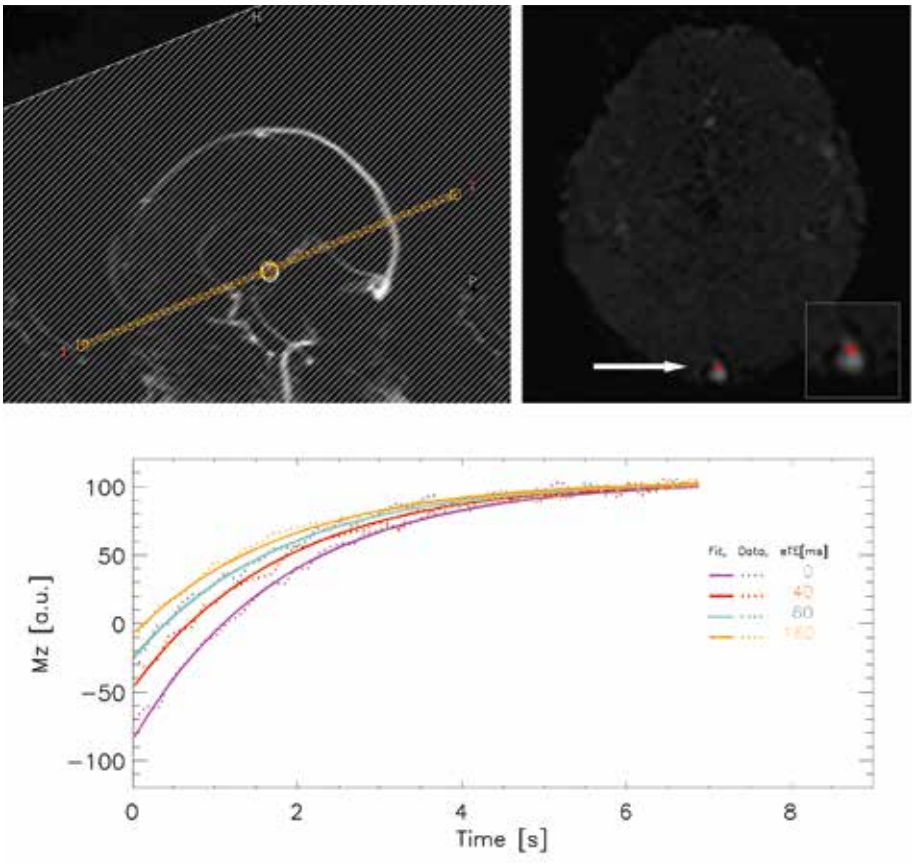
by applying a bipolar gradient and reversing this gradient when proceeding from the first to the second dataset. The flowing protons, or spins, in the blood thereby yield a net phase shift while stationary spins (e.g. tissue) do not gain a net phase. The magnitude of this phase shift is directly proportional to the velocity of the spins and can be expressed in cm/s. Multiplication of a vessel's cross-sectional surface area by the average blood velocity (in cm/s) in the same vessel, yields the actual quantity of blood (in ml/min) being transported by that vessel. Subsequently, blood flow towards the brain can be obtained by adding up the blood flow in the main feeding arteries: both internal carotid arteries and either both vertebral arteries or the basilar artery. The final step is calculating CBF (in ml/100*ml*/min) by dividing total blood flow by total brain volume derived from segmentation of anatomical MR images. Division by brain density (i.e. 1.06 g/ml in neonates) provides brain perfusion in more commonly reported units of ml/100*g*/min.[16]

The second technique, ASL is a subtraction technique where a perfusion weighted image is obtained by subtraction of a control image and a labelled image. In the labelled image, the blood signal has been inverted during the preparation phase. This is done by applying radiofrequency pulses in the neck region. The acquisition of the actual image starts at a certain delay after labelling (i.e. post-label delay), which allows the inverted spins to reach the brain tissue through the vasculature. The difference in signal between the control and labelled images is proportional to the CBF. In adults, CBF replaces ~1% of protons every second, yielding a signal difference (control-label) of only 1-2%, depending on the label duration and efficiency of the labelling. To increase the signal-to-noise ratio (SNR), multiple control-label sets (i.e. dynamics) are obtained. The signal difference of the subtracted image can be quantified using a general kinetic model which accounts for the size of the labelled bolus, the decay of the label, the efficiency of the labelling, and the proton exchange rate between blood and tissue.[17] The proton exchange rate can be considered a single constant. However, the size and decay of the label depend on the acquisition (e.g. label duration and post label delay), patient characteristics (i.e. transverse relaxation rate of blood T_{1b}), and the flow velocity of blood in the neck which affects label efficiency. Two research groups have measured the T_{1b} in neonates and found it to be higher and more variable in neonates compared to adults; mean 1.8s range with reported rages of 1.4-2s and 1.4-2.3s respectively.[18,19] For comparison, adults have a typical T_{1b} of around 1.6s.[20] These variations in neonatal T_{1b} arise from the more variable haematocrit observed in infancy. More importantly, assuming an incorrect T_{1b} can introduce perfusion errors ranging from -17% to 30%.[19] In addition, the label efficiency is more likely to vary in neonates and also depends on which labelling technique is being used. In general, two ASL techniques are used in neonates; pulsed ASL (PASL) and pseudocontinuous



← **Figure 8.1** (**A**) Labelling and imaging planes of pulsed arterial spin labelling (PASL); the labelling slab of PASL is around 10 cm and covers the neck region. (**B**) Labelling and imaging planes of pseudocontinuous ASL (pCASL); the labelling plane of pCASL is a thin slab just proximal of the brain on which a train of radiofrequency pulses is applied to label a pile of blood. (**C**) T_2-weighted images. (**D**) Perfusion-weighted images obtained with PASL. (**E**) Perfusion-weighted images obtained with pCASL. The images in (**C**), (**D**) and (**E**) are of the same infant.

ASL (pCASL). In PASL a thick slab (10 cm) of arterial hydrogen protons is inverted proximal to the imaging plane at a single time point using a short radiofrequency pulse (5-20ms), **figure 8.1A**.[21] In pCASL a train of discrete radiofrequency pulses is applied for a certain (label) duration (e.g. 1-2s) to label the protons in the neck region just proximal to the brain, **figure 8.1B**. Although pCASL has a higher SNR compared to PASL, its inversion efficiency is dependent on the blood's velocity, which is known



→ **Figure 8.2**
The image in the left upper corner shows the planning of the T_2-TRIR sequence. The T_2-TRIR sequence incorporates a whole-brain T_2 preparation scheme (white stripes) while the imaging plane (yellow) receives a presaturation pulse. This way the signal from all tissue within the imaging plane is suppressed and only T_2-prepared inflowing blood in the sagittal sinus is depicted (see right upper corner). An automatic region-of-interest is then chosen within the sagittal sinus and from the signal intensities within this area the T_2 of blood can be obtained.

to be quite variable in neonates.[22,23] A comparative study between PASL and pCASL in neonates demonstrated a strong correlation between the CBF values, but, the image quality score of pCASL images was higher than PASL images.[24] **Figure 8.1C** shows example perfusion images obtained with PASL and pCASL.

THE OXYGEN EXTRACTION FRACTION (OEF) AND THE OXYGEN SATURATION (SO_2)

The techniques to measure the SO_2 in neonates can be roughly divided into two groups; (1) the T_2-based measurements and (2) the susceptometry-based measurements.[25-30] The T_2-based measurements are T_2-relaxation-under-spin-tagging (TRUST), T_2-prepared tissue relaxation with inversion recovery (T_2-TRIR) and T_2-prepared blood imaging of oxygen saturation (T_2-BIOS).[25-29] The OEF is the difference between the arterial oxygen saturation (S_aO_2), obtained by pulse oximetry, and the venous oxygen saturation (S_vO_2) obtained in the sagittal sinus.

In the T_2-based measurements the transverse relaxation rate of pure blood (T_{2b}) is measured. The T_2 has a known relation with SO_2 and Hct, therefore the T_{2b} can be converted into SO_2 by means of calibration plots, which have been obtained through in vitro measurements on adult and neonatal blood.[25,31] The difference in TRUST, T_2-TRIR and T_2-BIOS lies in the approach used to isolate the signal coming from blood. Both TRUST and T_2-TRIR target the sagittal sinus and thus obtain venous T_{2b}, and thus S_vO_2. In TRUST the blood is magnetically labelled and isolated using a similar principle of control minus label image as is used in ASL MRI. Hereby contamination by signal from surrounding tissue and cerebrospinal fluid is avoided.[26] Instead, the T_2-TRIR uses an image saturation module to suppress signal from tissue types other than blood. The inflowing blood has not been suppressed previously and will be the only source of signal (**figure 8.2**). The T_2-BIOS technique on the other hand isolates the blood signal by exploiting "Intra-voxel incoherent motion imaging" (IVIM).[29,32,33] This way blood in arterial, capillary, and venous vessels is targeted and an overall T_{2b} of a mixed vascular compartment is obtained. In theory, this mixed compartment is comparable to the compartment that is being measured by NIRS. The advantage of the T_2-BIOS over NIRS is that it also provides a SO_2 estimate of the deeper grey and white matter.

The alternative approach, susceptometry-based measurements, relies on the relative magnetic susceptibility difference between intravascular blood and surrounding tissue.[34] As deoxygenated haemoglobin is paramagnetic, the extent to which the local magnetic field is disturbed depends on the blood oxygenation level. Intravascular protons sense a slightly larger magnetic field, which results in a susceptibility difference between intravascular and extravascular protons. The shifts in magnetic field can be quantified by measuring the phases of the MRI signal, and thereby the oxygenation level of blood can be quantified.

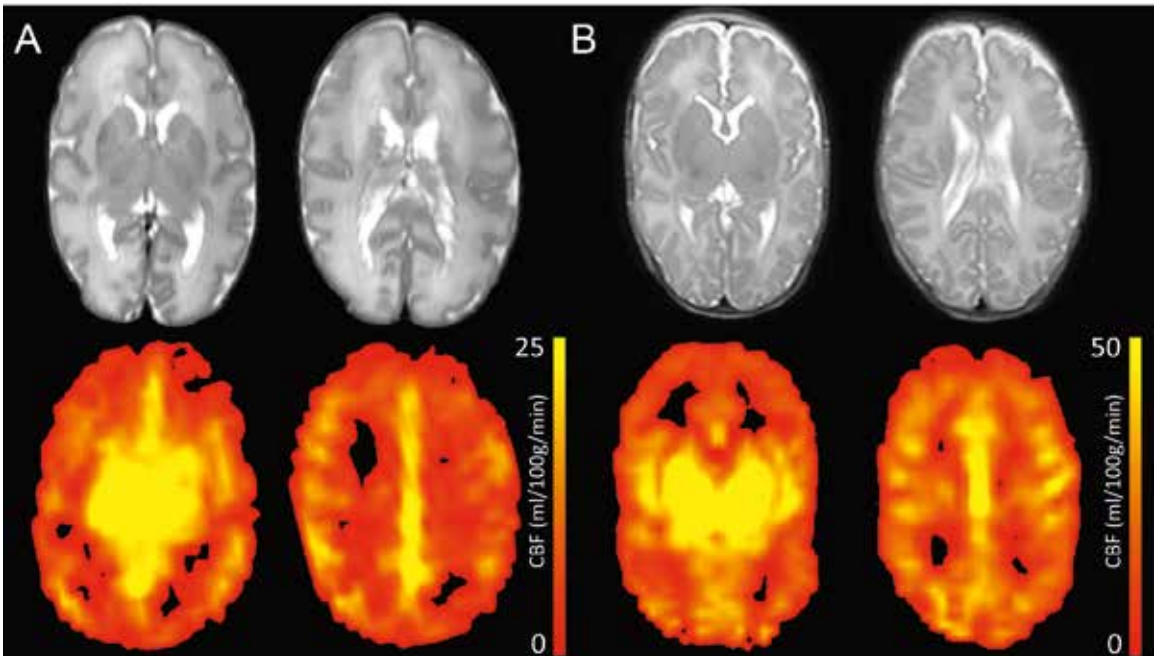
THE CEREBRAL METABOLIC RATE OF OXYGEN ($CMRO_2$)

The $CMRO_2$ (in μmol/100g/min) is the actual tissue consumption of oxygen and can be defined as the OEF times the CBF, while accounting for the oxygen carrying capacity of blood (i.e. Hb-bound O_2 and O_2 dissolved in plasma; C_a).[27] At a Hct of 0.44, 100 ml of blood can carry 8.97 μmol O_2.[28] The final equation for obtaining $CMRO_2$ is;

$$CMRO_2 = CBF \times OEF \times C_a$$

CLINICAL APPLICATIONS

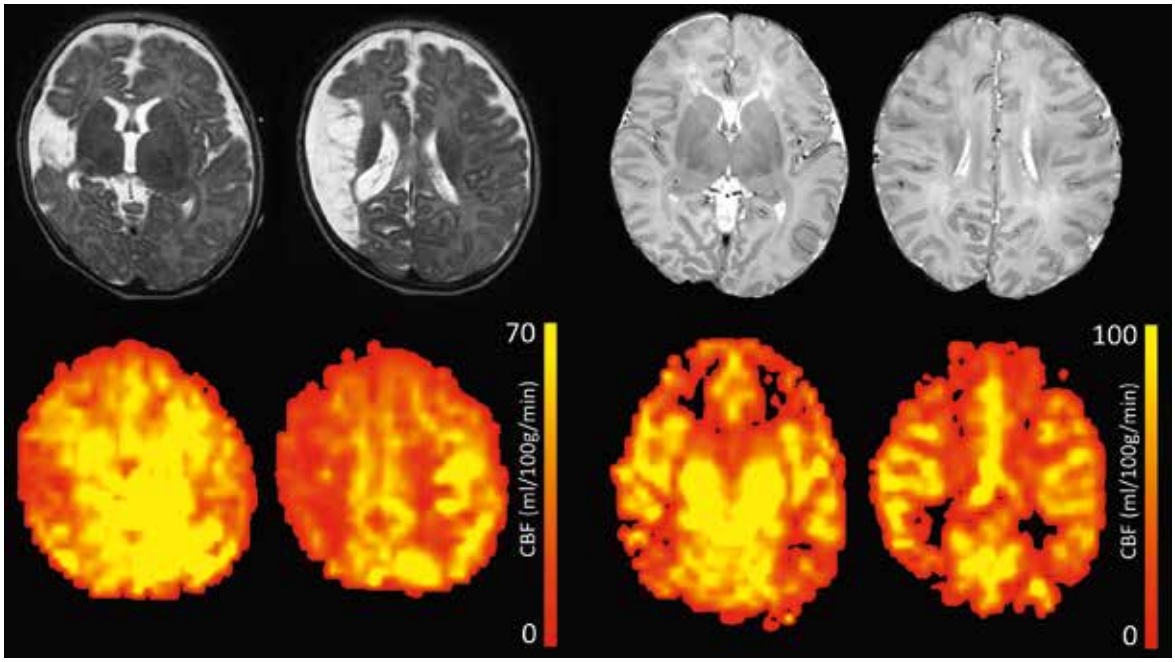
Thus far the main focus in terms of clinical applications has been on brain maturation in preterm infants, infants with PAIS, infants with HIE, and infants with congenital heart disease (CHD). The results of these studies are summarized in **Table 8.1.**



↑ **Figure 8.3**
Images of an infant born at preterm age (30 weeks postconceptional age). (**A**) T_2-weighted images and PASL images obtained at 31 weeks postconceptional age, the brain perfusion is shown in ml/100g/min (see colour bar). (**B**) T_2-weighted images and PASL images obtained at a postconceptional age of 38 weeks. Note that the colour bar has a higher scale than in (**A**), this was necessary because the perfusion was increased as compared to the images obtained at preterm age.

BRAIN MATURATION

Two studies evaluated CBF using PC-MRA and both of them found an increase in CBF with postconceptional age.[37,38] In the study of Benders et al., the CBF ranged from 11 to 48 ml/min/kg body weight in infants ranging from 30 to 51 weeks postconceptional age.[37] Varela et al. found CBF to vary from 24-56 ml/100g/min brain tissue in infants with a postconceptional of 30 to 95 weeks.[38] Moreover, in this last study, a steep rise in CBF was noticed between 47 and 62 weeks of postconceptional age.[38] A study performed using ASL found a similar increase in CBF with age; from 7 ml/100g/min to 29 ml/100g/min in infants ranging from 29 weeks up to 50 weeks postconceptional age.[39] In their study, CBF was highest in the deep grey matter. Similarly, Miranda et al. found a significant higher perfusion in the basal ganglia than in the cortical grey matter of term neonates and preterm neonates at term-equivalent age and it was hypothesized that these regional differences were caused by developmental processes.[40] The increase in CBF with postconceptional age reflects brain maturation and is consistent with previously performed positron emission tomography (PET) and xenon-enhanced computed



↑ **Figure 8.4**
(**A**) T_2-weighted images and PASL images of an infant with a perinatal arterial ischaemic stroke (PAIS) in the territory of the right middle cerebral artery (MCA). On the T_2-weighted images tissue loss is seen within the right MCA area. Within this same area, a lower perfusion is seen on the PASL images. (**B**) T_2-weighted images and PASL images of an infant with hypoxic-ischaemic encephalopathy. The brain tissue of this infant demonstrated a profound hyper perfusion; this is in particular noticeable on the scale of the colour bar, the upper limit had to be increased (i.e. 100ml/100g/min).

tomography studies (Xe-CT).[41-46] For an overview of these earlier found values, see **table 8.2. Figure 8.3** shows ASL images of a preterm infant imaged at preterm age and at term-equivalent age, the increase in perfusion with gestational age can be seen on these images. T_2-based measurements of S_vO_2 found mean values of 64% and 59% in infants with a postconceptional age of 33-40 weeks and 26-48 weeks respectively.[47,28] The mean $CMRO_2$ values found in these studies were 38 and 30 µmol/100g/min. Interestingly, both studies found an increase in the $CMRO_2$ with both postconceptional and postnatal age. Similar to the CBF measurements, the $CMRO_2$ measurements obtained by the newly developed non-invasive MRI techniques are in a similar order of magnitude as the values found earlier in PET and NIRS studies (**table 8.2**).[43-45,48]

PERINATAL ARTERIAL ISCHAEMIC STROKE

A study performed using PC-MRA demonstrated a higher blood flow in the ipsilateral ICA during the acute phase after unilateral PAIS. This increased blood flow towards the affected side disappeared after 3 months and no relation was found between this asymmetry in

blood flow and stroke size.[49] When using ASL, an increased CBF was found in 1 out of 4 patients. The remaining 3 patients demonstrated a decreased CBF in the stroke centre with increased CBF in the periphery of the stroke region.[50] Similar perfusion patterns were described in a second ASL MRI study.[51] In this study one patient presented with hyper perfusion, whereas the remaining three presented with hypo perfusion and only one of them had hyper perfusion in the periphery of the stroke area. Comparable to the PC-MRA study, perfusion at follow-up was comparable between the affected and unaffected side. It can be concluded that hyper perfusion is a common phenomenon after stroke in neonates. This is comparable to 'luxury perfusion' described in adult stroke. It has been suggested that the luxury perfusion represents physiologic transient reperfusion via recanalization and/or collateral flow, which might be a marker for tissue survival and protective for haemorrhagic transformation.[52-54] **Figure 8.4A** shows ASL images of an infant with PAIS.

HYPOXIC-ISCHAEMIC ENCEPHALOPATHY

Only ASL MRI has been used to evaluate cerebral haemodynamics in neonates with HIE. Two studies found a higher perfusion in the deep grey matter of the infants with HIE and poor outcome; De Vis et al. reported 63 vs. 28 ml/100g/min with a 85% sensitivity and 100% specificity for adverse outcome at a cut-off of 51 ml/100g/min.[55] Shi et al. did not quantify perfusion, but found significant higher signal intensity in the grey matter and basal ganglia of infants with HIE.[56] Pienaar et al. found a negative correlation between the apparent-diffusion-coefficient (ADC) and CBF in 9 neonates with clinical and imaging evidence of ischaemia, which suggests that cerebral tissue damage is associated with hyperperfusion.[57] A single study described the evolution of perfusion during the first week after the hypoxic-ischaemic event. Initial hypo perfusion on day 1 was followed by hyper perfusion in brain areas that subsequently exhibited brain injury on conventional images.[58] Another study evaluated the brain tissue perfusion in the second week after the hypoxic-ischaemic event and found global and regional CBF to still be higher in infants with HIE.[59] Amongst the infants with HIE, infants with injury on MR imaging had a lower CBF in the thalamus. This was contributed to pseudo normalization of CBF and low metabolic demand after progression to irreversible brain injury.[59] **Figure 8.4B** shows ASL images of an infant with HIE.

Thus far only one paper described MRI-based measurements of S_vO_2 and $CMRO_2$ in infants with HIE.[28] In this study the S_vO_2 in infants with HIE was found to be higher than in controls (66% vs. 50%), suggesting lower oxygen consumption caused by tissue injury. No significant differences in CBF and $CMRO_2$ were found, possibly due to the smaller sample size. Derangements in oxygen consumption in neonates with HIE, measured by magnetic resonance spectroscopy, have been found to be related to adverse neurodevelopmental outcome.[60]

CONGENITAL HEART DISEASE

Neonates with congenital heart disease are a particular difficult group to perform ASL perfusion measurements in, this is because vascular shunts between the pulmonary and arterial circulation decrease the body circulation time of labelled blood. This can cause labelled blood to appear in the imaging plane of control images causing negative perfusion voxels. Other factors which further increase this phenomenon are the small body size of neonates and the longer T_{1b} as compared to adults. Goff et al. demonstrated that the amount of negative voxels in neonates with CHD increases when the CBF is decreased, thus potentially introducing errors in the CBF measurements.[61] A means to decrease the number of negative perfusion voxels is by restricting the labelling volume.[62] In infants with CHD a decreased CBF and a smaller change in CBF under hypercarbia circumstances was shown to relate to periventricular leukomalacia.[63] Nagaraj et al found lower global and regional (basal ganglia) CBF in infants with CHD compared to controls. Within the group of infants with CHD, infants with cyanotic CHD had lower CBF in the basal ganglia, thalami and occipital white matter compared to infants with acyanotic CHD.[64] Susceptometry-based $CMRO_2$ measurements were performed in neonates with CHD but have thus far not been compared to measurements in control neonates.[65]

MULTIMODALITY STUDIES

Several studies have demonstrated associations between haemodynamic parameters assessed by different modalities (**table 8.3**). This cross-validates the different techniques and strengthens confidence in their use a measure of cerebral haemodynamics. Cerebral blood flow measured by PC-MRA correlated positively with CBF values obtained by Doppler ultrasound. Nevertheless, limits-of-agreement were wide (-78 to +68), most markedly at higher CBF.[66] NIRS-SO_2 has been found to have a strong correlation with both whole brain and frontal CBF.[29,65,67] Interestingly, the group of Massaro et al. did not find a strong relation between NIRS-SO_2 and CBF during the second week after HIE.[59] A good relation was found between NIRS-SO_2 and MRI-SO_2/S_vO_2 measured by either T_2-TRIR, T_2-BIOS or susceptometry-based measurements.[29,65] A cross-validation of the T_2-TRIR and T_2-BIOS technique found a moderate relation.[29]

FUTURE RESEARCH

Heterogeneity, arising both from clinical (e.g. different populations) and technical factors (e.g. different acquisitions), is the biggest concern in MR based haemodynamic imaging of the new born brain. In general, the reported (disease) populations are small and heterogeneous which makes direct comparison complex. In this regard, neonatal haemodynamic imaging could benefit from (inter)national collaborations in which

patients with specific diseases are pooled. More specifically, reference values with regard to postconceptional and postnatal age would be extremely useful. In PAIS, studies should be performed to investigate the relation between the different perfusion patterns (over time) and outcome. In addition, imaging of the SO_2 or OEF on a voxel-by-voxel basis with MRI sequences (as has been proposed in adults), combined with DWI could potentially identify the penumbra and thus could predict outcome.[68] In neonates with HIE, the course of the brain perfusion beyond the first week after the event is unclear. As well, the benefit of oxygen metabolism measurements and their relation with outcome should be investigated further. Haemodynamic evaluation of infants with CHD, both before and after surgery, should be compared to haemodynamic measurements performed in healthy controls. Currently the number of clinical conditions in which haemodynamic imaging have been used is relatively limited. Clinicians and researchers should be convinced to include these forms of imaging in their protocol. This can only be accomplished through a readily interpretable and accessible imaging protocol. From a technical point of view, it should be evaluated if pCASL does indeed perform better than PASL, as this investigation was thus far only performed in neonates with HIE and in non-random order. Furthermore, the actual labelling efficiency in pCASL should be investigated. More specifically, for neonates with CHD an optimal imaging protocol could reduce the number of negative perfusion voxels and thereby semi-standardise perfusion weighted imaging in this patient group. For imaging SO_2, the S_vO_2 is relatively established. It would be interesting to evaluate the performance of the different sequences. In addition, voxel-by-voxel methods should be improved to provide full brain coverage with sufficient SNR. Only then, these sequences can be assessed in terms of additional clinical value.

CONCLUSION

The three main non-invasive MRI techniques that are currently available to the neonatal population to image cerebral haemodynamics are arterial spin labelling, oxygen saturation assessment in the sagittal sinus, and the assessment of the global cerebral metabolic rate of oxygen. Thus far, only ASL during the first week of life in infants with HIE seems to have ascended from pure research tool to a tool that is applicable in daily clinical care. A higher CBF on day 1-7 predicts worse outcome and can be used to direct care and provide prognostic information. For most other conditions we can conclude that these haemodynamic tools clearly have additional value, but we need to increase the numbers, standardise research and imaging protocols to draw unequivocal conclusions that can be beneficial on an individual basis in daily clinical care.

↓ Table 8.1

MRI measurements of haemodynamic parameters.

Study	Method	Parameter	Age	N	Values
Brain maturation					
Benders et al., 2011 [37]	PC-MRA	CBF	30-51w PCA	30	25 (range: 11-48) ml/min/kg body weight
Varela et al., 2012 [38]	PC-MRA	CBF	30-95w PCA	21	range: 18-60 ml/100g/min
De Vis et al., 2013 [39]	ASL	CBF	29-50w PCA	29	range: 7-29 ml/100g/min
Miranda et al. 2006 [40]	ASL	CBF	PT-TEA	23	21.3 ± 5.1 ml/100g/min
Liu et al., 2014 [47]	PC-MRA	CBF	33-40w PCA	12	13.4 ± 4.2
	TRUST	S_vO_2			62.6 ± 8.3%
De Vis et al., 2014 [28]	T_2-TRIR	S_vO_2	26-48w PCA	42	59 ± 14%
Liu et al., 2014 [47]	TRUST/PC-MRA	$CMRO_2$	33-40w PCA	12	38.3 ± 17.7 µmol/100g/min
De Vis et al., 2014 [47]	T_2-TRIR/ASL	$CMRO_2$	26-48w PCA	22	30 ± 12 µmol/100g/min
PAIS					
Van der Aa et al., 2012 [49]	PC-MRA	Flow	w1/3mo	17	Asymmetry (ipsilateral /contralateral): 8.5% / -1.0%
De Vis et al., 2013 [51]	PC-MRA	Flow	w1	4	Asymmetry (ipsilateral/contralateral): 9.5%
De Vis et al., 2013 [51]	ASL	CBF	w1	4	Ipsilateral / contralateral: 11.5 ± 3.3 / 12.2 ± 2.1 ml/100g/min
HIE					
De Vis et al., 2015 [55]	ASL	CBF	HIE adverse outcome bgt	20	63 (28-108) ml/100g/min
			HIE good outcome bgt	8	28 (12-51) ml/100g/min
Shi et al., 2012 [56]	ASL	CBF	HIE gm/wm/bg	33	153 ± 12 /71 ± 10 /217 ± 13 SI
			Controls gm/wm/bg	7	125 ± 12/73 ± 12 /174 ± 15 SI
Massaro et al., 2013 [59]	ASL	CBF	HIE wb/bg/wm	18	24 ± 5 /52 ± 19/12 ± 3 ml/100g/min
			Controls wb/bg/wm	18	19 ± 2 /31 ± 5/10 ± 2 ml/100g/min
De Vis et al., 2014 [47]	ASL	CBF	HIE	9	12 ± 4 ml/100g/min
			Controls	10	14 ± 3 ml/100g/min
	T_2-TRIR	S_vO_2	HIE	11	66 ± 12%
			Controls	17	50 ± 11%
	T_2-TRIR/ASL	$CMRO_2$	HIE	9	24 ±12 µmol/100g/min
			Controls	10	30 ± 6 µmol/100g/min
CHD					
Licht et al., 2004 [63]	ASL	CBF	CHD	25	19.7 ± 9.2 ml/100g/min
		CBF HC			40.1 ± 20.3 ml/100g/min
Jain et al., 2014 [65]	SBM	S_vO_2	CHD	32	55.2 (IQR: 49.3, 60.2) %
		S_vO_2 HC			66.4 (IQR: 57.0,72.5)%
	ASL	CBF			9.6 (IQR: 7.5,15.1) ml/100g/min
		CBF HC			21.2 (IQR: 16.5,31.0) ml/100g/min
	SBM+ASL	$CMRO_2$			0.49 (IGR: 0.4, 0.79) ml O_2/100g/min
		$CMRO_2$ HC			0.53 (IQR: 0.4. 0.79) ml O_2/100g/min
Nagaraj et al, 2015 [64]	ASL	CBF	CHD	43	16.3 ml/100g/min
			Controls	58	19.3 ml/100g/min

The results are either presented as mean ± SD, median (range) or median (interquartile range, IQR). bg, basal ganglia; bgt, basal ganglia and thalami; gm, grey matter; HC, hypercapnia; PT-TEA, preterm at term-equivalent age; SBM, susceptometry-based measurements; SI, signal intensity; wb, whole brain; wm, white matter

↓ **Table 8.2**

Non-MRI measurements of haemodynamic parameters

Study	Method	Age	Category	N	Values
CBF (in ml/100g/min)					
Chiron et al., 1992 [41]	Xe-CT	2-45d PNA	Healthy	7	50 ± 3.4
		2-7m PNA	Healthy	7	55 ± 5.3
Pryds O et al., 1989 [42]	Xe-CT	<35w	RDS	22	8.4-11.5 (3.6-28.9)
Skov et al., 1993 [43]	NIRS	24-37 PCA	RDS	22	12.6 ± 6.4
			HIE	10	26.5 ± 17.9
Altman et al., 1993 [44]	PET	24-39 PCA	HIE, others	11	21.6 ± 21
Yoxall et al., 1998 [45]	NIRS	22-39 PCA	Seizures, others	20	9.3 (4.5 – 28.3)
Tyszuk et al., 1998 [46]	NIRS	24-34	Preterm	30	13.9 ± 6.9 / 12.3 ±6.4
S_vO_2 (in %)					
Skov et al., 1993 [43]	NIRS	24-37 PCA	RDS	22	53.44 ± 15.36
			HIE	10	67.3 ± 9.38
Yoxall et al., 1998 [45]	NIRS	22-39 PCA	Seizures, others	20	64.6 (76.1 – 46.8)
Zaramella et al., 2007 [48]	NIRS		HIE	22	75.3 (54.8-99)
			Controls	15	66.4 (55.9-88.8)
$CMRO_2$ (in µmol/100g/min)					
Skov et al., 1993 [43]	NIRS	24-37 PCA	RDS	22	44.7 ± 17.9
			HIE	10	62.6 ± 35.8
Altman et al., 1993 [44]	PET	24-39 PCA	HIE, others	11	21.4 ± 16.4
Yoxall et al., 1998 [45]	NIRS	22-39 PCA	Seizures, others	20	23.1 (8.6 -78.5)

This table gives an overview of the studies which have assessed the brain haemodynamic parameters with techniques other than MRI. The results of these studies are grouped per haemodynamic parameter; CBF, S_vO_2, and $CMRO_2$. HIE, hypoxic-ischaemic encephalopathy; RDS, respiratory distress syndrome.

↓ **Table 8.3**

Multimodality studies

Study	Modality 1	Modality 2	Population	Values
CBF				
Benders et al.[66]	wCBF (PC-MRA)	wCBF (Doppler)	Various	$R^2 = 0.25$, $p < 0.01$
Wintermark et al.[67]	wCBF (ASL)	SO_2 (NIRS)	Severe HIE	$R^2 = 0.77$, $p<0.01$
	wCBF (ASL)	SO_2 (NIRS)	Moderate HIE	$R^2 = 0.14$, $p = 0.74$
Alderliesten et al.[29]	wCBF (ASL)	SO_2 (NIRS)	Various	$R^2 = 0.50$, $p < 0.01$
	fCBF (ASL)	SO_2 (NIRS)	Various	$R^2 = 0.71$, $p <0.001$
Jain et al. [65]	wCBF (ASL)	SO_2 (NIRS)	CHD	$R^2 = 0.67$, $p <0.001$
Massaro et al. [59]	wCBF	SO_2 (NIRS)	HIE 2nd week	ns
SO_2				
Alderliesten et al.[29]	S_vO_2 (T_2-TRIR)	SO_2 (NIRS)	Various	$R^2 = 0.65$, $p <0.01$
	SO_2 (T_2-BIOS)	SO_2 (NIRS)		$R^2 = 0.64$, $p <0.001$
	SO_2 (T_2-BIOS)	S_vO_2 (T_2-TRIR)		$R^2 = 0.49$ $p <0.05$
Jain et al. [65]	S_vO_2 (SBM)	SO_2 (NIRS)	CHD	$R^2 = 0.69$, $p<0.001$
$CMRO_2$				
Jain et al. [65]	$CMRO_2$	SO_2 (NIRS)	CHD	$R^2 = 0.67$, $p<0.001$

Overview of the studies which have compared the MRI-hemodynamic measurements to non-MRI hemodynamic evaluation tools. CHD, congenital heart disease; fCBF, frontal CBF; HIE, hypoxic-ischaemic encephalopathy; ns, non-significant; SBM, susceptometry-based measurements; wCBF, whole brain CBF.

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Chapter 9

SIMULTANEOUS QUANTITATIVE ASSESSMENT OF CEREBRAL PHYSIOLOGY USING RESPIRATORY-CALIBRATED MRI AND NEAR-INFRARED SPECTROSCOPY IN HEALTHY ADULTS

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ABSTRACT

BACKGROUND Functional Near-Infrared Spectroscopy (fNIRS) and functional MRI (fMRI) are non-invasive techniques used to relate activity in different brain regions to certain tasks. Respiratory calibration of the blood oxygen level dependent (BOLD) signal, and combined fNIRS-fMRI approaches have been used to quantify physiological subcomponents giving rise to the BOLD signal. A comparison of absolute oxygen metabolism parameters between MRI and NIRS, using spatially resolved (SRS) NIRS and respiratory calibrated MRI, could yield additional insight in the physiology underlying activation.

MATERIALS AND METHODS Changes in BOLD signal, cerebral blood flow (CBF), and oxygen saturation (SO_2) were derived from a single MRI sequence during a respiratory challenge in healthy volunteers. These changes were compared to SO_2 obtained by a single probe SRS NIRS setup. In addition, concentration changes in oxygenated (O_2Hb), deoxygenated (HHb), and total haemoglobin (tHb), obtained by NIRS, were compared to the parameters obtained by MRI.

RESULTS NIRS SO_2 correlated with end-tidal CO_2 (0.83, $p < 0.0001$), the BOLD signal (0.82, $p < 0.0001$), CBF (0.85, $p < 0.0001$), and also MRI SO_2 (0.82, $p < 0.0001$). The BOLD signal correlated with NIRS HHb (-0.76, $p < 0.0001$), O_2Hb (0.41, $p = 0.001$), and tHb ($r = 0.32$, $p = 0.01$).

CONCLUSIONS Good correlations show that changes in cerebral physiology, following a respiratory challenge, go hand in hand with changes in BOLD signal, CBF, O_2Hb, HHb, NIRS SO_2, and MRI SO_2. Out of all NIRS derived parameters, the SO_2 showed the best correlation with the BOLD signal.

INTRODUCTION

Functional neuroimaging techniques are primarily used in research settings to relate activity in certain brain areas to specific tasks or functions.[1–3] Based on methodology, these techniques can be divided into two groups. Topographic-electroencephalography (EEG) and magnetoencephalography (MEG) map electrical activity and changes in local magnetism brought about by changes in electrical activity to brain anatomy, respectively.[4,5] On the other hand, techniques such as positron emission tomography (PET), functional Near-InfraRed Spectroscopy (fNIRS) and functional Magnetic Resonance Imaging (fMRI), rely on visualizing haemodynamic changes related to neuronal activation. fNIRS and fMRI are of special interest as they do not require the injection of a tracer substance.[6,7] Both fNIRS and fMRI have their own advantages and disadvantages regarding costs, mobility, spatial and temporal resolution.[8]

Localized changes in tissue oxidative metabolism, blood volume (CBV), and cerebral blood flow (CBF) are responsible for the contrast generated in blood oxygen-level dependent (BOLD) fMRI.[9–13] Calibration of the resting-state BOLD signal can be done by either performing a hypercapnia, or a hyperoxia experiment.[14–16] This calibration allows for the quantification of specific physiological subcomponents of the BOLD response. More recently, combined approaches of hypercapnia and hyperoxia were introduced.[17,18] These approaches enable a more accurate and quantitative assessment of local changes in the cerebral metabolic rate of oxygen ($CMRO_2$).

In contrast to fMRI, NIRS relies on the translucency of biological tissue to near-infrared light (~650-950nm). Light in this wavelength range is attenuated by different compounds (chromophores), including deoxygenated (HHb) and oxygenated haemoglobin (O_2Hb). Therefore, NIRS can be used to estimate changes in HHb and O_2Hb concentration.[19–21] In fNIRS, multiple transmitters and receivers (channels) are used to record signal changes arising from different brain regions. Subsequently, the point of origin of these signal changes can be mapped to brain anatomy by using spatial mapping tools.[6] NIRS can also be used to assess the tissue oxygen saturation (SO_2). This can be done by using spatially resolved spectroscopy (SRS), time resolved spectroscopy (TRS), or phase modulation spectroscopy (PMS).[22,23]

Simultaneous fMRI-fNIRS studies have been performed to identify the relation between BOLD signal changes and the haemodynamic response observed by NIRS.[8,24–27] This has led to a better understanding of the different physiological parameters contributing to the BOLD response.

A detailed comparison of absolute oxygen metabolism parameters between MRI and NIRS has not yet been performed. The purpose of this study was to compare quantitative data obtained by respiratory calibrated MRI to quantitative data obtained by SRS NIRS.

THEORY

MRI

Respiratory calibration of the BOLD signal

The changes in BOLD signal detected in fMRI depend on localized changes in tissue oxygen content.[10,12,28] The tissue oxygen content influences HHb and O_2Hb concentrations. As HHb is paramagnetic, the HHb concentration influence the transversal relaxation rate R_2^*.[29] Therefore, changes in HHb concentration can be visualized on T_2^*-weighted images.

Calibration of the BOLD signal is possible by estimating the theoretical maximal BOLD signal change (*M*) that would occur during a complete washout of HHb. During a hypercapnia experiment, the CBF is increased by CO_2-induced vasodilatation. The increase in CBF causes a washout of HHb and thus a change in BOLD signal. When this signal change, and the change in CBF are recorded, *M* can be estimated.[14] Subsequently, *M* can be expressed in HHb concentration in relation to cerebral blood volume (CBV).[15] Using both the Grubb power law relationship and Fick's mass conservation principle, the full hypercapnia calibration model can be expressed in terms of BOLD, $CMRO_2$ and CBF.[30,31]:

EQUATION 1

$$\frac{\Delta BOLD}{BOLD_0} = M\left(1-\left(\frac{CMRO_2}{CMRO_{2_0}}\right)^{\beta}\left(\frac{CBF}{CBF_0}\right)^{\alpha-\beta}\right)$$

Where $BOLD_o$, $CMRO_{2|o}$ and CBF_o represent the values obtained at baseline. The corresponding variables without subscript represent the values measured during hypercapnia. The β is a constant that depends on magnetic field strength, typically set at 1.3 for 3.0 Tesla field strengths.[16] The α_v is the Grubb coefficient, which was set at 0.18 to represent the non-arterial blood volume.[32,33] The calibration model can also be expressed in terms of BOLD, CBF and HHb concentration.[18]:

EQUATION 2

$$\frac{\Delta BOLD}{BOLD_0} = M\left(1-\left(\frac{CBF}{CBF_0}\right)^{\alpha}\left(\frac{[HHb]_v}{[HHb]_{v_0}}\right)^{\beta}\right)$$

Here $[HHb_v]$ represents the HHb concentration in the venous compartment. A dual echo pseudocontinuous Arterial Spin Labelling (pCASL) sequence can be used to obtain changes in BOLD signal and CBF simultaneously.[34] After estimating *M* (Eq. 1), changes in BOLD signal and CBF can be transformed to changes in [HHb] in the venous compartment by using Eq 2.

Total Cerebral Blood Volume

The measured changes in CBF can be directly related to changes in tCBV according to the Grubb power law by using the original value of α_t = 0.38 for the Grubb exponent.[30]:

EQUATION 3 $$\frac{CBV}{CBV_0} = \left(\frac{CBF}{CBF_0}\right)^{\alpha}$$

The change in tCBV (in ml/100g) can be calculated when Eq. 3 is combined with an assumed baseline blood volume fraction of 0.05.[35]

Oxygen saturation

Eq. 3 can be used to relate CBF to tCBV and non-arterial CBV (vCBV) by using an α_t of 0.38 and an α_v of 0.18, respectively. In turn, tCBV and vCBV are directly proportional to tHb and total haemoglobin in the non-arterial compartment (tHb_v), respectively.[30,32] Changes in blood volume fractions over time can be assessed when baseline arterial (0.2) and non-arterial (0.8) blood volume fractions are combined with the estimated values of tCBV and vCBV (Eq. 3).[36] Assuming that the HHb concentration in the arterial compartment is negligible, total HHb is represented by $[HHb]_v$ in Eq. 2. HHb_v can be related to tHb_v by using a baseline oxygen extraction fraction (OEF), which was set at 0.4.[37] Calculation of the SO_2 is then possible by determining the ratio of O_2Hb (1-HHb) vs. tHb:

EQUATION 4 $$SO_2(\%) = \frac{(1\text{-}vCBV) + vCBV*(1\text{-}HHb_V)}{tHb}$$

Where the term (1-vCBV) represents the arterial blood volume and vCBV represents the non-arterial blood volume.

NEAR-INFRARED SPECTROSCOPY

Modified Lambert-Beer law

In NIRS it is assumed that the contribution to total attenuation caused by oxygen independent light losses (static tissue) remains constant over time. Therefore, a change in attenuation, from one point in time to another, is caused by a change in concentration of oxygen dependent chromophores. These concentration changes can be expressed in terms of optical densities (OD) according to the modified Lambert-Beer law.[21]:

EQUATION 5 $$\Delta c = \frac{\Delta OD_\lambda}{\varepsilon_\lambda * L * B}$$

Where Δc represents the change in chromophore concentration, ΔOD_λ is change in optical density at a certain wavelength, and ε_λ is the molecular extinction coefficient of a chromophore at a certain wavelength. The L represents the distance between the light source and the detector, and B is the differential path length factor (DPF). The DPF accounts for the fact that photons travel a longer distance (than L) through tissue due to scatter. As ε_λ, L, and B are known, measured changes in ODs can be converted into concentration changes when one wavelength per observed chromophore is used.[21]

Spatially Resolved Spectroscopy

Scatter of photons becomes homogeneous at sufficiently large distances (> 3.0 cm) from a light source.[38] Therefore, the contribution to attenuation caused by scatter is equal at multiple (light source vs. detector) distances. Multiple distances can be created by using a single source with multiple detectors, or vice versa. With data from multiple distances it is possible to calculate the relative attenuation coefficients of O_2Hb and HHb.[23] When O_2Hb is expressed as a ratio of tHb (O_2Hb + HHb) the tissue SO_2 can be calculated:

EQUATION 6

$$SO_2\,(\%) = \frac{O_2Hb}{O_2Hb + HHb}$$

The SO_2 is an absolute value that represents the weighted average of O_2Hb as a ratio of tHb (O_2Hb + HHb) in arterial, capillary and venous vessels.

Total Cerebral Blood Volume

Total cerebral haemoglobin is directly proportional to tCBV. Therefore, changes in tHb can be converted to changes in tCBV.[39]

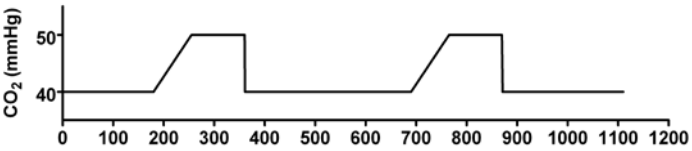
EQUATION 7

$$\Delta CBV = \frac{\Delta[tHb] * MW_{Hb}}{[Hb]_{blood} * D_{bt} * R}$$

Where ΔtCBV is the change in tCBV (ml per 100g brain tissue), MW_{Hb} is the molecular weight of haemoglobin (64500 g/Mol), $[Hb]_{blood}$ is the haemoglobin concentration in the blood (in g/dl), D_{bt} is the density of brain tissue set at 1.05 g per ml, and R is the large to small vessel haematocrit ratio set at 0.69.[40,41]

MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Review Board. Informed consent was obtained in 7 (3 female, 4 male) healthy, non-smoking volunteers.



← **Figure 9.1**
Schematic of the respiratory paradigm

Volunteers were instructed not to drink any caffeine containing substances, and not to perform heavy exercise directly before the experiment.[42] MR imaging and NIRS were performed simultaneously during a respiratory challenge.

COMPUTERIZED RESPIRATORY CHALLENGE

A computerized end-tidal gas targeting system (RespirAct™, Thornhill Research Inc, Toronto, Canada) was used to supply varying gas concentrations to the subject's mask via a single tube system.[43] To ensure an airtight seal, transparent medical dressings (Tegaderm; 3M, St Paul, MN) were used to fixate the mask over the subject's nose and mouth.

Before each experiment, the RespirAct™ was calibrated using a calibration gas (9.01/90.99% CO_2/ N_2). Subsequently, gas concentrations of the four connected gasses (gas A: 21 O_2 /79% N_2, gas B: 100% O_2, gas C: 10% O_2 / 90% N_2 and gas D: 20% CO_2, 10% O_2 / 70% N_2) were validated. Tidal CO_2 and O_2 partial pressures were continuously sampled and recorded by the RespirAct™. This enabled the automatic identification of respiratory rate, end-tidal CO_2 (P_{ETCO_2}), and end-tidal O_2 (P_{ETO_2}). Baseline parameters (i.e. P_{ETCO_2}, P_{ETO_2}, respiratory rate, and gas breathing volume) of each individual were recorded before the experiment. These parameters were used to tailor the settings of the RespirAct™. Thereafter, the P_{ETCO_2} was targeted at 50 mmHg for 2 minutes to let the subjects get accustomed to hypercapnic breathing.

The actual respiratory paradigm started with a 180s baseline period (**figure 9.1**). The paradigm contained two hypercapnic segments where P_{ETCO_2} was gradually ramped up from the individual's baseline to 50 mmHg in 75s, and then maintained at 50 mmHg for 105s. The hypercapnic segments were separated by a 330s baseline period. The paradigm ended with 240s baseline. The total duration of the respiratory challenge was 18 minutes 30 seconds.

MRI

MR imaging was performed using a 3.0 Tesla Phillips scanner (Phillips Achieva, Phillips Heathcare, Best, The Netherlands) with a 8-channel head coil. The MR imaging protocol contained a T_1-weighted magnetization prepared rapid acquisition echo (MP-RAGE), a T_2-weigthed fluid attenuation inversion recovery (T_2-FLAIR), a 2D phase contrast magnetic resonance angiography (PC-MRA), and a respiratory-calibrated

dual echo pCASL sequence.[17] All sequences, except for the dual echo pCASL sequence, were performed during regular breathing.

A multislice dual-echo gradient-echo pCASL sequence was used with an echo-planar imaging (EPI) readout. Scan parameters were: EPI factor 35, matrix 80x80, FOV 240x240mm, TR/TE1/TE2: 4000/13.79/36.25 ms, and a 90° EPI readout flip angle. Eleven axial slices were prescribed, with 7 mm slice thickness and 1 mm slice gap (3x3x7 mm^3 voxel). A labelling duration of 1650 ms was used with a post label delay of 1550 ms. To ensure an optimal label efficiency, the labelling slab was positioned perpendicular to the internal carotid and vertebral arteries using a sagittal PC-MRA image as a reference. A total of 135 dynamics, equivalent to 18:15 (mm:ss) scan time, were performed. The sequence was started 15s after the start of the respiratory paradigm. The MPRAGE and T_2-FLAIR sequences were used for anatomical reference.

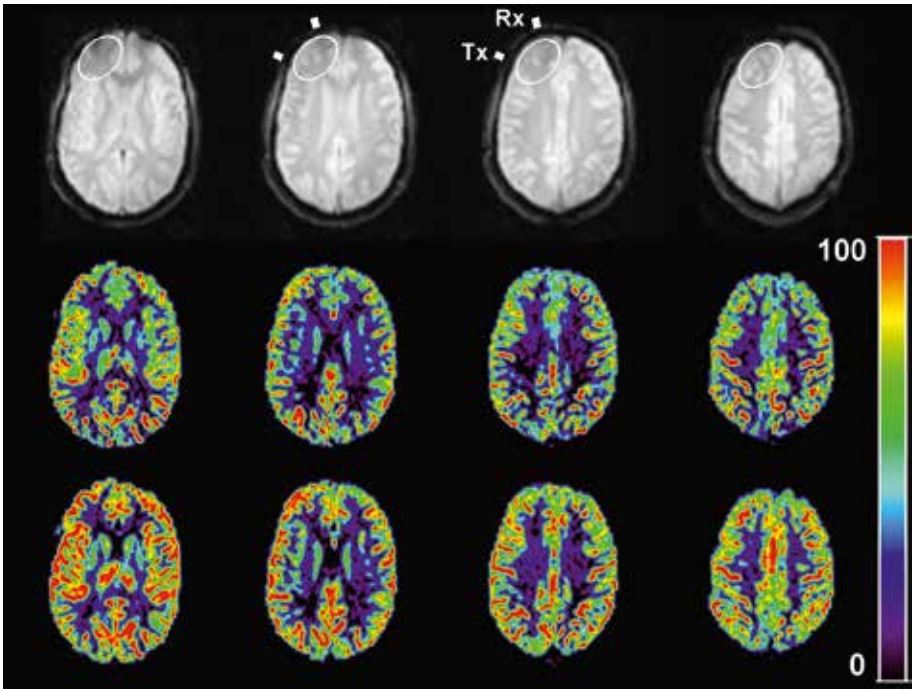
NEAR-INFRARED SPECTROSCOPY

An Oxymon Mk III continuous wave NIRS system (Artinis Medical Systems, Zetten, The Netherlands) was used to determine SO_2 (in %), and also absolute changes in O_2Hb, HHb and tHb (O_2Hb + HHb) concentration (in μM). This system uses three laser sources, each combining two wavelengths (i.e. 764nm, and 857nm), and a single receiver. The average inter-optode distance was 40 mm, with a source separation of 4 mm (δρ).

A fibre optic MR compatible probe with 10m fibres was positioned on the right side of the subject's forehead. The probe was carefully positioned to avoid contact with hair, and to avoid the measurement of cerebral venous sinuses. The probe position approximately corresponded to the Fp2 and F8 positions according to the international 10-20 EEG system (**figure 9.2**). The probe position was marked with a vitamin D capsule that was visible on MRI. The NIRS device was calibrated before each experiment to assure equal signal quality between experiments. The DPF was varied according to age.[44] NIRS data was obtained continuously during all MRI sequences.

DATA ANALYSIS

All MR data was analysed using IDL 6.1 for Windows (ITT Visual Information Solutions, Boulder, CO, U.S.A.). The paired labelled and non-labelled ASL images were surround subtracted to produce ASL subtraction (ΔM) images.[34,45] Perfusion was quantified on the ΔM images using the following assumptions: a longitudinal relaxation time of tissue of 1.5s, a longitudinal relaxation time of blood of 1.6s, and an average water partition coefficient between blood and grey-white matter (λ) of 0.91. The average labelling efficiency was set at 0.90. The fully relaxed magnetization of arterial blood (Mob) was estimated from the data. BOLD signal changes were simultaneously acquired from the second TE. Drift of the BOLD signal was removed by a quadratic fit to the baseline points.

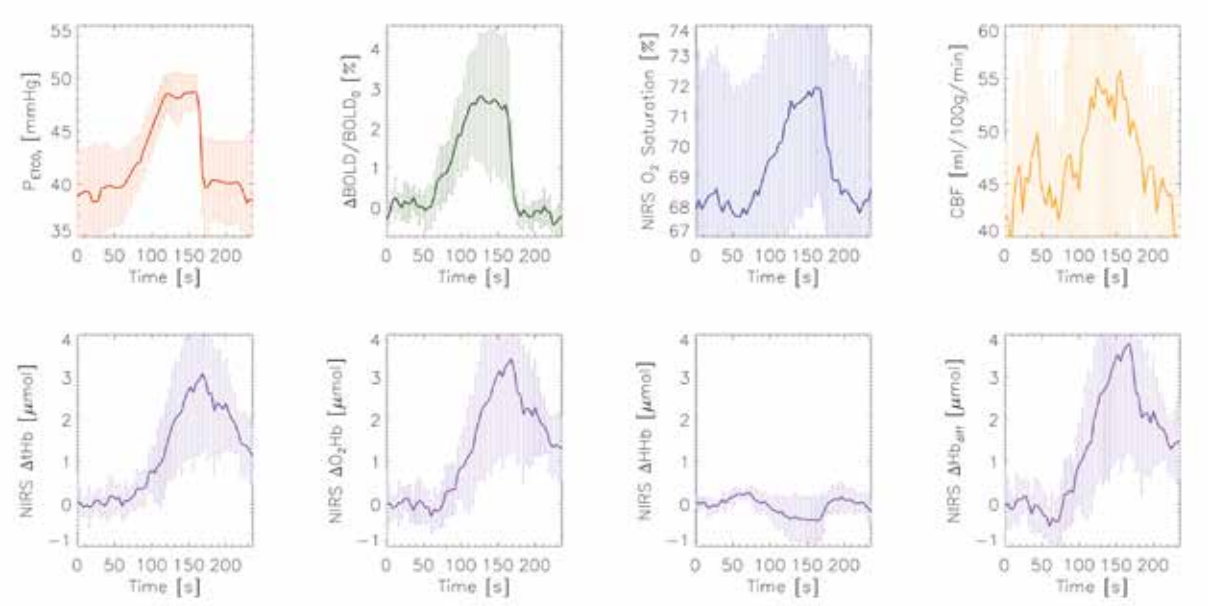


← **Figure 9.2**
Four MR slices obtained with the pCASL sequence in a representative subject. Top row: raw EPI data during baseline, middle row: CBF (in ml/100g/min) map at baseline and bottom row: CBF map at hypercapnia. Representative ROIs and a schematic representation of the NIRS optodes (second and third image from the left) are shown on the raw EPI images.

Changes in CBF (ΔCBF) and BOLD signal (ΔBOLD), from baseline to hypercapnia, were used to estimate *M* (Eq. 1). To increase the signal-to-noise ratio (SNR), and to ensure more reliable estimation of *M*, data from both hypercapnic episodes were used.[46] After the estimation of *M*, the HHb_v concentration could be calculated (Eq. 2). A temporal SO_2 was generated (Eq. 4) by combining a baseline OEF (0.4) with vCBV and tCBV estimates (Eq. 3). **Table 9.1** lists the standard physiological parameters that were used to calculate MR and NIRS derived parameters. An error analysis was performed to calculate the minimum and maximum possible values of these derived parameters. To do so, standard physiological parameters were allowed to vary in a pre-specified physiological range based on the literature (**table 9.1**).

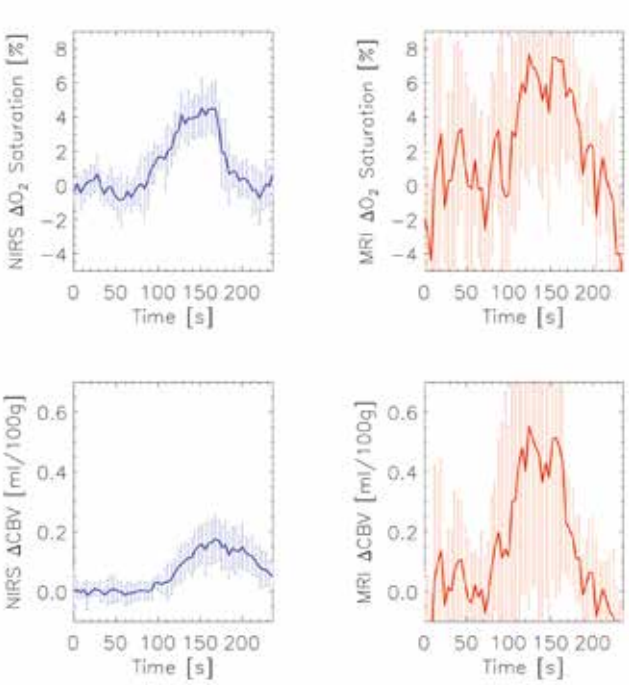
For comparison with NIRS data, regions of interest (ROIs) were manually drawn by one observer (MRicro version 1.4, www.mricro.com). The vitamin D capsule was identified on the MR images and subsequently used as a reference for drawing the ROIs. In order to obtain sufficient SNR, data from four ROIs placed on the 4 slices surrounding the NIRS optode were averaged (**figure 9.2**). For NIRS, the absolute concentration changes in O_2Hb and HHb recorded from the three different inter-optode distances (channels) were averaged.

At the time of the first pCASL dynamic, an event was inserted simultaneously on the NIRS device and the RespirAct™ to temporally align NIRS, RespirAct™, and MRI data.



↑ **Figure 9.3**
Group average with error bars of induced changes in P_{ETCO_2}, data obtained with MRI, and data obtained with NIRS.

→ **Figure 9.4**
Group average with error bars of calculated MRI (i.e. SO_2 and tCBV) and NIRS parameters (i.e. tCBV). NIRS SO_2 is shown as the absolute change in % with respect to a subject's individual baseline.



Absolute values and normalised values (normalized to an assumed change of 10mmHg P_{ETCO_2}) were calculated in order to facilitate comparison between subjects. Values of all subjects were grouped and are displayed as a group mean with error bars.

RESULTS

The subjects had a mean age of 28 years (range 25-33). Data of both hypercapnia episodes were used in all 7 subjects. **Figure 9.2** displays ASL images of a representative subject. **Figure 9.3** displays the group average of the actual measured data during the respiratory challenge both for MRI and NIRS. **Figure 9.4** presents the ΔSO_2 and ΔtCBV estimates. **Table 9.2** lists the absolute values (per subject) of all measured parameters obtained during normocapnia (NC) and hypercapnia (HC), as well as the absolute difference (NC-HC) and the values standardized to an increase in P_{ETCO_2} of 10 mmHg (CP10). **Table 9.3** presents all calculated values for the individual subjects.

Mean increase in SO_2, normalised per 10 mmHg CO_2 change, was 3.8% ($p < 0.001$) for NIRS and 11.3% for MRI ($p < 0.001$). Mean change in CBF and BOLD signal were 12.2 ml/100g/min ($p < 0.001$) and 3.2% ($p < 0.001$) per 10 mmHg CO_2, respectively. Temporally, the onset of the increase in tCBV coincided between both methods. The return to baseline was delayed in NIRS, as compared to MRI. The mean change in tCBV was higher in MRI (0.56 ml/100g vs. 0.12 ml/100g).
The BOLD signal correlated with changes in HHb ($r = -0.76$, $p < 0.0001$), O_2Hb ($r = 0.41$, $p = 0.001$), and tHb ($r = 0.32$, $p = 0.01$) concentration. NIRS SO_2 correlated with the BOLD signal ($r = 0.82$, $p < 0.0001$), CBF ($r = 0.85$, $p < 0.0001$), and MRI SO_2 ($r = 0.82$, $p < 0.0001$). P_{ETCO_2} correlated with NIRS SO_2 ($r = 0.83$, $p < 0.0001$), BOLD ($r = 0.97$, $p < 0.0001$), and CBF ($r = 0.85$, $p < 0.0001$).
Table 9.4 presents the results of the error analysis. By varying the standard physiological parameters, the MRI-based ΔSO_2 ranged from 5.9 to 14.6% per 10 mmHg CO_2. The minimum and maximum values of ΔtCBV obtained by MRI were 0.16 and 1.30 ml per 100g, respectively. The possible range of ΔtCBV obtained by NIRS was 0.07 to 0.20 ml per 100g.

DISCUSSION

This is the first study to compare cerebral oxygen saturation, and tCBV estimates between MRI and NIRS during a respiratory challenge. In addition, more conventional signal changes were compared between the two modalities (e.g. ΔBOLD vs. ΔHHb).
During a respiratory challenge, the CO_2 concentration in the breathing air can be increased. A higher CO_2 concentration increases CBF across all brain regions while

$CMRO_2$ remains relatively stable.[32,47] In contrast, during functional activation there is a relative overshoot of CBF, as compared to the increase in $CMRO_2$. Moreover, the changes in cerebral physiology following functional activation are spatially restricted to the regions of activation. Despite these differences, the two approaches have in common that there is an increase in CBF, as compared to $CMRO_2$. This makes that the manner of generating a BOLD signal change is rather comparable between the two.

In fNIRS experiments, spatial mapping tools are essential to pinpoint the origin of the changes in cerebral physiology.[6,48] In this study, a respiratory challenge with hypercapnia was used to eliminate the need for spatial mapping tools. In addition, only a single SRS NIRS probe was used. Good spatial and temporal agreements have been found in qualitative comparisons between fMRI and fNIRS.[8,25,27,49–55]

Out of the three haemoglobin species that were measured (i.e. HHb, O_2Hb, tHb), HHb had the best correlation with the BOLD signal. This has also been found by several others.[50,54–56] Conversely, the BOLD signal has been shown to have the best correlation with O_2Hb while others found no distinct difference between the haemoglobin species.[52,53] Interestingly, we initially observed a small change in O_2Hb and tHb concentrations at the onset of the rise in P_{ETCO_2}. This change was followed by an accelerated and more prolonged rise in O_2Hb and tHb concentrations.

The average amplitude of the ΔtCBV estimated by NIRS was half that of ΔtCBV estimated by MRI. The onset of the rise in tCBV was comparable between the two modalities. However, the return to baseline of NIRS tCBV was delayed.

The baseline and the temporal alignment of the SO_2 were found to be comparable between NIRS and MRI. Nevertheless, the average amplitude of the ΔSO_2 estimated by MRI was almost 3 times higher. An excellent correlation was found between NIRS SO_2 and CBF measured by MRI. In fact, out of all parameters measured by NIRS (i.e. SO_2, HHb, O_2Hb, tHb), the SO_2 was found to correlate the best both with the BOLD signal and CBF. Therefore, the use of NIRS SO_2 as a biomarker for changes in cerebral haemodynamics seems justified. It should be realised that this SO_2 is calculated from a combination of arteries, veins, and capillaries. As a consequence, changes both in the oxygen supply (i.e. arterial saturation, and CBF), and the oxygen demand (i.e. $CMRO_2$) can result in an altered SO_2.

Before final interpretation of the results can be done, several aspects regarding the used techniques and the applied models need to be discussed.

CALIBRATION OF THE BOLD SIGNAL

The Davis model was the first model to use hypercapnia for the calibration of the BOLD signal. This model relies on three main assumptions.[14] Firstly, that only extravascular BOLD effects contribute to the BOLD signal changes. Secondly, that signal changes are brought about by changes in venous oxygenation and venous

CBV.[28] Finally, that CBF will rise while $CMRO_2$ remains stable.[32,47] It has been shown that more refined models can increase accuracy.[57,58] Moreover, recent work suggests that $CMRO_2$ decreases during hypercapnia.[59] Although, the Davis model might be an oversimplification of physiology, it has been shown to be a reasonable approximation of more complex models.[57] The Davis model has also been reported to be relatively insensitive to fluctuations in common physiological parameters.[17] Recently, the use of calibrated BOLD methods in fMRI has been discussed in great detail.[60]

ESTIMATION OF THE M PARAMETER AND REGION OF INTEREST

The outcome of neurovascular coupling studies can be predicted based on the value of the calibration parameter *M*.[46] Accuracy of the estimation of *M* can be improved by using multiple hypercapnia segments, increasing the ΔCBF change, or by combining hypercapnia with hyperoxia in a single experiment.[17,18] We found a mean value of *M* of 10.9%, which is in the upper range of earlier reported values.[17,58,61,62]. Remarkably, the *M* values of subjects 1, 2, and 4 were high (i.e. 15.5%, 22.8%, and 11.7%). These three subjects also showed the lowest increase in CBF in response to hypercapnia (i.e. 1.3, 2.2, and 4.9 ml/100g/min per 10 mmHg CO_2).

As a consequence of low perfusion in white matter, ASL has a low sensitivity for detecting perfusion changes within the white matter. A partial-volume effect of white matter or CSF in the ROI could have resulted in an underestimation of CBF. An underestimation would subsequently lead to an overestimation of *M* (Eq. 1). Another factor could be a relatively large contribution of veins in the ROI. Reviewing data on the ROI selection, and pCASL data quality did not reveal any distinct differences between subjects. As such, these two factors can probably not explain the marked difference between the subjects. Nevertheless, we cannot exclude these factors from biasing the overall estimation of *M*. Due to the global effect of hypercapnia during the respiratory challenge, we do not expect that averaging of 4 ROIs (slices) per subject was a major source of bias.

SPATIAL RESOLUTION, EXTRA-CEREBRAL CONTAMINATION, AND OPTICAL FIBRES

The spatial resolution of NIRS is in the order of 1-3 cm, while the spatial resolution of (functional) MRI scanners nowadays is around 1-2 mm.[63] This difference in spatial resolution is mostly a concern when functional data is compared between the two modalities.

However, an aspect that should not be overlooked is the difference in vascular sensitivity between the two. As stated before, the BOLD signal arises both from extra- and intra-vascular compartments. Furthermore, it originates largely from tissue surrounding larger venous structures.[28,64,65] In contrast, NIRS is susceptible to all vascular compartments (i.e. arterial, capillary, and venous), with a decreased

sensitivity to vessels with a diameter above 1 mm.[66] The difference in vascular sensitivity is likely to contribute to the observed differences between the two modalities, both in amplitude and temporal alignment. In addition, the NIRS signal is obtained from all tissue layers underneath the probe. This makes NIRS prone to contamination by signal originating from extra-cerebral tissue, e.g. the pial vessels. It has been shown that tHb and O_2Hb are less affected by pial vein contribution than HHb.[25] Surprisingly, we found the best correlation between HHb and the BOLD signal.

Another possible source of bias is the penetration depth of near-infrared light, which is approximately $\frac{1}{2}$ the inter-optode distance.[63] With an inter-optode distance of 40mm, considering the thickness of the scalp and skull, the sensitivity of NIRS is limited to the most superficial regions of the brain. Variations in skull thickness, scalp thickness, and inter-optode distance could thereby influence the amount of cerebral tissue that is measured, as compared to extra-cerebral tissue.[8,63] Interestingly, stronger correlations were found between the BOLD signal and the haemoglobin species when only data of the largest inter-optode distance was used, as compared to the average of the three inter-optode distances that is reported here (data not shown).

It has been suggested that optical fibres longer than 10m give a lower SNR due to higher signal attenuation.[67] During calibration of the NIRS device, we found no difference in SNR between 3m and 10m fibres.

BASELINE ASSUMPTIONS ON STANDARD PHYSIOLOGY

The importance of the baseline assumptions is stressed by the results of the error analysis (**table 9.4**). By varying these baseline assumptions, MRI SO_2 could be closely matched to NIRS SO_2. This was also seen for the two tCBV estimates. However, instead of finding the optimal agreement between the two methods, we chose to use values on standard physiology that have been reported in similar literature on calibrated BOLD.[17,57]

CROSS TALK BETWEEN HHB AND O_2HB IN NIRS

Cross talk of O_2Hb into HHb can occur when a change in O_2Hb concentration is large, as compared to a change in HHb concentration.[52,68] There are four possible sources of error when NIRS is used to determine changes in HHb and O_2Hb concentrations.[68,69] Firstly, the absolute magnitudes and relative differences in path length factors as a function of wavelength. Secondly, the location of the change in absorption with respect to the position of the optical probe. Thirdly, possible differences in spatial distribution of the haemoglobin species (e.g. HHb). Finally, the possibility of measuring multiple regions of activation at once.

In the current study, cross talk was minimized by three elements of the study design: (i) wavelengths of 764 nm and 857 nm were selected, (ii) the DPF was set at approximately 6 in all 7 subjects by varying the DPF according to age, and (iii) a respiratory challenge

was used to induce global changes in cerebral physiology, as opposed to focal changes during functional activation. [44,52,68]

All things considered, a partial-volume effect of white matter or CSF in the ROI selection, the differences in vasculature sensitivity between the two modalities, the measurement of extra-cerebral tissue by NIRS, and the baseline assumptions could bias the results. These potential sources of bias might explain the differences in SO_2 and tCBV that were found between MRI and NIRS.

IMPLICATIONS AND DIRECTIONS FOR FUTURE RESEARCH

Two approaches can be used to quantify cerebral SO_2 by MRI. The calibration of the BOLD signal is one approach.[14,17,18] A different approach is to directly estimate SO_2 by measuring the T_2 of pure blood.[70–73] The advantages of measuring the T_2 of pure blood are that a respiratory challenge is not required, and that fewer assumptions are required. Knowledge of cerebral SO_2, estimated by either one of these approaches, could make the interpretation of the BOLD signal more straightforward.

A different application of the quantification of cerebral SO_2 by MRI is the validation of NIRS in (preterm) neonates. Most of the NIRS devices that are used in clinical settings have been validated against jugular saturations. Moreover, this has almost exclusively been done in non-neonatal populations. As a consequence, NIRS currently lacks the single time-point quantitative precision to guide treatment.[22,74] Nevertheless, NIRS can be used as a reliable individual trend monitor in the neonatal population.

Our future research will focus on the translation of the proposed framework to the neonatal population. We will also continue our efforts to develop a MRI based method for direct SO_2 assessment.[72,73]

CONCLUSIONS

This paper has highlighted that cerebral SO_2 can be obtained by using respiratory calibrated MRI. A good correlation was found between SO_2 determined by NIRS and MRI. Out of all parameters determined by NIRS (i.e. HHb, O_2Hb, tHb, and SO_2), it was found that the SO_2 had the best correlation both with CBF and the BOLD signal. These results are of importance, as absolute values play a key role in the quantitative interpretation of fNIRS and fMRI. Moreover, this initial framework for MRI SO_2 evaluation might be used for validating NIRS in neonates.

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↓ Table 9.1

Standard physiological parameters

Variable	Value	Description
ω_a	0.05	Blood volume fraction at baseline
ω_c	0.2	Arterial fraction of blood volume at baseline
ω_v	0.4	Capillary fraction of blood volume at baseline
V_0	0.4	Venous fraction of blood volume at baseline
V_a	0.016	Arterial blood volume during hypercapnia
V_c	0.021	Capillary blood volume during hypercapnia
V_v	0.022	Venous blood volume during hypercapnia
OEF	0.4	Resting oxygen extraction fraction
SaO_2	0.98	Arterial oxygen saturation
Hct	0.44	Resting haematocrit in large arteries and veins

↓ Table 9.2

Acquired data CP10 = change per 10mmHg P_{ETCO_2} increase.

Subject (Gender, Age)	P_{ETCO2} [mmHg] NC/HC	CBF [ml/100g/min] NC / HC / CP10	ΔBOLD/$BOLD_0$ [%] NC-HC / CP10	NIRS O_2sat [%] NC / HC / CP10	NIRS HHb [µmol] NC-HC / CP10	NIRS HbO_2 [µmol] NC-HC / CP10	NIRS tHb [µmol] NC-HC / CP10
1 (F, 30)	35.8 / 45.7	50.4 / 51.7 / 1.3	3.5 / 3.5	69.3 / 73.6 / 4.3	-0.71 / -0.75	3.57 / 3.64	2.86 / 2.89
2 (F, 33)	36.7 / 47.6	40.9 / 43.3 / 2.2	6.0 / 5.7	63.5 / 67.5 / 3.7	-1.21 / -1.12	5.22 / 4.82	4.00 / 3.70
3 (M, 27)	46.9 / 51.8	49.7 / 55.7 / 12.2	0.9 / 2.0	69.1 / 71.4 / 4.9	-0.24 / -0.51	1.11 / 2.47	0.88 / 1.96
4 (M, 28)	37.6 / 47.4	41.9 / 46.8 / 4.9	1.9 / 2.1	65.2 / 69.8 / 4.7	-0.09 / -0.07	2.98 / 3.02	2.89 / 2.95
5 (M, 26)	43.0 / 48.3	42.9 / 54.0 / 21.1	2.1 / 4.2	76.9 / 78.8 / 3.5	-0.38 / -0.69	1.11 / 2.03	0.73 / 1.34
6 (M, 25)	40.4 / 47.5	30.8 / 35.4 / 6.6	1.4 / 1.9	68.9 / 70.1 / 1.7	-0.41 / -0.57	0.31 / 0.34	0.71 / 0.92
7 (F, 26)	36.5 / 46.2	54.2 / 90.1 / 36.9	2.7 / 2.8	64.6 / 68.6 / 4.1	-0.18 / -0.17	3.29 / 3.38	3.11 / 3.22
Total: Mean (SD)	39.6(4.1) / 47.8(2.0)	44.4(7.8) / 53.9(17.4) / 12.2(12.9)	2.7(1.7) / 3.2(1.4)	68.2(4.5) / 71.4(3.8) / 3.8(1.1)	-0.34(0.51) / -0.39(0.55)	2.51(1.73)/ 2.82(1.41)	2.17(1.36) / 2.43(1.03)

NC = Normocapnia, HC = Hypercapnia
NC-HC = Change from normocapnia to hypercapnia.
CP10 = change per 10mmHg P_{ETCO2} increase.

↓ Table 9.3

Estimated parameters

Subject	M [%] NC-HC	MRI O_2sat [%] NC/HC/CP10	NIRS O_2sat [%] NC/HC/CP10	MRI ΔCBV [ml/100g] NC-HC/CP10	NIRS ΔCBV [ml/100g] NC-HC/CP10
1	16.6	70.4 / 75.3 / 4.9	69.3 / 73.6 / 4.3	0.19 / 0.24	0.15 / 0.15
2	24.7	71.7 / 75.2 / 3.2	63.5 / 67.5 / 3.7	0.27 / 0.23	0.19 / 0.18
3	4.8	75.3 / 77.6 / 4.9	69.1 / 71.4 / 4.9	0.20 / 0.40	0.03 / 0.08
4	12.3	71.9 / 78.9 / 7.0	65.2 / 69.8 / 4.7	0.32 / 0.33	0.14 / 0.14
5	6.7	72.4 / 80.2 / 14.6	76.9 / 78.8 / 3.5	0.57 / 1.09	0.04 / 0.07
6	5.7	72.3 / 76.7 / 6.3	68.9 / 70.1 / 1.7	0.32 / 0.47	0.03 / 0.03
7	5.5	72.1 / 83.9 / 12.1	64.6 / 68.6 / 4.1	1.12 / 1.17	0.17 / 0.16
Total: Mean (SD)	10.9(7.5)	72.3(1.5) / 78.2(3.1) / 7.6(4.2)	68.2(4.5) / 71.4(3.8) / 3.8(1.1)	0.43(0.33) / 0.56(0.40)	0.10(0.05) / 0.12(0.06)

NC = Normocapnia, HC = Hypercapnia
NC-HC = Change from normocapnia to hypercapnia.
CP10 = change per 10mmHg P_{ETCO2} increase.

↓ Table 9.4

Error analysis

	MRI ΔO_2sat		MRI ΔCBV		NIRS ΔCBV	
	Parameters	[%] CP10	Parameters	[ml/100g] CP10	Parameters	[ml/100g] CP10
Minimum within search range	α_v = 0.18 α_t = 0.28 ω_a = 0.3 OEF = 0.25	5.9	α_t=0.28 V_0=0.02	0.16	[Hb] = 18 R = 0.92	0.07
Reported	α_v = 0.18 α_t = 0.38 ω_a = 0.2 OEF = 0.4	11.3	α_t=0.38 V_0=0.05	0.56	[Hb] = 15 R = 0.69	0.12
Maximum within search range	α_v = 0.38 α_t = 0.58 ω_a = 0.1 OEF = 0.45	14.6	α_t=0.58 V_0=0.07	1.30	[Hb] = 12 R = 0.50	0.20

CP10 = change per 10mmHg P_{ETCO2} increase.

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Chapter 10

BRAIN OXYGEN SATURATION ASSESSMENT IN NEONATES USING T_2-PREPARED BLOOD IMAGING OF OXYGEN SATURATION (T_2-BIOS) AND NEAR-INFRARED SPECTROSCOPY (NIRS)

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ABSTRACT

INTRODUCTION Neurological sequelae in neonates are often related to disturbances in the cerebral oxygen and blood supply. Near-InfraRed Spectroscopy (NIRS) is increasingly being used to monitor cerebral oxygenation in neonates. However, NIRS has limited penetration depth. A newly developed, T_2-prepared Blood Imaging of Oxygen Saturation (T_2-BIOS), MR sequence provides an oxygen saturation (SO_2) estimate on a voxel-by-voxel basis in a mixed (venous-arterial-capillary) vascular bed.

MATERIALS AND METHODS In 15 neonates, who underwent MR examination for clinical reasons, brain SO_2 was measured by T_2-BIOS ($SO_{2\text{-}T2\text{-}BIOS}$) and NIRS ($rScO_{2\text{-}NIRS}$). Regions-of-interest on T_2-BIOS images were matched to the NIRS sensors, which were placed on the left and right fronto-parietal region of the head. Cerebral blood flow (CBF) was measured by means of a pulsed arterial spin labelling sequence, and venous SO_2 ($S_vO_{2\text{-}T2\text{-}TRIR}$) by means of a T_2-prepared Tissue Relaxation Inversion Recovery (T_2-TRIR) sequence.

RESULTS $SO_{2\text{-}T2\text{-}BIOS}$ correlated positively with $rScO_{2\text{-}NIRS}$ (R^2 0.643, $p < 0.001$). Both $SO_{2\text{-}T2\text{-}BIOS}$ and $rScO_{2\text{-}NIRS}$ correlated with haematocrit, and haematocrit correlated with the observed difference between these two methods (R^2 0.470, $p < 0.001$). $rScO_{2\text{-}NIRS}$ correlated with frontal CBF ($n=10$, R^2 0.712, $p < 0.001$), whole brain CBF ($n=10$, R^2 0.500, $p < 0.01$), and $S_vO_{2\text{-}T2\text{-}TRIR}$ ($n=10$, R^2 0.646, $p < 0.01$). $SO_{2\text{-}T2\text{-}BIOS}$ correlated with $S_vO_{2\text{-}T2\text{-}TRIR}$ ($n=10$, R^2 0.494, $p < 0.023$).

CONCLUSIONS Measuring SO_2 by T_2-BIOS is feasible, non-invasive, and does not require a respiratory calibration experiment. Good correlations between SO_2 estimates obtained by MR and NIRS works as a cross validation and confirms the use of both techniques for determining cerebral oxygenation.

INTRODUCTION

In neonates, brain injury is often related to disturbances in cerebral blood flow (CBF) and/or cerebral oxygenation. Examples are hypoxic-ischaemic encephalopathy following perinatal asphyxia, perinatal arterial ischaemic stroke (PAIS), peri-intraventricular haemorrhages, and periventricular leukomalacia.[1–6] Near-InfraRed Spectroscopy (NIRS) is increasingly being used on the neonatal intensive care unit (NICU) to monitor the balance between the oxygen supply and oxygen consumption of the brain. In addition it can be used as a surrogate for CBF.[7] NIRS exploits the relative permeability of biological tissue to NIR light. The combination of multiple wavelengths and multiple emitter-receiver distances enables the estimation of relative concentrations of oxygenated (O_2Hb) and deoxygenated haemoglobin (HHb), which can subsequently be converted into a regional cerebral oxygen saturation ($rScO_{2\text{-NIRS}}$).[8] The $rScO_{2\text{-NIRS}}$ is an absolute value that represents the weighted-average of O_2Hb as a ratio of total Hb (O_2Hb + HHb) concentration in a mixed vascular bed (i.e. arterial-capillary-venous). For the brain, the distribution between the arterial and a combined capillary-venous compartment is generally assumed to be approximately 25%/75%.[9,10] The obtained information depends on sensor location, in infants this usually means uni- or bilateral fronto-parietal regions.[7] Although regional information is sufficient for monitoring the trend of oxygenation and CBF in the clinical setting, knowledge of oxygen saturation (SO_2) in different regions of the brain, including deep brain matter, can be invaluable in monitoring disease progress and can possibly provide new insights into the pathophysiology of diseases.

In the neonatal population, MRI seems to be the designated tool to provide a whole brain SO_2 estimate, as MRI is non-invasive and does not require the use of ionizing radiation. Recent advances in MRI have brought forward multiple techniques to measure SO_2 in the brain. These techniques can be roughly divided into two approaches: the first is respiratory-calibration of the blood oxygen level dependent (BOLD) signal, the second approach is modelling the decay of transverse magnetization following spin-spin relaxation only (T_2) or in combination with magnetic field inhomogeneity (T_2^*).[11–14] The former is far less feasible in neonates as a respiratory challenge has both ethical and practical limitations in this population. In the latter, SO_2 quantification is based on modelling T_2 and T_2^* in a two-compartment model, in which extravascular and intravascular compartments correspond to brain tissue and blood vessels, respectively. A method that could measure blood T_2 (T_{2b}) more directly would reduce sensitivity to field-inhomogeneity, and model- assumptions needed for the estimation of Y could be limited. With appropriate knowledge of T_{2b}, SO_2 can be derived by using previously determined relationships between T_2 and SO_2.[13,15–17]

In this work we present a method which combines "Intra-voxel incoherent motion

imaging" (IVIM) to yield blood-volume-weighted images with a standard Malcolm Levitt (MLEV) T_2-preparation.[18–20] These two techniques are combined in the "T_2-Prepared Blood IVIM Imaging of Oxygen Saturation" (T_2-BIOS) sequence, which enables the estimation of T_{2b} on a voxel-by-voxel basis in a single slice of brain tissue.[21,22] The $SO_{2\text{ - }T2\text{-BIOS}}$ derived from T_{2b} should in theory correspond to SO_2 in a mixed (arterial-capillary-venous) vascular bed, comparable to the $rScO_{2\text{-NIRS}}$ obtained by NIRS. For example, QUIXOTIC would be less suitable for the comparison with NIRS, as it is mostly targeted at post capillary venular blood and thus provides a pure venous estimate.[23] In addition it suffers from low a low signal-to-noise ratio (SNR), which is particularly a concern in neonates. Inclusion of the entire vasculature as proposed here could significantly improve SNR.

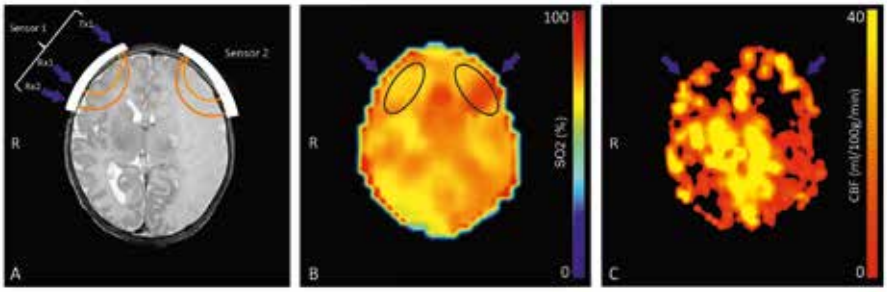
The primary aim of this study was to assess feasibility of measuring SO_2 in neonates by means of T_2-BIOS and subsequently investigate its association with $rScO_{2\text{-NIRS}}$ obtained by using a commercially available NIRS device.

METHODS

The ethics committee of the University Medical Center Utrecht, which adheres to the "Medical Research Involving Human Subjects Act (WMO)", approved this retrospective study and waived the requirement to obtain written parental informed consent for the use of data for research purposes (protocol 15-568/C). A total of 15 infants admitted between October 2011 and March 2012, in whom NIRS monitoring was performed as part of standard clinical care and who subsequently underwent an MRI examination for clinical indications, were included in this study. One infant with PAIS was examined twice, yielding 16 datasets for analysis. Basic clinical characteristics are shown in **table 10.1**.

NIRS MEASUREMENTS

The $rScO_{2\text{-NIRS}}$ was monitored for at least 15 minutes directly before and directly after the MR examination, during which the infants were already sedated and still sedated, respectively. Monitoring was performed by using a 2-distance (i.e. 30mm and 40mm) and 2-wavelength (i.e. 730 and 810 nm) NIRS device (INVOS 5100c, Covidien, Mansfield, MA, USA) in combination with a small-adult sensor (SAFB-SM, Covidien, Mansfield, MA, USA). This device uses a single light emitting diode and two distant sensors to calculate the $rScO_{2\text{-NIRS}}$ (**figure 10.1**, panel A). Two sensors, one placed on the left and one placed on the right fronto-parietal region of the head, were fixated by using an elastic bandage. The sensors were removed before the MR examination, and the position was marked with a vitamin D capsule to visualize the sensor position on structural MR images, and to assure a comparable sensor placement after the MRI.



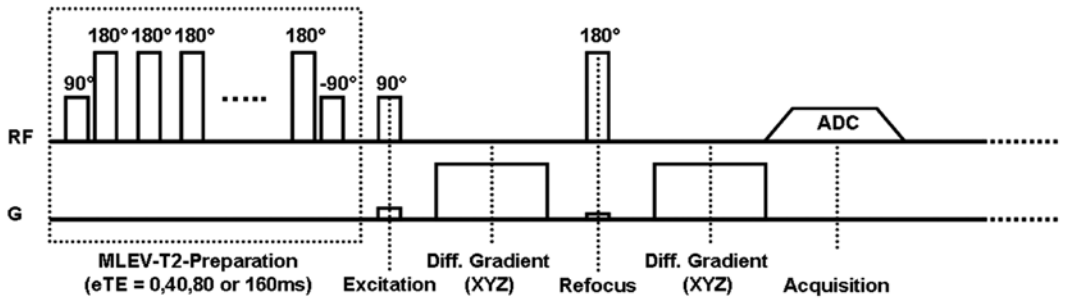
← **Figure 10.1** Neonate with an infarction of the left medial cerebral artery: A) Conventional T_2 weighted image with schematic of the two NIRS sensors. The blue arrows indicate the light emitter (Tx_1) and two detectors (Rx_1+Rx_2). The orange arches indicated the presumed path that light travels through the brain; B) $SO_{2\text{-}T_{2\text{-}BIOS}}$ map with ROIs and blue arrows indicating the centre of the NIRS sensors; C) a CBF map with blue arrows indicating the centre of the NIRS sensors.

MR-IMAGING

All MR-imaging was performed on a 3.0 Tesla system (Philips Healthcare, Best, The Netherlands) with a quadrature body coil for transmission and an 8-element phased-array SENSE head coil as a signal receiver. Preterm born infants scanned at term-equivalent age (TEA) were sedated by chloralhydrate (50-60mg/kg, oral). Term infants were sedated by an intramuscular injection which combines pethidine (2mg/kg), chlorpromazine (0.5mg/kg) and promethazine (0.5mg/kg). This injection was given after local application of lidocaine/prilocaine cream for 45 minutes to anesthetize the skin and while giving some oral sucrose for comfort. A vacuum cushion was used to provide comfort and to minimize motion during imaging. MiniMuffs (Natus Europe, Münich, Germany) and closed headphones (Philips Achieva, Philips Healthcare, Best, The Netherlands) were used for noise insulation. Heart rate and arterial oxygen saturation were monitored by pulse-oximetry (Nonin Medical, Plymouth, MN, USA) and respiratory rate by an abdominal transducer (Philips Healthcare, Best, The Netherlands). A neonatologist was always present throughout the examination.

Conventional MR imaging included a sagittal T_1-weighted, and either coronal (i.e. preterm infants at TEA) or axial (term infants) T_1- and T_2-weighted sequences. Thereafter, T_2-BIOS, Pulsed Arterial Spin Labelling (PASL), and "T_2-prepared Tissue Relaxation Inversion Recovery" (T_2-TRIR) sequences were performed.[16,21,22]

In the T_2-BIOS sequence, blood-volume-weighted images are obtained by exploiting IVIM effects. Perfusion-related parameters obtained by IVIM imaging have previously been compared with cerebral blood volume (CBV) and CBF, and showed reasonable agreement with each other.[18,19] The key element for reliable fitting of T_{2b} on the blood volume weighted images is T_2-preparation of the longitudinal magnetization by using a MLEV-preparation.[15,20] Two schemes were applied to minimize imperfections in the T_2-preparation pulses: composite pulses were used for both the 180° (i.e. $90°_x 180°_y 90°_x$) and -90° (i.e. $270°_x$-$360°_x$) pulses, and the signs of the pulses were then arranged in a MLEV pattern (i.e. 1 1 -1 -1). This preparation is repeated in groups of 4 (**figure 10.2**), corresponding to 0, 4, 8, or 16 refocusing pulses with an inter-pulse time (τ_{CPMG}) of 10ms. These groups of refocusing pulses translate into effective MLEV TE (eTE)



↑ **Figure 10.2**
MR sequence chart of the T_2-BIOS sequence

preparations of 0, 40, 80 or 160 ms. After T_2-preparation, a standard Stejskal-Tanner diffusion sequence is played out in one of x,y or z directions, and low and higher b-value (i.e. 0 and 50 s/mm^2) images are acquired in all desired directions for each eTE. Magnetization is allowed to fully recover between repetitions. Scan parameters were: TR 8000ms, TE 41ms, 64x64 matrix, FOV=240x240 mm^2, flip-angle=90°, 6mm slice thickness, SENSE=2.5, eTE=0,40,80 and 160ms, b=0 and b=50 s/mm^2 in x,y,z directions. A single slice was prescribed and positioned based on the vitamin D capsules placed on the forehead. Total scan time was 4:32 min.

The T_2-TRIR sequence enables simultaneous measurement of T_{1b} and T_{2b} of venous blood in the sagittal sinus.[16] It uses a pre-saturation pulse with subsequently a MLEV T_2-preparation scheme and a non-selective inversion pulse thereafter. Scan parameters were: TR 15 s, TE 20 ms, ΔTI 140 ms, TI$_1$ 20 ms, scan matrix 128 × 128, FOV 160 × 160 mm^2, flip angle 95°, slice thickness 3mm, SENSE=2.5, eTE= 0,40,80 and 160 ms, and total scan time was 1:30 min. A sagittal 2D phase contrast MR angiography was used to make sure that the imaging plane was planned perpendicular to the sagittal sinus.

For PASL, a PULSAR pulse sequence was used.[24] Scan parameters were: TR 2500, TE 20ms, matrix 64 × 64, FOV 240 × 240 mm^2, SENSE 2.5, slice thickness 6mm, 1 mm slice gap, inversion delay (TI) 1500 ms, 70 averages, EPI factor 35, Q2TIPS 600 ms, labelling slab of 150mm, and the total scan time was 3:05 min.[25] The number of slices varied from 8 to 11 according to the head size of the infant and the labelling slab was aligned parallel to the imaging plane with a gap of 10mm.

DATA ANALYSIS

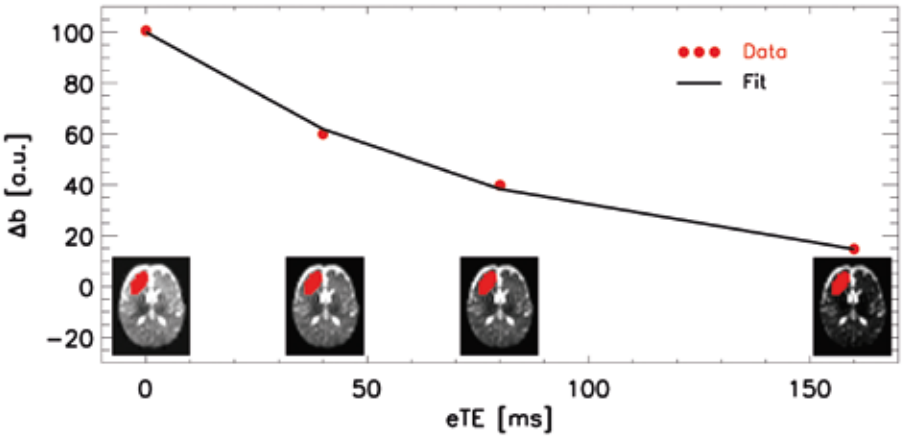
Data analysis was performed by using IDL 6.1 for Windows (ITT Visual Information Solutions, Boulder, CO, USA). For T_2-BIOS, the signal from the high b-value scans was first subtracted from the low b-value scans (i.e. b=0 and b=50 s/mm^2), then the individual diffusion directions were averaged, essentially forming a Δb image per eTE.

These images are strongly vascular weighted as long as the higher b-value is chosen within the IVIM regime (i.e. < b ≈ 150 s/mm²). The higher b-value of 50 s/mm² was chosen as a compromise between SNR and inclusion of static tissue in the Δb images. As the blood flow velocity is in general lower in neonates than in adults, a higher b-value ensures inclusion of the entire blood pool (incl. the smallest capillaries) in neonates and thereby yields a higher SNR. A b-value higher than 50 mm/s² was considered undesirable because of increasing signal contributions coming from static tissue.
The relationship between T_{2b} and the eTE of every Δb image can be described as follows:

EQUATION 1: $$\Delta b(eTE) = V_b * M_{0b} * e^{-\frac{eTE}{T_{2b}}}$$

Here, eTE is the effective MLEV echo time, T_{2b} the T_2 of blood, and $V_b * M_{ob}$ is a pseudo blood volume times bloods equilibrium magnetization, which can be considered as a single constant for this purpose. Alternatively, with appropriate knowledge of M_{ob}, a pseudo blood volume can be obtained.[19] The four eTE's allow robust fitting of T_{2b} on a voxel-by-voxel basis. Earlier work determined the relationship between T_{2b}, Hct, and SO_2 by fitting data to a two-compartment exchange model proposed by Wright et al. and Golay et al.[13,26,27]:

EQUATION 2: $$\frac{1}{T_{2b}} = A + B * (1- SO_2) + C * (1- SO_2)^2$$
$$A = a_1 + a_2 * Hct + a_3 * Hct^2$$
$$B = b_1 * Hct + b_2 * Hct^2$$
$$C = c_1 * Hct * (1-Hct)$$



← **Figure 10.3** Δb images for each eTE (i.e. 0, 40, 80, and 160ms), with a single ROI and corresponding curve fit for an exemplary subject.

When using the characterized relationship between T_{2b}, Hct, and SO_2, the SO_2 can be estimated by combining T_{2b} from T_2-BIOS with Hct obtained either by T_2-TRIR or from a recent blood sample in case T_2-TRIR data was unavailable.[13,16,17,28] Two regions-of-interest (ROI), one in the left and one in the right frontal lobe, were drawn manually (MRIcro version 1.4, www.mricro.com) for the comparison with $rScO_{2\text{-NIRS}}$ (**figure 10.1B**). Care was taken to avoid inclusion of central (i.e. ventricles) and peripheral cerebro-spinal fluid (CSF) in the ROI as much as possible. **Figure 10.3** shows the four Δb(eTE) images and corresponding curve fit for a single ROI in a representative subject.

For the T_2-TRIR an automated localizer tool was used to identify the blood signal in the sagittal sinus. From this signal four different inversion recovery curves were fitted, one for each eTE, from which T_{1b} and T_{2b} were fitted simultaneously.[16] The T_{1b} can be used to obtain haematocrit [Hct], and in a way similar to the T_2-BIOS, the T_{2b} can then be converted into venous oxygen saturation ($S_vO_{2\text{ - T2-TRIR}}$), see Eq 2.[28]

To calculate CBF, ASL images were motion corrected by using a 6 parameter affine transformation between (control- label) image pairs followed by a 12 parameter affine transformation to align all control-label volumes. The ASL imaging pairs were subtracted to generate ΔM images. The mean and standard deviation of the difference signal over the subtracted images pairs was calculated and image pairs with a difference signal larger than 2 standard deviations were automatically discarded.[29] Finally, CBF was quantified on the average ΔM images.[25,30] The inversion efficiency (α) was assumed to be 0.95, and the brain–blood partition coefficient (λ) 1.1 mL/g.[31] The T_{1b} was obtained by T_2-TRIR or derived from a recent blood sample when T_2-TRIR data was unavailable.[16,28] Subsequently, CBF was calculated both using a whole brain mask (wbCBF) and averaged over several ROIs drawn in the frontal lobes (fCBF).[32].

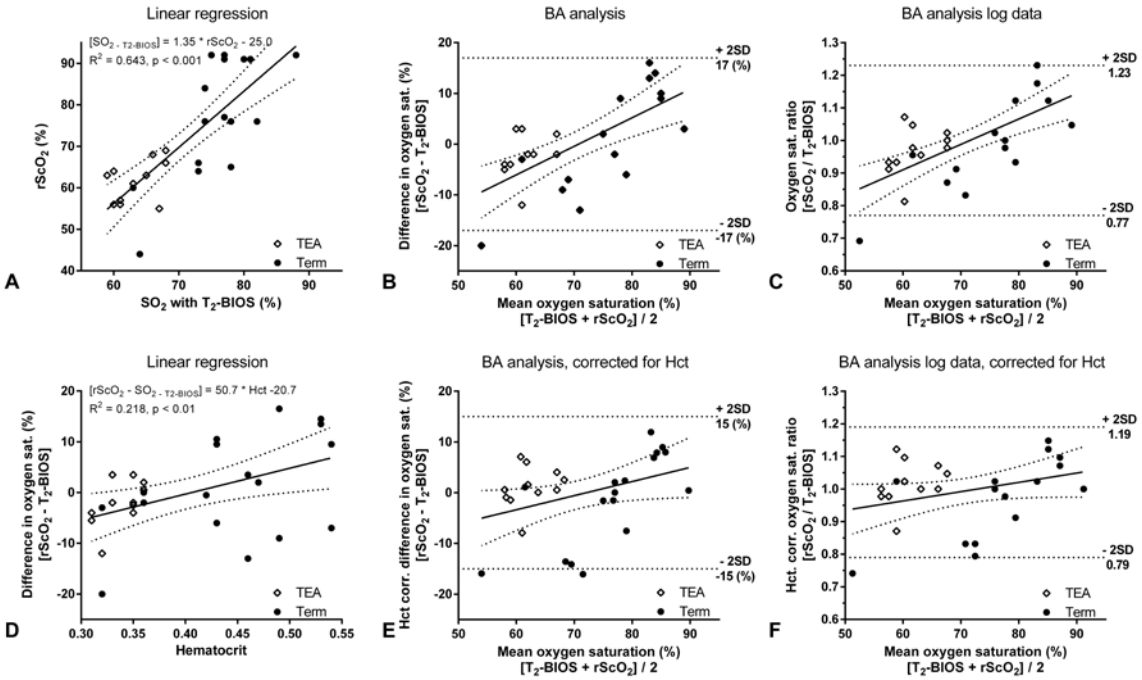
For statistical analysis, IBM SPSS Statistics (v22, SPSS Inc, Chicago, IL) was used. Linear regression and Bland-Altman plots were used for comparison of left and right $rScO_{2\text{-NIRS}}$ values with left and right $SO_{2\text{ - T2-BIOS}}$.[33,34] For the comparison with $S_vO_{2\text{ - T2-TRIR}}$ in the sagittal sinus, left and right $rScO_{2\text{-NIRS}}$ values were averaged. For comparison of $SO_{2\text{ - T2-BIOS}}$ with $S_vO_{2\text{ - T2-TRIR}}$, values obtained from left and right ROI's on T_2-BIOS images were averaged. A p-value <0.05 was considered statistically significant.

As both oxygen saturation estimates are influenced by Hct to a different degree, the influence of Hct on the bias between the two methods was investigated.[13,17,35] The actual bias between the two methods was corrected for variations in Hct, thereby it was avoided to confer that status of a gold-standard on either one of the two methods. The slope derived from linear regression analysis between Hct and the bias (**figure 10.4**, panel D) was used to calculate a correction factor per subject. This was accomplished by subtracting the reference Hct (set at a common value of 0.4) from the patient's actual Hct, the difference was then multiplied by the earlier derived slope, and the product was subsequently deducted from the actual bias between the two methods in

that patient. Thereafter, a conventional Bland-Altman plot was created.
On top of the influence of Hct, simulations were run to investigate the possible error in $SO_{2\text{ - T2-BIOS}}$ arising from the height of the upper b-value, and possible partial-volume-inclusion of CSF in the ROI.

RESULTS

Hct was significantly different between preterm infants scanned at TEA and term infants (med 0.34 , IQR [0.32-0.36] vs. 0.47 [0.43-0.52], p <0.0001). Left and right $SO_{2\text{ - T2-BIOS}}$ were obtained in all 16 examinations. In addition, $S_vO_{2\text{ - T2-TRIR}}$ data was successfully obtained in 10, and CBF data also in 10 examinations. The $rScO_{2\text{-NIRS}}$ values obtained before and after the MRI were comparable (mean pre-MRI 68.4%, SD±12.9% vs. post-MRI 70.3% ± 15.8%, p = 0.167) and there was an excellent correlation between values obtained before and after the MRI (R^2 0.857, p <0.0001). Therefore, values obtained before and after the MRI were averaged. **Figure 10.1** shows a schematic of the sensor placement, a representative $SO_{2\text{ - T2-BIOS}}$ map, and a CBF map in an infant with PAIS. In this infant, NIRS revealed asymmetric oxygen saturation with a $rScO_{2\text{-NIRS}}$ of 91% on the left and 67% on the right, and $SO_{2\text{ - T2-BIOS}}$ of 86% and 71%, respectively
Figure 10.4 panel A displays the correlation between $rScO_{2\text{-NIRS}}$ and $SO_{2\text{ - T2-BIOS}}$. Bland-Altman analysis between $rScO_{2\text{-NIRS}}$ and $SO_{2\text{ - T2-BIOS}}$ revealed an average bias of 0.3 % (95% limits of agreement -17% to 17%), which was found to be significantly different between preterm infants scanned at TEA and term infants (median -4 [-7; 1] vs. 2 [-4; 7], p = 0.03). There was a linear association (R^2 0.454, p<0.001) between the difference and the average of the two methods (**figure 10.4**, panel B). Log-transformation of the data did not remove this association (**figure 10.4**, panel C; R^2 0.462, p <0.001).[33,34] The average ratio between the two was 1.0 (95% limits of agreement 0.77 to 1.23), indicating that bias varies between 23% under and 23% overestimation (**figure 10.4**, panel C). The highest discrepancy (i.e. absolute difference -20%) was observed in an infant who had a unilateral enlarged ventricle on that side, this infant also had a low Hct of 0.32.
Both $rScO_{2\text{-NIRS}}$ (R^2 0.492, p<0.001) and $SO_{2\text{ - T2-BIOS}}$ (R^2 0.470, p<0.001) were significantly related to Hct. The difference between the two methods correlated positively with Hct (**figure 10.4**, panel D). The correlation between this difference and Hct showed a positive trend when evaluating preterm infants scanned at TEA (R^2 0.304, p = 0.08) and term infants (R^2 0.226, p = 0.05) separately. The bias after correcting for Hct was 0.2% (95% limits of agreement -15 to +15%). Although less, there was still a significant linear association between the difference and the average of the two methods (R^2 0.174, p=0.02), see **figure 10.4**, panel E. After log-transformation the mean ratio between the two was 1.0 (95% limits 0.79-1.19) and there was no significant relation anymore between the mean and the ratio of the two methods (R^2 = 0.106, p = 0.09; **figure 10.4**

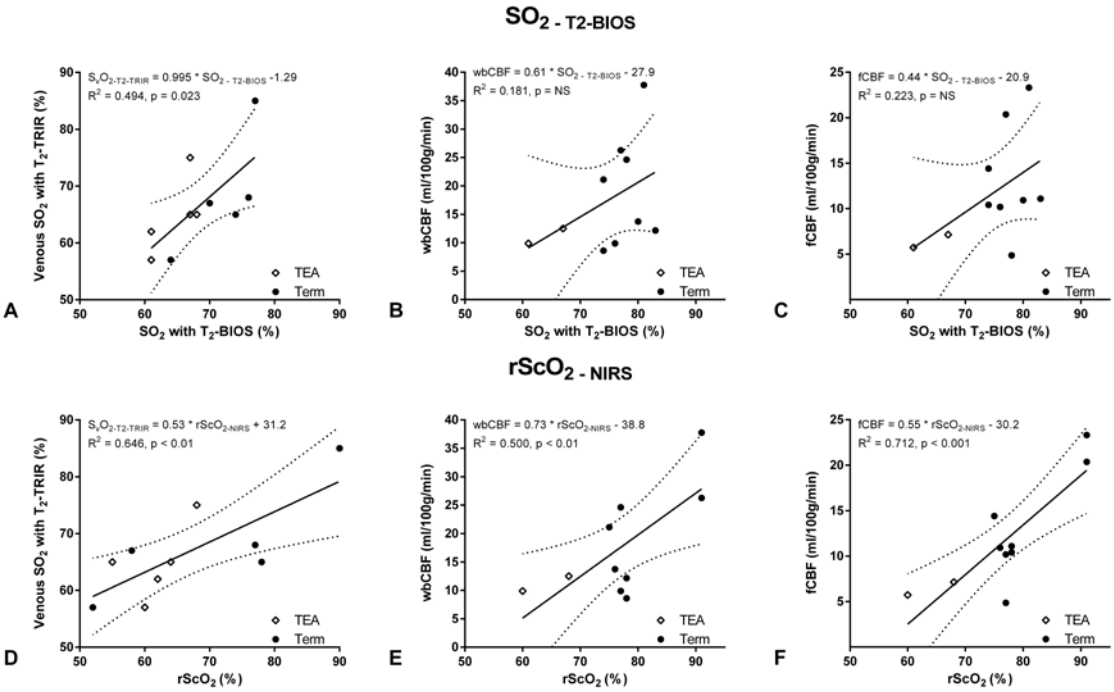


↑ **Figure 10.4**
A) Linear regression plot between $SO_{2\text{ - T2-BIOS}}$ and $rScO_{2\text{-NIRS}}$, B) Bland-Altman plot with regression line between the average and difference between the two methods, C) Bland-Altman plot of log-transformed data as mean vs. ratio's, D) Linear regression plot between haematocrit (Hct) and the observed difference bias between $SO_{2\text{ - T2-BIOS}}$ and $rScO_{2\text{-NIRS}}$, E) Bland-Altman plot where bias data was corrected for Hct variation, and F) Bland-Altman plot of log-transformed data where the bias was corrected for Hct variation.

panel F). The results of the error-analysis on the effect of potential CSF inclusion in the ROI and the effect of the chosen higher b-value are presented in **table 10.2**. This is done for two different SO_2 levels while fixing Hct at 0.4. **Figure 10.5** displays the results of linear regression analysis between $SO_{2\text{ - T2-BIOS}}$ (panels A-C) or $rScO_{2\text{-NIRS}}$ (panels D-F) on one end and $S_vO_{2\text{ - T2-TRIR}}$, wbCBF, and fCBF on the other end.

DISCUSSION

We present results obtained with a new MR sequence that enables estimation of cerebral oxygen saturation on a voxel-by-voxel basis in a full slice of brain tissue. Although NIRS and T_2-BIOS are clearly two completely different techniques, the results show that frontal cerebral oxygen saturation measured by NIRS and T_2-BIOS



↑ **Figure 10.5**
Scatter plots with regression lines between $SO_{2\text{ - T2-BIOS}}$ and A) $S_vO_{2\text{ - T2-TRIR}}$, B) whole brain CBF and C) frontal brain CBF, and scatter plots between $rScO_2$ and D) $S_vO_{2\text{ - T2-TRIR}}$, E) whole brain CBF and F) frontal brain CBF.

are strongly correlated. This shows that estimating SO_2 by MRI is feasible in neonates, and to a limited extent also works as a two-way validation that confirms that both NIRS and T_2-BIOS can be used as a measure of cerebral oxygenation.

There is, however, bias between the methods for which there are several possible explanations. It is striking that there is a linear association between the bias (i.e. $rScO_{2\text{-NIRS}}$ - $SO_{2\text{ - T2-BIOS}}$) and the average SO_2 (i.e. [$rScO_{2\text{-NIRS}}$ + $SO_{2\text{ - T2-BIOS}}$]/2) of both methods (**figure 10.4B**). This persisted after log-transformation of the data.[33,34] The three most likely explanations for this linear association seem to be related to: Hct/Hb, partial-volume-inclusion of CSF in the ROI, and the selected upper b-value. First of all, Hct is known to be quite variable in neonates. After birth neonates experience a physiological decrease in Hct, partly because the production of adult Hb cannot keep up with the degradation of foetal Hb.[36] This decline is even more pronounced

in preterm neonates due to haemodilution following rapid body growth, smaller iron reserves, the liver being the main erythropoietin producing organ, and a shorter erythrocyte lifespan.[37,38] The $rScO_{2\text{-NIRS}}$ is known to be associated with Hb and thereby Hct.[35] Likewise, the very framework that forms the foundation for estimating $SO_{2\text{ - T2-BIOS}}$ also depends on Hct, for example overestimation of Hct would result in underestimation of T_{2b}.[13,17] Interestingly, correction for Hct of the data removed the statistical significance of the linear association after log-transformation (**figure 10.4C** vs. **10.4F**), but it only marginally decreased the limits of agreement (**figure 10.4B+C** vs. **figure 10.4E+F**). In this study, the calibration curve for adult haemoglobin ,that relates $T2_b$ to SO_2 and Hct, was used as opposed to the published curve for foetal blood.[13,17] This was done because many infants were examined multiple weeks after birth and because all infants received multiple erythrocyte transfusions before the MR examination. Neonates have a circulating volume of approximately 80ml/kg, and a single transfusion (i.e. 20ml/kg) already introduces significant amounts of adult Hb on top of the physiological breakdown of foetal Hb. It is unlikely that the neonates still had a significant amount of foetal type haemoglobin present in their circulation during the MR examination and therefore the use of the curve based on the adult haemoglobin is warranted. Nevertheless, we do recommend the use of the foetal curve in relatively healthy neonates who are examined shortly after birth. Other explanations for the linear relation between the bias and the average SO_2 could be CSF inclusion in the ROIs and the selection of the upper b-value.

The error-analysis (**table 10.2**) indeed demonstrates that these two factors could very well explain the bias and the linear trend associated with SO_2. The initial T_2-BIOS implementation presented in this paper does not include CSF suppression pulses. Despite the chosen b-values (i.e. 0 and 50 mm/s^2), CSF still shows up on the Δb images (**figure 10.3**) as it has a high diffusion coefficient. Therefore, a partial-volume-effect of CSF cannot be excluded and would cause overestimation of SO_2 at lower levels of oxygenation, while it compensates the negative bias at higher levels of oxygenation. However, it seems unlikely to be a major source of bias, as great care was taken to avoid CSF while drawing the ROIs. The ventricles were avoided at all times and when we purposely included as much peripheral CSF as possible in the ROI, the %CSF in the ROI was 7.8% at most. Please note that the deliberate inclusion of CSF was only done to estimate the absolute worst case scenario and these ROIs were not used in analysis. Finally, the chosen b-values (i.e. 0 and 50) should ensure vascular-weighting to be based on all but the capillaries with the lowest flow (mostly the smallest capillaries on the venous side). The upper b-value is a compromise between obtaining adequate SNR and avoiding static tissue contributions. A higher b-value will include more static tissue in the Δb-images. As tissue T_2 is relatively constant between individuals, a larger inclusion of tissue would result in decreased variation in T_2, and thus $SO_{2\text{ - T2-}}$

$_{BIOS}$, arising from actual oxygenation differences. Reduction of the upper b-value (**table 10.2**), combined with an improved readout to boost SNR, could reduce the bias that is theoretically present due to the currently implemented upper b-value of 50 mm/s$_2$.

A less likely source of bias is the variation in CBF, which could potentially influence $SO_{2 - T_2\text{-BIOS}}$ estimates as vascular weighting is based on flow velocity. A low CBF, either global or regional, would result in lower SNR and could bias the $SO_{2 - T_2\text{-BIOS}}$ estimates. This is important when comparing data obtained in preterm infants to data obtained at term/TEA, as CBF is known to increase with age.[32] In the current study, all infants were either examined near term or at TEA, making bias arising from age-related variations in CBF unlikely. We did not find a clear relation between post-menstrual age at scan and CBF in the current population (data not shown). We can, however, not exclude pathological conditions associated with differences in CBF, such as hypoxic-ischaemic encephalopathy and PAIS, from influencing the results.[1,39,40]

The largest bias (i.e. $rScO_{2\text{-NIRS}} - SO_{2 - T_2\text{-BIOS}} = -20\%$) was shown on the ipsilateral side in the infant with unilateral ventricular dilatation. We can only speculate on the actual cause of this bias. Although the selected upper b-value should ensure adequate vascular weighting even in pathological conditions, one explanation could be that increased intra-ventricular pressure reduced the overall/regional blood pool and thereby SNR.[41,42] Unfortunately, no CBF data is available due to severe motion during the PASL sequence. In terms of actual flow velocity, the venous flow will suffer the most in case of increased intra-ventricular pressure, as the venous system has a lower pressure and lower flow velocity to begin with. A change in blood volume should be picked up by NIRS and T_2-BIOS in a similar way.[43,44] However, an impact on actual flow velocity theoretically affects the contribution of venous blood to the T_2-BIOS signal and therefore could yield a higher $SO_{2 - T_2\text{-BIOS}}$ estimate. NIRS on the other hand, is not influenced by the actual flow velocity of the blood. That being said, NIRS can by no means be seen as a gold-standard and the large bias could just as well have arisen from there. It has been shown that the extent of ventricular dilatation affects the $rScO_{2\text{-NIRS}}$ estimate.[45,46] Thus far it remains unresolved if this reflects real changes in oxygenation, or if this is a measurement error. An increased CSF fraction on the dilated side is another possible explanation. However, review of all available data (e.g. T_1, DWI, potential partial-volume-inclusion of CSF in the ROIs) did not reveal any apparent differences between the left and right hemisphere, except for the dilated ventricle.

The approach for vascular weighting chosen here is the strength of the proposed method, as it reduces sensitivity to field inhomogeneity, requires relatively few assumptions, and increases SNR by using the entire blood pool. Moreover, the $rScO_{2\text{-NIRS}}$ and $SO_{2 - T_2\text{-BIOS}}$ should be very comparable in terms of vascular compartments as both methods will include arterial and venous compartments in their estimates. NIRS most likely includes more of the smallest capillaries, and a bit less of the larger vessels

as light gets trapped in them.[47] NIRS is also slightly biased due to scalp contributions, which should have minimal effect in neonates as the scalp is still thin.[48–50] The T_2-BIOS does not suffer from scalp contributions at all, but on the other hand might lack some sensitivity to the smallest venous capillaries (i.e. lowest flow velocity), as discussed in the previous paragraphs. Altogether, we believe that differences in vascular compartments are a minimal source of bias.

Besides the correlation with $rScO_{2\text{-NIRS}}$, $SO_{2\text{ - T2-BIOS}}$ was also found to be strongly correlated with $S_vO_{2\text{ - T2-TRIR}}$, which is based on the same principle of estimating SO_2 from T_{2b}.[51,52] In a way, the T_2-TRIR uses a less complicated approach as it relies on selection of a ROI in the sagittal sinus instead of vascular weighting to obtain T_{2b}. The fact that $SO_{2\text{ - T2-BIOS}}$ has a good relation with $S_vO_{2\text{ - T2-TRIR}}$ strengthens the confidence in the more complicated approach of the T_2-BIOS even further. As expected, the $S_vO_{2\text{ - T2-TRIR}}$ values are slightly lower than $SO_{2\text{ - T2-BIOS}}$, as T_2-TRIR is strictly venous because of the ROI selection in the sagittal sinus.

As far as NIRS is concerned, the strong correlations between $rScO_{2\text{-NIRS}}$ on one hand, and $SO_{2\text{ - T2-BIOS}}$, $S_vO_{2\text{-T2-TRIR}}$, fCBF and wbCBF obtained by MRI on the other hand, enforces the use of NIRS both as a measure of cerebral oxygenation and as a surrogate measure for CBF. The correlation between $rScO_{2\text{-NIRS}}$ and CBF has been demonstrated in neonates before.[40] The $rScO_{2\text{-NIRS}}$ has also been shown to correlate with SO_2 obtained by means of a respiratory-calibrated MRI experiment.[11] However, a direct comparison with cerebral oxygenation obtained by MRI without requiring a respiratory calibration, as reported here, is novel.

Despite possible sources of bias, results of NIRS and $SO_{2\text{ - T2-BIOS}}$ are strongly correlated, which strengthens confidence in both techniques. Knowing SO_2 in the brain on a voxel-by-voxel basis can have both technical and clinical applications. From a technical point of view, T_2-BIOS might be used to validate NIRS in neonates. Currently, a true gold-standard for cerebral oxygen saturation measurements in neonates is lacking. NIRS devices intended for neonatal use have mostly been validated against values obtained from extra-corporal membrane oxygenation machines, or against jugular bulb saturations during cardiac procedures.[53,54] A different research area where the T_2-BIOS sequence could be of use is fMRI, as fMRI results are not only modulated by CBF, but also by SO_2.[55,56] From a clinical point of view, the T_2-BIOS sequence provides an oxygenation parameter without the need for a respiratory-calibration experiment, which makes it feasible to use in neonates. This could yield additional insight into the pathophysiology of certain diseases / types of brain injury. In addition, the T_2-BIOS might be used for prognostic purposes and help to decide whether or not to start additional treatment to try and save potentially viable tissue. In PAIS for example, it is very relevant to know if there is tissue that has been injured but is still potentially viable (i.e. the penumbra).[1] For this purpose, the $SO_{2\text{ - T2-BIOS}}$ could be overlaid on diffusion

weighted images. Regions with restricted diffusion but low $SO_{2\text{ - }T_2\text{-BIOS}}$ (i.e. high oxygen extraction) would suggest potentially viable tissue, whereas regions with actual dead tissue would show restricted diffusion and high $SO_{2\text{ - }T_2\text{-BIOS}}$ as a consequence of decreased O_2 utilization.[57] In the former, efforts to save the still viable tissue might be helpful, while it might not be worthwhile in the latter.

CONCLUSION

We demonstrate the feasibility of measuring cerebral oxygen saturation on a voxel-by-voxel basis in a mixed vascular bed by using a new MRI sequence, the T_2-BIOS. The great advantages are that it has relatively high SNR as takes the entire blood pool into account, and it does not require a respiratory-calibration experiment, which makes the technique also applicable in neonates. The good correlation between estimates obtained by NIRS and MRI confirms the use of both techniques for determining cerebral oxygenation, as the different underlying frameworks yield comparable results. Future research will focus on the exploration of clinical applications of the T_2-BIOS, as well as on the implementation of CSF suppression pulses and full brain coverage into the sequence.

↓ **Table 10.1**
Basic clinical characteristics

	N	GA birth med [IQR]	PMA at MRI med. [IQR]	Reason for MRI	Hematocrit med. [IQR]
TEA	6	26^{+4} [25^{+6}-28^{+3}]	41^{+0} [40^{+4}-41^{+2}]	GA <30w	0.34 [0.32-0.36]
Term	9				
HIE	5	40^{+2} [39^{+2}-40^{+6}]	40^{+6} [39^{+6}-41^{+2}]	HIE	0.43 [0.40-0.50]
PAIS	2	$37^{+1,a}$, 40^{+0}	$37^{+6,a}$, $38^{+6,a}$, 40^{+5}	PAIS	0.42[a], 0.41[a], 0.46
Other	2	36^{+6}, 39^{+5}	37^{+5}, 44^{+5}	Seizures[b]/ PHVD	0.42; 0.32

GA: gestational age in weeks^{+days}, HIE: Hypoxic-ischemic encephalopathy, IQR: inter-quartile range, PAIS: perinatal arterial ischemic stroke; PHVD: post-hemorrhagic ventricular dilatation, PMA: post-menstrual age at scan in weeks^{+days}, TEA: Preterms scanned at term-equivalent age. [a]Infant was scanned twice, [b] clinical seizures (not during MRI)

↓ **Table 10.2**
Results of the error analysis on % partial volume inclusion of CSF and chosen b-value at two levels of oxygenation.

	b-value in s/mm²					
	SO_2 = 50%			SO_2 = 90%		
CSF PV%	10	30	50	10	30	50
0	1.3 %	3.2 %	4.6%	-4.4%	-9.8%	-13.4%
5	6.1 %	14.0%	18.8%	-2.4%	-5.6%	-7.7%
10	10.4 %	22.7%	30.0%	-0.2%	-0.8%	-1.5%

PV%: percentage of CSF included in the region-of-interest, SO_2: oxygen saturation
Assumed parameters: tissue (T_1/T_2/ADC): 1400ms/60ms/$0.6*10^{-3}$; CSF (T_1/T_2/ADC); 4000ms / 500ms / $3.5*10^{-3}$; Hct = 0.4, cerebral blood volume = 5%

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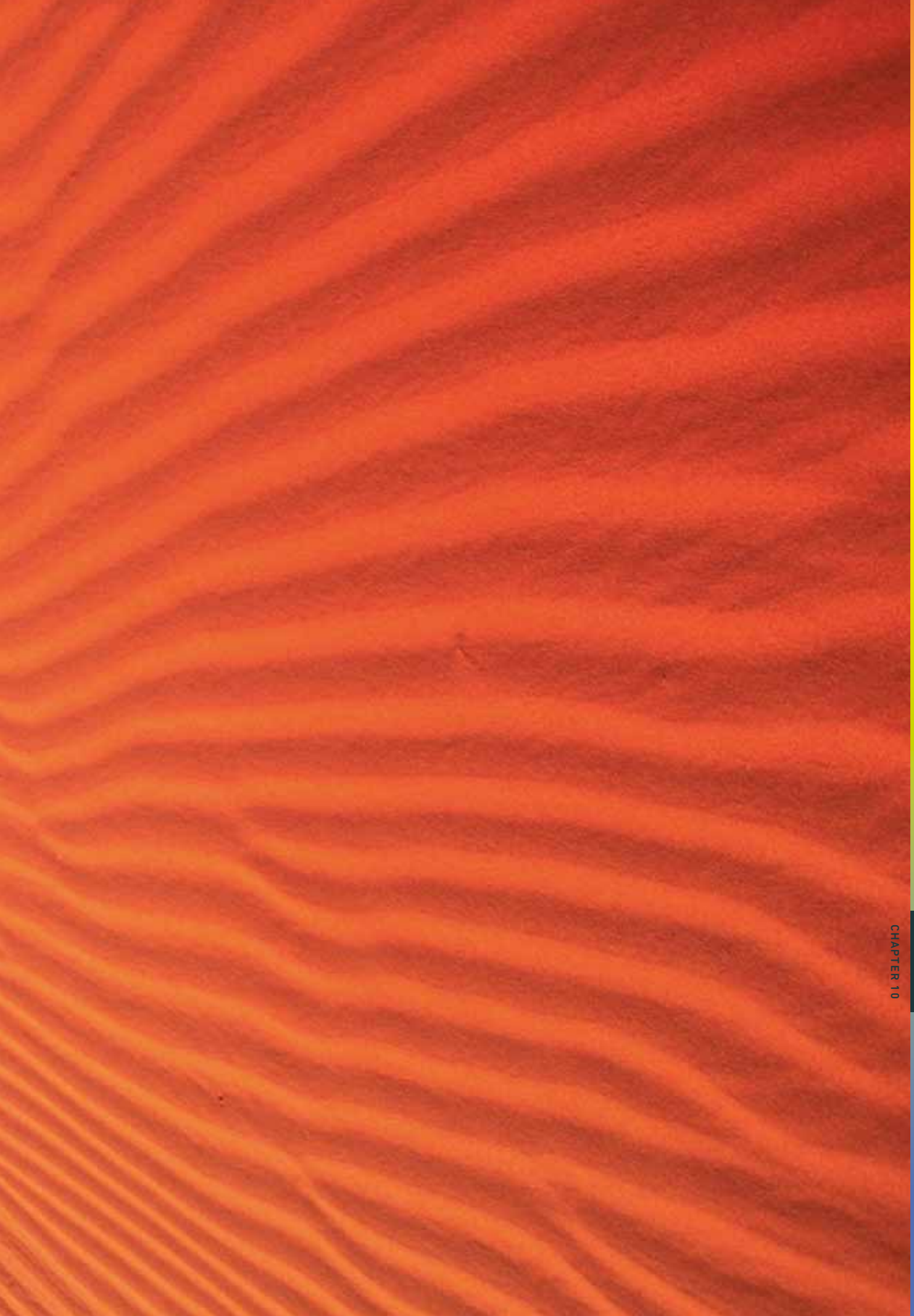

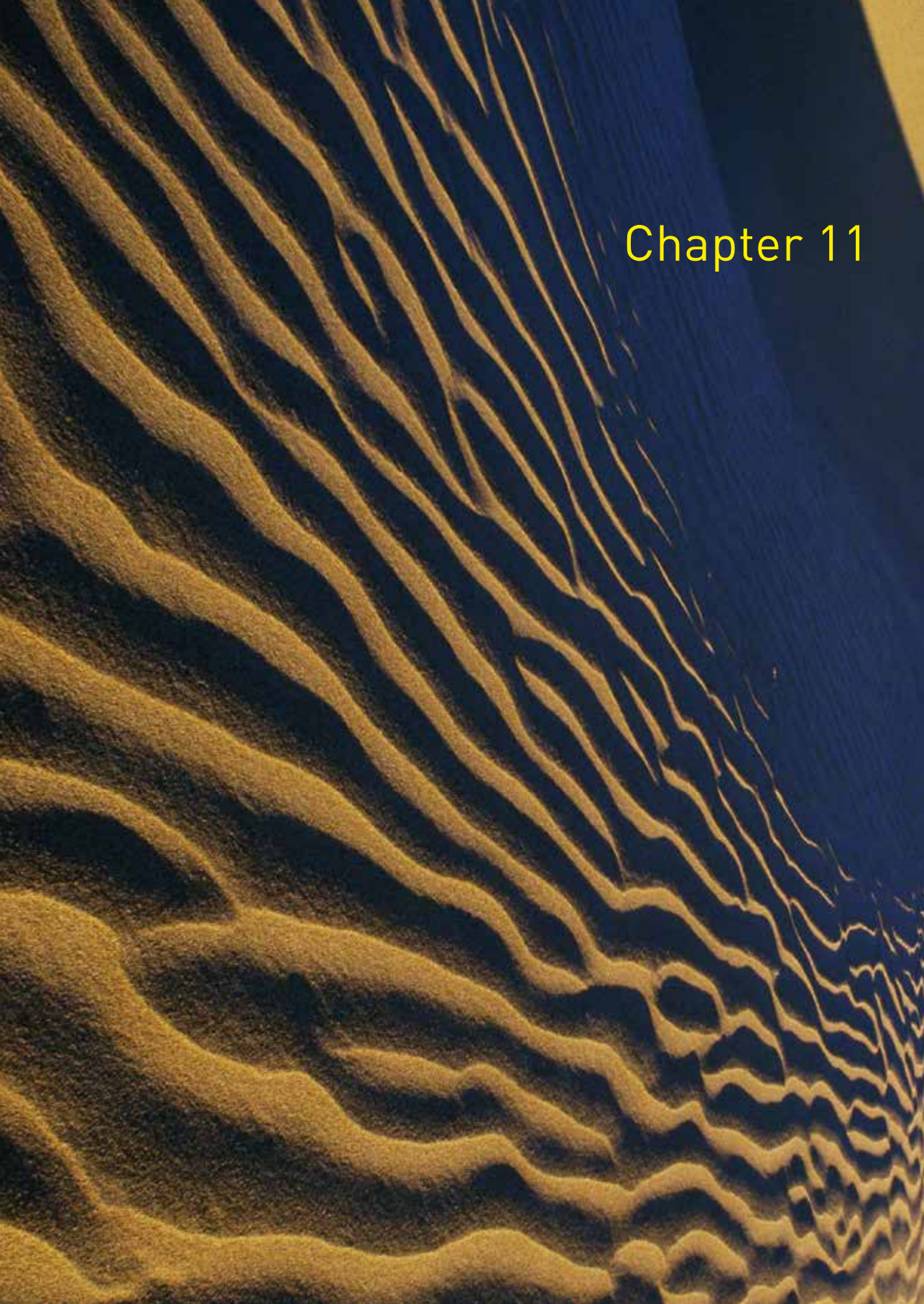
Chapter 11

FULL-BRAIN MAPPING OF OXYGEN SATURATION AND PSEUDO BLOOD VOLUME USING T_2-PREPARED VELOCITY SELECTIVE LABELLING DURING A RESPIRATORY CHALLENGE

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Submitted

ABSTRACT

BACKGROUND AND AIM Disturbances in cerebral oxygenation saturation (SO_2) have been linked to adverse outcome both in adults and neonates. On the neonatal intensive care, the cerebral SO_2 is often monitored by Near-InfraRed Spectroscopy (NIRS). Unfortunately NIRS has a limited penetration depth. The "modified T_2-prepared Blood Imaging of Oxygen Saturation" (T_2-BIOS) MR sequence provides cerebral SO_2 with full brain coverage.

MATERIALS AND METHODS A pseudo cerebral blood volume, tissue SO_2, and venous SO_2 (S_vO_2) were obtained simultaneously by T_2-BIOS during a respiratory challenge in ten healthy volunteers. These three measures were compared to cerebral blood volume and SO_2 that were obtained by a single probe MR-compatible NIRS setup, and to cerebral blood flow and venous SO_2 that were obtained by arterial spin labelling and "T_2-prepared Relaxation Imaging with Inversion Recovery" (T_2-TRIR), respectively.

RESULTS $SO_{2\text{-T2-BIOS}}$ and $SO_{2\text{-NIRS}}$ had a mean bias of -0.5% (95% CI -9.6% to 8.8%). $S_vO_{2\text{-T2-BIOS}}$ correlated with $SO_{2\text{-NIRS}}$ (R^2 = 0.41, p=0.002) and $S_vO_{2\text{-T2-TRIR}}$ (R^2 = 0.87, p=0.002). In addition, $SO_{2\text{-NIRS}}$ correlated with $S_vO_{2\text{-T2-TRIR}}$ (R^2 = 0.85, p=0.003) Frontal cerebral blood flow correlated with $SO_{2\text{-T2-BIOS}}$ (R^2 = 0.21, p=0.04), but was not significant in relation to $SO_{2\text{-NIRS}}$

DISCUSSION/CONCLUSION The strong agreement between two methods that rely on an entirely different framework provides confidence in measuring cerebral SO_2 by either one of them. Full brain SO_2 assessment as provided by the T_2-BIOS can help validating NIRS in infants, and may prove useful in guiding the clinical management of cerebral injury following hypoxic-ischaemic events.

INTRODUCTION

Blood oxygen saturation (SO_2) can be an important parameter in the management of disease. In adults, SO_2 has relevance in diseases like ischaemic stroke. In stroke, SO_2 is related to the Oxygen Extraction Fraction (OEF) and Cerebrovascular Reserve (CVR), both markers for tissue viability with impaired CVR resulting in high OEF.[1–3] In children, neonates in particular, knowledge of the cerebral SO_2 and insight into oxygen metabolism can also be of great clinical value. Cerebral injury in neonates is often related to a disturbance in the blood and/or oxygen supply of the brain.[4–6] In the neonatal intensive care unit, it is becoming more and more common to use Near-InfraRed Spectroscopy (NIRS) to estimate the regional cerebral oxygenation and perfusion.[7] However, the interpretation of NIRS is not always straightforward and the technique is limited by the penetration depth of the near-infrared light (~2cm).[8]

Recent advances in MRI have provided various approaches to non-invasively estimate oxygen metabolism parameters of the brain. One approach is the respiratory calibration of the BOLD signal.[9,10] Although calibrated BOLD approaches can provide invaluable information, the experimental setup is complex, the framework relies on several assumptions (e.g. fixed cerebral blood volume – cerebral blood flow (CBV-CBF) relation), and the respiratory paradigm itself is already challenging for healthy subjects, let alone patients. The need for a respiratory paradigm also provides ethical concerns for the use in neonates.

A second approach is the modelling of the transverse relaxation time of blood (T_{2b}).[11,12] Examples are the "T_2-prepared Relaxation Imaging with Inversion Recovery" (T_2-TRIR) and "T_2-Relaxation-Under-Spin-Tagging" (TRUST) sequences that both measure venous oxygen saturation (S_vO_2) in the sagittal sinus.[12,13] The downside is that neither of these two sequences provides a voxel-by-voxel measurement of SO_2 or S_vO_2. A more direct estimation of S_vO_2 has been proposed in the form of the QUIXOTIC sequence. The advantage of this technique is that it is not sensitive to field inhomogeneity and it does not require model assumptions.[11] While S_vO_2 is ideal for true OEF estimation, the QUIXOTIC suffers from a low signal-to-noise ratio (SNR). In reality, the oxygenation levels have dropped already significantly even before blood reaches the capillaries. The low SNR, in particular a concern in neonates, can be improved by including the entire vasculature as has been done in the recently proposed "T_2-prepared Blood Imaging of Oxygen Saturation" (T_2-BIOS) approach.[14,15] The T_2-BIOS sequence measures T_{2b} on a voxel-by-voxel basis. Subsequently, SO_2 can be estimated from T_{2b} and Hct using previously determined relationships between T_2, SO_2 and haematocrit (Hct).[12,16] The T_2-BIOS provides SO_2 in a mixed arterial-venous compartment and thereby an overall estimate of tissue wellbeing, irrespective of CBF and arterial oxygen saturation (S_aO_2), which are both known to be quite variable in neonates.[17] However,

the initial implementation was limited by the fact that it only provided a single slice and did not include any radio frequency pulses to suppress signal from cerebro-spinal fluid (CSF).

In this work we present a modified version of the T_2-BIOS sequence, which now uses velocity selective labelling, offers full brain coverage, and includes CSF suppression pulses. Thereby T_{2b} and pseudo cerebral blood volume (pCBV) are obtained on a voxel-by-voxel basis. Data obtained using the T_2-BIOS sequence is compared to oxygenation parameters obtained with a commercially available NIRS device, to S_vO_2 measured in the sagittal sinus by means of the T_2-TRIR sequence, and to CBF measured by arterial spin labelling (ASL).

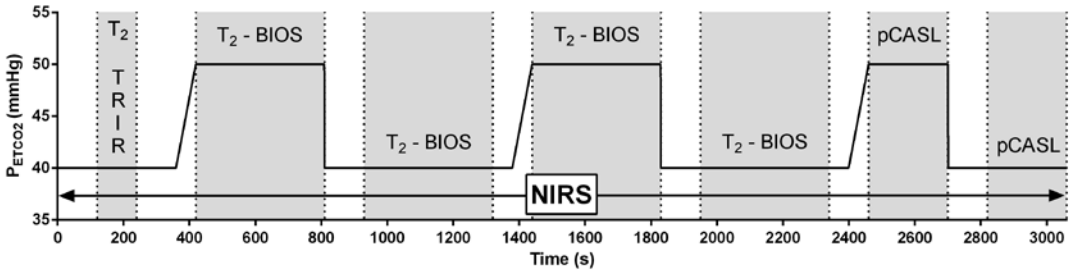
MATERIALS AND METHODS

SUBJECTS

The experimental protocol was approved by the Institutional Review Board of the University Medical Center Utrecht (Utrecht, the Netherlands), protocol number NL39070.041.11. Written informed consent was obtained in 10 (6 female, 4 male) healthy, non-smoking volunteers. Volunteers were instructed not to drink any caffeine containing substances and not to perform heavy exercise during the hours before the experiment.[18] MR imaging and NIRS were performed simultaneously during a respiratory challenge.

COMPUTERIZED RESPIRATORY CHALLENGE

In this work, a respiratory challenge was performed to assess two levels of oxygenation per subject. As cerebral metabolic rate of oxygen ($CMRO_2$) remains relatively stable during hypercapnia (HC), cerebral oxygen saturation should follow an increase in CBF induced by HC.[19,20] Hypercapnia was induced by using a computerized end-tidal gas targeting system (RespirAct™, Thornhill Research Inc, Toronto, Canada) as described before.[9,21] Before each experiment the subjects' individual baseline respiratory parameters (P_{ETCO_2}, P_{ETO_2}, respiratory rate and gas breathing volume) were determined and subsequently used to tailor the settings of the RespirAct™. The respiratory challenge was performed in a block design by aiming for a P_{ETCO_2} that was 10 mmHg higher at HC than during normocapnia (NC). After every HC 'block' there was a 2 minute baseline period before the next sequence was started to allow for P_{ETCO_2} to return to baseline. In **figure 11.1** a schematic of the respiratory paradigm is presented. P_{ETO_2} was maintained at 120 mmHg throughout the experiment. For temporal alignment between MR, NIRS and the respiratory challenge, a synchronization point was inserted on both the NIRS and RespirAct™ device at the beginning of the experiment and at the start of each MR sequence.



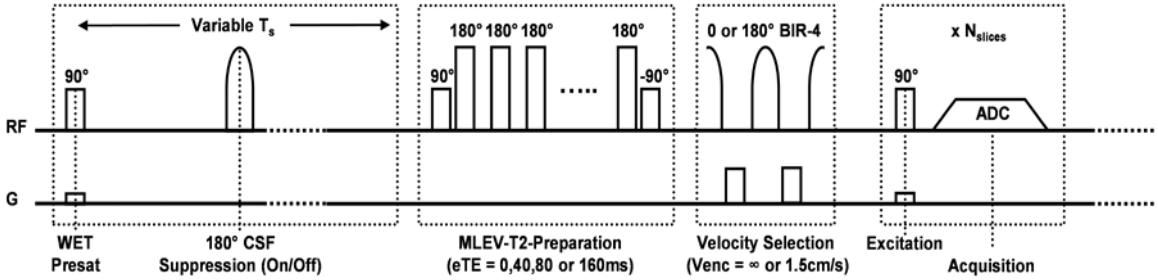
↑ **Figure 11.1**
Schematic of the respiratory paradigm

MR ACQUISITION

MR imaging was performed using a 3.0 Tesla Phillips system (Philips Achieva, Phillips Healthcare, Best, The Netherlands) with a quadrature body coil for transmission and an 8-channel phased-array SENSE head coil as a signal receiver. The MR imaging protocol consisted of T_1-weighted magnetization prepared rapid acquisition echo (MP-RAGE), T_2-weigthed fluid attenuation inversion recovery (T_2-FLAIR), 2D phase contrast magnetic resonance angiography (PC-MRA), T_2-TRIR, a pseudo-continuous arterial spin labelling (pCASL) sequence and the T_2-BIOS sequence.[13]

The MPRAGE and the T_2-FLAIR images were used for anatomical reference. The PC-MRA was used for optimal positioning of the labelling slab of the pCASL sequence, which was positioned perpendicular to the internal carotid and vertebral arteries. In addition, the PC-MRA was used to plan the T_2-TRIR imaging slab perpendicular to the sagittal sinus just above the confluence with the straight sinus. The T_2-BIOS was used to determine SO_2, pCBV, and S_vO_2, the T_2-TRIR was used to determine Hct, OEF, and S_vO_2 in the sagittal sinus, and the pCASL sequence was used to measure CBF. All sequences except every second T_2-BIOS or pCASL sequence were performed during normocapnia breathing.

The T_2-BIOS sequence starts with a pre-saturation followed by a recovery time with a CSF suppression inversion pulse. Then the longitudinal magnetization is T_2 prepared using a standard Malcolm Levitt (MLEV) preparation, corresponding to 0, 4, 8, and 16 refocussing pulses using an interpulse time of (τ_{CPMG}) of 10ms.[16,22] Two schemes were applied to minimize imperfections in the T_2-preparation pulses: composite pulses were used for both the 180° (i.e. $90°_x180°_y90°_x$) and -90° (i.e. $270°_x$-$360°_x$) pulses, and the signs of the pulses were then arranged in a MLEV pattern (i.e. 1 1 -1 -1). Subsequently, a BIR-4 based velocity selective labelling sequence is played out right before a conventional multi-slice EPI readout. The sequence is repeated in groups of 4, each with different effective TE preparations in the range 0-160 ms. For each eTE, both a label and a control experiment (i.e. V_{enc}= 1.5 cm/s and V_{enc}=∞) is performed. A



↑ **Figure 11.2** Sequence chart

graphical representation of the T_2-BIOS sequence is presented in **figure 11.2**. The scan parameters of the T_2-BIOS sequence were: TR/TE=7600/7ms, matrix = 40 x 40, FOV = 240 x 240mm², flip-angle=90°, 11 slices of 7mm, SENSE=2.0, eTE=0,40,80 and 160ms, V_{enc}= 1.5 cm/s and V_{enc}=∞, and total scan time 6:32 min. In relation to the respiratory challenge, the T_2-BIOS was started as soon as P_{ETCO_2} was in the target range. The T_2-BIOS was performed 4 times (i.e. two times at HC and two times at NC, **figure 11.1**).

For CBF measurement, a multi-slice gradient-echo pCASL sequence was used with an EPI readout. Scan parameters were: TR/TE: 4000/13.79ms, matrix = 80 × 80, FOV = 240 × 240 mm², flip-angle=90°, 11 slices of 7mm, SENSE=2.5, a labelling duration of 1650 ms, and a post label delay of 1550 ms. A total of 30 dynamics, equivalent to 4:05 (mm:ss) scan time, were performed. The pCASL sequence was performed twice (i.e. at NC and at HC).

The T_2-TRIR consists of a presaturation pulse in the imaging plane to supress surrounding tissue and just get the signal from inflowing blood in the sagittal sinus, followed by an MLEV T_2-preparation and an non-selective adiabatic inversion pulse.[13] Scan parameters were: TR/TE/ΔTI/TI_1: 15s/20ms/140ms/20ms, matrix = 128 x 128, FOV = 240 x 240 mm², flip-angle 95°, single 3mm slice, SENSE=2.5, 50 phases, eTE 0, 40, 80 and 160ms. Total scan time 2:00 min.

NEAR-INFRARED SPECTROSCOPY

NIRS data was obtained continuously throughout the whole MRI session as described before.[9] In summary, an Oxymon Mk III continuous wave NIRS system (Artinis Medical Systems, Zetten, The Netherlands) was used to determine regional oxygen saturation (SO_2 in %) and absolute changes (in µM) in oxygenated (O_2Hb), deoxygenated (HHb) and total haemoglobin (tHb = O_2Hb + HHb) concentration with respect to an arbitrary baseline. A fibre optic MR compatible probe with 10m fibres, 1 receiver optode, and 3 emitting optodes (avg. inter-optode distance 40mm, separation 4mm) was positioned on the right side of the patient's forehead. The probe was carefully positioned to avoid contact with hair and to avoid measurement of cerebral venous sinuses. The position of the probe was marked with a vitamin D capsule (visible on structural MRI). Before

every data acquisition the device was calibrated against a known standard to avoid small variations in fibre connections from influencing results.

DATA ANALYSIS

All images were analysed using IDL 6.1 for Windows (ITT Visual Information Solutions, Boulder, CO, U.S.A.).

T_2-BIOS: SO_2, S_vO_2, OEF, and pseudo CBV

Subtracting velocity encoded (label) from non-velocity encoded (control) images yields a single ΔeTE image per eTE and thereby 4 images in total (i.e. MLEV TE preparations of 0, 40, 80 and 160ms). These images are strongly vascular weighted, for which the signal relationship of T_{2b} follows:

EQUATION 1: $$\Delta S(eTE) = V_b * M_{0b} * e^{-\frac{eTE}{T_{2b}}}$$

Where eTE is the effective MLEV echo time and $V_b * M_{ob}$ is blood volume fraction times bloods equilibrium magnetization. The latter can be considered a single constant for estimating T_{2b}. Fitting of the MR signal as a function of the T_2 preparation duration yields T_{2b}. Knowing T_{2b} and Hct, the oxygen saturation can be estimated by using eq. 1-4 from (Lu et al., 2012), which shows that by knowing two parameters out of T_2, SO_2 and Hct, the third can be estimated:

EQUATION 2: $$\frac{1}{T2_b} = A + B * (1\text{-}SO_2) + C * (1\text{-}SO_2)^2$$

$$A = a_1 + a_2 * Hct + a_3 * Hct^2$$

$$B = b_1 * Hct + b_2 * Hct^2$$

$$C = c_1 * Hct + (1\text{-}Hct)$$

The Hct can be obtained in three ways: in healthy adults it can be assumed, it can be obtained from a blood sample, or it can be derived from T_{1b} (e.g. by T_2-TRIR). Knowing T_{1b}, large vessel Hct can be determined using the previously described relationship between T_{1b} and Hct.[23]

EQUATION 3: $$\frac{1}{T1_b} = 0.5 * Hct + 0.37$$

In the current study, data of the T_2-TRIR was used to estimate Hct or by assuming a value (i.e. 0.45 for male and 0.40 for female subjects) in case T_2-TRIR data was unavailable.

With appropriate knowledge of M_{ob} (Eq. 1) from a pure blood ROI in the sagittal sinus, a pCBV can be obtained.[24] This is not a true CBV estimate, because Venc=1.5cm/s

excludes the capillary compartment from the pCBV estimate. From the ROI in the sagittal sinus the venous oxygen saturation (S_vO_2) can be determined following the steps described above (Eq. 1-2). Combined with an assumed S_aO_2 of 98% (in healthy adults), the global OEF can be calculated ($[S_aO_2-S_vO_2]/S_aO_2$).

T_2-TRIR: Hct, S_vO_2 and OEF

The data-analysis of the T_2-TRIR sequence has been described in more detail elsewhere.[13] In short, a pure blood ROI is automatically selected in the sagittal sinus. Four different inversion recovery curves with eTE's of 0, 40, 80 and 160 ms are plotted based on data obtained from this ROI, from which T_{1b} and T_{2b} are simultaneously fitted:

EQUATION 4: $$M_b(TI) = M_{0b} * \left[1 - \left(1 + e^{-\frac{eTE}{T_{2b}} * IE}\right) * e^{-\frac{TI}{T_{1b}}}\right]$$

Here, $M_b(TI)$ is the longitudinal magnetization of blood at each inversion time (TI), M_{ob} is the equilibrium magnetization of blood, eTE is the effective echo time and IE is the inversion efficiency. The obtained T_{1b} and T_{2b} can be converted into Hct and S_vO_2, respectively, as described above.

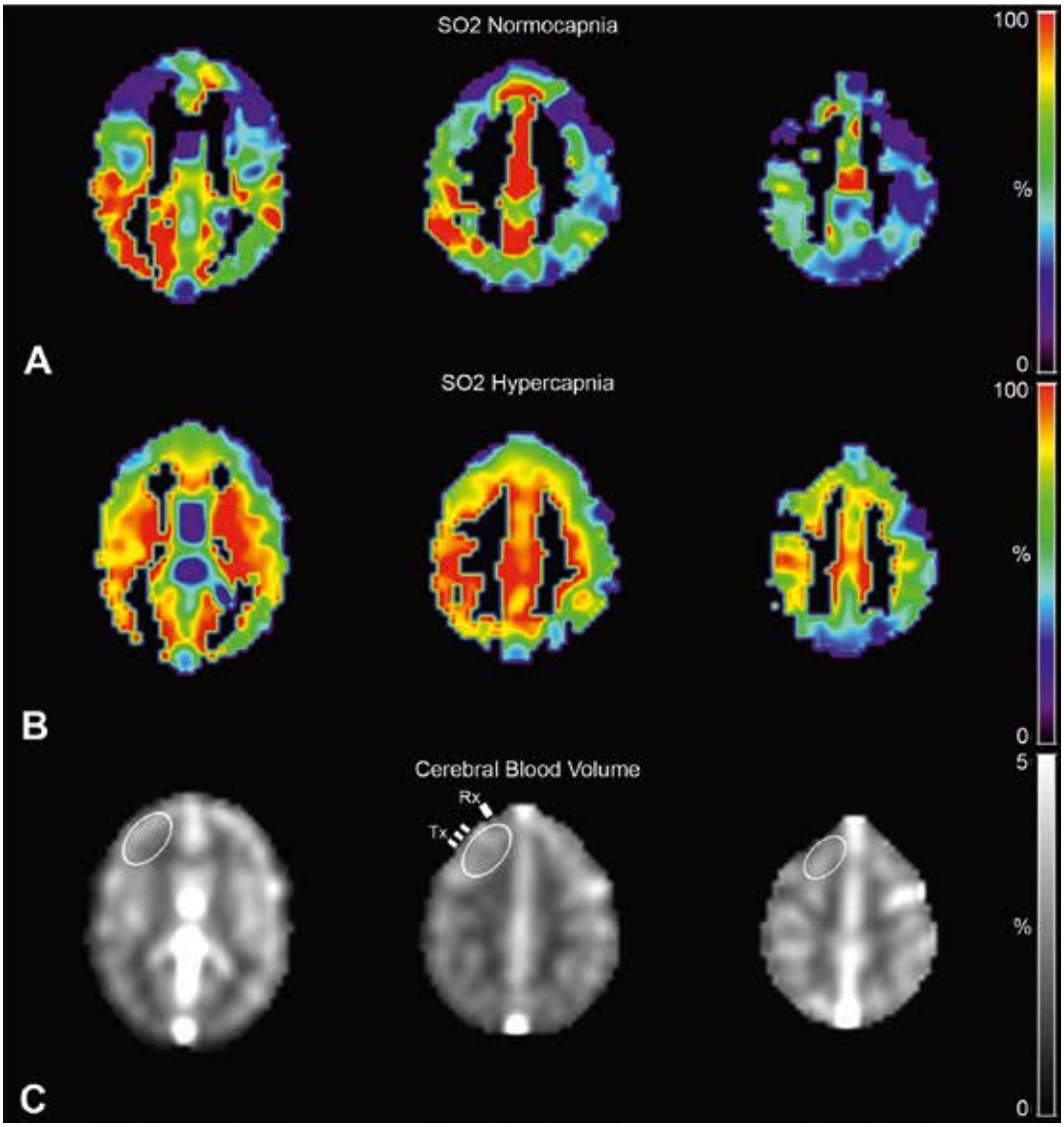
pCASL: CBF

For obtaining CBF, the paired labelled and non-labelled ASL images were surround subtracted to produce ASL subtraction (ΔM) images.[25,26] Perfusion was quantified on the ΔM images based on the following assumptions: a longitudinal relaxation time of tissue of 1.5s, a longitudinal relaxation time of blood of 1.6s and an average water partition coefficient between blood and grey-white matter (λ) of 0.91. The average labelling efficiency was set at 0.90. The fully relaxed magnetization of arterial blood was estimated from the data.

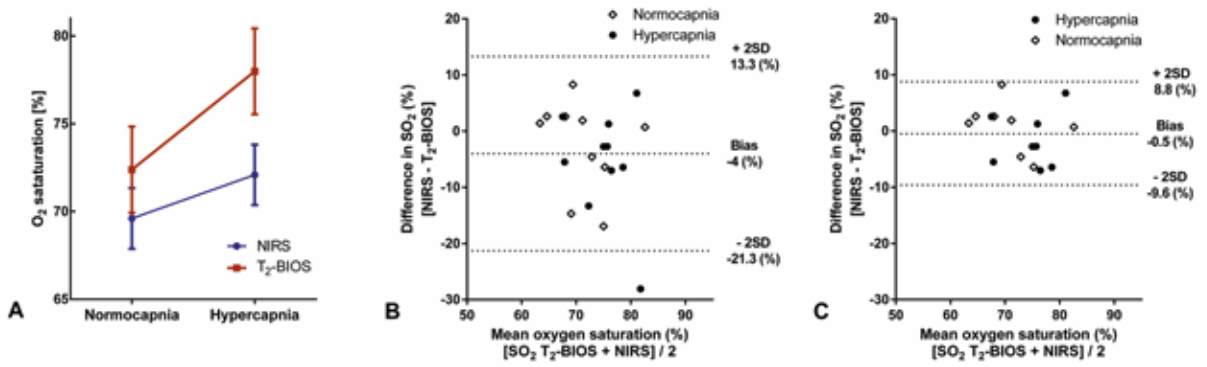
NIRS: SO_2, HHb, O_2Hb, tHb (HHb+O_2Hb), and CBV

The SO_2 was obtained by means of spatially resolved spectroscopy which enables the calculation of the relative attenuation coefficients of O_2Hb and HHb.[27,28] When O_2Hb is expressed as a ratio of tHb, the SO_2 (in %) is obtained, which represents the weighted average of O_2Hb as a ratio of tHb in arterial, capillary and venous vessels.

The absolute changes in [O_2Hb], [HHb], and [tHb] were obtained by using the modified Lambert-Beer equation and combining the data from the three different inter-optode distances.[29] Part of this equation is the differential path length factor (DPF), which accounts for the fact that a photon does not travel in a straight line through tissue due to scatter. The DPF in this study was varied according to the age of the subject.[30] As tHb is directly proportional to tCBV, changes in tHb can be converted to changes in tCBV.[31]



↑ **Figure 11.3**
Three exemplary slices in a single subject of the oxygen saturation maps during A) normocapnia and B) hypercapnia, and C) pseudo CBV map at baseline with a schematic of the NIRS probe and regions-of-interest. Rx: NIRS receiving optode, Tx: three NIRS emitter optodes.



↑ **Figure 11.4** The SO_2 measured by T_2-BIOS and NIRS: A) absolute values at normocapnia and hypercapnia, B) bias between the two methods, and 3) the bias but when excluding 4 outliers.

EQUATION 5: $$\Delta tCBV = \frac{\Delta[tHb] * MW_{Hb}}{[Hb]_{blood} * D_{bt} * R}$$

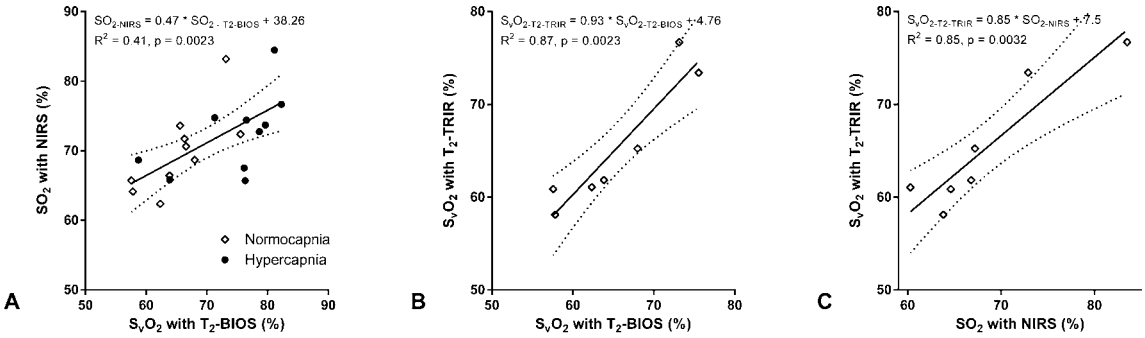
Where ΔtCBV is the change in tCBV (ml per 100g brain tissue), MW_{Hb} is the molecular weight of haemoglobin (64500 g/Mol), $[Hb]_{blood}$ is the haemoglobin concentration in the blood (in g/dl), D_{bt} is the density of brain tissue set at 1.05 g per ml, and R is the large-to-small vessel haematocrit ratio set at 0.69.[32,33]

For comparison with NIRS data, regions of interest (ROIs) were manually drawn on $SO_{2\text{-}T2\text{-}BIOS}$ and CBF maps by one observer (ETP). The vitamin D capsules positioned on the NIRS optode was used as a reference for ROI drawing. In order to obtain sufficient SNR, data from three ROIs placed on the 3 slices surrounding the NIRS optode were averaged. Data are displayed as absolute values and absolute changes (delta) from NC to HC. In addition, data are presented as normalised values (normalized to an assumed change of 10mmHg P_{ETCO_2}) in order to facilitate comparison between subjects. Values of individual subjects were grouped and are displayed as a group mean with error bars.

RESULTS

Subjects had a mean age of 30 years (range 25-40). All 10 datasets were complete, except for data obtained with T_2-TRIR, which was missing in 3 subjects due to technical issues. Data of both NC-HC blocks during which the T_2-BIOS was performed, were used for analysis. **Figure 11.3** presents an oxygen saturation map at NC and HC in a representative subject, together with a schematic of the NIRS sensor placement over a pseudo CBV map.

The SO_2 values obtained by NIRS and the T_2-BIOS, both at NC and HC, are displayed in **figure 11.4A**. On average, NIRS measured 4% lower values (bias -4.0%, 95% limits of agreement -21.3 to 13.3, **figure 11.4B**). When excluding 4 outliers where a suboptimal fit



↑ **Figure 11.5**
The S_vO_2 in comparison: A) $S_vO_{2\text{-T2-BIOS}}$ vs. $SO_{2\text{-NIRS}}$, B) $S_vO_{2\text{-T2-BIOS}}$ vs. $S_vO_{2\text{-T2-TRIR}}$, and C) $SO_{2\text{-NIRS}}$ vs. $S_vO_{2\text{-T2-TRIR}}$.

was obtained for the T_2-BIOS data, the bias was -0.5% (95% limits of agreement -9.6% to 8.8%), see **figure 11.4C**. No linear association was found between the bias and the average of the two techniques. (p = 0.19). **Table 11.1** lists the absolute values per subject of all the parameters that were obtained during NC and HC. **Table 11.2** lists all values that were calculated based on the acquired data from **table 11.1**.
In **figure 11.5** the two S_vO_2 estimates (T_2-BIOS and T_2-TRIR) are compared against each other and against $SO_{2\text{-NIRS}}$. The $S_vO_{2\text{-T2-BIOS}}$ and $S_vO_{2\text{-T2-TRIR}}$ had an average bias of -0.14% (95% limits of agreement -5.3% to 5%). The mean change in pCBV was higher than the mean change in CBV recorded by NIRS (1.76 ml/100g vs. 0.69 ml/100g). Frontal CBF correlated with $SO_{2\text{-T2-BIOS}}$ (R^2 = 0.21, fCBF = 0.5943 * $SO_{2\text{-T2-BIOS}}$ + 19.74, p = 0.04) but not with $SO_{2\text{-NIRS}}$.

DISCUSSION

A technique is presented that enables the measurement of SO_2 in a mixed vascular compartment (i.e. arterial-venous) without the need for a respiratory calibration but while providing voxelwise full brain coverage. In this work the performance of the sequence was tested by using a respiratory challenge which yielded two levels of cerebral oxygenation through the manipulation of CBF. The sensitivity the modified T_2-BIOS sequence to both arterial and venous structures has three advantages: 1) the use of a large blood pool increases SNR, 2) it provides an overall 'tissue status' even without knowledge of CBF or S_aO_2, and 3) the $SO_{2\text{-T2-BIOS}}$ should in theory be comparable to the SO_2 obtained by NIRS, which can be used to cross-validate both techniques.
The main aim was to compare SO_2 parameters obtained by T_2-BIOS and NIRS. After exclusion of four data points with a bad fit, the average bias was -0.5%, which can hardly

be deemed clinically significant. In addition, the ±10% limits of agreement are in the same neighbourhood of what has been documented for bilateral NIRS measurements in neonates.[34] Compared to the initial implementation of the T_2-BIOS (chapter 10), we did not observe a linear association between the bias and the average of the two methods.[15] This suggests that the implementation proposed in this paper is indeed less affected by signal contributions from CSF and static tissue.

The pCBV estimates that we observed at baseline (mean [SD] 3.95 [0.91] ml/100g) are quite comparable to results that were obtained by others through MR (3.77 [1.05] ml/100g) and Positron Emission Tomography (cortical grey matter 5.5 [0.6] ml/100g and white matter 2.1 [0.5] ml/100g, especially when considering that our data were obtained from a combined cortical grey-matter / white matter ROI.[35,36] However, the pCBV values obtained at HC are unrealistic (5.71 [1.52] ml/100g). In particular when comparing to the CBV change in NIRS, which is almost three times lower. In addition the %pCBV change per 10mmHg clearly surpasses the %CBF changes (mean 69.3% [43.2] vs. 37.6% [19.0]), whereas this usually is the other way around.[37] This can probably be attributed to either partial volume issues of selecting a whole blood voxel in the sagittal sinus to determine M_{ob} or from the velocity selective properties of the T_2-BIOS at hypercapnia (see below).

The $S_vO_{2\text{-}T2\text{-}BIOS}$ had a minimal bias and demonstrated an excellent correlation with $S_vO_{2\text{-}T2\text{-}TRIR}$. Essentially this is a comparison between readouts to obtain T_{2b} in the sagittal sinus, as the underlying framework to estimate S_vO_2 from T_{2b} is the same. This confirms that the T_2-BIOS is as reliable for T_{2b} and thus S_vO_2 estimation as the T_2-TRIR, and strongly suggests that the partial volume effect caused by the relatively crude resolution of the T_2-BIOS (i.e. matrix 40x40 vs. 128x128) is minimal, at least in the sagittal sinus during baseline conditions.

Although NIRS and T_2-BIOS are comparable in that they both measure the SO_2 in a mixed vascular compartment, they are clearly different and do have their own limitations. The limitations of NIRS have been discussed in far more detail by us and others.[9,38–40] The main sources of bias are extra-cerebral contamination and cross-talk between HHb and O_2Hb. The very nature of NIRS makes that it is sensitive to attenuation and scattering originating from extra-cerebral tissue.[38,41] In addition, the penetration depth of NIR light is approximately ½ the inter-optode distance.[8] When increases in SO_2 mostly occur in brain tissue, extra-cerebral contributions could attenuate the absolute changes in haemoglobin species as recorded by NIRS and thereby affect SO_2 and CBV. This could in part explain the decreased amplitude of these estimates compared to T_2-BIOS. To minimize the second concern, cross-talk between HHb and O_2Hb, three actions were taken in this study: 1) wavelengths of 764 and 857nm were selected, 2) the differential path length factors were varied according to age, which was close to 6 in all 10 subjects, and 3) global (changes in) SO_2 were looked at instead of performing a functional NIRS-MRI comparison.[39,40,42]

For the T_2-BIOS the limitations roughly come down to SNR constraints based on the velocity selective labelling of the cerebral blood pool. By including both the arterial and venous blood pool we tried to increase SNR.[11] Nevertheless, voxels had to be relatively large to yield sufficient SNR. These SNR constraints might have caused a suboptimal fit of the T_{2b}, resulting in the 4 outliers (**figure 11.4B**). In addition, the large voxel size could introduce unwanted partial volume effects. Although this was avoided by carefully selecting the ROIs, it did prohibit separate white- and grey-matter estimates. The velocity selective labelling is both the strength and a limitation of the T_2-BIOS. As a limitation it can potentially explain the relatively large step change in SO_2 and pCBV from NC to HC. The slope in blood flow velocity, from larger vessels to capillaries, is steeper on the arterial side than on the venous side.[43] An overall increase in CBF and thus flow velocity will include a larger blood pool overall, and possibly shifts more towards the capillaries on the arterial side than it does on the venous side. In theory the labelling approach could therefore contribute to the relatively larger increases in pCBV and SO_2 at HC level that were observed in comparison to NIRS. On the other hand, NIRS has a slight bias towards the smaller vessels and is sensitive to changes in blood volume (e.g. when the arterial-capillary-venous ratio changes), but not influenced by changes in blood flow velocity.[44]

One of the aims of this study is to provide reliable full brain coverage for the assessment of SO_2 without a respiratory calibration, both in adults and in neonates. The T_2-BIOS and NIRS seem to be measuring SO_2 in the same vascular compartment. Where NIRS has the advantage of providing longitudinal measurements, the advantage of the T_2-BIOS is that it provides full brain SO_2, including deeper white- and grey-matter. The T_2-BIOS can be calibrated against an external standard (e.g. artificially oxygenated blood flowing through a phantom) and can subsequently serve as a validation tool for NIRS in (pre)term infants, for whom validation studies have mostly been performed by using animal models and infants on cardio-pulmonary bypass.[45,46] In addition, from a clinical perspective, SO_2 in deeper brain structures might be useful for prognostic purposes in infants with hypoxic-ischaemic encephalopathy and perinatal arterial ischaemic stroke, and also in adults with ischaemic stroke.[1–3,6,47,48] To provide full brain coverage in neonates, some SNR constraints need to be overcome. Future research will focus on improving the readout to improve both SNR and spatial resolution. In addition the optimal cut-off for the velocity encoding will be determined. Lowering this cut-off would theoretically include a larger blood pool, increase SNR, and possibly equalize the shift towards the capillary compartment on the arterial and venous side. Another addition to the T_2-BIOS that we will explore is the inclusion of multiple saturation durations at the shortest eTE. This will allow for the T_{1b} to be measured simultaneously with T_{2b}, and therefore a separate measurement of the T_{1b} and thus Hct would not be necessary.

CONCLUSIONS

In the sagittal sinus conclusion, simultaneous measurement of full brain SO_2 on a tissue level and S_vO_2 the sagittal sinus is possible without needing a respiratory calibration experiment. We provide a comprehensive set of parameters that were obtained simultaneously by NIRS and MRI. These two techniques are entirely different, but the various parameters still showed a good agreement. This adds to the confidence, both in NIRS and SO_2 assessment through MRI.

↓ Table 11.1
Acquired data

	MRI					NIRS			
						During T_2-BIOS			
Subject (Gender, Age)	RespirAct P_{ETCO2} [mmHg] NC / HC / HC-NC	pCASL fCBF [ml/100g/min] NC / HC / CP10 / %CP10	T_2-BIOS T_2 [ms] NC / HC	T_2-BIOS SS T_2 [ms] NC/ HC	T_2-TRIR SS T_1 / T_2 [ms] NC	SO_2 [%] NC / HC / CP10	HHb [µmol] NC-HC / CP10	O_2Hb [µmol] NC-HC / CP10	tHb [µmol] NC-HC / CP10
1 (F, 27)	38.2 / 44.7 / 6.5	51.3 / 63.5 / 28.7 / 55.9	97 / 108	79 / 83	1814 / 90	65.7 / 68.7 / 4.5	-0.66 / -1.01	1.52 / 2.34	0.87 / 1.33
2 (M, 28)	35.5 / 39.4 / 3.8	47.2 / 59.6 / 32.6 / 69	113 / 139	66 / 94	1508 / 62	66.4 / 67.5 / 2.8	-0.67 / -1.76	0.86 / 2.24	0.18 / 0.48
3 (F, 30)	35.6 / 39.2 / 3.5	62.7 / 71.5 / 20.8 / 33.2	84 / 147	71 / 87	1724 / 71	64.1 / 65.8 / 4.8	-0.88 / -2.48	1.06 / 3.00	0.19 / 0.52
4 (M, 29)	38.9 / 47.1 / 8.2	48.6 / 52.8 / 5.4 / 11.1	68 / 92	69 / 110	n/a	73.6 / 76.7 / 3.7	-0.72 / -0.88	2.56 / 3.11	1.84 / 2.23
5 (M, 40)	32.3 / 38.3 / 6.0	52.9 / 54.2 / 2.1 / 4.0	100 / 109	71 / 82	n/a	71.7 / 74.7 / 5.1	-0.50 / -0.84	1.37 / 2.29	0.87 / 1.45
6 (F, 28)	43.9 / 51.8 / 7.9	62.6 / 78.6 / 27.7 / 44.2	91 / 96	71 / 95	n/a	70.6 / 74.4 / 4.8	-0.05 / -0.07	3.85 / 4.89	3.8 / 4.83
7 (M, 26)	33.1 / 41.8 / 8.7	63.6 / 77.9 / 16.1 / 25.3	107 / 95	84 / 104	1547 / 93	83.2 / 84.5 / 1.4	-0.01 / -0.01	2.09 / 2.39	2.08 / 2.38
8 (F, 30)	32.1 / 39.8 / 7.7	66.3 / 79.3 / 28.2 / 42.6	77 / 92	90 / 100	1531 / 85	72.4 / 73.7 / 1.7	-1.15 / -1.49	-0.02 / -0.03	-1.17 / -1.52
9 (M, 31)	37.9 / 46.9 / 9.1	46.4 / 62.3 / 24 / 51.7	72 / 104	74 / 100	1530 / 68	68.7 / 72.7 / 4.5	-1.49 / -1.64	0.78 / 0.86	-0.71 / -0.79
10 (F,25)	33.7 / 41.5 / 7.8	54.9 / 71.4 / 21.4 / 38.9	118 / 98	75 / 118	1686 / 72	62.4 / 65.7 / 4.2	-1.39 / -1.79	4.02 / 5.15	2.63 / 3.37
Mean (SD)	36.1 (3.5) / 43.1 (4.2) / 6.9 (1.8)	55.6 (7.1) / 67.1 (9.5) / 20.7 (9.6) / 37.6 (19.0)	93 (16.5) / 108 (18.4)	75 (7.0) / 97 (11.1)	1620 (111) / 77 (11)	69.9 (5.7) / 72.4 (5.5) / 3.8 (1.2)	-0.75 (0.47) / -1.2 (0.74)	1.81 (1.26) / 2.62 (1.50)	1.06 (1.46) / 1.43 (1.79)

NC = Normocapnia, HC = Hypercapnia
NC-HC = Change from normocapnia to hypercapnia.
CP10 = change per 10mmHg P_{ETCO2} increase
%CP10 = Percentage change in respect to baseline per 10mmHg P_{ETCO2} increase

↓ Table 11.2
Estimated parameters

Subject	T_2-TRIR SS Hct / S_vO_2 [%]/ OEF [%] NC	NIRS – T_2-TRIR SO_2 [%] NC	T_2-BIOS SS S_vO_2 [%] NC / HC / CP10	T_2-BIOS SS OEF [%] NC / HC / CP10	T_2-BIOS SO_2 [%] NC / HC / CP10	NIRS – T_2-BIOS SO_2 [%] NC / HC / CP10	NIRS – T_2 BIOS ΔCBV [ml/100g] NC-HC / CP10	T_2-BIOS ΔpCBV [ml/100g] NC / HC / CP10 / %CP10
1	0.29 / 60.9 / 37.9	64.6	57.6 / 58.7 / 1.8	41.3 / 40.1 / -1.8	63.3 / 66.2 / 4.5	65.7 / 68.7 / 4.5	0.64 / 0.99	3.72 / 4.74 / 1.58 / 42.5
2	0.44 / 61.8 / 36.9	66.8	63.8 / 76.1 / 32.1	34.9 / 22.3 / -32.8	83.5 / 95.8 / 32.3	66.4 / 67.5 / 2.8	0.12 / 0.30	5.10 / 5.87 / 2.01 / 39.5
3	0.32 / 58.1 / 40.7	63.8	57.8 / 63.9 / 17.1	41.0 / 34.8 / -17.5	62.7 / 78.9 / 45.8	64.1 / 65.8 / 4.8	0.12 / 0.33	3.10 / 4.29 / 3.36 / 108.4
4	n/a	73.1	65.6 / 82.3 / 20.3	33.1 / 16.1 / -20.7	65.2 / 75.3 / 12.2	73.6 / 76.7 / 3.7	1.09 / 1.32	4.72 / 7.33 / 3.18 / 67.4
5	n/a	70.7	66.3 / 71.3 / 8.3	32.3 / 27.3 / -8.5	78.4 / 81.8 / 5.6	71.7 / 74.7 / 5.1	0.52 / 0.86	4.20 / 7.12 / 4.87 / 116.0
6	n/a	68.6	66.6 / 76.5 / 12.6	32.0 / 21.9 / -12.8	75.2 / 77 / 2.4	70.6 / 74.4 / 4.8	2.41 / 3.07	5.42 / 8.70 / 4.17 / 77.0
7	0.46 / 76.7 / 21.7	83.5	73.2 / 81.1 / 9.1	25.4 / 17.2 / -9.3	82.2 / 77.7 / -5.2	83.2 / 84.5 / 1.4	1.24 / 1.41	3.33 / 3.26 / -0.09 / -2.6
8	0.46 / 73.4 / 25.1	72.9	75.6 / 79.6 / 5.3	22.9 / 18.8 / -5.4	70.2 / 76.4 / 8.1	72.4 / 73.7 / 1.7	-0.74 / -0.97	3.48 / 5.32 / 2.40 / 69.0
9	0.44 / 65.2 / 33.4	67.2	68.0 / 78.6 / 11.7	30.6 / 19.8 / -12	66.7 / 79.9 / 14.6	68.7 / 72.7 / 4.5	-0.42 / -0.47	2.32 / 5.45 / 3.45 / 148.9
10	0.35 / 61.1 / 37.7	60.3	62.3 / 76.3 / 17.9	36.4 / 22.1 / -18.3	76.4 / 70.6 / -7.4	62.4 / 65.7 / 4.2	1.95 / 2.50	4.14 / 5.02 / 1.12 / 27.0
Total: Mean (SD)	0.4 (0.07) / 65.3 (6.5) / 33.3 (6.6)	69.2 (6.1)	65.7 (5.5) / 74.4 (7.3) / 13.6 (8.2)	33 (5.6) / 24 (7.4) / -13.9 (8.4)	72.4 (7.4) / 78 (7.4) / 11.3 (15.6)	69.9 (5.7) / 72.4 (5.5) / 3.8 (1.2)	0.69 (0.95) / 0.94 (1.17)	3.95 (0.91) / 5.71 (1.52) / 2.61 (1.42) / 69.3 (43.2)

NC = Normocapnia, HC = Hypercapnia
NC-HC = Change from normocapnia to hypercapnia.
CP10 = change per 10mmHg P_{ETCO2} increase.
%CP10 = Percentage change in respect to baseline per 10mmHg P_{ETCO2} increase

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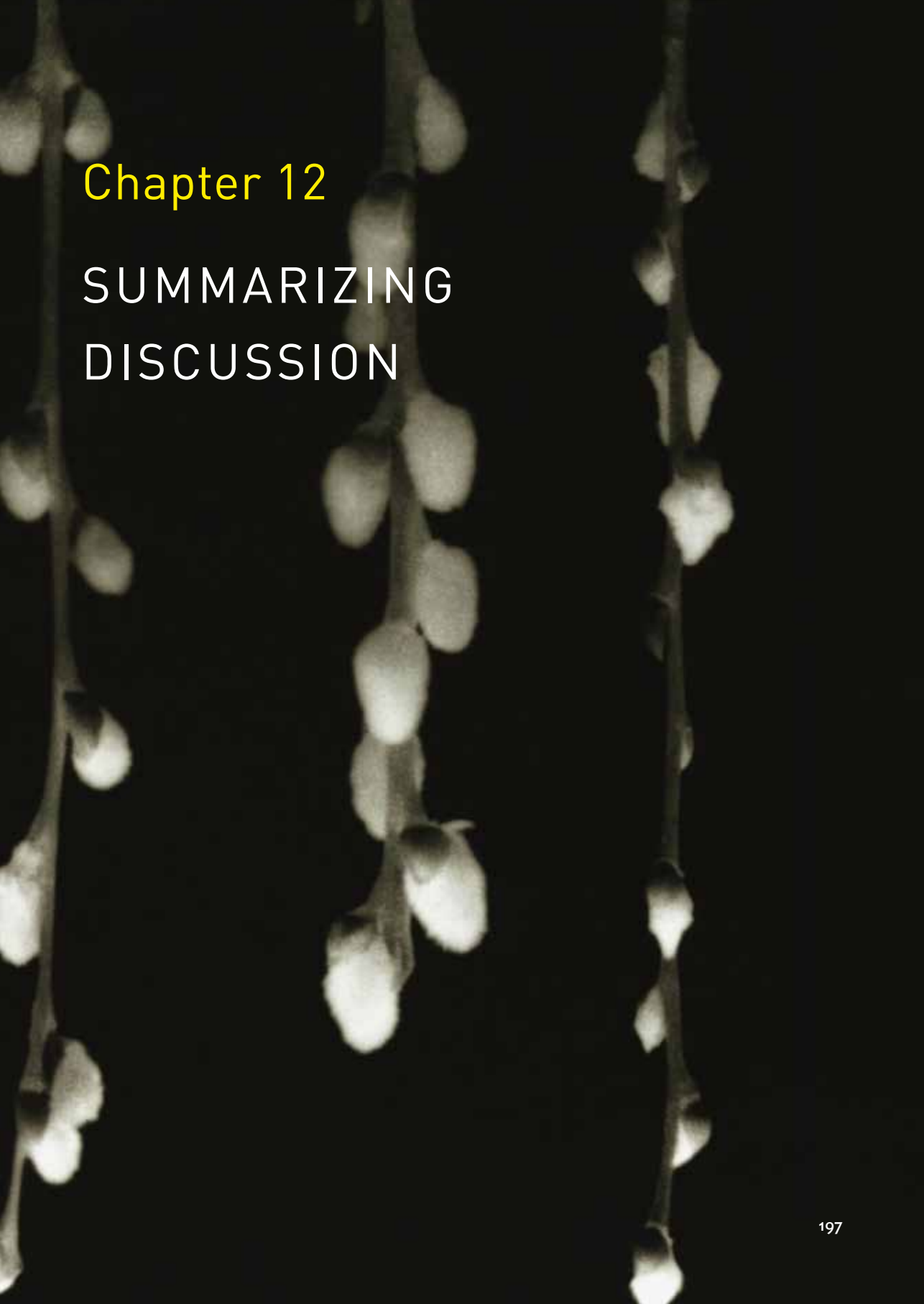

Chapter 12

SUMMARIZING DISCUSSION

The care for premature born neonates has improved considerably over the past few decades. In the Netherlands, the limit of viability is considered to be 24 weeks of gestation (GA), while some other developed countries go as low as 22 weeks of gestation.[1] Despite a decrease in mortality, morbidity is still a considerable burden, not only for the child and its parents, but also for society.

Ideally we would be able to reduce morbidity in such a way that more preterm infants survive without severe disability. The question remains, to which extent are these morbidities an expression of events (e.g. inflammation) that took place before birth, and how much is related to circumstances after birth.[2,3] In other words, is there still room for improvement in the postnatal care we are providing? For the most severe cerebral injuries: peri- and intraventricular haemorrhage (PIVH), associated periventricular haemorrhagic infarction (PVHI), and cystic white-matter injury (WMI) there is a clear association with perinatal disturbances in cerebral oxygenation and blood supply.[4–7] Although this is less straightforward for more global WMI, reports do suggest that postnatal events are related to decreased brain volumes, for instance.[8] Both hypoxia and hyperoxia have been associated with neuronal injury and therefore may contribute to WMI.[9,10] These findings suggest that we could improve neurocognitive outcome by ensuring adequate and stable blood and oxygen supply to the brain.

Simply put, all we are trying to do on an NICU is to ensure an adequate and stable supply of oxygen and other nutrients to the vital organs (e.g. brain, kidneys, gut), similar to what happens during the last trimester of pregnancy. We try to maintain cellular homeostasis through monitoring and managing neonatal haemodynamics. For many years now, the mean arterial blood pressure (MABP) has been one of the most commonly used monitoring parameters as it is continuous, an absolute measure, and shown to be *associated* with neurological sequalae.[11–13] The MABP increases with GA and is the product of cardiac output and systemic vascular resistance and is thus related to systemic perfusion, which also indicates its greatest pitfall.[14,15] It is a macrovascular measure and does not directly represent oxygenation *or* perfusion of the vital organs.[16,17] Over the recent years, evidence is accumulating that the MABP on its own is not sufficient to assess whether or not anti-hypotensive treatment is necessary.[18–20] It has even been suggested that anti-hypotensive treatment itself might be harmful, altogether pointing to the fact that we need additional tools to monitor infant circulatory well-being.[16,21,22]

Near-InfraRed Spectroscopy was introduced as the designated tool to monitor (cerebral) haemodynamics in neonates, as it is non-invasive, continuous, and provides microvascular information.[23] Because NIRS is sensitive to arterial, capillary, and venous compartments, it can also be used as a surrogate of perfusion.[24] Despite numerous studies that showed its relevance in relation to common clinical issues such as PDA, RDS and presumed hypotension, (cerebral) NIRS still is not as commonly

used as the SaO_2, MABP, or aEEG monitoring.[25–27] Presumably the slow acceptance of NIRS is the result of the heterogeneity in 'reference values' reported in literature, and because clear-cut thresholds that are associated with clinically relevant outcomes are lacking. The off-set between adult and neonatal/paediatric sensors only adds to this heterogeneity.[28,29] This off-set probably results from limited options to validate NIRS in neonates, but is perhaps less of a problem as long as one is aware of this off-set and uses sensor-specific reference values and thresholds. However, which sensor provides us with most accurate estimation of the true cerebral oxygenation remains to be seen. The first part of this thesis is focussed on bedside monitoring of preterm infants by means of near-infrared technology as an adjunct, or even a replacement, of the MABP. All in an effort to identify infants whose (cerebral) circulation is compromised. In the second part the focus is shifted from bedside monitoring towards assessing cerebral haemodynamics by MRI, trying to gain knowledge from combining different modalities, and to go beyond the restricted spatial specificity and limited validation of NIRS in the preterm brain.

PART I: BEDSIDE MONITORING

Despite the relatively well known association of haemodynamic instability with PIVH, few studies have reported on continuously measured haemodynamic parameters in relation to the occurrence of PIVH. In **chapter 2**, we describe the patterns of regional cerebral oxygenation ($rScO_2$), cerebral fractional tissue oxygen extraction (cFTOE) and MABP in reference to PIVHs that developed postnatally (i.e. were not present at admission). Each infant with PIVH was matched with two infants who never developed PIVH. The requirement was that infants had $rScO_2$ and MABP data available for at least 24h before, and 24h after the first diagnosis of PIVH on cUS. Infants who developed a severe PIVH, but not mild/moderate PIVH, had higher $rScO_2$ and inversely lower cFTOE values compared to their matched counterparts. This suggests that hyper perfusion, or transition from a low perfusion state to a normal or high perfusion state, to play a key role in the progression from mild/moderate to severe PIVH. In addition, the higher correlation coefficient between MABP and $rScO_2$ suggest that infants who developed a PIVH have a more pressure passive cerebral circulation. In healthy humans, the cerebral circulation regulates its own perfusion, dampening swings in perfusion pressure (i.e. the MABP). So ideally, the cerebral circulation is *not* pressure passive and the MABP would not correlate with the $rScO_2$ at all. A pressure passive circulation can be harmful, especially in the presence of blood pressure swings, which can for example be the consequence of administering inotropes. Indeed, infants with PIVH received more inotropes than their matched controls. All three parameters, the $rScO_2$, cFTOE, and MABP-$rScO_2$ correlation, had relatively good negative predicting values. Although, this population is the largest with continuous parameters and

relatively precise timing of the PIVH (i.e. within 24h), future research with even more frequent cUS's and a more elaborate assessment of cerebral autoregulation could potentially yield clear indicators of an increased risk of PIVH.[30]

In **chapter 3**, low blood pressure was related to $rScO_2$ and especially to long term neurodevelopmental outcome. This was again done using a case-control design, excluding infants with a haemodynamically significant patent ductus arteriosus (*hs*PDA), as a *hs*PDA is known to influence the $rScO_2$. Although the MABP was lower in cases, no differences in $rScO_2$ or neurodevelopmental outcome could be demonstrated between cases and controls. Low $rScO_2$ (i.e. <50% for more than 10% of time) was found to be associated with neurodevelopmental outcome. This effect was not significantly different between infants with and without low blood pressure. A low MABP was by no means related to impaired cerebral autoregulatory capability. The use of inotropics on the other hand showed a dose-response effect with higher doses being associated with less cerebral autoregulatory capability. Together with the association between inotropes and PIVH as described in chapter 2, this raises the question whether we are treating hypotension to vigorously and should rely more on measures of (organ) perfusion. However, to unequivocally state this there is a need for randomised controlled trials proving that treatment based on 'circulatory-status' is equally good or better than treatment based on using a single (MA)BP threshold.

A study that was designed to elucidate this is the "Treatment of Hypotension of Prematurity (TOHOP)" trail. In the TOHOP-trial infants are randomised to receive treatment when the MABP in mmHg is below the GA in weeks, or to receive treatment when there are signs of low organ perfusion or when MABP is <GA-5mmHg. In **chapter 4** an interim-analysis of the short-term results is presented of the infants that were included in the TOHOP trial thus far. The number of infants receiving inotropes was higher in the regular treatment group, but the MABP, cerebral oxygenation and renal oxygen saturation were comparable. At this time, no significant differences could be demonstrated for the adverse events (i.e. ROP, NEC, IVH, or death), or for white- and grey-matter injury as seen on cerebral MRI performed at term-equivalent age. A coincidental finding was a significantly lower renal rSO_2 in infants with a *hs*PDA, even before the cerebral rSO_2 decreased. Although no definite conclusions can be drawn as recruitment is still ongoing, there are no signs that treatment based low MABP alone is better than treatment based on perfusion. Full recruitment, and assessment of outcome at 24 months of age needs to be awaited before definite conclusions can be drawn. In the meantime, renal rSO_2 as a measure of circulatory compromise is worth exploring.

The first step to implement a new parameter in daily clinical care for preterm infants is not only to explore how it behaves in relation to other clinical parameters, but also to see how it varies according to gestational and postnatal age. In **chapter 5**, another circulatory monitoring parameter that uses light in the near-infrared spectrum is investigated, the perfusion-index. The perfusion-index has the advantage that it is readily available on every pulse-oximeter. Infants with a *hs*PDA were found to have a higher PI, suggesting a hyperdynamic circulation. Furthermore, the PI was found to be negatively related to the MABP and administration of inotropes, but positively to volume expansion. The responsiveness of the PI to circulatory parameters suggests that it might be used in the circulatory management of (preterm) infants. This was a retrospective observational study and future research has to determine whether or not it can be used to distinguish the need for therapeutic interventions on the NICU (e.g. fluids vs. inotropes), as already has been demonstrated for fluid responsiveness in infants during surgery.[31]

In **chapter 6**, the evolution of the $rScO_2$ and cFTOE over the first 3 days after birth is studied in more detail. Both parameters traverse a parabolic curve during the first 72h after birth. Gestational age, birth weight, and *hs*PDA are shown to influence the $rScO_2$ and cFTOE. We provide a comprehensive set of parameters that can be used to generate reference curves for every GA. In all cases, the ±2SD bandwidth for the $rScO_2$ was found to be 30% and a single SD encompasses roughly 7%. The variation in $rScO_2$ according to GA and PA shows that one cannot simply use a single target value across the entire preterm population. This is especially relevant when designing studies that use the $rScO_2$ as an observational, or even as an interventional tool. Based on one SD, we recommend assessing an infants' clinical variables when encountering a change in $rScO_2$ >7%, but only after checking that the measurement setup did not change (e.g. sensor position). We provide reference curves for adult and neonatal sensors, and show that when taking into account GA, postnatal age and type of sensor, that values published in literature are not as heterogeneous as previously thought. These results provide reference values for a NICU population, but do not provide clear thresholds that identify infants at risk of adverse outcome. To do so a sweep has to be made through a wide range of fixed thresholds (i.e. not depending on GA or postnatal age) and thresholds that do vary according to GA and postnatal age.

This is what was done in **chapter 7**. In this chapter, the $rScO_2$ during the first three days after birth is studied as a predictor of neurodevelopmental outcome at 15 and 24 months of age, corrected for prematurity. Infants who died or whose neurodevelopmental outcome score was one SD below the mean had significantly lower $rScO_2$ values overall and spend more time below 55%, which was the most predictive fixed threshold. For

GA and postnatal age specific reference values, values below -1.5SD had the strongest association with adverse outcome. High levels of $rScO_2$ were not predictive on their own, but when combined with low levels of $rScO_2$ into time spend outside (e.g. below -1.5SD or above +1.5SD), this proved to be more predictive at 15 months and for motor outcome at 24 months than just time spend below a threshold. Thereby suggesting that high levels of oxygenation are also associated with outcome, be it to a lesser degree than low levels or $rScO_2$. These observations were most obvious on day 3 of life, but could already be demonstrated on the first day of life. Intriguingly, values on day 2 were slightly higher in general and found not to be related to adverse outcome. The fact that both the $rScO_2$ and the associations with outcome vary over time suggest we can still improve outcome and that we do not merely monitor a "static state" brought about by prenatal events. The SafeBoosC study demonstrated that the $rScO_2$ can be influenced by using an intervention guideline designed to stabilise the $rScO_2$.[32]
The levels of $rScO_2$ reported here are slightly higher than values previously thought to be associated with adverse outcome. These thresholds can serve as indicators to assess an infant's circulatory parameters (e.g. blood pressure, haemoglobin levels, cardiac ultrasound), and may prove useful in future studies to assess whether we can actually improve outcome by targeting $rScO_2$ values that are a bit higher and within a slightly narrower bandwidth.

Altogether, these chapters prove that near-infrared technology can be of benefit in daily clinical care. The $rScO_2$, $rSrO_2$, and perfusion-index are all associated with relevant clinical parameters. The $rScO_2$ was previously thought to only be useful as a trend monitor. We showed that, as long as the same position and same sensor type is used in every infant, that even clear thresholds can be identified that are clinically relevant. This body of evidence makes that NIRS can be used clinically as long as one is aware of the possible off-set of a certain device/sensor combination. Two questions remain, can we actually modify outcome by targeting different $rScO_2$ and which device/sensor combination most closely represents the real cerebral oxygenation? [28,29,32] To address the latter issue, there is a need for a gold-standard of cerebral oxygenation that can also be used in preterm neonates. For this, Magnetic Resonance Imaging (MRI) might be of help. Moreover, it could help relating $rScO_2$ of more superficial brain structures to physiology of deeper grey- and white-matter.

PART II: BEDSIDE TO BENCH

The first application of nuclear magnetic resonance to produce two-dimensional images was proposed in 1973.[33] Since then, great advances have been made in terms of spatial resolution and acquisition speed, enabling imaging of the whole brain in just a few minutes. These advances make MRI the designated tool for detailed imaging of the newborn brain. More recently, techniques such as arterial spin labelling (ASL), functional MRI, and respiratory calibrated MRI have introduced the possibility to take a closer look at cerebral haemodynamics.[34–36] Imaging cerebral haemodynamics by MRI has the great advantage that it is non-invasive and does not required ionizing radiation, as is the case with Xenon clearance and positron-emission-tomography, which are currently considered to be the gold-standards to evaluate brain-haemodynamics.[37,38] Despite some challenges due to intrinsically lower signal-to-noise ratio in neonates, ASL has made its transition to the neonatal population.[39,40] The core principle in ASL is that the arterial blood is magnetically labelled, this labelled blood can be imaged and subsequently quantified by applying a general kinetic model.[41] As cerebral blood flow (CBF) is directly related to the amount of HbO_2 delivered to the brain, it is not surprising that NIRS measures of cerebral oxygenation correlate quite nicely with CBF as measured by other modalities.[42,43] Nevertheless, without specifically designed experiments, NIRS in its basic form is only a surrogate for CBF, as it provides measures of cerebral oxygenation.

Providing a true quantitative measure of cerebral oxygenation by MRI is rather complicated. In functional MRI, changes in the Blood Oxygen-Level Dependent (BOLD) signal, mostly arising from tissue surrounding larger venous structures, are visualised.[44–46] The BOLD signal is a function of HHb, which is influenced by tissue oxygen content. As HHb is paramagnetic, it influences the transversal relaxation rate (R_2*) and changes in HHb concentration can thus be visualized in T_2*-weighted images.[47] Unfortunately, the BOLD signal is relative and does not provide a quantitative measure. Respiratory calibrated MRI was introduced to overcome this limitation, and can provide quantitative measures of cerebral oxygen metabolism.[36] By modelling CBF and BOLD data, combined with the appropriate assumptions, it should be possible to determine the cerebral tissue oxygen saturation, comparable to NIRS, but then simultaneously throughout the entire brain. An alternative approach still harnesses the dependency of R_2* on HHb concentration, but does not model the BOLD signal. Instead, the aim is to measure pure blood T_2 (T_{2b}). The main challenge here is to obtain sufficiently blood-weighted images that that are not 'contaminated' too much by signal from other tissue types. As long as the contamination is low, this approach relies on fewer assumptions, and with appropriate knowledge of haematocrit, the measured T_2 can be converted into an oxygen saturation.[48] This has been shown both in adults and neonates in large vessels, but not on a tissue level.[49–51] A step closer to the $rScO_2$ provided by NIRS is the QUIXOTIC approach, which uses venular-targeted velocity-selective

labelling to obtain pure (venous) blood maps.[52] However, this technique suffers from a low signal-to-noise ratio, which makes it less feasible in neonates who have lower CBF and thus a lower signal-to-noise ratio to begin with. In addition, QUIXOTIC is strictly venous and thus ignores the arterial compartment that is included in the $rScO_2$ by NIRS. Targeting both the arterial and venous compartments would overcome these two drawbacks and would match nicely with the oxygen saturation in a mixed arterial-capillary-venous compartment represented by the $rScO_2$ obtained by NIRS. In that respect, these MRI techniques can provide more spatial detail and insight into SO_2 of deeper brain structures, and have the potential to be used to validate NIRS in the neonatal population. In part II of this thesis, the focus lies on the development and application of MRI sequences that visualize cerebral haemodynamics, the cerebral oxygenation in particular. These sequences were compared to results obtained with commercially available NIRS devices.

Chapter 8 provides an overview of the MR techniques that are currently available to quantify cerebral physiology in neonates and discusses the potential clinical applications. The two main implementations of arterial spin labelling (ASL), pulsed ASL and pseudo-continuous ASL are the two most commonly used MR techniques to assess cerebral haemodynamics in neonates. From a technical perspective, there is still a lot of heterogeneity with different in-plane resolutions, labelling approaches, label durations and post-label delays on the acquisition side, and studies correcting for haematocrit variability and those who do not on post-processing side. In that respect, neonatal ASL could benefit from a white-paper, as has been published for adults.[53] The current main application of ASL is in infants with perinatal arterial-ischaemic stroke, hypoxic-ischaemic encephalopathy or congenital heart-disease, and to study brain development. It has been shown that CBF can have good prognostic value in infants with HIE, possibly even slightly better than diffusion-weighted imaging.[54] Studies combining different modalities to gain additional insight into cerebral physiology, and especially studies evaluating parameters of cerebral oxygenation, are still sparse. Therefore, implementation of these oxygen-modalities in daily clinical care is a bridge too far, for now.

Chapter 9 describes an approach that requires a hypercapnia-MR experiment to obtain quantitative oxygen metabolism parameters and combines this with simultaneous NIRS measurements. Healthy adult volunteers were given a face mask through which they were breathing a slightly higher concentration of CO_2. CO_2, a potent vasodilator, causes an increase in CBF and thereby increases the amount of oxygenated haemoglobin but decreases deoxygenated haemoglobin. By simultaneously measuring CBF and the BOLD signal through a dual echo pseudo-

continuous ASL sequence, estimation of the calibration parameter M is possible. Knowing M, representing the theoretically maximal BOLD signal change, the BOLD signal can be used as a quantitative measure of cerebral oxygenation. The oxygen saturation determined by MRI showed a good correlation with the $rScO_2$ that was obtained by NIRS, suggesting that determining oxygenation in a mixed vascular compartment by MRI is feasible. Nevertheless, this approach relies heavily on assumptions and the need for a respiratory calibration experiment makes it impossible to perform in a neonatal population.

In **chapter 10** a novel MR-sequence was used to obtain oxygen saturations in full slice of brain without needing a hypercapnia experiment. This approach relies on obtaining blood-weighted images sensitive to all vascular compartments (i.e. arterial, capillary, and venous). When the signal is T_2-prepared, the measurement of blood T_2 is possible. We used this sequence in 15 neonates and compared data to other measures of cerebral haemodynamics, including cerebral blood flow, venous oxygenation, and also $rScO_2$ as determined by NIRS. The cerebral oxygen saturation determined by means of this T_2-BIOS sequence showed good correlations with CBF, venous oxygenation in the sagittal sinus, and also $rScO_2$. Results show that measuring cerebral oxygen saturation by MRI in neonates is feasible. This is done without the need of a respiratory challenge, which is essential as a respiratory challenge cannot be performed in neonates due to practical and ethical constraints. The current implementation only enables a single slice acquisition. Also there was a bias between the NIRS and MRI measurements for which several sources can be identified: variation in haemoglobin concentration between subjects, and contamination of the blood volume weighted images arising from signal coming from cerebro-spinal fluid and brain matter.

Chapter 11, takes the T_2-BIOS from chapter 10 one step further. The sequence is modified to provide full-brain coverage and now includes a suppression pulse to avoid contamination by signal coming from cerebro-spinal fluid. This sequence is tested in healthy volunteers, who were again subjected to a respiratory challenge. However, this challenge was not necessary to calculate the oxygen saturation, instead it was used to validate the new approach by creating two levels of CBF (i.e. baseline and during hypercapnia) and thus also two levels of cerebral oxygen saturation. The oxygen saturation was compared to CBF obtained by ASL, and parameters of cerebral oxygenation that were simultaneously obtained by using an MR compatible NIRS device. This improved implementation has the great advantage that it is multi-slice and insensitive to signal from cerebrospinal fluid. However, there is no such thing as a free lunch. These two steps forward go at the cost of signal, which needs to be boosted before the sequence can be translated to the neonatal population.

CONCLUSIONS

The following conclusions can be drawn from this thesis

- Hyper perfusion, or an abrupt transition from a low to a higher state of cerebral perfusion is related to the development of severe PIVH. Continuous monitoring of the cerebral circulation might help identifying infants at risk of developing a severe peri- or intraventricular haemorrhage (**chapter 2**).
- Hypotension by definition of the most commonly used treatment threshold, a mean arterial blood pressure below the infants gestational age in weeks, is not associated with a lower neurodevelopmental outcome score at 2 years of age (**chapter 3**).
- The cerebral and renal oxygenation, number of adverse events, and the extent of white- and grey matter injury were all comparable between neonates treated for hypotension by using more liberal treatment threshold and neonates who were treated using the most commonly used treatment threshold (**chapter 4**).
- The perfusion-index was strongly associated with blood-pressure itself and anti-hypotensive treatment. Therefore it is a good candidate to complement monitoring of the cerebral oxygenation in the management of the neonatal circulation (**chapter 5**).
- Values of cerebral oxygenation increase with gestational age and differ according to postnatal age. When taking this into account, results reported in literature are less heterogeneous than previously thought (**chapter 6**).
- Low values of cerebral oxygenation are associated with adverse outcome. This was already demonstrated on the first day after birth. The absolute values are slightly higher than reported in literature thus far, suggesting that more infants are at risk than anticipated (**chapter 7**).
- Measuring cerebral oxygenation by means of a respiratory-calibrated MR experiment is possible and showed good correlations with indices of cerebral oxygenation as measured by NIRS (**chapter 9**).
- Measuring cerebral oxygenation in a mixed vascular bed, comparable to NIRS, without needing a respiratory challenge is feasible and shows good correlations with indices of cerebral oxygenation as measured by NIRS, as well as with perfusion measured by MRI. Thereby the application of this technique is feasible in (preterm) neonates (**chapter 10 and 11**).

FUTURE DIRECTIONS OF RESEARCH

"HYPOTENSION"

Low MABP does not necessarily affect oxygenation and perfusion of the vital organs (chapter 4).[26,27] Neither does it seem to affect short- and long term outcome (chapter 3 and 4).[19,55] Nevertheless, no long-term outcome results from randomised trials that compare conventional treatment thresholds to treatment based on oxygenation and perfusion have been published so far. In addition, it might be that there are more subtle effects of hypotension on grey- and white-matter that can only be seen using advanced MRI techniques, such as micro-structural analysis, cortical morphology analysis, and connectomics.[56,57] We will continue recruitment in the TOHOP-trial and will prepare the acquired MRI data for micro-structural and morphological analysis. For future studies we would suggest including resting state fMRI in the imaging protocol. More efficient networks have been related to cognitive outcome, at least in adults.[58] As far as the monitoring and management of hypotension is concerned, both the regional renal oxygenation and perfusion-index are worth exploring. For instance, the pleth-variability index (i.e. a derivate of the perfusion-index) has been shown to indicate hypotension responsive to volume during surgery.[31] These two parameters might identify infants who are (at that moment) still able to maintain cerebral oxygenation, but are the verge of full-blown circulatory compromise and could benefit from specifically targeted circulatory support.

BEDSIDE MONITORING OF CEREBRAL OXYGENATION

The variable associations over time of $rScO_2$ with outcome (i.e. day 1 and 3 vs. the lack of associations on day 2, chapter 7) hint that we are not merely monitoring events that were set in motion before birth, but that we can still take action to influence outcome. Recently, it has been shown that the burden of hypoxia, as recorded by NIRS, can actually be modified.[32] To unequivocally state that cerebral NIRS is of benefit for the individual infant, there is a need for a large multi-center interventional trial which uses clinically relevant outcomes, including long-term follow-up, and advanced MRI to assess cerebral injury and connectivity. For future trials, we suggest the use of reference values that are specific for gestational-age and postnatal-age (chapter 6+7), instead of using fixed thresholds.

Besides monitoring the cerebral oxygenation, NIRS can also be used a surrogate for cerebral blood flow. When combining NIRS with MABP, assessment of cerebral autoregulation becomes possible.[59,60] Reliable, bedside monitoring of cerebral autoregulatory capacity is considered the 'holy grail' in neonatal neuromonitoring. The rationale for this is to establish the optimal MABP to ensure maximal vasoreactivity and thus optimal cerebral autoregulation. However, currently there is controversy as to the best methodology to assess cerebral autoregulation, be it simple running linear correlation analysis as applied in chapters 2 and 3, frequency domain analysis, or even the use of HR instead of MABP.[61,62] Moreover, current studies were all retrospective data-analyses. To make monitoring of cerebral autoregulation truly viable for clinical application, on-the-fly methods need to be developed that incorporate both time- and frequency domain analyses. The biggest challenge will be automated artefact identification and rejection that need to be performed instantaneous.

MEASURING OXYGENATION BY MRI

Although we have proven that is it possible to measure cerebral oxygenation in a non-invasive way and demonstrated good correlations with $rScO_2$, the next step would be calibration of the T_2-BIOS against a true gold-standard. Only then it can serve as true middle-man to sort-out what the true cerebral oxygenation is in reference to the different NIRS sensors. To accomplish this, blood of varying levels of oxygenation and haematocrit is necessary, similar to what has been done in vitro calibration study for the TRUST sequence.[48] The latter can be accomplished by a membrane oxygenator, which at the same time can be used to vary the flow velocity of the blood.[29] As soon as the calibration has been performed, work needs to be done to translate the multi-slice approach (chapter 11) to the neonatal population. To do so, some SNR constraints need to be overcome. This can be done in several ways, one approach would be improving the readout, an alternative would be the use of a neonatal 32-channel head

coil. Dedicated head coils have proven to deliver a considerable SNR increase over adult 32-channel head coils, let alone 8-channel which have been used in the studies in this thesis.[63]

A second research track for the oxygenation sequence is the exploration of its clinical applications, especially when combining multiple modalities. For instance, in perinatal arterial ischaemic stroke it is relevant to know how large the penumbra is.[64] The T_2-BIOS could be overlaid on diffusion weighted images. Regions with restricted diffusion but low SO_2 (i.e. high oxygen extraction) would suggest potentially viable tissue, whereas regions with actual dead tissue would show restricted diffusion and high SO_2 as a consequence of decreased O_2 utilization.[65] In the latter, efforts to save tissue that is still viable might not be worthwhile, while these efforts might be beneficial in the former.

FROM BIG BROTHER TO BIG DATA: MULTI-MODALITY AND MULTI-SITE MONITORING

Monitoring of cerebral autoregulation is already a wonderful example on what can be accomplished by combining different modalities. The benefit, but also the complexity of the $rScO_2$ lies in the fact that it combines supply, consumption, and outflow in a single measure. The combination with amplitude integrated electroencephalography could help making a better distinction in the actual cause of changes in $rScO_2$ and subsequently better targeting of therapy.[66] A slightly different approach would be multi-site NIRS monitoring.[67–69] We have shown in chapter 4 that renal NIRS might be more sensitive to pick-up changes in haemodynamics related to a (developing) *hs*PDA. It would be of interest to study how renal NIRS relates to cerebral NIRS in a large group of preterm infants, see how it relates to clinical variables (e.g. fluids, inotropes, ventilation), and know how it responds to drops in arterial saturation.

Currently, most studies focus on maybe a couple of parameters, at most. It would be interesting to combine a multitude of parameters into one single monitoring system and subsequently try to automatically point out infants who are at risk for adverse events. The increasing inter-connected design of modern intensive care units makes the NICU an ideal setting for such a monitoring system. However, there are several problems with a hypothetical setup like this. First of all, how would we select the variables to include? The number of possible parameters is enormous to begin with, and their interdependency is overwhelming. Combined with their potential derivatives, for instance MABP-$rScO_2$ combined to assess autoregulation giving rise to several more parameters, this yields a list of variables that is beyond comprehension. In addition to the number of variables, there are also technical challenges: the amount of data that is collected in a single day is already tremendous,

the collected data is very divers (e.g. sample rates >200Hz for aEEG vs. daily cranial ultrasound, and monitored parameters vs. human observations), and how does one apply weighting as not every parameter is equally important? Data-sets obtained by this hypothetical setup would be perfectly suited to analyse with a "big-data" approach that tries to discover previously unknown relations in datasets that are too large to be managed with conventional data management systems.[70,71]

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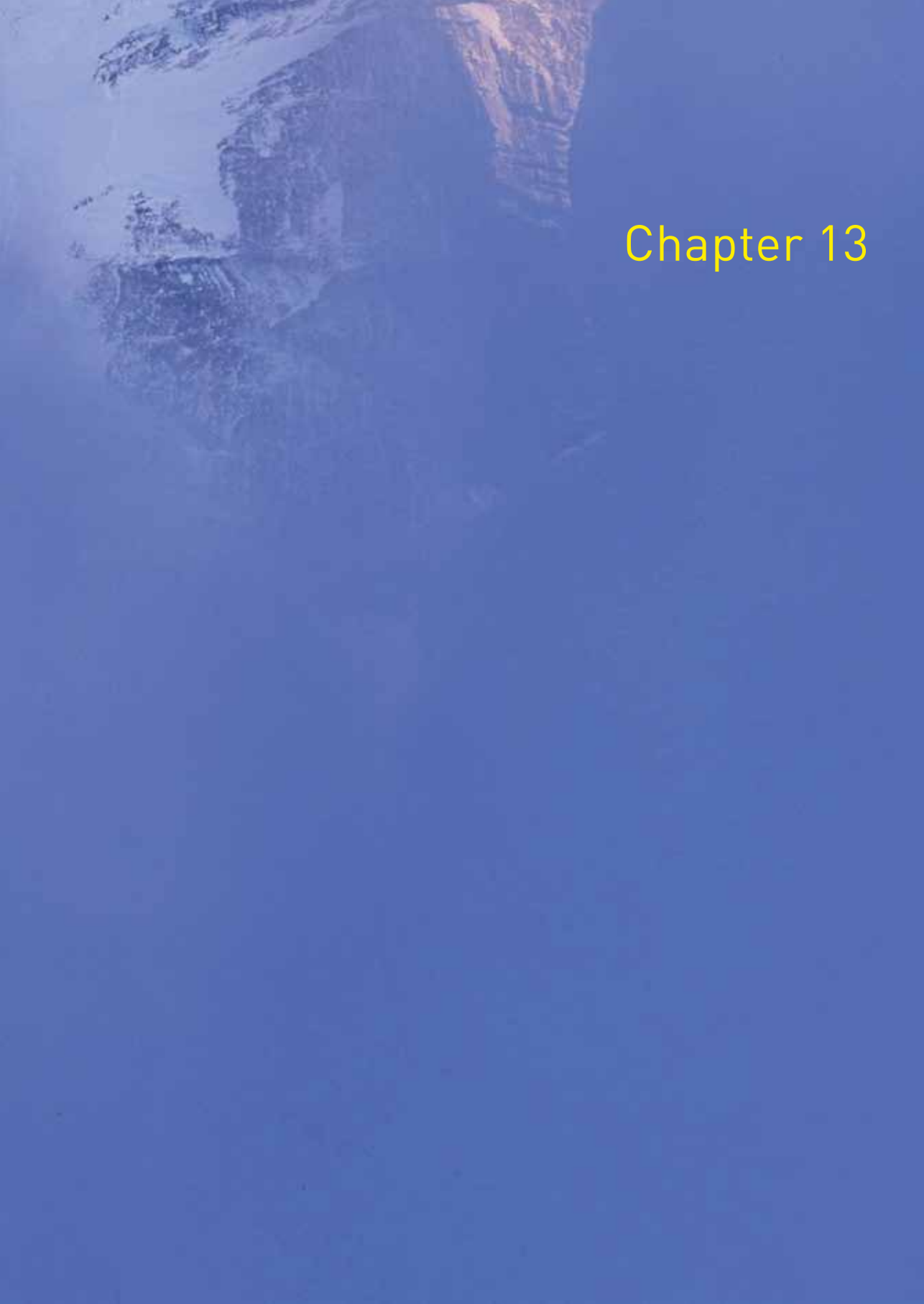

Chapter 13

NEDERLANDSE SAMENVATTING

De afgelopen decennia is de zorg voor prematuur geboren kinderen sterk verbeterd. Echter ondanks dat de mortaliteit is afgenomen gaat prematuriteit nog steeds gepaard met veel complicaties.[1] Deze complicaties zijn met name een belasting voor het kind, de ouders, maar daarnaast ook voor de maatschappij.
In een ideale wereld zouden we in staat zijn om ook deze complicaties terug te dringen, waardoor een groter aantal te vroeg geborenen overleeft zonder ernstig restletsel en dan met name zonder ernstig hersenletsel. De grote vraag blijft echter of en in welke mate deze complicaties een uiting zijn van gebeurtenissen die zich hebben afgespeeld voor de geboorte en hoe sterk deze gerelateerd zijn aan gebeurtenissen die na de geboorte hebben plaatsgevonden[2,3] Anders gezegd, is het mogelijk om hersenschade na de geboorte van premature pasgeborenen te voorkomen door verbeteringen in zorg? De meest voorkomende vormen van hersenletsel bij deze groep kinderen zijn die door bloedingen in de hersenventrikels en schade aan de witte stof van de onrijpe hersenen. Er zijn vele factoren die een rol spelen bij de ontwikkeling van hersenschade bij de prematuur geborene, maar in veel gevallen spelen verstoringen in de zuurstof- en bloedvoorziening van de hersenen een belangrijke rol.[4–10] Dit suggereert dat we complicaties kunnen verminderen en de neuromotorische ontwikkeling van deze prematuur geboren kinderen mogelijk kunnen verbeteren door ervoor te zorgen dat de bloed en zuurstofvoorziening naar de hersenen niet alleen afdoende is, maar ook niet teveel schommelingen vertoont.
Dit vormt de basis van de zorg op de een neonatale intensive care unit (NICU). Op de NICU wordt gestreefd naar een geleidelijke overgang van het leven in de baarmoeder naar het leven buiten de baarmoeder zo geleidelijk mogelijk te laten gaan en zo de bloed en zuurstofvoorziening naar de vitale organen optimaal te houden. Om dit te bewerkstelligen worden verschillende aspecten van de circulatie en zuurstofvoorziening gemonitord, zoals de hartslag, de zuurstofsaturatie in het slagaderlijke bloed en de bloeddruk. De bloeddruk is één van de belangrijkste en meest gemeten parameters. De bloeddruk is een absolute parameter die continue gemeten kan worden en die een directe relatie heeft met de pompfunctie van het hart. De bloeddruk vormt een belangrijke maat omdat lage bloeddruk en neurologische complicaties met elkaar in verband lijken te staan.[11–13] De bloeddruk is een resultante van de hoeveelheid bloed die het hart rondpompt en de weerstand die heerst in de bloedvaten. Normaliter stijgt de bloeddruk in de eerste dagen tot weken na de geboorte.[14,15] Dat de bloeddruk een resultante is van pompfunctie en vaatweerstand is ook meteen een beperking; het is geen directe maat voor de absolute hoeveelheid bloed en zuurstof dat uiteindelijk de organen bereikt.[16,17] De afgelopen jaren is er steeds meer bewijs gekomen dat het behandelen van een bloeddruk onder een bepaalde grens (passend bij de leeftijd van een prematuur geboren kind) geen behandeldoel op zich moet zijn.[18–20] Er zijn zelfs aanwijzingen dat behandeling van lage bloeddruk

mogelijk schadelijk is voor met name de hersenen. Derhalve hebben we eigenlijk andere hulpmiddelen nodig om de zuurstof en bloedvoorziening van de organen van zieke pasgeborenen in de gaten te houden, en waar nodig bij te sturen.[16,21,22]

"Near-InfraRed Spectroscopy (NIRS)", is reeds vele jaren beschikbaar en lijkt dé aangewezen techniek om bij pasgeborenen de circulatie van de hersenen in de gaten te houden.[23] Deze techniek maakt gebruik van de doorgankelijkheid van vrijwel alle weefsels voor (nabij)infrarood licht én de eigenschap dat het zuurstofdragende molecuul in rode bloedcellen (hemoglobine) dit licht in verschillende mate absorbeert en reflecteert. De mate van absorptie en reflectie is vooral afhankelijk van het al dan niet aan zuurstof gebonden zijn van het hemoglobinemolecuul. Door gebruik te maken van minimaal twee golflengten en een redelijke afstand tussen de lichtbron en de detector (3-4cm), is het mogelijk om de relatieve concentraties hemoglobine mét en zonder zuurstof uit te drukken als een zuurstof saturatie. Hierbij wordt het aan zuurstof gebonden hemoglobine uitgedrukt als een percentage ten opzichte van de totale hoeveelheid hemoglobine, de regionale weefsel zuurstof saturatie ($rStO_2$). Er zijn door de verschillende fabrikanten van NIRS apparatuur verschillende termen beschreven die allen de regionale zuurstofsaturatie weergeven,: "Tissue-Oxygenation Index" (TOI), "Tissue Saturation Index" (TSI), "Oxygen Saturation (SO_2)", of de "regional tissue oxygen saturation" (rS_tO_2). In deze samenvatting maken we gebruik van de $rStO_2$, waarbij de 't' varieert afhankelijk van de locatie van de sensor: $rScO_2$ voor de saturatie van de hersenen en $rSrO_2$ voor de saturatie van de nieren.

In tegenstelling tot de arteriële saturatie monitor geeft NIRS-monitoring de zuurstofvoorziening weer in de weefsels en niet alleen in het zuurstofrijke arteriële bloed. In de weefsels bevindt het grootste deel van het bloed zich in het veneuze, zuurstofarme, zuurstofarme systeem. De $rStO_2$ wordt dus vooral bepaald door de zuurstofconcentratie in het veneuze systeem. In de hersenen bevindt ongeveer 70% van het bloedvolume zich in de veneuze vaten. Wanneer men vervolgens deze relatief veneus gewogen $rScO_2$ combineert met de arteriële zuurstof saturatie (SaO_2), is mogelijk om de zuurstofextractie van de hersenen te berekenen (cFTOE): $cFTOE = [SaO_2 – rScO_2]/ SaO_2$. Deze afgeleide maat beweegt doorgaans in tegengestelde richting van de $rScO_2$. Als voorbeeld: wanneer de zuurstoftoevoer (de $rScO_2$) toeneemt, maar het verbruik gelijk blijft, dan zal er procentueel minder zuurstof uit het bloed gehaald worden om absoluut dezelfde hoeveelheid aan het weefsel te leveren. Hierdoor daalt dus de cFTOE. Een bijkomstig voordeel van NIRS is dat de verschillende oxygenatie parameters ook gebruikt kunnen worden als surrogaat maat voor cerebrale doorbloeding.[24]

Ondanks dat er vele studies zijn geweest die de relevantie van NIRS hebben aangetoond in relatie tot veel voorkomende klinische problemen, is NIRS nog steeds niet zo geïntegreerd in de dagelijkse zorg als het monitoren van bijv. de SaO_2,

hartslag of bloeddruk.[25–27] Deze relatief trage integratie van NIRS in de dagelijkse praktijk komt vooral door afwezigheid van eenduidige referentiewaarden en het ontbreken van grenswaarden die een duidelijke relatie hebben met weefselschade. Bijkomende problemen zijn de relatief hoge prijs van de sensoren en het feit dat volwassen sensoren enerzijds en de kinder/baby sensoren anderzijds, verschillende waarden meten.[28,29] Dit verschil is hoogstwaarschijnlijk het resultaat van de beperkte mogelijkheid om NIRS te valideren in pasgeborenen. In volwassenen is het mogelijk om de zuurstofsaturatie te bepalen in afvoerende (veneuze) bloedvaten dichtbij de hersenen. Dit is bij pasgeborenen op de NICU, die soms niet meer wegen dan 500-800 gram, niet mogelijk. Hierdoor is validatie van normale waarden in deze groep zeer moeilijk, en is dit met name gebeurd in diermodellen en bij ernstig zieke kinderen aan de hart-long machine. Deze twee methoden lijken beiden niet representatief voor prematuur geborenen op een NICU. Dus welke sensor geeft ons de meest exacte afspiegeling van de daadwerkelijke zuurstofsaturatie in de hersenen van prematuur pasgebornene?

In het eerste deel van dit proefschrift wordt met name het monitoren van cerebrale oxygenatie van te vroeg geboren neonaten met behulp van NIRS beschreven. Het focus van onderzoek ligt met name op het gebruik van de NIRS techniek in de dagelijkse klinische praktijk als toevoeging, of zelfs vervanging, van de bloeddrukmonitoring met als doel een adequate bloed- en zuurstofvoorziening van de kwetsbare onrijpe hersenen te garanderen. In het tweede deel ligt de focus op het meten van de cerebrale oxygenatie met behulp van geavanceerde MRI technieken. Door gebruik te maken van MRI wordt gepoogd om enkele tekortkomingen van NIRS te overkomen, te weten: de beperkte validatie van NIRS in pasgeborenen en het feit dat nabij-infrarood licht ongeveer 2 cm het weefsel indringt alvorens het uitdooft en daarmee dus geen informatie geeft over diepere hersen structuren.

DEEL I: CONTINUE MONITORING OP DE NICU

Er is nog weinig bekend in de literatuur over de relatie tussen de continue gemeten parameters betreffende zuurstof en bloedvoorziening van de hersen en het ontstaan van peri- en intraventriculaire bloedingen. In **hoofdstuk 2** worden de patronen van de $rScO_2$, cFTOE en de bloeddruk beschreven in relatie met het ontstaan van deze bloedingen. Terugkijkend in reeds verzamelde data werden prematuren onderzocht die een hersenbloeding hadden ontwikkeld en waarbij minimaal 24 uur voor én 24 uur na het vaststellen van de bloeding betrouwbare $rScO_2$ en bloeddrukdata beschikbaar waren. Als controlegroep werden prematuren geselecteerd zonder hersenbloeding, maar met vergelijkbare zwangerschapsduur en geboortegewicht.
Kinderen die een ernstige bloeding ontwikkelde, dat wil zeggen een bloeding

die >50% van de hersenkamers vulde en/of gepaard ging met en infarct van het omliggende hersenweefsel, hadden een hogere $rScO_2$ en lagere cFTOE direct voor de bloeding in vergelijking met de controlegroep zonder bloeding. Dit doet vermoeden dat hier sprake was van een verhoogde doorbloeding van de hersenen, of een overgang van verlaagde doorbloeding naar een hoog-normale doorbloeding op dat moment. Tevens werd er een sterkere relatie gevonden tussen de bloeddruk en de $rScO_2$ in kinderen met een bloeding, wat zou kunnen passen bij een verminderde cerebrale autoregulatie. Cerebrale autoregulatie is het proces waarbij de hersenen de eigen bloedtoevoer reguleren en stabiliseren, onafhankelijk van de bloeddruk. In het meest ideale geval hebben de bloeddruk en $rScO_2$ dus geen relatie met elkaar, maar wanneer dit wel zo is dan worden schommelingen in de bloeddruk rechtstreeks doorgegeven aan de hersenen, waardoor er een verhoogd risico is dat de bloedvaten onder te grote druk komen te staan of dat de doorbloeding te kort schiet.
Het was opvallend dat de kinderen met bloedingen ook meer bloeddruk ondersteunende medicatie hebben gekregen en meer schommelingen in bloeddruk vertoonden. Tot nu toe is dit de grootste groep prematuren waarbij continue monitoringsparameters en relatief precieze timing van de bloeding (een marge van 24u) werden gecombineerd in één onderzoek.[30]

In **hoofdstuk 3** werd onderzocht of lage bloeddruk (een gemiddelde bloeddruk die lager was dan de zwangerschapsduur in weken) een relatie had met $rScO_2$ en in het bijzonder met de mentale ontwikkeling van het kind op de leeftijd van 18-24 maanden. Zoals verwacht hadden kinderen met een lage bloeddruk meer bloeddruk ondersteunende medicijnen nodig, echter er werden geen aanwijzingen gevonden dat lage bloeddruk een relatie had met een lagere $rScO_2$ of een slechter ontwikkeling op 18-24 maanden. Daarentegen was een lage $rScO_2$ (<50% gedurende meer dan 10% van de bestudeerde periode) wel geassocieerd met een slechtere ontwikkeling. Er werd geen relatie gevonden tussen een lage bloeddruk volgens bovenstaande definitie en verminderde cerebrale autoregulatie, gemeten als de correlatie tussen gemiddelde bloeddruk en $rScO_2$. Wel was er een duidelijke relatie tussen hogere doseringen bloeddruk ondersteunende medicatie en verminderde cerebrale auto regulatie. De resultaten uit hoofdstuk 2 en 3 leidden tot de vraag of de lage bloeddruk wel behandeld moet worden met medicatie die potentieel gevaarlijke bijwerkingen kan hebben, zeker als parameters die weefseldoorbloeding weergeven normaal en stabiel zijn. Om deze vraag goed te kunnen beantwoorden zal eerst door middel van een gerandomiseerd onderzoek moeten worden aangetoond dat het starten van circulatoire ondersteuning op basis van weefseldoorbloeding op zijn minst net zo veilig als het starten van behandeling op geleide van alleen de bloeddruk.

Met dit doel voor ogen is de "Treatment of Hypotension of Prematurity (TOHOP)" studie opgezet. In deze studie worden kinderen die op het moment dat ze lage bloeddruk hebben, gerandomiseerd voor conventionele behandeling (alleen op geleide van de bloeddruk) danwel behandeling op geleide van maten van orgaandoorbloeding. In **hoofdstuk 4** wordt een tussentijdse analyse van de korte termijn resultaten van de deelnemers aan de TOHOP studie tot nu toe beschreven. De hoeveelheid gebruikte bloeddruk-ondersteunende medicatie blijkt hoger in de conventionele groep, echter de bloeddruk, de $rScO_2$, de cFTOE en de zuurstof voorziening van de nieren ($rSrO_2$) waren uiteindelijk niet verschillend tussen de twee groepen. Op dit moment zijn er geen verschillen wat betreft ongewenste complicaties. Tevens werden er geen verschillen gezien tussen de twee groepen wat betreft de schade aan witte- en grijze stof, zoals werd gezien op een MRI-scan die werd uitgevoerd op de uitgerekende datum. Hoewel er nog geen harde conclusies mogen worden getrokken voordat er 150 kinderen in de studie zijn geïncludeerd, zijn er op dit moment geen aanwijzingen dat behandeling op geleide van doorbloeding nadelige effecten heeft.
Binnen de patiënten van de TOHOP studie die een openblijvende ductus arteriosus hadden werd ook een belangrijke bevinding gedaan. De ductus is een bloedvat dat de longslagader en lichaamsslagader met elkaar verbindt en heeft een belangrijke functie in de bloedsomloop van het kind tijdens de zwangerschap. Dit vat sluit zich na de geboorte als de longen gaan functioneren. De kinderen met een ductus die open bleef na de geboorte hadden een evident lagere rSrO, wat een maat is voor de nier doorbloeding. Dit verschil was al statistisch significant voordat er een verschil ontstond in de $rScO_2$, welke normaliter wordt meegewogen in de behandeling van een openblijvende ductus op de NICU in het UMC Utrecht.

Bij elke nieuwe monitorparameter die wordt geïntroduceerd in de dagelijkse klinische praktijk is het niet alleen belangrijk om te weten hoe deze zich gedraagt in relatie tot andere klinische parameters, maar ook hoe deze samenhangt met de zwangerschapsduur en postnatale leeftijd. In **hoofdstuk 5**, wordt een andere parameter bestudeerd die ook gebruik maakt van licht in het infrarode spectrum, de perfusie-index. In tegenstelling tot NIRS geeft de perfusie-index meer inzicht in de algehele circulatie en niet op orgaan niveau. Een voordeel is dat deze parameter beschikbaar is op vrijwel iedere arteriële saturatiemonitor en daarmee een ideale kandidaat is om te gebruiken bij de integrale beoordeling van de circulatie, naast o.a. de $rScO_2$ en de bloeddruk. In deze studie werden 311 kinderen bestudeerd en werden referentiewaarden van de perfusie-index vastgesteld gedurende de eerste 3 dagen na de geboorte. Kinderen met een openblijvende ductus arteriosus bleken een aanzienlijk hogere perfusie-index te hebben dan degenen zonder open ductus, wat past bij het feit dat het hart meer bloed moet rondpompen om het lichaam van

voldoende bloed te voorzien. Voorts bleek de perfusie-index een negatieve relatie met de bloeddruk en toegediende bloeddruk ondersteunende medicatie te hebben , maar een positieve relatie met het toedienen van extra vocht via het infuus bij verlaagde bloeddruk. De reactiviteit van de perfusie-index op verschillende circulatoire parameters geeft aan dat we deze maat in de toekomst kunnen gaan gebruiken om de circulatoire toestand van prematuur geboren kinderen beter te kunnen monitoren en tevens beter te evalueren of een behandeling het gewenste effect heeft.[31]

In **hoofdstuk 6**, wordt de bloeddruk losgelaten en wordt dieper ingegaan op het verloop van zowel de $rScO_2$ als de cFTOE gedurende de eerste 3 dagen na de geboorte. Dit werd gedaan door $rScO_2$ en cFTOE data die continu werden verzameld gedurende 72 uur na de geboorte in 999 prematuren te analyseren voor het creëren van referentiewaarden. Beide parameters bleken te verlopen volgens een parabolische curve, de $rScO_2$ met een piek en de cFTOE met een dal op 36 uur na de geboorte. Zowel zwangerschapsduur, geboortegewicht, als een openblijvende ductus arteriosus waren van invloed op de $rScO_2$ en cFTOE. Een model wordt gepresenteerd voor de $rScO_2$ en cFTOE waarmee het mogelijk is om referentie waarden te genereren, ongeacht de zwangerschapsduur. Voor alle zwangerschapsduren was de bandbreedte tussen -2 standaard deviaties en +2 standaard deviaties, zo'n 30% voor de $rScO_2$ en 0.32 voor de cFTOE. De sterke relatie met zwangerschapsduur en postnatale leeftijd toont aan dat het minder wenselijk is om een vaste afkapwaarde te gebruiken voor een populatie kinderen met variërende zwangerschapsduur. Dit is in het bijzonder van belang wanneer de $rScO_2$ wordt gebruikt als observationele of zelfs interventie parameter in huidige en toekomstige studies. Een enkele standaard deviatie in $rScO_2$ is ongeveer 7-8%, in het verlengde hiervan zouden we willen adviseren om de klinische parameters van het kind (beademing, bloeddruk, lab waarden, echografie van het hart) na te lopen wanneer er een relatief plotse verandering plaatsvind in $rScO_2$ >7%. Echter moet altijd eerst nagegaan worden of er niets is veranderd aan de meetopstelling, bijvoorbeeld of de sensor niet is verschoven. Om zowel tegemoet te komen aan NICU's die volwassen sensoren gebruiken, als zij die de nieuwere neonatale sensoren gebruiken werden referentie curves gemaakt voor beide typen sensoren. De resultaten in dit hoofdstuk geven ook aan dat de "referentie waarden" in de literatuur lang niet zo heterogeen zijn als men rekening houdt met de zwangerschapsduur, postnatale leeftijd en de gebruikte sensor. De volgende stap is het identificeren van heldere afkap waarden voor de $rScO_2$ die op individueel niveau gebruikt kunnen worden. Om dat te doen zal er een breed scala aan afkapwaarden moeten worden onderzocht, zowel vaste afkapwaarden als afkap waarden die variëren aan de hand van referentiewaarden zoals die hier zijn gegeven.

Bovenstaande wordt bestudeerd in **hoofdstuk 7**. In dit hoofdstuk wordt beschreven de of de $rScO_2$ gedurende de eerste 3 dagen na de geboorte een relatie heeft met de motorische en mentale ontwikkeling op de leeftijd van zowel 15 als 24 maanden, gecorrigeerd voor prematuriteit. De hypothese hierbij was dat zowel lage als hoge $rScO_2$ waarden schadelijk kunnen zijn voor de hersenen en dus geassocieerd zijn met een ongunstige ontwikkeling. Kinderen die overleden in de eerste levensweken of meer dan één standaard deviatie onder het gemiddelde scoorden tijdens ontwikkelingstests bleken een lagere $rScO_2$.waarden te hebben in de eerste dagen na de geboorte. Wanneer één vaste afkap waarde werd gebruikt voor alle prematuur geborenen bleek een $rScO_2$ <55% het meest discriminerend tussen kinderen met een ongunstige en gunstige uitkomst. Gebaseerd op de referentiewaarden uit hoofdstuk 6, is een $rScO_2$ beneden 1.5 standaard deviatie het sterkst voorspellend voor ongunstige uitkomst. Dit was ook een sterkere voorspeller dan de vaste afkapwaarde van 55%. Een hoge $rScO_2$ was alleen voorspellend wanneer zij werd gecombineerd met de lage waarden in één maat (tijd boven 1.5 *of* onder -1.5 tezamen). Hiermee kan geconcludeerd worden dat hoge waarden ook een relatie hebben met ongunstige ontwikkeling, hoewel in mindere mate dan lage waarden. Al deze bevinding waren het meest uitgesproken voor de $rScO_2$ op dag 1 en 3. Dat zowel de $rScO_2$, als de associaties met neuromotorische ontwikkeling varieert over dag 1,2 en 3 suggereert dat we nog winst kunnen boeken en dat we niet simpelweg een statisch geheel aan het monitoren zijn dat het resultaat is van gebeurtenissen voor de geboorte. De gevonden afkap waarden zijn enigszins hoger dan voorheen gedacht, wat een indicatie is dat een grotere groep kinderen risico lopen om zich ongunstig te ontwikkelen op basis van een verminderde zuurstof en bloedtoevoer naar het brein. De identificatie van deze afkapwaarden is stap 1, de volgende stap is bekijken of we met interventies gericht op de $rScO_2$ ook daadwerkelijk de neuromotorische ontwikkeling kunnen verbeteren. Het is reeds aangetoond dat een interventie richtlijn in ieder geval de $rScO_2$ kan stabiliseren.[32] Toekomstige studies kunnen gebruik maken van de meer specifieke afkapwaarden die hier geven worden.

Samenvattend geven hoofdstuk 2-7 aan dat technologie die gebruik maakt van licht in het infrarode spectrum zeker van toegevoegde waarde kan zijn in de dagelijkse klinische zorg op een NICU. Tot op heden werd de $rScO_2$ vooral gezien en gebruikt als een trend monitor van de zuurstofvoorziening naar de hersenen. Echter de hier beschreven studies laten zien dat, zolang de sensor positie en het type sensor hetzelfde zijn in ieder kind, er ook absolute grenswaarden zijn vast te stellen die een sterke relatie hebben met klinisch relevante eindpunten. Echter er is op dit moment nog geen antwoord op een drietal vragen: 1) kunnen deze eindpunten daadwerkelijk beïnvloedt worden door te streven naar een gewenste $rScO_2$, 2) hoe verhoudt de relatief oppervlakkig gemeten $rScO_2$ zich tot de zuurstof saturatie in dieper gelegen

hersen structuren en, 3) welk apparaat in combinatie met welke sensor geeft de meest accurate weergave van de werkelijke zuurstofsaturatie in de hersenen?[28,29,33] Deze laatste twee vragen kunnen mogelijk of deels worden beantwoord door gebruik te maken van nieuwe MRI technieken. Deze technieken zijn veelbelovend en mogelijk in staat om zowel een gouden standaard te bieden voor het meten van cerebrale oxygenatie in neonaten, alsmede inzicht te geven in de zuurstof saturatie van dieper gelegen hersen structuren.

DEEL II: VAN KLINIEK NAAR EXPERIMENTELE SETTING

De eerste toepassing van het gebruik magnetische velden om tweedimensionale beelden te produceren stamt uit 1973.[34] Sindsdien zijn erg grote sprongen gemaakt op het gebied van resolutie en snelheid van het vervaardigen van deze beelden, wat ervoor heeft gezorgd dat "Magnetic Resonance Imaging (MRI)" bij uitstek geschikt is om de hersenen van pasgeborenen in beeld te brengen. Het grote voordeel van MRI is dat het niet-invasief is en er geen stralingsbronnen nodig zijn om beelden te vervaardigen.[35,36] Recentere ontwikkelingen zoals "Arterial Spin Labelling (ASL)", functionele MRI (fMRI) en respiratoir gekalibreerde MRI hebben het zelfs mogelijk gemaakt om te kijken naar circulatoire aspecten van het brein.[37–44]

Hoewel tegenwoordig veel aspecten van de cerebrale fysiologie in beeld gebracht kunnen worden, blijft het een uitdaging om de cerebrale oxygenatie in beeld te brengen. Bij fMRI onderzoek wordt er wel gebruik gemaakt van het feit dat de hoeveelheid gemeten signaal afhangt van de hoeveelheid hemoglobine dat *niet* is gebonden aan zuurstof. Hemoglobine bevat ijzeratomen welke magnetische eigenschappen hebben. De mate waarin deze ijzeratomen wel (gebonden aan zuurstof) of niet worden afgeschermd beïnvloedt daardoor het lokale magneetveld.[45–47] Zodoende kunnen veranderingen in niet gebonden hemoglobine worden gevisualiseerd met MRI.[48] Echter, zonder kennis van de concentratie ongebonden hemoglobine aan het begin van het experiment blijft fMRI slechts kwalitatief. Om deze reden werd respiratoir gekalibreerde MRI ontwikkeld.[39] Hierbij wordt tegelijkertijd de doorbloeding (d.m.v. ASL) als het fMRI signaal geregistreerd en is het mogelijk om de theoretische maximale signaal verandering vast te stellen, waardoor het fMRI signaal omgezet kan worden naar een kwantitatieve maat. Met de juiste aannames is het vervolgens mogelijk om de zuurstofsaturatie in het brein te bepalen die vergelijkbaar is met de met NIRS gemeten $rScO_2$, maar dan niet regionaal, maar simultaan in het gehele brein.

Waar fMRI gebruik maakt van lokale veranderingen in het magneetveld, richt een andere aanpak zich op het rechtstreeks meten van magnetische eigenschappen van bloed. De grote uitdaging hier is om het signaal afkomstig van bloed te scheiden van signaal afkomstig van het hersenweefsel. Echter, wanneer dergelijke opnames zijn verkregen is het grote voordeel dat het omzetten van deze magnetische

eigenschappen in een zuurstofsaturatie relatief eenvoudig is, zonder dat er al te veel aannames moeten worden gedaan.[49] Dat deze aanpak werkt, is aangetoond voor de grotere vaten in zowel volwassenen als neonaten. Helaas is dit alleen in de grote vaten en dus nog steeds niet op micro vasculair niveau waarbij het arteriële en veneuze bloed gecombineerd wordt in één parameter, zoals dat bij NIRS het geval is.[50–52] In deel II van dit proefschrift ligt de focus op de ontwikkeling en toepassing van MRI technieken die de cerebrale hemodynamiek in beeld brengen en daarbij met name de zuurstofsaturatie in het brein. Deze geavanceerde MRI technieken worden steeds vergeleken met resultaten van commercieel verkrijgbare NIRS apparaten.

Hoofdstuk 8 geeft een overzicht van de verschillende MRI technieken die op dit moment beschikbaar zijn om de fysiologie van het neonatale brein in beeld te brengen en bediscussieert de mogelijke klinische toepassingen daarvan. "Arterial Spin Labelling (ASL)" is de meest gebruikte techniek en vanuit een technisch perspectief is er nog steeds veel heterogeniteit wat betreft de gebruikte resolutie, de aanpak van het labelen van het bloed en hoe het signaal wordt omgezet in een fysiologisch betekenisvolle maat. Een uniforme protocol zoals voorgesteld in de white-paper voor volwassenen zou veel bij kunnen dragen aan de uniformiteit van ASL onderzoek in pasgeborenen.[53] De voornaamste toepassing van ASL in pasgeborenen ligt momenteel bij kinderen met een perinataal arterieel ischemisch infarct, globale hypoxische-ischemische hersenschade of aangeboren hartafwijkingen en ter bestudering van de ontwikkeling van de hersenen. Er is nog weinig literatuur waarin verschillende modaliteiten gecombineerd worden om zo meer inzicht te verkrijgen in de fysiologie dan dat elke modaliteit afzonderlijk kan leveren. In het bijzonder is er nog weinig onderzoek gedaan naar de klinische toepassing van MRI technieken die de cerebrale oxygenatie in kaart brengen.

In **hoofdstuk 9** werd een respiratoir gekalibreerd MRI experiment uitgevoerd om zo in één keer de zuurstof saturatie in het gehele brein te kunnen meten. Dit werd gedaan terwijl tegelijkertijd de $rScO_2$ werd gemeten met een MRI compatibel NIRS apparaat. Gezonde volwassen vrijwilligers kregen een masker op waardoor ze een verhoogde CO_2 concentratie inademden. Een verhoogd CO_2 geeft een toename in bloeddoorstroming én een positief fMRI signaal, waardoor kalibratie van het fMRI signaal mogelijk is. De zuurstof saturatie die met MRI werd bepaald toonde een goede correlatie met de $rScO_2$. Deze resultaten zijn veelbelovend omdat ze laten zien dat het mogelijk is om met MRI een zuurstof saturatie te bepalen waarin het arteriële en veneuze bloed in één maat wordt gecombineerd en daarmee in theorie vergelijkbaar zou moeten zijn met hoe met NIRS de $rScO_2$ gemeten wordt. Desondanks leunt deze aanpak zwaar op aannames en zijn er evidente ethische en praktische bezwaren om dergelijk respiratoir experiment uit te voeren in pasgeborenen.

In **hoofdstuk 10** wordt een andere aanpak gebruikt om de zuurstofsaturatie in de hersenen te meten. Er wordt een geheel nieuwe MRI techniek beschreven waarmee het mogelijk is om de zuurstof saturatie te bepalen in een hele plak hersenweefsel, maar nu zonder dat er een respiratoir experiment voor nodig is. Met deze methode wordt alleen het verval van signaal vastgelegd in (arterieel en veneus) bloed. De snelheid van dit verval kan vervolgens worden omgezet in een zuurstofsaturatie.[54] Deze techniek werd onderzocht in 15 pasgeborenen waarbij ook de doorbloeding van de hersenen (ASL) en de zuurstof saturatie in veneuze drainerende systeem werden gemeten met MRI. Tevens werd de $rScO_2$ gemeten. Er waren goede correlaties tussen al deze parameters onderling wat laat zien dat het meten van de cerebrale zuurstofsaturatie goed mogelijk is met MRI zonder een respiratoir experiment, zelfs in neonaten. Dit onderzoek toont tevens aan dat het signaal dat afkomstig is van hersenvocht een afwijking kan veroorzaken in de zuurstof saturatie die wordt gemeten met MRI. De volgende stap is het verbeteren van deze techniek zodat het gehele brein in één keer in beeld kan worden gebracht en dat het signaal afkomstig van hersenvocht een kleinere invloed heeft op het gemeten signaal.

In **hoofdstuk 11** wordt er voortgebouwd op de techniek die beschreven wordt in hoofdstuk 10. Er zijn verbeteringen doorgevoerd die ervoor zorgen dat nu het gehele brein in één keer in beeld kan worden gebracht en er zijn aanpassingen gemaakt waardoor het signaal afkomstig van het hersenvocht wordt onderdrukt. Bij een experiment vergelijkbaar met hoofdstuk 9 ademden gezonde vrijwilligers een verhoogde concentratie CO_2 in door een masker. Doordat de gebruikte MRI techniek anders is dan in hoofdstuk 9, was dit niet nodig om überhaupt een kwantitatieve maat te verkrijgen, wel werd dit respiratoire experiment gebruikt op twee doorbloedingsniveaus (basaal niveau en verhoogd niveau) te creëren en daarmee ook twee niveaus van cerebrale zuurstofsaturatie. De gemeten zuurstofsaturatie werd vergeleken met de doorbloeding van de hersenen (met behulp van ASL) en de zuurstofsaturatie in de grote drainerende veneuze vaten. Daarnaast werd tegelijkertijd weer de $rScO_2$ geregistreerd met NIRS.
Er was een goede relatie tussen de $rScO_2$ en de zuurstofsaturatie gemeten met MRI. Er was slechts een minimale afwijking tussen beide methoden en bovendien leek de grootte van deze afwijking niet samen te hangen met de hoogte van de zuurstofsaturatie, zoals dat wel het geval was in hoofdstuk 10. Dit wijst erop dat deze verbeterde MRI techniek specifieker gericht is op signaal afkomstig van bloed en minder gevoelig is voor afwijking afkomstig van statische weefsel (witte en grijze stof) of hersenvocht.
Hoewel de correlaties tussen de verschillende parameters veelbelovend zijn blijven er nog uitdagingen bestaan om dit ook in neonaten toe te passen. Deze

implementatie kent twee grote voordelen, het in beeld brengen van het hele brein en de onderdrukking signaal afkomstig van hersenvocht, maar helaas is de signaalsterkte op dit moment onvoldoende om dit 1-op-1 toe te passen in neonaten. De volgende uitdaging wordt dan ook om juist dit aspect te verbeteren alvorens we dit toe kunnen passen in neonaten.

CONCLUSIES

De volgende samenvattende conclusies kunnen worden getrokken.

- Het monitoren van de cerebrale zuurstof saturatie kan helpen om prematuren te identificeren die risico lopen op het ontwikkelen van een ernstige intraventriculaire bloeding (**hoofdstuk 2**).
- "Lage" bloeddruk, gedefinieerd als een bloeddruk beneden de zwangerschapsduur in weken, is niet geassocieerd met ongunstige neuromotorische ontwikkeling op de leeftijd van 2 jaar (**hoofdstuk 3**).
- Er zijn geen aanwijzingen dat behandeling van verlaagde bloeddruk op geleide van perfusie parameters in de eindorganen minder veilig is dan behandeling op basis van een vaste grens per zwangerschapsduur (**hoofdstuk 4**).
- De perfusie-index is een goede parameter om het moment van starten en het effect van circulatoire ondersteuning vast te stellen (**hoofdstuk 5**).
- De gemeten waarden van cerebrale zuurstof saturatie is afhankelijk van de zwangerschapsduur en postnatale leeftijd, wanneer men deze in acht neemt zijn resultaten uit literatuur minder heterogeen dan aanvankelijk gedacht (**hoofdstuk 6**).
- Lage cerebrale saturatie is geassocieerd met ongunstige motorische en mentale ontwikkeling op 2-jarige leeftijd. Dit verhoogde risico kan al op de eerste dag na de geboorte worden aangetoond (**hoofdstuk 7**).
- Het is mogelijk om de cerebrale zuurstofsaturatie te meten in een gecombineerd arterieel-veneus compartiment door middel van respiratoir-gekalibreerd MRI experiment (**hoofdstuk 9**).
- Ook zonder respiratoir-kalibratie experiment is het mogelijk om de zuurstof saturatie te meten, wat deze techniek toepasbaar maakt in neonaten. Zuurstof saturatie gemeten met MRI toonde een goede overeenkomst met de zuurstofsaturatie gemeten door middel van NIRS (**hoofdstuk 10 en 11**).

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LIST OF ABBREVIATIONS

aEEG	Amplitude integrated electroencephalography
AGA	Appropriate for gestational age
ASL	Arterial spin labelling
BOLD	Blood oxygen level dependent
BPAP	Bi-level positive airway pressure
BSID-II-NL	Bayley Scales of Infant Development, Second (Dutch) Edition
BSITD-III-NL	Bayley Scales of Infant and Toddler Development, Third (Dutch) Edition
BW	Birthweight
CA	Corrected age
CBF	Cerebral blood flow
CBV	Cerebral blood volume
CCS	Corticosteroids
cFTOE	Cerebral fractional tissue oxygen extraction
CHD	Congenital heart disease
$CMRO_2$	Cerebral metabolic rate of oxygen
CO	Cardiac output
CP_{10}	Values standardized to a CO_2 change of 10mmHg
$\%CP_{10}$	Percentage change in respect to baseline per 10mmHg P_{ETCO_2} increase
CPAP	Continuous positive airway pressure
CRIB-II	Clinical risk index for babies version II
CSF	Cerebro-spinal fluid
cUS	Cranial Ultrasound
CVR	Cerebrovascular reserve
D_{bt}	Density of brain tissue
DPF	Differential path length factor
DQ	Developmental quotient
DWI	Diffusion Weighted Imaging
eCS	Emergency caesarean section
EEG	Electroencephalography
EPI	Echo-planar imaging
eTE	Effective echo time
fCBF	Frontal brain CBF
fMRI	Functional magnetic resonance imaging
fNIRS	Functional Near-InfraRed Spectroscopy
FOV	Field-of-View
GA	Gestational Age
GAMLSS	Generalized Additive Models for Location, Scale and Shape
GMDS	Griffiths Mental Development Scales
Hb	Haemoglobin
HELLP	Haemolysis Elevated Liver enzymes and Low Platelets
HC	Hypercapnia
Hct	Resting haematocrit of large arteries and veins
HFOV	High-frequency oscillatory ventilation
HHb	Deoxygenated haemoglobin
HIE	Hypoxic-ischaemic encephalopathy
HR	Heart-rate
*hs*PDA	Haemodynamically significant patent ductus arteriosus
InSurE	Intubation-Surfactant-Extubation
IQR	Inter-quartile range
IVIM	Intra-voxel incoherent motion
LED	Light-emitting diode
L	Average light-source / detector separation distance
M	Theoretical maximal BOLD signal change
ΔM	ASL subtraction images
M_{ob}	Fully relaxed magnetization of blood
MABP	Mean-arterial blood pressure
MLEV	Malcolm Levitt's composite-pulse-decoupling
MRI	Magnetic resonance imaging
MW_{Hb}	Molecular weight of haemoglobin
NC	Normocapnia
NDO	Neurodevelopmental outcome
NEC	Necrotising enterocolitis
NICU	Neonatal Intensive Care Unit

NIRS	Near-InfraRed Spectroscopy
ω_a	Arterial fraction of blood volume at baseline
ω_c	Capillary fraction of blood volume at baseline
ω_v	Venous fraction of blood volume at baseline
O_2Hb	Oxygenated Haemoglobin
OD	Optical density
OEF	Oxygen extraction fraction
PA	Postnatal age
PA-sq	The square of postnatal age, for modelling time
PAIS	Perinatal arterial ischaemic stroke
PASL	Pulsed arterial spin labelling
pCASL	Pseudo-continuous arterial spin labelling
PC-MRA	2D phase contrast magnetic resonance angiography
pCBV	Pseudo cerebral blood volume
PE	Pre-eclampsia
PET	Positron emission tomography
P_{ETCO_2}	End-tidal CO_2
P_{ETO_2}	End-tidal O_2
PI	Perfusion index
PIVH	Peri-Intraventricular Haemorrhage
PMS	Phase modulation spectroscopy
pPROM	Preterm premature rupture of membranes
$\Delta\rho$	Optical source separation distance
R	Large-to-small vessel haematocrit ratio
RDS	Respiratory distress syndrome
ROC	Receiver operator characteristics
ROI	Region of Interest
SafeBoosC	Safeguarding the Brains of our Smallest Children
SD	Standard deviation
rSO_2	Regional oxygen saturation
$rScO_2$	Regional cerebral oxygen saturation
$rSrO_2$	Regional renal oxygen saturation
S_aO_2	Arterial oxygen saturation
SO_2	Oxygen saturation
SES	Socioeconomic status
SGA	Small for gestational age
SIMV	Synchronised intermittent mandatory ventilation
SNR	Signal-to-noise ratio
SRS	Spatially resolved spectroscopy
SVC	Superior vena cava
S_vO_2	Venous oxygen saturation
SVR	Systemic vascular resistance
T_1	Longitudinal magnetization
T_{1b}	T_1 of blood
T_2	Transverse magnetization
T_{2b}	T_2 of blood
T_2-BIOS	T_2-prepared Blood Imaging of Oxygen Saturation
T_2-FLAIR	T_2-weigthed fluid attenuation inversion recovery
T_2-TRIR	T_2-prepared Tissue Relaxation with Inversion Recovery
tHb	Total haemoglobin
TEA	Term-equivalent age
TI	Inversion time
TOHOP	Treatment of Hypotension of Prematurity
TR	Repetition time
TRS	Time resolved spectroscopy
TRUST	T_2-Relaxation-Under-Spin-Tagging
V_o	Venous fraction of blood volume at baseline
V_a	Arterial blood volume during hypercapnia
V_c	Capillary blood volume during hypercapnia
V_v	Venous blood volume during hypercapnia
wbCBF	Whole brain CBF
WMI	White matter injury

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2 De Vis JB, **Alderliesten T**, Hendrikse J, Petersen ET, Benders MJ. Magnetic Resonance Imaging based non-invasive measurements of brain haemodynamics in neonates; a systematic review. [Submitted]

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Op een gegeven moment is het dan zover, je bent de oudste onderzoeker. Door de jaren heen zijn er heel wat promovendi langsgekomen op de neo. Kristin, ofwel "de professor". Dat is natuurlijk een inside joke tussen ons twee. Die "professor" is niet meer en we hebben elkaar in de loop der jaren beter leren kennen, met als hoogtepunt de vakantie in de VS. Hoe gaaf was dat? Dat doe ik graag nogmaals. Inmiddels in de onderzoeksgroep bij de psych, waar je compleet nieuwe materie leert, maar zeker 'je mannetje' staat. Succes met je promotietraject. Nienke, eigenlijk kende we elkaar helemaal niet zo goed tot we bij elkaar

op de kamer kwamen. Je bent enorm toegewijd en weet waar je over praat. Ik heb het idee dat je altijd weet wat er speelt bij de mensen om je heen en je bent heel attent. Heel leuk vond ik het bijv. het etentje en de 'speurtocht naar de moordenaar van onze geliefde actrice'. Dat moeten we vooral nog eens doen. Ik heb met veel plezier een werkplek met jou gedeeld. Nathalie, daar aan de overkant van de gang. Heel regelmatig waren wij de laatste twee onderzoekers die de neo verlieten, beide wachten tot die verrekte file's waren opgelost. Jij heb een lastige knoop moeten doorhakken over hoe jouw promotietraject er verder uit zou gaan zien. Nu ga je samen met de neo, kinder ic en cardiologie je tanden zetten in een fantastisch nieuwe project. Succes hiermee, ik heb er geen twijfels over dat je dit kunt. Lisanne, altijd vrolijk en eerlijk. Ik heb het idee dat je altijd zegt wat je denkt en dat vind ik echt heel prettig. Druk bezig met het chirurgie project en dat loopt als een zonnetje, dat is voor een groot deel jouw verdienste. Goed bezig! Lex, ik heb jou gewoon onder het kopje promovendi geschoven. In mijn afwezigheid was jij opeens de enige man op de neo. Ik weet nog goed dat je zei 'dat komt wel goed', toch zei je wat anders aan het einde van je stage ;D. Op het werk hebben we elkaar weinig gezien, maar daar buiten des te meer, super leuk. Laura, jij bent toch wel de grootste (import) Amsterdammer(t) die ik ken. Dat er buiten 020 ook nog leven bestaat, daar verbaas jij je nog wel eens over. Zonder dollen, je hebt je zaakjes goed voor elkaar en weet wat je wilt, dat bewonder ik. Ik vond het super gezellig om bij jou te BBQ-en. Wanneer weer? Lauren, daar zaten we dan in Tampa in die grote rode auto, wat was de omgeving daar verder saai hè? Vrij kort daarop gingen we naar Canada, dat was een hele belevenis. Van Vancouver naar Calgary, wat was het soms nog koud. Van beren tot gletsjers, we hebben het allemaal gezien daar. Terug in Nederland moest je plots verhuizen, wat toch wel even tegenviel. Tijdens het werk voor je promotie voer je ook nog de Anser studie uit, die heeft je wel een paar extra grijzen haren opgeleverd, niet? Nu net terug van je avontuur in Leuven en op naar jouw promotie! Als je eens even moet 'ventileren', Rotterdam-Utrecht is echt niet zo ver als het lijkt, zeker niet met die mooie nieuwe auto van je! Bovendien kom ik nu bijna dagelijks langs Utrecht, dus een kleine tussenstop is zo gemaakt. Heel veel succes met het afronden van jouw promotietraject, dat gaat helemaal lukken. Daar heb ik alle vertrouwen in! Inge-Lot, jij hebt al heel wat promovendi zien komen en gaan. Je bent ontzettend precies en dat is één van de dingen die ik enorm waardeerde als jij een van mijn mede auteurs was. Ik bewonder je inzet en toewijding. Verder ben je altijd in voor een praatje, heel gezellig. Johanneke, wat kan ik toch altijd hartelijk lachen op jouw opmerkingen die soms compleet uit het niets kwamen (voor mij). Het ene moment ben je hard en nauwgezet aan het werk en het andere moment komt er een heerlijke oneliner, fantastisch! Nu alweer enige tijd in opleiding tot kinderarts in Leiden, heel veel succes daar! Karina, na hard werken was daar een schitterend proefschrift en nu ga je aan de slag in het AMC. Ik wil je heel veel plezier en succes wensen, ik heb er alle vertrouwen in dat je een geweldige kinderarts wordt. Britt, wij hebben elkaar maar heel kort meegemaakt op de neo, maar des te vaker tijdens alle promoties en feestje daarna, altijd gezellig. Nu alweer ruim een jaar in opleiding tot psychiater, volgens mij zit je daar op je plek. Margaretha, vorig jaar was het zo ver, je promotie was een feit. Je bent de rust zelve en maakt vaak gevatte opmerkingen, heel prettig om me samen te werken. Ook bewonder ik je doorzettingsvermogen, met een kleine thuis en een kleine onderweg toch je proefschrift afmaken, chapeau! Hilde, ondanks enkele tegenslagen tijdens jouw promotietraject heb ik je zelden niet vrolijk gezien. Nu na afronding van je proefschrift en succesvolle verdediging ook jij aan de bak als assistent in opleiding tot kinderarts. Super leuk! En natuurlijk Julia, dacht je dat ik jou zou vergeten? Ik heb er werkelijk van genoten om een werkkamer met jou te delen. Vooral alle grappige afbeeldingen op de muur, 'the wall of great ideas', onze kunstwerken van post-it's en ga zo maar door. Jij maakte 'werk' een stuk leuker. Af en toe kon ik je wel achter het behang plakken als je weer een hele zak wortels of iets anders hard en knapperigs aan het eten was, maar daar kan ik nu alleen maar hartelijk om lachen! De gezelligheid mis ik af en toe wel. Verder kom ik graag een keer langs om jouw 'casa' te bewonderen in Amsterdam!

Dear Caterina, Maria-Luisa, Simona, Matteo, Silvia and Monica, throughout the years a lot of Italians visited the WKZ and we were always sharing the office. Ti voglio bene (to all of you). My love for Italy has grown a lot, all thanks to you guys. We discovered that we are quite different (Cate and ML, remember Miami ;D), but also that we are so similar at the same time. More than one of you told me that I was somehow born Italian and switched at birth. Caterina, your stay in the Netherlands was hard at first but we became good friends and still everytime we see eachother it is like you never left. Maria-Luisa, we had so many good conversations about all things in life. At work, at the pancake house, everywhere. Please tell your mother that I still want a special 'ML mother phone' on which she can call me every day just like she calls you (at least 5 times). Simona, I had so much fun with you. In the beginning, we sometimes misunderstood eachother which led to hilarious situations (ML can confirm this). We had a lot of fun togheter, the pictures in the office confirm this (looking at them right now). Matteo, the only guy that dared to make the trip to the Netherlands. I think you are very funny and your view of life is inspiring. You have a talent for spotting character

traits in an instant, very impressive. Selfie Silvia, I think it is very special that you seem always happy, even when life is not so kind to you. I think you are a wonderful person, It is a shame that I've missed 6 months of your stay with us because I was not there. The same goes for Monica, or Moni as we like to call you, I missed most of you time with us, which is sad. The more reason for me to come to Siena and visit you all! And for when you guys are giving me a hard time, I will never forget: Non mi rompere I coglioni!

Except from a 'cohort' of Italians there were always other visitors. Firdose and Aneta, you two stayed for a bit longer than a couple of weeks. It was an absolute pleasure to have you with us. Also, I found it very interesting to hear about life in your respective countries. Firdose, I still don't understand why you refused to cycle ;).

Dear Jo, David, Serena, Julia, Marian, Matthew, Joanna, Joseph, Helen, Marjam, Serena, Allesandro, Dafnis (big D), Kay, Piergiorgio, Luca, Anita, Max, Fran, Lorenzo, Giulio, Jana, Chris, Emer, Rui, Paul, Giovanni, Michelle H, Michelle K, David C, Gareth, the six months in London have been absolutely fantastic. We've had so many good laughs, conversations, drinks, … the list goes on. You've made me feel at home and part of the group. Without you guys I couldn't have done it! Now, you're all invited to come over to the Netherlands (not all at the same time though, that would be complicated). And of course, Dr. Tomoki Arichi, dear Tom, I couldn't have wished for a better supervisor than you. You've taught me so much about fMRI. Together we've made combined functional MRI/ASL work, we've proved David wrong ;D! And we also accomplished the ultimate goal in life, a picture together with Prof. Buxton! I am amazed by your approach to life. You just go for it (everything) and everyone loves you! You really bring a good vibe to every occasion, not a thing many people can say. Also It was lovely to meet your kids, James and Hanako, and your wife Judy. I'm hoping we can keep collaborating in the future. It was great!

'Even naar de overkant', dat gebeurde met grote regelmaat. Vrijwel altijd ging dat over MRI's. Ik wil alle laboranten hartelijk bedanken voor de hulp bij het maken van de MRI's en alle leuke gesprekken die we hebben gehad. Beste Prof. Dr. J. Hendrikse, beste Jeroen, je hebt zoveel bereikt en tegelijkertijd toch zo met beide benen op de grond blijven staan. Je hebt een geweldig gevoel voor humor en heel leuk hoe je altijd even langs kwam om te kijken hoe het ging als Esben, Jill en ik weer aan het stoeien waren met de 'RespirAct'. Jeroen (Siero), ik wil je enorm bedanken voor je hulp bij het werken met Matlab. Ik begon als echte 'dummy' en ben nu toch echt in staat om mijn eigen code te schrijven en code van anderen te interpreteren. Super bedankt! Succes met je verdere carrière, ik hoop dat we elkaar in de toekomst nog eens tegenkomen en misschien wel eens iets samen kunnen doen op het gebied van fMRI bij neonaten? En natuurlijk niet te vergeten, Jill. Wat kan jij toch ontzettend hard werken. In no-time heb jij een schitterend proefschrift geproduceerd waar veel mensen jaloers op kunnen zijn. Met jouw kennis en kunde kan het niet anders dat je een geweldige Radioloog wordt. Ik heb genoten van alle experimenten die we samen hebben uitgevoerd. Verbazingwekkend toch hoe snel we steeds dingen voor elkaar krijgen als we ons ergens op focussen? Goede voorbeelden zijn te vinden in deel 2 van dit proefschrift. De volgende uitdaging wordt T_2-metingen in liquor samen met Liza, ik heb er nu al zin in!

Charlotte, Anna, Juliette, Yara, Liza, Eline en Melina. Ik vond het heel leuk om met jullie samen te werken, hopelijk vinden jullie dat ook. Anna, op sokken over de gang, fantastisch. Juliette, volgens mij ben je nooit echt vrienden geworden met het echo apparaat, maar nu op je plek bij de Genetica in Groningen. Charlotte, super ijverig en secuur waardoor het in het begin lastig was de rode draad in alle literatuur voor de review te ontdekken. Je leerde echter snel en je had de smaak daarna goed te pakken, super. Yara, mooi werk met als eindresultaat een artikel, daarna naar de VS, ik ben benieuwd hoe het daar is. Liza, heen en weer naar de 3T standalone, het hield niet op, maar je deed het met overtuiging. Eline, even tussendoor onderzoek doen op de afdeling van je vader. We hadden zo graag een artikel gehad met zowel de eerste als laatste auteur 'van Bel'. Je ben heel spontaan, vrolijk en gezellig. Super leuk. Melina, steeds maar worstelen met SignalBase op een trage PC, wat zeg ik meerdere trage pc's tegelijk toch? Daar bovenop ook nog klinische data die niet altijd perfect was, een hele opgave. Ik heb je er echter nooit over horen klagen, respect!

René, Ben, Bram, Roland, John en Luuk bedankt voor al jullie hulp tijdens mijn onderzoek. Jullie waren altijd bereid om 'out-of-the-box' te denken en zo hebben we samen leuke en goede oplossingen verzonnen voor problemen. In het bijzonder wil ik

René, Ben en Luuk bedanken. René zonder jou hadden we SignalBase niet gehad en zonder jouw constante bereidwilligheid om functies aan SignalBase toe te voegen was ik allang gek geworden van het analyseren van alle data. Ben, bedankt voor al je hulp bij het verzamelen/zoeken/herstellen van data, je hebt altijd creatieve ideeën. Luuk, jij was altijd bereid om mee te denken en je hebt een belangrijke rol gespeeld om het BedBase project van de grond te krijgen op de NICU. Zonder de computers die jij voor ons hebt geregeld hadden we nu nog met de oude 'toren' gemeten.

Beste dr. N.J.G. Jansen, beste Koos, je hebt ons meer dan eens uit de brand geholpen wanneer op de NICU alle aEEG en NIRS apparaten in gebruik waren. Ik mocht je altijd bellen en altijd mochten we apparatuur lenen bij de PICU.

Beste verpleging, van TOHOP en Thomas was het natuurlijk een kleine stap naar TOHOP-Thomas. Sommigen van jullie dachten zelfs dat ik de afkorting van de studie zo had bedacht of andersom dat Petra mij had aangenomen vanwege mijn voornaam. Beide zijn niet het geval (voor zover ik weet). Hoe dan ook, zonder jullie inspanningen was het onmogelijk geweest om alle NIRS apparaten aan te sluiten bij alle kinderen en al net zo onmogelijk om een gerandomiseerde studie uit te voeren bij deze populatie. Bedankt voor jullie inzet, jullie zijn top!

Ha Wilma, Els, Monique en Fuad, ik heb genoten van jullie gezelligheid en vooral ook de begroetingen geroepen vanaf unit 4 terwijl ik bij unit 1 (of vice versa) liep

Beste Hanneke, Ineke, Karin, Jacqueline, Marjan, Erica, Mariska, hoe vaak heb ik niet bij jullie gestaan al glimlachend met mijn 'puppy blue eyes' als ik weer iets geregeld moest hebben of als ik gewoon koekjes/dropjes kwam eten? Volgens mij ontelbare keren. Enorm bedankt voor jullie hulp en gezelligheid!

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Beste kinderartsen, arts-assistenten, verpleegkundigen en al het paramedisch personeel van het Meander Medisch Centrum in Amersfoort. Na 4 jaar onderzoek doen was het toch echt weer tijd om de kliniek in te gaan. Ik heb daar met spanning, maar vooral veel plezier naar uitgekeken. Hoe leuk is het dat ik, na mij semi-arts stage in het oude ziekenhuis, mijn eerste baan als assistent kindergeneeskunde bij jullie mag starten in het nieuwe ziekenhuis. Ik wil jullie bedanken voor al het geduld en begrip dat jullie hadden. Een mooi nieuw ziekhuis, met leuke collegae en een prettige werksfeer, ik had me geen betere plek kunnen wensen.

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met dat mooie blokjes rooster in Excel. Dat laatste heb ik mee meerdere malen voorgenomen, maar is nooit gelukt. Het mooie van onze vriendschap vind ik dat, ondanks dat we de deur niet platlopen bij elkaar, het is altijd meteen 'goed' wanneer we elkaar zien. Ook geniet ik keer op keer van jouw passie voor lekker eten en goede wijn. Volgens mij hebben we al jaren het voornemen om een keer te gaan fietsen en dan niet alleen naar een BBQ, laten we dat nu toch eens een keer doen.

Jolien, wij kennen elkaar al meer dan 20 jaar, hoe gek is dat? Van ik hangend achter jouw brommer (de TOMOS) tot jij hangend in mijn wiel op de fiets, super leuk. Jacco, ik heb jou leren kennen toen jij en Jolien 'wat met elkaar kregen'. We hebben veel vergelijkbare interesses en zijn al snel goede vrienden geworden. Jullie zijn nu getrouwd, maar wonen gelukkig samen in het mooie Rotterdam. Ik geniet altijd met volle teugen van jullie aanwezigheid, of dat het nu op de fiets is of met een glas bier of wijn in de hand. Meer dan eens hebben jullie me geholpen om dingen te relativeren, misschien niet altijd direct gerelateerd aan dit proefschrift, maar daardoor niet minder belangrijk om dit proefschrift tot een goed einde te kunnen brengen. Jullie zijn altijd vrolijk en positief, daar kunnen veel mensen een voorbeeld aan nemen. Paul, jij hoefde eigenlijk geen seconde na te denken toen ik vroeg of ik 'even' bij jou mocht wonen, dat 'even' werden meer dan 6 maanden. Ik heb me altijd thuis gevoeld aan de Viruly en het heeft me enorm geholpen om met je te kunnen te praten en zo mijn leven weer een beetje op orde te krijgen. Zonder die duwtjes in de rug had dit boekje er nu zeker nog niet geweest. We gingen van een koekje bij de thee naar cask strength Whisky, aan beide heb ik zeer goede herinneringen en mis ik ze stiekem wel. En het straatje achter is nog steeds vlak, dat hebben we toch goed dichtgelegd met z'n 2! Nu samen met Mijke en de camper, heel gezellig en jullie passen perfect bij elkaar. Ik ben benieuwd waar jullie terecht komen met jullie zoektocht naar een nieuw huis. Ingrid, ook wij kennen elkaar al lange tijd. Laatst kwam ik nog een klassenfoto tegen uit de brugklas, wat waren we daar nog jong zeg! Van feestjes in de achtertuin als tieners tot gezellig borrelen, fietsen en wintersport. We staan bekend als dat we af en toe lekker kunnen botsen met elkaar. Ik vind dat juist wel mooi dat we zo nu en dan een compleet ander standpunt hebben. Ik vind het altijd leuk en gezellig om je te zien. Jij nu in opleiding tot huisarts en net een nieuwe woning in gebruik genomen, echt een eigen plek, dat moet heel fijn voelen. Marten en Linda, ik heb jullie voor het eerst leren kennen naar aanleiding van een kast en hoewel hij de verhuizingen niet geheel top heeft overleefd heb ik hem nog steeds. Ik vind het super om met jullie te kunnen discussiëren over van alles en nog wat en wat hebben we toch steeds een plezier met z'n allen in de bergen. Of dat nu ploeterend op een fiets is of in de sneeuw. Bedankt! Sawadi, zo'n groot deel van ons leven hebben we samen doorgebracht. Ik heb je leren kennen als dat vrolijke meisje met een mooie glimlach, haast van oor tot oor. Ik ben er van overtuigd dat ik zonder jou nooit zo ver was gekomen. Je stond altijd voor me klaar en hebt me keer op keer gestimuleerd om steeds een sprongetje verder te maken. Zo lief, kundig en attent als jij bent ben ik er van overtuigd dat je een geweldige huisarts zult worden. Zoveel leuke momenten hebben we met elkaar gehad. Helaas is het niet zo gelopen als we hadden gehoopt. Je blijft altijd in mijn gedachten. Ik hoop dat we ondanks alles altijd onderdeel van elkaars leven zullen blijven uitmaken.

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*Met watervaste stift, op uw linker *of* rechter wang