Dissertation

Peripheral fractional tissue oxygen extraction and infection in term and preterm neonates: a prospective pilot observational study

submitted by

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Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the "Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz".

Graz, 23.11.2023 *Christina Helene Wolfsberger eh.*

Disclosures

Parts of this thesis were accepted for publication in the journal “Frontiers in Pediatrics - Neonatology”. Tables and figures of the accepted manuscript were not used in the thesis.

“Peripheral muscle fractional tissue oxygen extraction in stable term and preterm neonates during the first 24 hours after birth”

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All co-authors have agreed to use the data of this publication for the thesis. Written statements are submitted together with the dissertation.

List of my published articles, which are cited in this thesis:

Wolfsberger C, Baik-Schneditz N, Schwaberger B, Binder-Heschl C, Höller N, Mileder L, Bruckner M, Avian A, Urlesberger B, Pichler G. Changes in peripheral muscle oxygenation measured with near-infrared spectroscopy in preterm neonates within the first 24 h after birth. Physiol Meas. 2020; 41(7):075003-075003. Doi: 10.1088/1361-6579/ab998b

Wolfsberger CH, Bruckner M, Baik-Schneditz N, Schwaberger B, Mileder LP, Avian A, Urlesberger B, Pichler G. Fetal inflammatory response syndrome and cerebral oxygenation during immediate postnatal transition in preterm neonates. Front Pediatr. 2020; 8:401. Doi: 103389/fped.2020.00401

Wolfsberger CH, Bruckner M, Schwaberger B, Mileder LP, Urlesberger B, Pichler G. Impact of carbon dioxide on cerebral oxygenation and vital parameters in stable preterm and term infants immediately after birth. Neonatology. 2022; 119(1):10-17. Doi: 10.1159/000525847

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Pfurtscheller D, Wolfsberger CH, Höller N, Schwaberger B, Mileder LP, Baik-Schneditz N, Urlesberger B, Schmölzer GM, Pichler G. Cardiac output and regional-cerebral-oxygen-saturation in preterm neonates during immediate postnatal transition: An observation

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Table of Content

List of Abbreviations

ANC	absolute neutrophil count
BE	base excess
BPD	bronchopulmonary dysplasia
BSID	Bayley Scales of Infant Development
cFTOE	cerebral fractional tissue oxygen extraction
CO	cardiac output
COest.	estimated cardiac output
COest/adj.	estimated/adjusted CO
CRP	C-reactive protein
$crSO_2$	cerebral regional oxygen saturation
DABP	diastolic blood pressure
deoxy-Hb	deoxygenated haemoglobin
E.coli	Escherichia coli
ECG	electrocardiography
EOS	early-onset sepsis
FIRS	fetal inflammatory response syndrome
GBS	Group B streptococcus
Hbflow	blood flow
HbO_2	oxygenated haemoglobin
Hb_{tot}	total haemoglobin
HCO3-	bicarbonate
HR	heart rate
IL-6	Interleukin 6
IRDS	infant respiratory distress syndrome
IT-ratio	immature-to-total-neutrophil ratio
IVH	intraventricular haemorrhage
LOS	late-onset sepsis
MABP	mean arterial blood pressure
NCPAP	nasal continuous positive airway pressure
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
NIRS	near-infrared spectroscopy

pCO_2	partial pressure of carbon dioxide
PCT	procalcitonin
PDA	patent ductus arteriosus
p-deoxy-Hb	peripheral deoxygenated haemoglobin
pDO_2	peripheral oxygen delivery
pFOE	peripheral fractional oxygen extraction
pFTOE	peripheral fractional tissue oxygen extraction
pH	potential of Hydrogen
pHbflow	peripheral haemoglobin flow
$pHbO_2$	peripheral oxygenated haemoglobin
PI	perfusion index
pO_2	partial pressure of oxygen
pOE	peripheral oxygen extraction
PP	pulse pressure
$prSO_2$	peripheral regional oxygen saturation
$pSvO_2$	peripheral mixed venous oxygen saturation
pTOI	peripheral tissue oxygenation index
PVH	periventricular haemorrhage
PVL	periventricular leukomalacia
pVO_2	peripheral oxygen consumption
ROP	retinopathy of prematurity
rSO2	regional tissue oxygen saturation
SABP	systolic blood pressure
SD	standard deviation
SIP	spontaneous intestinal perforation
SpO_2	arterial oxygen saturation
SvO_2	mixed venous oxygen saturation
TOI	tissue oxygenation index
VO_2	oxygen consumption
WHO	World Health Organisation

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Zusammenfassung

Einleitung

Periphere Muskeloxygenierung ermöglicht die frühzeitige Erkennung mikrozirkulärer Dysfunktionen bei Infektionen/Entzündungen und/oder Sepsis. Die periphere fraktionierte Gewebesauerstoffextraktion (pFTOE) stellt die relative Extraktion vom arteriellen zum venösen Kompartiment dar, die Informationen über den Sauerstoffverbrauch und die Sauerstoffabgabe an das Gewebe liefert. Ziel der vorliegenden Studie war es zu untersuchen, ob sich pFTOE innerhalb der ersten sechs Stunden nach der Geburt, bei Reifgeborenen und Frühgeborenen mit und ohne laborchemischen Zeichen einer Infektion unterscheidet.

Methodik

Diese Studie wurde als prospektive Beobachtungsstudie an der Abteilung für Neonatologie Graz durchgeführt. In die Studie wurden Reif- und Frühgeborene ≥ 30 Schwangerschaftswochen, die an der neonatologischen Intensivstation mit Atemnotzeichen und < 6 h postnatalem Alters, aufgenommen wurden, inkludiert. Innerhalb der ersten sechs Stunden nach der Geburt wurden periphere und zerebrale NIRS-Messungen durchgeführt, die durch fünf kurze Reapplikationen am rechten Unterarm bzw. an der linken Stirn durchgeführt wurden. Die routinemäßige Überwachung der arteriellen Sauerstoffsättigung (SpO_2), Herzfrequenz und mittlerem arteriellen Blutdruck wurde im Zeitrahmen der NIRS-Messungen dokumentiert. pFTOE wurde nach folgender Formel aus dem peripheren Gewebeoxygenierungsindex (pTOI) und der SpO_2 berechnet: pFTOE = (SpO_2-pTOI)/SpO_2. Es wurden routinemäßige Blutproben (C-reaktives Protein, Leukozyten und IT-Verhältnis) während der ersten 48 Stunden nach der Geburt dokumentiert. Neugeborene mit Zeichen einer Infektion, definiert als CRP > 10 mg/l, Leukozyten < 6000/µl oder > 30000/µl, IT-Verhältnis > 0,2 und/oder positiver Blutkultur, wurden der Infektionsgruppe zugeteilt. Neugeborenen mit unauffälligen Laborparametern wurden der Gruppe ohne Infektion zugeteilt. Neugeborene der Infektionsgruppe wurden mit der Gruppe ohne Infektion verglichen. Reifgeborene und Frühgeborene wurden getrennt analysiert.

Ergebnisse

Insgesamt wurden 80 Neugeborene, 32 Reifgeborene (Infektion n=15, keine Infektionsgruppe n=17) und 48 Frühgeborene (n=6, n=42) in die vorliegende Studie inkludiert. Gestationsalter und Geburtsgewicht betrugen 39,6 ± 1,7 Wochen und 3543 ± 615 Gramm bzw. 38,5 ± 1,4 und 3221 ± 592 Gramm bei reifen Neugeborenen der Infektionsgruppe bzw. der Gruppe ohne

Infektion. Es gab keine Unterschiede in pFTOE von 0,229 ± 0,064 in der Infektionsgruppe und 0,235 ± 0,032 in der Gruppe ohne Infektion (p = 0,293).
Bei Frühgeborenen betrugen das Gestationsalter und das Geburtsgewicht 34,8 ± 1,7 Wochen und 2476 ± 720 Gramm bzw. 34,3 ± 1,6 und 2284 ± 474 Gramm in der Infektionsgruppe bzw. in der Gruppe ohne Infektion. Es gab keine Unterschiede im pFTOE von 0,235 ± 0,050 in der Infektionsgruppe und 0,224 ± 0,051 in der Gruppe ohne Infektion (p = 0,306).

Konklusion

In der vorliegenden Studie wurde bei Reifgeborenen und Frühgeborenen mit Infektion kein Unterschied in pFTOE, gemessen durch fünf kurze Reapplikationen innerhalb der ersten sechs Stunden, im Vergleich zu Neugeborenen ohne Infektion beobachtet. Dennoch ist pFTOE als früher Marker für Mikrozirkulationsstörungen bei Neugeborenen mit Atemnotsymptomen innerhalb der ersten Stunden nach der Geburt weiterhin von Interesse.

Abstract

Introduction

Peripheral muscle oxygenation enables early recognition of microcircular dysfunction in cases of infection/inflammation and/or sepsis. Peripheral fractional tissue oxygen extraction (pFTOE) represents the relative extraction from arterial to venous compartment, which provides information about oxygen consumption and oxygen delivery to tissue. Primary aim of the present study was to investigate, whether pFTOE measured within the first six hours after birth differs in term and preterm neonates, with laboratory signs of infection and without infection.

Methods

This study was performed as a prospective observational study performed at the Division of Neonatology Graz. Term and preterm neonates ≥30 weeks of gestational age with respiratory distress, admission to the NICU and age < 6 hours were included in the present study. Within the first six hours after birth, peripheral and cerebral NIRS measurements, performed by five short (re-)applications on the right forearm and on the left forehead, respectively, were conducted. Routine monitoring of arterial oxygen saturation (SpO_2), heart rate (HR), mean arterial blood pressure (MABP) were documented in the time frame of NIRS measurements. pFTOE was calculated by the formula using peripheral tissue oxygenation index (pTOI) and SpO_2: pFTOE = (SpO_2-pTOI)/SpO_2. Routine blood samples, including C- reactive protein, leukocytes and IT-ratio during the first 48 hours after birth were collected. Neonates with signs of infection, defined as CRP >10mg/l, leukocytes <6000/µl or >30000/µl, IT ratio >0.2 and/or positive blood culture were stratified to the infection group. Those neonates with inauspicious laboratory parameter were stratified to the no-infection group. Neonates of the infection group were compared to the no-infection group. Term and preterm neonates were analysed separately.

Results

A total of 80 neonates, 32 term neonates (infection n=15, no-infection group n=17) and 48 preterm neonates (n=6, n=42), were included in the present study. Gestational age and birth weight were 39.6 ±1.7 weeks and 3543 ±615 grams and 38.5 ±1.4 and 3221 ±592 grams in term neonates of the infection group and of the no-infection group, respectively. There were no differences in pFTOE 0.229 ±0.064 in the infection group and 0.235 ±0.032 in the no-infection group (p=0.293).

In preterm neonates, gestational age and birth weight were 34.8 ±1.7 weeks and 2476 ±720 grams and 34.3 ±1.6 and 2284 ±474 grams in the infection group and in the no-infection group,

respectively. There were no differences in pFTOE 0.235 ±0.050 in the infection group and 0.224 ±0.051 in the no-infection group (p=0.306).

Conclusion

In the present study, in term and preterm neonates with infection no difference in pFTOE measured by five short reapplications within the first six hours were observed compared to neonates without infection. Nevertheless, pFTOE as an early marker for microcirculatory dysfunction is still of interest in neonates with respiratory distress symptoms within the first hours after birth.

1. Introduction

1.1. Neonates

1.1.1. Term neonates

Term neonates are defined as neonates who are born ≥ 37 weeks of gestation (1). Further subcategories among term neonates include “early term” born neonates, with a gestational age of 37 and 38 weeks of gestation and “full term” neonates, with a gestational age of 39 to 41 weeks of gestation (2)(3)(4). According to these subcategories among term neonates, “early term” have a higher risk for neonatal morbidities compared to “full term” neonates (3). In Austria, a prevalence study described that 25.5% neonates were born between 37 and 38 weeks of gestation, including only live born and singletons (3).

1.1.2. Preterm neonates

Preterm neonates are defined as neonates who are born < 37 weeks of gestation (3). There is another stratification into categories according to their gestational age, introduced by the World Health Organisation (WHO): “extremely preterm” neonates are defined as born between 22 and 27 weeks of gestation, “very preterm” neonates are defined as born between 28 and 31 weeks of gestation and “moderately preterm” neonates are born between 32 and 36 weeks of gestation (5). Preterm neonates born between 34 and 36 weeks of gestation are characterised as “late preterm” neonates (6).

According to data presented by the WHO (5), 15 million neonates are born as preterm neonates each year. As preterm birth is associated with numerous neonatal morbidities and the death rate of preterm neonates is higher than in term neonates, with rates varying between low-income and high-income countries: death rates in high-income countries within the first days after birth vary between 10 and 15% (5)(7).

1.1.2.1. Morbidities of preterm neonates

Preterm birth is associated with increased risk for neonatal morbidity and mortality, whereby neonates with lower gestational age are of a higher risk compared to higher gestational age (8). Short-term morbidities include morbidities occurring during early neonatal period until 40 weeks of corrected gestational age or discharge home from neonatal intensive care unit

(NICU). Short-term morbidities can affect respiratory, cardiocirculatory, neurological, intestinal, ophthalmic and infectious conditions (9). Respiratory morbidities in preterm neonates include infant respiratory distress syndrome (IRDS), pulmonary hypertension, or bronchopulmonary dysplasia (BPD) (9). The latter is defined as chronic neonatal lung disease, whereby diagnosis is made at corrected 36 weeks of gestational age and include criteria such as respiratory support and/or need for oxygen supply (10). The patent ductus arteriosus (PDA) is a very common cardiac problem in preterm neonates. With lower gestational age of preterm neonates, the risk for PDA increases: 90% of preterm neonates with a gestational age of less than 24 weeks were diagnosed with PDA, whereby only 10% of neonates with a gestational age of 30 to 37 weeks (11)(12). Neurological morbidities including intraventricular haemorrhage (IVH), periventricular haemorrhage (PVH) and cystic periventricular leukomalacia (PVL) may be associated with impaired neurodevelopment and/or the development of cerebral palsy and therefore, play an important role on long-term neonatal outcome (13)(14). Necrotizing enterocolitis (NEC) and spontaneous intestinal perforation (SIP) are intestinal conditions during neonatal period that deteriorate neonatal outcome (9)(15). Retinopathy of prematurity (ROP) is described as a vasoproliferative disease of the retina of preterm born neonates and is mainly caused by oxygen therapy (16). Further, infections during neonatal period are causative for several consecutive conditions, increasing the risk for mortality and morbidity (17).

1.1.3. Routine monitoring at the neonatal intensive care unit

Term and preterm neonates, who were admitted to the NICU were routinely monitored. Routine monitoring consists of measurement of arterial oxygen saturation (SpO_2), heart rate (HR) and blood pressure (invasive and non-invasive) (18).

1.1.3.1. Pulse oximetry

The principle of pulse oximetry bases on the attenuation of light through tissues. In conventional pulse oximetry, there are two diodes, which transmit light at 660nm (red light) and at 940nm (infrared light) (19). These are differently absorbed by oxygenated and deoxygenated haemoglobin.

Pulse oximetry enables non-invasive monitoring of SpO_2, HR and perfusion index (PI).

Detecting hypoxemia and hyperoxemia is of special interest in neonates, as several morbidities in neonatal period are influenced by abnormal SpO_2, including IVH, NEC, ROP and BPD (19). Beside SpO_2, pulse oximetry provides information about the PI. PI is calculated out of pulsatile

to non-pulsatile components of the infrared light (pulsatile/non-pulsatile) (20). This may provide information about peripheral perfusion (21) and can therefore be a useful tool in different situation including congenital heart defect or PDA. PI, however, can be effected easily be surroundings: It has been described that prone sleeping position show higher PI values compared to other sleeping position (21).

1.1.3.2. Electrocardiography (ECG)

Monitoring of HR can be performed clinically (auscultation, pulse palpation) and by the use of ECG or pulse oximetry. In very low birth weight preterm neonates, monitoring of HR is preferred by pulse oximetry due to potential skin damage and trauma (burn ulcers) as a result of adhesive ECG electrodes (22). Generally, due to a higher accuracy, ECG is the gold standard at the NICU concerning HR assessment (23).

1.1.3.3. Blood pressure

Blood pressure can be measured either non-invasive, using a pneumatic cuff, or invasive through a catheter placed in an artery. The latter is more accurate, compared to the pneumatic cuff, especially in cases of mean arterial blood pressure (MABP) < 30 mmHg (24). Nevertheless, invasive blood pressure measurements can be associated with complications including thrombosis or infections. However, an artery catheter cannot only be used for invasive blood pressure measurements, but also for frequent blood samplings (25). Beside the invasive method, the non-invasive method, using oscillometry technique, also provides MABP. For the non-invasive method, the pneumatic cuff is initially inflated beyond the systolic pressure. As the cuff slowly deflates, the amplitude of arterial pulsations increases until it reaches its peak, which is defined as the MABP. This process includes using estimates for pulse pressure and computational algorithms, ultimately determining both systolic blood pressure (SABP) and diastolic blood pressure (DABP) (24). Accurate measurements of non-invasive blood pressure using pneumatic cuff is dependent on the use of an appropriate sized cuff (24).

1.2. Infection in neonatal period

1.2.1. Definition

Neonatal infection and/or possible neonatal infection are leading complaints for neonatal admission on the NICU. It is estimated that every year about 1.3 million neonates were diagnosed with neonatal sepsis, with a lower prevalence in high-income countries (26)(27). Definition of neonatal sepsis is not homogenous. The diagnosis of bacterial infection in the newborn period is very rare, whereby, neonatal sepsis is the dominated diagnosis for neonates with presence of indicators for infection (28). In neonates with normal laboratory results and sterile blood cultures, however, with clinical signs of infection, the term “clinical sepsis” has been established (29). Furthermore, neonates who initially presented as septic neonates during admission to NICU, with quick recovery and no laboratory signs of infection are commonly diagnosed as “rule out sepsis” (30).

1.2.2. Sepsis in neonates

Neonatal sepsis is a condition of systemic infection and it can be defined on the one hand as early-onset sepsis (EOS) and on the other hand as late-onset sepsis (LOS) (31). The former is defined as occurrence of symptoms within the first 72 hours after birth and it is acquired by vertical acquisition from mothers. The latter is defined as a presentation of sepsis after the first 72 hours after birth and it is mainly acquired horizontally after birth, however, also a vertical acquisition combined with a late onset of symptoms is possible (32). Beside characteristics of time when symptoms were diagnosed, sepsis is defined as life-threatening organ dysfunction (33). A distinction between neonatal infection and neonatal sepsis (EOS, LOS) is not based on a consensus definition. Definitions of neonatal sepsis include in many cases the need for inotropes or vasopressors, the isolation of bacterial pathogens from blood or the length of the treatment with antibiotics (33)(34)(35).

1.2.3. Pathogenes

Pathogenes that cause EOS differ from those that trigger LOS due to differences in aetiology. EOS is mainly caused by pathogenes transmitted vertically by the mother. These include Group B streptococcus (GBS) and Escherichia coli (E. coli). The former causes 40 to 45% of EOS in term neonates, whereby E. coli is isolated in 10 to 15% (32). Although EOS with GBS

is more frequent, E. coli is associated with a higher rate of mortality and morbidities (36). In contrast, in cases of LOS, coagulase-negative Staphylococci are isolated in about 50%, followed by Staphylococcus aureus in about 7% (32)(37). Beside these gram-positive bacteria, gram-negative bacteria including E. coli, Klebsiella pneumonia, Serratia marcescens and Enterobacter spp. are causative for LOS in 20% to 42% (32)(37). Bacterias are the most common pathogenes isolated in neonates with LOS, nevertheless, Fungi, including Candida albicans and Candida parapsilosis, also may cause LOS in 5% to 28%, with a higher percentage in very low birth weight preterm neonates (32)(38).

1.2.4. Risk factors for infection

Risk factors include on the one hand maternal conditions that favour the development of neonatal infection and on the other hand neonatal characteristics. Maternal conditions mainly increase the risk for EOS. Conditions include maternal fever during labour and maternal elevated infectious laboratory variables, rupture of membranes and the duration between rupture of membranes and delivery and/or amniotic fluid infection/chorioamnionitis (32). Chorioamnionitis/amniotic fluid infection/intra-amniotic infection is a condition that affects about one to four percent of neonates in the United States (39)(40). Characteristics of chorioamnionitis are maternal fever and maternal elevated leukocyte counts or purulent cervical discharge or pathological fetal CTG (tachycardia) (40)(41). Maternal infection has the potential to also affect fetuses and further influence neonatal morbidity and mortality. This is called fetal inflammatory response syndrome (FIRS) and is defined as elevated interleukin 6 values in umbilical cord blood above 11 pg/ml (42). Further, maternal positive GBS screening during pregnancy may also increase the risk for neonatal infection (43). The development of LOS is mainly influenced by neonatal characteristics including preterm birth with increasing risk with decreasing gestational age and birth weight, congenital anomalies, invasive ventilation, central venous catheters, peripheral inserted central catheters, delayed enteral feeding and medications that affect the immune system and microbiome of the neonate (32)(38)(44).

1.2.5. Diagnostic of infection

Diagnosis of infection in neonatal period remains challenging. Diagnostic procedures include interpretation of clinical signs, elevated laboratory parameters of the neonate and blood culture.

1.2.5.1. Clinical signs

Clinical symptoms of infection during early neonatal period include all organ systems. Neonates with infection present with pallor, mottling, jaundice, bruising, petechiae and/or instability in body temperature including low temperature and fever (32)(38)(45). Neurological symptoms include irritability, lethargy, seizures, bulging fontanelle, reduced reflexes, jitteriness, tremors, abnormal crying and abnormalities in muscle tone (34). Low blood pressure, bradycardia or tachycardia, reduced heart rate variability, prolonged capillary refill time or cyanosis may represent impaired cardiovascular system in cases of an infection (32)(45). Neonates with infection often show respiratory symptoms including tachypnea (respiratory rate > 60 breaths/min), apnea, desaturations, grunting, nasal flaring, retractions and/or the need for respiratory support and/or supplemental oxygen (31)(32)(46). Further, gastrointestinal symptoms can also provide a hint on neonatal infection. These symptoms include abdominal distension, emesis, feeding intolerance and/or diarrhea. In cases of hypotension renal symptoms, including oliguria, may represent centralisation (32)(45).

1.2.5.2. Laboratory parameters

Laboratory parameters can be obtained on the one hand from cord blood and on the other hand from the neonate. Cut-off values of interleukin 6 (IL-6) and procalcitonin (PCT) IL-6 in cord blood differ in literature (45)(47). A cut-off value of IL-6 of 15.85 ng/l showed a sensitivity of 73.7% and specificity of 84.2%, whereby for PCT the cut-off value was 0.235 µg/l (sensitivity 78.6%, specificity 86.3%) (48). Further, it was observed that the combination of IL-6 and PCT increases sensitivity and specificity in the prediction of EOS. Elevated IL-6 values above 11 pg/ml in umbilical cord blood are defined as FIRS, which is further associated with increased risk for neonatal morbidity and mortality (49).

Numerous of laboratory parameters can provide information about neonatal sepsis, however, the low predictive values have to be taken into account (46). Further, clinical settings, including asphyxia or gestational age, as well as the time, when blood samples were performed, may influence laboratory parameters (32). Abnormal leukocyte counts (<6000/µl and >30.000/µl within the first days after birth) can be predictive of sepsis (50). Leukocytes, however, can also be affected by meconium aspiration syndrome, maternal fever, intraventricular haemorrhage, pneumothorax, convulsion and crying (50). Neutrophil counts, defined by absolute neutrophil count (ANC), especially neutropenia (ANC < 1000/µl at ≥ 4 hours), have a high specificity for neonatal sepsis. Nevertheless, ANC varies between different gestational ages: lower limit of ANC with lower gestational age (50). Another parameter that has a wide variation with gestational age and postnatal age, is immature-to-total-neutrophil ratio (IT-ratio). IT-ratios have

the highest sensibility for predicting neonatal sepsis among laboratory parameters (50). In literature, different cut-offs for IT-ratio exist. The most common cut-off for IT-ratio is > 0.2 (32)(50). Thrombocytopenia < 140000/µl can also be associated with neonatal sepsis (50)(51). C-reactive protein (CRP) plays an important role in the diagnosis of neonatal sepsis. It has been described, that about 10 to 12 hours after the onset of an infection, a rise in CRP levels can be expected (52). This delay and the fact that an elevation of CRP is also likely in various non-infectious inflammatory conditions including maternal fever, fetal distress, asphyxia, meconium aspiration and or intraventricular haemorrhage, lead to the low sensitivity within the first 24 hours after birth (52). Situation leading to elevated CRP values can also affect leukocytes (53). Taking these into account, elevated CRP values alone cannot predict an infection (54). In literature, CRP levels ≥ 10mg/l are suspected for neonatal infection in preterm and term neonates (54). However, there are differences in CRP levels according to gestational age and postnatal age. CRP values in term neonates reach a peak at about 21 hours after birth (55). A large secondary outcome analysis showed, that a CRP value about 10mg/l predicts low to moderate risk of sepsis, however, CRP values ≥ 20mg/l predict high risk of sepsis. Sepsis can only be proven by positive blood cultures (56). In contrast in preterm neonates, the peak of CRP was reached at 27 to 36 hours after birth (55). Further, there are differences in CRP levels between EOS and LOS: higher CRP values were observed in LOS compared to EOS (57).

Beside CRP, PCT taken from the neonate can also predict an infection in neonatal period. Comparable to CRP, PCT is also affected by gestational age. PCT increases in stable term neonates, reaching the peak level at 24 hours after birth. At the peak level, PCT is 2.9 (0.4-18.7)µg/l. After 24 hours a decrease is observed until 80 hours after birth. In preterm neonates, a rapid increase is described until 21 to 22 hours after birth, 6.5 (0.9-48.4.) µg/l. Afterwards, a declining is observed until five days after birth (55).

1.2.5.3. Blood culture

According to guidelines, blood culture is the most accurate method to detect pathogenes, which further leads to the diagnosis of sepsis and is therefore defined as gold standard (45). Nevertheless, sampling blood cultures is not always feasible in clinical routine as a certain amount of blood is necessary. This is even more challenging in preterm neonates. Studies have shown, that too little amount of blood (0.5 ml) are associated with false negative results (32). False positive results can be caused by contamination (32)(58). Beside the amount of blood, the time until the results of blood cultures are available are between three to five days in best cases, however, clinicians have to decide on starting antibiotic therapy before these

results are available (46). According to clinical symptoms, especially the occurrence of neurological symptoms, including seizures, culture of cerebral spinal fluid obtained by lumbar punctures, can be necessary in diagnosis of neonatal sepsis (32)(59).

1.2.6. Treatment

The start of an antibiotic therapy depends on risk factors, clinical symptoms and results of laboratory tests. What is more, a risk calculator can also be used in clinical routine to support the decision concerning starting an antibiotic therapy (60). Antibiotic therapy is routinely administered intravenously.

In cases of risk factors and clinical appearance suspected for an EOS, an initial empiric antibiotic therapy is recommended to be started even before the first results of laboratory tests and blood culture are available. The recommended antibiotic regimen for initial empiric therapy consists of ampicillin and aminoglycoside, or ampicillin and cephalosporin. The former combination should be preferred over the latter (61). Higher doses of ampicillin are necessary in cases of meningitis (61).

A comparable empiric therapy is recommended for LOS, however, ampicillin in combination with cephalosporin have a higher priority compared to the management of EOS (61).

The duration of antibiotic therapy varies according to response to the therapy and to the result of blood culture. In neonates with positive blood culture, antibiotic therapy should be administered for 10 days. In contrast, if the blood culture is negative, however, the neonate still presents with symptoms of a suggested sepsis, antibiotic therapy should last between five to 10 days. If a neonatal infection is unlikely (no clinical symptoms, no risk factors) and blood culture results are negative, antibiotic therapy should be stopped after 36 to 48 hours (54).

1.2.7. Morbidity and mortality of infection

Neonates with infection in early neonatal period are at a high risk for cerebral morbidities, with a high percentage in the development of PVL (62). Especially, direct infection of the brain, neonatal meningitis, can cause local inflammation and cytokine release. These lead to excitotoxicity due to release of reactive oxygen and nitrogen species (63). As a results of several complex mechanism, oligodendroglial injury and further inhibition of maturation and myelination till neuronal loss occur (64). These mechanism, however, have not only be described in meningitis, but also in infection in neonatal period without a direct invasion of the brain (63).

Short-term morbidities of neonatal infection include the development of cerebral palsy, BPD, seizures, ROP > stage III and/or need for oxygen support at time point of discharge (32)(36). Several studies have further investigated, that neonates with an infection with a positive blood culture are at a higher risk for abnormal results in neuroimaging (white matter abnormalities) and impaired neurodevelopmental outcome (65)(66)(67). Impaired neurodevelopmental outcome include motor, hearing and visual impairment and/or cognitive delays, whereby a high rate of these morbidities was observed in preterm neonates with an infection and with a birth weight less than 1500 g (32)(67)(68). Nevertheless, it is still unclear whether neonatal infection or prematurity itself, is the main contributor to poor long-term outcome.
Mortality of neonatal infection has a wide variation in accordance to gestational age. Mortality rates in term neonates vary between 2-3% and 0.3% for EOS and LOS, respectively (32). In extremely preterm neonates the rate is between 50% and 4% for EOS and LOS, respectively (32).

1.3. Near-infrared spectroscopy

1.3.1. Method of near-infrared spectroscopy

Near-infrared spectroscopy (NIRS) enables continuous and non-invasive measurement of tissue oxygenation of different regions of interest, including cerebral, peripheral muscle tissue, intestinal or renal/splanchnic tissue (69). The principles of NIRS have been described by Jobsis in 1977 (70). This principle is based on the one hand on the relative transparency of biological tissue to near-infrared light (wavelength 700 -1000 nm) and on the other hand on the availability of chromophores in tissue. When near-infrared light propagates through tissues it is differently absorbed by oxygenated (HbO_2) and deoxygenated (deoxy-Hb) haemoglobin, myoglobin and cytochrome oxidase (71). Myoglobin and cytochrome oxidase contribute to the signal only about 10% or less and can be neglected. The absorption of light take place at 760nm and 800nm. At 760nm the absorption is mainly dominated by deoxy-Hb, whereas at 800nm HbO_2 and deoxy-Hb absorb light. The changes in absorption of the near-infrared light can further be converted into changes in the concentration of HbO_2 and deoxy-Hb. These provide information about the oxygenation of the haemoglobin and the blood volume, expressed by total haemoglobin (70)(71)(72). Out of the relative absorption of light of HbO_2 and deoxy-Hb, tissue oxygenation can be calculated: regional tissue oxygen saturation (rSO_2) measured with INVOS 5100 and tissue oxygenation index (TOI) measured with NIRO

300/200NX. The rSO_2 reflect tissue oxygenation in veins (70-80%), arteries (15-25%) and capillaries (5%) (73).

There exist different techniques of NIRS (74) including continuous wave, spatially resolved spectroscopy, time resolved spectroscopy and phase modulation spectroscopy. In the continuous wave technique, changes in the light intensity are measured, whereby the spatially resolved spectroscopy (multidistance spectroscopy) describe the technique when the light intensity is measured at various different source-detector distances. In contrast, the time resolved spectroscopy (domain spectroscopy) is based on the measurement of the time of flight in addition to the light intensity. It uses a ultrashort pulse light source (picosecond pulse) and the time of photon flight corresponds to the pathlength (74).

1.3.2. Cerebral near-infrared spectroscopy

During the last decades, there have been increasing interest in monitoring cerebral oxygenation using near-infrared spectroscopy in neonates (75). There have been several studies describing cerebral oxygenation during initial fetal-to-neonatal transition period (76)(77)(78).

Urlesberger et al. (79) observed the difference in cerebral regional oxygen saturation ($crSO_2$) between neonates delivered by Caesarean section and vaginal delivery and observed that there were no differences in $crSO_2$ between the two groups, although, SpO_2 and HR were significantly lower in neonates delivered by Caesarean section.

Neonatal brain, especially in preterm neonates, is one of the most vulnerable organs to hypoxia. Therefore, growing interest exist in observing if monitoring $crSO_2$ during initial fetal-to-neonatal transition period can influence cerebral morbidities in neonates. Baik et al. (80) observed, that preterm neonates who developed IVH showed significantly lower $crSO_2$ values within the first 15 minutes after birth, with no differences in other routine vital parameters, compared to neonates without IVH. A similar result was observed by Wolfsberger et al. (81). They described the potential influence of cerebral oxygenation during initial fetal-to-neonatal transition period in preterm neonates ≤ 32 weeks of gestation or ≤ 1500g birth weight, on long-term outcome, evaluated by Bayley Scales of Infant Development (BSID) at a corrected age of two years. Preterm neonates with adverse outcome (BSID ≤70 or testing not possible due to death or severe cognitive impairment), showed significantly lower $crSO_2$ values and significantly higher cerebral fractional tissue oxygen extraction (cFTOE) values compared to preterm neonates with favourable outcome.

A multicenter, multinational randomised controlled phase 3 study, the COSGOD III trial, investigated if $crSO_2$ guided immediate postnatal transition and resuscitation can increase survival without cerebral injury compared to preterm neonates with routine resuscitation (82). Sixhundred-seven preterm neonates <32 weeks of gestational age were included. No significant difference in survival without cerebral injury was observed between the two groups, however, survival without cerebral injury increased by 4.3% in the NIRS-group.

A study comparable to the COSGOD III trial is the SafeBoosC III trial (83). They investigated in preterm neonates <28 weeks of gestational age whether $crSO_2$ guided treatment during the first 72 hours after birth can result in lower incidence of death or survival with severe brain injury at 36 weeks of corrected gestational age. A total of 1579 preterm neonates were eligible for final analysis. They observed no difference in death or survival with severe brain injury between the two groups.

For interpretation of rSO_2 values, reference ranges are of great importance. Pichler et al. (84) described references ranges for $crSO_2$, measured with the INVOS 5100 monitor, in term and preterm neonates. The 50th centile of $crSO_2$ in minute two was 41.1%, in minute five 68.4%, in minute ten 79.4% and in minute 15 77.5%. Comparable, Baik et al. (85) provided, reference ranges of stable term neonates with no need for respiratory support after Caesarean section, measured with the NIRO 200NX. Centile charts including the 10th, 25th, 50th and 90th percentile were calculated. The 50th centile of cTOI in minute two was 55.9%, in minute five 65.6%, in minute ten 74.5% and in minute 15 72.7%.

1.3.2.1. Cerebral fractional tissue oxygen extraction

Beside $crSO_2$, cFTOE provides additional information about cerebral oxygenation and perfusion in neonates. In the majority of above mentioned studies (79)(81)(84)(85), cFTOE has been calculated. cFTOE describes the balance between oxygen delivery and the oxygen consumption (86)(87). Higher FTOE values were observed in situation of lower oxygen delivery and stable oxygen consumption. Naulaers et al. (87) tested 2007 whether TOI can be used for measurement of FTOE. They first stated the formula of FTOE:

$$cFTOE = \frac{(SpO2 - TOI)}{SpO2}$$

They observed that decreasing TOI was associated with an increase in FTOE (87). Pichler et al. (84) provided reference ranges for cFTOE measured with the INVOS 5100 monitor in stable

term and preterm neonates during initial postnatal transition period. In minute two the 50th centile of cFTOE was 0.33, in minute five 0.21, in minute ten 0.15 and in minute 15 0.18.
Baik et al. (85) calculated reference ranges for cFTOE, beside cTOI, in stable term neonates after elective Caesarean section, using the NIRO 200NX: in minute two the 50th centile of cFTOE was 0.24, in minute five 0.20, in minute ten 0.21 and in minute 15 0.24.

1.3.3. Peripheral near-infrared spectroscopy

Peripheral muscle oxygenation measured with NIRS has the potential to provide information of sepsis or shock at early stages, due to changes in microcirculation. These changes in peripheral muscle oxygenation can occur before changes in routine vital parameters, including SpO_2, HR or MABP (88)(89). Several studies have already investigated peripheral muscle oxygenation in different clinical relevant situations (90-96).
Peripheral muscle oxygenation can be performed on the one hand in combination with occlusion (arterial or venous occlusion), or without occlusion.

1.3.3.1. Arterial occlusion method

Peripheral muscle oxygenation in combination with arterial occlusion, combine the measurement of peripheral muscle NIRS measurements with a pneumatic cuff, which is inflated to a level above the systolic arterial pressure, leading to interrupted arterial inflow and venous outflow (97)(98)(99)(100). This method provides information about peripheral muscle oxygen consumption (VO_2). The arterial occlusion method can be easily influenced by movement artefacts, leading to lower reliability. Further arterial occlusion causes more discomfort to the neonates due to higher inflation pressure, when compared to other methods in peripheral NIRS measurements.

1.3.3.2. Venous occlusion method

When combining peripheral muscle NIRS measurements with the venous occlusion method, a pneumatic cuff is placed around the upper arm or thigh. The NIRS probe is placed on the same limb around the lower arm or calf. Afterwards the pneumatic cuff is inflated to a level above diastolic pressure and below systolic pressure, when arterial inflow is not disturbed, however, venous outflow is interrupted. These lead to changes in HbO_2, deoxy-Hb and total haemoglobin (Hb_{tot}). Further, blood flow (Hbflow), mixed venous oxygen saturation (SvO_2) and peripheral fractional oxygen extraction (pFOE) can be calculated (101)(102). Peripheral NIRS measurements in combination with venous occlusion method are difficult to be implemented

into clinical routine, due to the inflation time of 20 sec in combination with a rest period of at least 40 sec between the inflations. Further, the occlusion methods are quite prone to movement artefacts and are limited by low gestational age and low birth weight due to difficulties on application of the NIRS sensor at the limb of the neonate (97)(103)(104).

1.3.3.3. Calculation of peripheral muscle NIRS parameters in combination with venous occlusion

The base of all calculation are changes in peripheral HbO_2 ($\Delta pHbO_2$) and peripheral deoxy-Hb (Δp-deoxy-Hb) (97)(100)(105)(106)(107).

pFOE is calculated out of peripheral VO_2 (pVO_2) and peripheral oxygen delivery (pDO_2):

$$pFOE = \frac{pVO2}{pDO2}$$

pVO_2 is calculated out of peripheral haemoglobin flow (pHbflow/min), SpO_2 and peripheral SvO_2 ($pSvO_2$). Unit of pVO_2 is µmol/l/min.

$$pVO2 = pHbflow/\min * 4 * (\frac{SpO2}{100 - pSvO2})$$

pDO_2 is calculated out of pHbflow/min and SpO_2. Unit of pDO_2 is µmol/l/min.

$$pDO2 = pHbflow/\min * 4 * (\frac{SpO2}{100})$$

pHbflow/min is calculated out of $\Delta pHbO_2$ and Δp-deoxy-Hb and represents the increase in total haemoglobin ΔpHbtot within one minute. Unit of pHbflow/min is mol/l/min.

$$pHbflow/\min = \Delta p - deoxy - Hb + \Delta \mathrm{pHbO2}$$

$pSvO_2$ is calculated out of $\Delta pHbO_2$ and ΔpHbtot.

$$pSvO2 = \frac{\Delta \mathrm{pHbO2}}{\Delta \mathrm{pHbtot}}$$

1.3.3.4. Peripheral NIRS measurements without occlusion method

Peripheral muscle NIRS measurements without occlusion method provide the peripheral rSO_2 ($prSO_2$) (INVOS 5100) or peripheral TOI (pTOI) (NIRO 300/ 200NX). Using the $prSO_2$ or pTOI and SpO_2, peripheral fractional tissue oxygen extraction (pFTOE) can be calculated. For measurements, the NIRS probe is placed on the forearm or calf and is hold in position without applying circular pressure. A pneumatic cuff is not necessary for this technique (69). This method is less prone to movement artefacts and easier to perform in clinical routine due to less effort in the implementation, however, less information about the peripheral muscle oxygenation and perfusion status can be gained.

1.3.3.5. Influencing parameters of peripheral muscle NIRS measurements

Pichler et al. described potential associations between peripheral muscle NIRS measurements and different parameters (108). They observed a positive correlation of gestational age and birth weight, as well as actual weight, diameter calf and subcutaneous fat with pTOI and a negative correlation with pFOE and pFTOE. This is in contrast with postnatal age: postnatal age has been described with a negative correlation with pTOI and with a positive correlation with pFOE and pFTOE, which can be explained by changes in muscle tone.

Differences in peripheral muscle oxygenation according to the position of measurement, comparing forearm and calf tissue oxygenation, were investigated by Pichler et al. (102). They observed no differences in FOE (0.32 ±0.07 and 0.32 ±0.0.7 on the forearm and on the calf, respectively), in SvO_2 and in TOI comparing measurements on the forearm and on the calf. DO_2 and VO_2, however, showed lower values on the forearm compared to the calf, which can be due to differences in limb size.

1.3.4. Oxygen extraction

1.3.4.1. Peripheral oxygen extraction

Peripheral oxygen extraction (pOE) gives information about oxygen consumption and delivery and is calculated out of SpO_2 and SvO_2.

$$pOE = SpO2 - SvO2$$

In neonates, pOE is described in two studies (97)(109). Bay Hansen et al. (109) observed the potential relation between peripheral and central venous saturation. The NIRS device they used was the Radiometer and they measured in term and preterm neonates. They only

described values in case series for each patient at different time points of postnatal age. Therefore, these values cannot be compared with other values. Pichler et al. (97) described the calculation of pOE and defined quality criteria for peripheral muscle NIRS measurements based on pOE, however, no exact values of pOE were presented.

1.3.4.2. Peripheral fractional oxygen extraction

Peripheral fractional oxygen extraction (pFOE) represents the relative difference/extraction from arterial to venous compartment and is obtained by peripheral muscle NIRS measurements in combination with venous occlusion. pFOE is calculated out of pVO_2 and pDO_2 (105)(100).

$$pFOE = \frac{pVO2}{pDO2}$$

pFOE has been described in several studies in term and in preterm neonates (101).

1.3.4.3. Peripheral fractional oxygen extraction in healthy neonates

Several studies described pFOE in stable term and preterm neonates without any respiratory or medical support and without pathological findings (93)(95)(110)(111)(112)(113)(114). These values can be seen as normal values. pFOE values of the calf in term neonates were the highest values when being compared to measurements on the forearm or in preterm neonates. During the first 18 hours after birth a slight decrease was observed in pFOE in preterm neonates (110). However, comparing values during the first 24 hours after birth with pFOE obtained after 48 to 72 hours after birth, an increase in pFOE can be investigated (101). Normal values, however, measured in the same cohort on the same position (calf or forearm), with an observational period of three or more days are missing.

1.3.4.4. Peripheral fractional oxygen extraction in neonates with pathological findings

Beside, pFOE values in stable neonates, many studies described pFOE in sick neonates or neonates with exposure to risk factors, including tobacco exposure during pregnancy, asphyxia, PDA, elevated CRP values, neonates with anaemia or hypotension (90)(92)(93)(96)(101)(107)(111)(114)(115).

1.3.4.5. Peripheral fractional tissue oxygen extraction (pFTOE)

Peripheral fractional tissue oxygen extraction (pFTOE) represents the relative difference/extraction from arterial to tissue compartment. These include smaller arterial and

venous vessels and capillaries. It is calculated out of SpO_2 and pTOI (NIRO 200NX/ 300) or $prSO_2$ (INVOS 5100) (87).

$$pFTOE = \frac{(SpO2 - pTOI)}{SpO2}$$

The comparability of pFOE and pFTOE has been investigated by Hoeller et al. (116). Further they observed the potential for providing information at early stages of disturbances in microcirculation. They described that pFOE and pFTOE cannot be equated, however, the showed the same trend. pFOE values were higher than pFTOE values. Further, pFOE might have a higher potential to detect early stage of disturbances in microcirculation.

pFTOE have been described in several studies, focussing on the first 15 minutes after birth in term and preterm neonates (69)(78)(117). Urlesberger et al. (78) observed the potential influence of a left-to-right shunt via PDA on peripheral muscle oxygenation during the first 15 minutes after birth. There were no significant differences in pFTOE, measured preductal and postductal, between the shunt group compared to the no shunt group. Further, Bruckner et al. (117) studied the association between body temperature on cerebral and peripheral oxygenation. They found no correlation of pFTOE with body temperature neither in term nor in preterm neonates during the first 15 minutes after birth.

Eriksen et al. investigated the effect of different modus of respiratory support in preterm neonates on rSO_2 and pFTOE after the first week after birth (118). No differences in pFTOE were observed after changes in respiratory support modus.

Wolfsberger et al. (119) described normal values of pFTOE during the first 24 hours after birth in stable term and preterm neonates.

1.3.5. Peripheral NIRS measurements during the first day after birth

Several studies have been published, performing peripheral muscle NIRS measurements within the first 24 hours after birth (93)(97)(110)(111)(119). Wolfsberger et al. (110) described parameters of peripheral muscle oxygenation and perfusion in stable preterm neonates within the first 24 hours after birth and investigated changes of these parameters comparing the first six hours after birth with time periods 7-12 hours, 13-18 hours and 19-24 hours after birth. They observed an increase in Hbflow, DO_2 and SvO_2, and a decrease of pFOE. pFOE showed values of 0.35 (0.29-0.40) during the first six hours after birth and decreased to 0.29 (0.22-0.34) in the last time period, 19-24 hours after birth. In contrast, pTOI showed only a slight increase over time, without reaching significance. These values were measured on the

forearm. Comparable results were provided in term neonates by Pichler et al. (93) who investigated the impact of smoking on peripheral muscle oxygenation. In the control group with no tobacco exposure during pregnancy, pFOE was 0.30 ±0.04 during the first 24 hours after birth. Mileder et al. (111) described the influence of PDA on peripheral muscle oxygenation in preterm neonates, with measurements on the calf. Again, the provided values of pFOE in the control group (preterm neonates without signs of PDA) were comparable to other published values during the first 24 hours after birth (pFOE 0.3 [0.3-0.3]).

Normal values of pFTOE in stable term and preterm neonates during the first 24 hours after birth are described by Wolfsberger et al. (119). In term neonates “0-6h after birth”, “7-12h after birth”, “13-18h after birth” and “19-24h after birth” pFTOE was 0.264 (0.229-0.300), 0.228 (0.192-0.264), 0.237 (0.200-0.274) and 0.220 (0.186-0.254). In preterm neonates pFTOE was 0.229 (0.213-0.246), 0.225 (0.209-0.240), 0.226 (0.210-0.242) and 0.238 (0.222-0.255) in the first, second, third and fourth time period. pFTOE exhibited no notable alterations during the specified time intervals, whether in term or preterm neonates.

1.3.6. Ratio of peripheral and cerebral near-infrared spectroscopy

Simultaneous measurements of peripheral und cerebral oxygenation and perfusion by NIRS have been described by a few studies yet (94)(120)(121).

Simultaneous measurements of cTOI and pTOI was performed by Grossauer et al. in term and preterm neonates (121). They measured in mean 16 days after birth and observed significantly higher cTOI values compared to pTOI. The cTOI/pTOI ratio was 1.14 ±0.14. Hoeller et al. (120) investigated the ratio of cTOI/pTOI in stable preterm neonates during the first 24 hours after birth. The mean cTOI/pTOI during the first 24 hours after birth was 0.96 ±0.02. Further, they described that the ratio of cTOI/pTOI decreased within the first hours after birth and showed significantly lower values between five to 15 hours after birth compared to the time period before. As these values of the ratio of cTOI/pTOI were described in stable preterm neonates, it can be seen as normal values.

1.3.7. Precision of NIRS measurements

Precision and reproducibility of measurements of cerebral and peripheral haemodynamic parameters is of great importance. Several studies have already described techniques to improve the precision and reproducibility in NIRS measurements (97)(104)(122)(123)(124).

1.3.7.1. Reapplications

Reapplications of the NIRS sensor can improve precision and reproducibility, whereby reapplication between 30 seconds and 25 minutes have been described in several studies (97)(122)(124)(125). Avian et al. (122) performed five reapplications, with one lasting for one minute, to evaluate the precision of rSO_2 measured with time-resolved spectroscopy using the tNIRS-1 (Hamamatsu Photonics). They observed a within-patient variation of the rSO_2 below a threshold of 5% and can be stated to be precise. In contrast to five reapplications, Menke et al. (125) performed 20 reapplications with a duration of 30 seconds of each reapplication. Sorensen and Greisen (124) investigated the precision of cTOI measured with the NIRO 300. If one single measurement was performed low precision and great within variations were observed. Five to eight reapplications, in contrast, increased the reproducibility comparable to the precision of pulse oximetry.

A method that is similar to reapplication has been described by Hyttel-Sorensen et al. (126) who describe the use of two measurement channels simultaneous and to calculate the mean of these two values. This technique enables to reduce the effect of optical heterogeneity.

1.3.7.2. Quality criteria

Pichler et al. (97) described quality criteria to increase the reproducibility of peripheral NIRS measurements. When performing peripheral muscle NIRS measurements with venous occlusion, the criterion “linear changes of Hbtot with R^2>0.95 assessed with linear regression analysis” (97) has to be fulfilled. What is more, TOI has to be greater than SvO_2, as SvO_2 is only measured in the venous compartment, whereby, TOI represents the oxygen saturation of the venous, capillary and arteriolar compartments. Further, they described that the elimination of implausible values improves the within-patient variance from 46.6% to 35%.

1.3.7.3. Recommendations

In a consensus statement, Pichler et al. (104), described standardised procedures to achieve more comparable results in peripheral muscle NIRS measurements. The neonates should be at rest with the position of the upper or lower limb just above mid-sternal level. The exact place on the limb where the NIRS probe is placed should be documented. The interoptode distance should not be greater than the diameter of the limb. Measurements of subcutaneous fat should be performed by ultrasound. In cases of venous occlusion in combination with peripheral muscle NIRS, the pressure of the pneumatic cuff should be less than 10mmHg lower than the diastolic arterial pressure and an inflation time of 30sec is sufficient. Nevertheless, between occlusions, a rest period should be performed.

1.4. NIRS and infection

Several studies have already observed the influence of infection and/or infectious parameters in neonates on cerebral and peripheral oxygenation (49)(92)(114).

The influence of FIRS on $crSO_2$ and cFTOE within the first 15 minutes after birth in preterm neonates has already been described. IL-6 in the FIRS group was in mean 32.0 pg/ml and in the non-FIRS group 5.4 pg/ml and there were no differences in $crSO_2$ between the two groups. cFTOE, however, was significantly lower in the FIRS group compared to the non-FIRS group during the first five minutes. A potential compromised oxygen consumption and delivery might be the reason for lower cFTOE within the first minutes in the FIRS group (49). Peripheral muscle NIRS measurements were performed and compared also in neonates with and without elevated CRP values, in cases of normal routine haemodynamic variables (114). In 33 neonates with elevated CRP values (mean CRP 25.6 ±16.5mg/dl) pTOI, SvO_2, DO_2 and VO_2 were significantly lower compared to the control group. No significant differences were observed in pFOE and Hbflow. Binder et al. (92) observed in their study a potential influence of leukocyte counts on peripheral muscle NIRS parameters. They described a negative correlation of leukocytes with pTOI and a positive with vascular resistance.

2. Objectives

2.1. Study rationales

Admission to the NICU after birth due to respiratory distress is very common in term and preterm neonates, whereby the diagnosis of the underlying disease is often challenging, especially, to rule out an early onset infection. Therefore, there is growing interest in methods enabling to recognise subtle early signs like microvascular dysfunction due to infection. NIRS enables measurements of cerebral and peripheral muscle oxygenation and perfusion and has a great potential for early recognition of compromised microcirculation and oxygenation. NIRS measurements performed as short reapplications within the first hours after birth are feasible in clinical routine and have a high precision.

In the present study we wanted therefore to evaluate in term and moderate-to-late preterm neonates with respiratory distress, if peripheral muscle and cerebral FTOE measured by five short reapplications of the NIRS sensors within the first six hours after birth enables to recognise early microvascular dysfunction due to inflammation/infections. Therefore, we wanted to investigate prospectively, if there are significant differences in peripheral muscle and cerebral FTOE within the first hours after birth between neonates, who develop clinical and laboratory signs of early onset infection, and neonates without signs of infection.

2.2. Aim

2.2.1. Primary aim

The primary aim of the present study was to assess, if pFTOE measured by five short NIRS (re-)applications within the first six hours after birth differs in neonates with early onset infection and neonates without infection.

2.2.2. Secondary aims

Secondary aims of the present study were to assess, if cFTOE and the ratio cFTOE/pFTOE measured by five short NIRS (re-)applications within the first six hours after birth differ in neonates with early onset infection and neonates without infection.

2.2.3. Third aims

Third aims were to assess, if there are differences in pFTOE, cFTOE and cFTOE/pFTOE between term and preterm neonates.

2.3. Hypothesis

2.3.1. Primary hypothesis

We hypothesised that term and preterm neonates with early onset infection show higher pFTOE compared to neonates without infection.

2.3.2. Secondary hypotheses

We hypothesised that term and preterm neonates with early onset infection show higher cFTOE and a higher ratio of cFTOE/pFTOE compared to neonates without infection.

2.3.3. Third hypotheses

We hypothesised that term neonates show higher values in pFTOE, cFTOE and cFTOE/pFTOE compared to preterm neonates.

2.4. Outcome parameters

2.4.1. Primary outcome parameter

- pFTOE within the first six hours after birth

2.4.2. Secondary outcome parameters

- cFTOE and cFTOE/pFTOE within the first six hours after birth
- gestational age

2.4.3. Explanatory outcome measures

- pTOI, cTOI
- cardiac output (CO): estimated CO (COest.) and estimated/adjusted CO (COest./adj) and CO
- routine monitoring data (SpO_2, PI, HR, blood pressure, temperature)
- laboratory parameters and blood cultures
- demographic data
- diagnoses
- therapy and medication

3. Methods

3.1. Study design

The present study was performed as a part of a prospective observation single center pilot study (pFTOE Trial; Clinical Trials.gov identifier: NCT04818762) and was performed at the Division of Neonatology, Department of Pediatrics and Adolescent Medicine, Medical University of Graz between February 2021 and February 2023. The study was approved by the Regional Committee on Biomedical Research Ethics with the EC number: 33-161 ex 20/21. Before inclusion in this prospective study, written parental consent had been.

3.2. Study population

Term and preterm neonates with a gestational age ≥30+0 weeks with respiratory distress admitted to the NICU of the Division of Neonatology Graz, after vaginal delivery or caesarean section on the first day after birth were eligible for the study.

3.2.1. Inclusion criteria

Inclusion criteria were signs of respiratory distress at time-point of inclusion (tachypnoea > 60/min, grunting, intercostal/subcostal/jugular retractions, nasal flaring, supplemental oxygen or respiratory distress, transient respiratory distress of the newborn), age < six hours, decision to conduct full life support and written informed consent.

3.2.2. Exclusion criteria

Excluded were neonates with a gestational age < 30+0 weeks of gestation, age > six hours, severe congenital malformations, severe asphyxia (umbilical cord artery pH <7.00), no decision to conduct full life support or no written informed consent.

3.3. Patient groups

Term and preterm neonates were investigated separately. There were four study groups:

1) Term neonates (≥37+0 weeks of gestation) with clinical and laboratory signs of early onset infection (within 48 hours after birth).
2) Term neonates (≥37+0 weeks of gestation) without infection.
3) Preterm neonates (30+0 – 36+6 weeks of gestation) with clinical and laboratory signs of early onset infection (within 48 hours after birth).
4) Preterm neonates (30+0 – 36+6 weeks of gestation) without infection.

3.4. Laboratory signs of infection

Routine blood samples were performed within 24 and 48 hours after birth in each neonate with the suspicion of early onset infection. Blood culture sampling was recommended, and the sampling was tried in each neonate. Laboratory signs of infection were defined as elevated CRP value >10mg/l, elevated leukocytes >30000/µl or decreased leukocytes <6000/µl, elevated IT ratio >0.2 or positive blood culture. At least one of the mentioned criteria has to be fulfilled to be defined as infection.

3.5. NIRS measurements

The NIRO 200 NX (Hamamatsu Photonics K.K, Hamamatsu City, Japan) was used for peripheral muscle and cerebral oxygenation measurements **(Figure 1)**. For peripheral muscle NIRS measurements different interoptode distance were used, according to the birth weight of the neonate: an interoptode distance of 3.0 cm in neonates with a birth weight > 1500g and 2.0 cm in preterm neonates with a birth weight < 1500g. Measurements of cerebral oxygenation were performed using optodes with a distance of 4.0 cm.

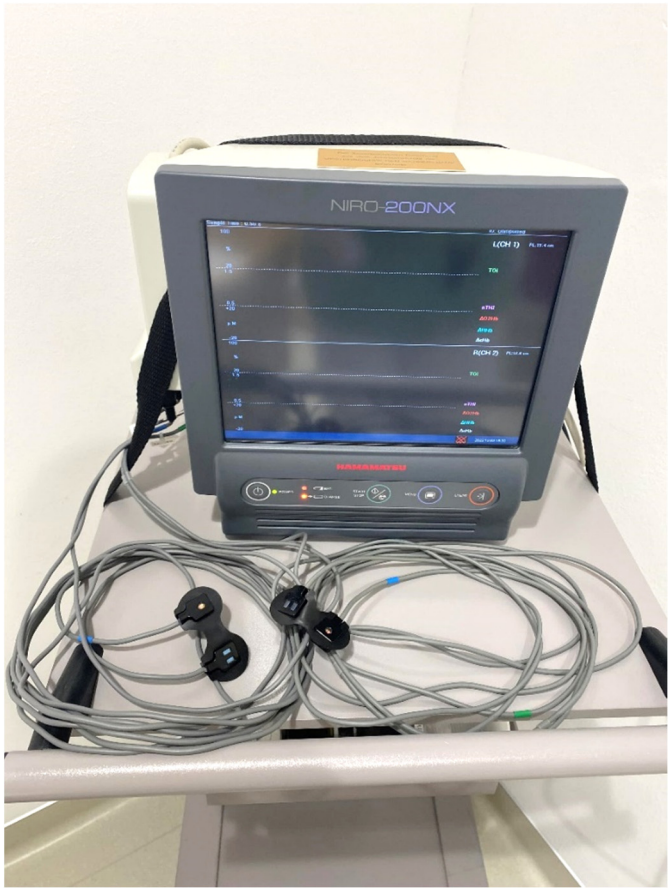

Figure 1. NIRS 200NX (Hamamatsu Photonics K.K., Hamamatsu City, Japan) Near-infrared spectroscopy monitor with two sensors for cerebral and peripheral NIRS measurement.

3.6. Routine measurements

Measurements of SpO_2 and PI were performed using pulse oximetry by the IntelliVue MP70 or MX750 monitor (Philips, Netherlands). HR was measured with ECG electrodes placed on the chest of the neonates. Non-invasive measurements of blood pressure were also measured with the IntelliVue MP70 or MX750 monitors (Philips, Netherlands). Peripheral and central temperature were measured with a skin probe placed under the neonate and with a rectal probe, respectively.

3.7. Procedure

3.7.1. Medical History

Patients' medical history and demographic data including gestational age, birth weight, gender, Agpar score, blood gas analysis from the umbilical artery were documented. Patients' medical history included main neonatal diagnosis, patients' blood gas analysis and laboratory results and provided therapy including respiratory and medical support. Diameter, subcutaneous fat and circumference of the right arm were evaluated by ultrasound of the right arm (diameter, subcutaneous fat) using the GE Logiq S8 (Chicago, United States) and by tape measure (circumference). To evaluate risk factors, maternal history including markers of systemic inflammation (clinical, laboratory results) and main maternal diagnosis were documented.

3.7.2. Laboratory investigations and blood culture

In neonates with respiratory distress, blood samples for laboratory investigations on the first and second day after birth were routinely performed. Further, in neonates with respiratory distress and risk factors for infection it was routine to take blood cultures before beginning with antibiotics. Laboratory findings included leucocytes, CRP values and IT ratio. The time point of the first and the second blood sample was documented.

3.7.3. Procedure of measurements of peripheral muscle and cerebral oxygenation

After obtaining the informed consent from the parents, the measurements were performed once within the first six hours after birth. For NIRS measurements the NIRO 200NX was used and NIRS sensors with appropriate interoptode distances, were applied by hand on the right forearm (pTOI) **(Figure 2)** and on the left forehead (cTOI) **(Figure 3)** until stable signals were obtained for approximately 30 seconds, respectively. Then the sensors were removed for 10 seconds rest period. After that, the sensors were reapplied in approximately the same position. This procedure was repeated five times. The mean of the five peripheral muscle measurements and the mean of the five cerebral NIRS measurements were calculated. Simultaneously, SpO_2, HR and PI were recorded in the time period when peripheral muscle and cerebral NIRS measurements were performed. The mean of SpO_2, HR and PI during the time period of peripheral muscle NIRS measurements and the mean of SpO_2, HR and PI during the time period of cerebral NIRS measurements were calculated. Before and after NIRS

measurements, blood pressure was measured non-invasively. Further, peripheral body temperature, obtained by a skin sensor was documented before and after NIRS measurements and rectal body temperature was documented only after NIRS measurements. Central capillary refill time, measured on the chest of the neonate, was also documented immediately after NIRS measurements.

Figure 2. NIRS sensor with an interoptode distance of 3.0 cm was applied by hand on the right forearm of the neonate for measurements of peripheral muscle oxygenation.

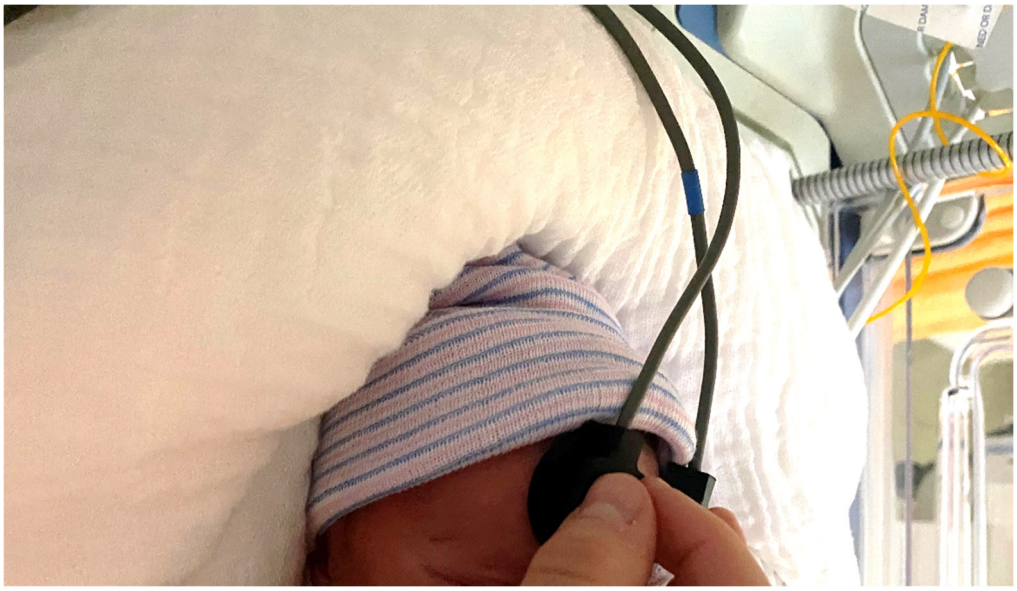

Figure 3. NIRS sensor with an interoptode distance of 4.0 cm was applied by hand on the left forehead of the neonate for measurements of cerebral oxygenation.

3.8. Calculations and statistical analysis

3.8.1. Calculations NIRS parameter and routine vital parameters

Term and preterm neonates were analysed separately. In each neonate pTOI, cTOI, pFTOE, cFTOE, pPI (PI during peripheral NIRS measurements), cPI (PI during cerebral NIRS measurements), SpO_2 during pTOI, SpO_2 during cTOI, HR during pTOI and HR during cTOI were recorded during measurements. Mean values of pTOI, cTOI, pFTOE, cFTOE, pPI, cPI, SpO_2 during pTOI, SpO_2 during cTOI, HR during pTOI and HR during cTOI, were calculated and used for further analysis.
pFTOE was calculated out of SpO_2, during pTOI, and pTOI (87):

$$pFTOE = \frac{(SpO2 - pTOI)}{SpO2}$$

cFTOE was calculated out of SpO_2, during cTOI, and cTOI (87):

$$cFTOE = \frac{(SpO2 - cTOI)}{SpO2}$$

3.8.2. Calculations cardiac output

Cardiac output (CO) was calculated for each group (127). For calculations of CO mean SABP and DABP was calculated out of SABP / DABP before and after measurements. Mean HR during pTOI and cTOI was calculated and used for CO calculations.
Estimated cardiac output (COest.) was calculated by the formula from Liljestrand and Zander (128). COest. consists of pulse pressure (PP), sum of SABP and DABP and HR. COest. has no unit.

$$COest. = \frac{PP}{(SABP + DABP) * HR}$$

To obtain adjusted estimated CO (COest./adj.), the formula of COest. was adapted with the calibration factor (k). The unit of COest./adj is l/min.

$$COest./adj = k * [\frac{PP}{(SABP + DABP) * HR}]$$

Further, CO can be calculated out of COest./adj. and birth weight in kilogram. Unit of CO is ml/kg/min.

$$CO = \frac{COest./adj}{birth\ weight}$$

3.8.3. Statistical analysis

Data were presented as mean and standard deviation (SD) for normally distributed continuous variables or median and minimum/maximum if the distribution was skewed. NIRS data and patient's characteristics of neonates with infection and without infection were compared according their distribution using t-test or Mann-Whitey-U-Test for continuous variables and $\chi 2$ or Fisher's exact test for categorical variables in term and preterm neonates. A p-value <0.05 was considered statistically significant. Multiple testing corrections were performed using the Bonferroni correction. The statistical analyses were performed using SPSS Statistics 25 (IBM Corporation; Armonk, New York, USA).

4. Results

Eighty neonates, 32 term neonates and 48 preterm neonates, with signs of respiratory distress within the first six hours after birth were screened for inclusion in the pFTOE trial. All screened neonates were included and analysed in the pFTOE trial (**Figure 4**, Flow chart). Out of 32 included term neonates, 15 neonates were stratified to the infection group and 17 were stratified to the no-infection group. Out of 48 preterm neonates, six neonates were stratified to the infection group and 42 were stratified to the no-infection group. None of the initially included neonates had to be excluded from final analysis.

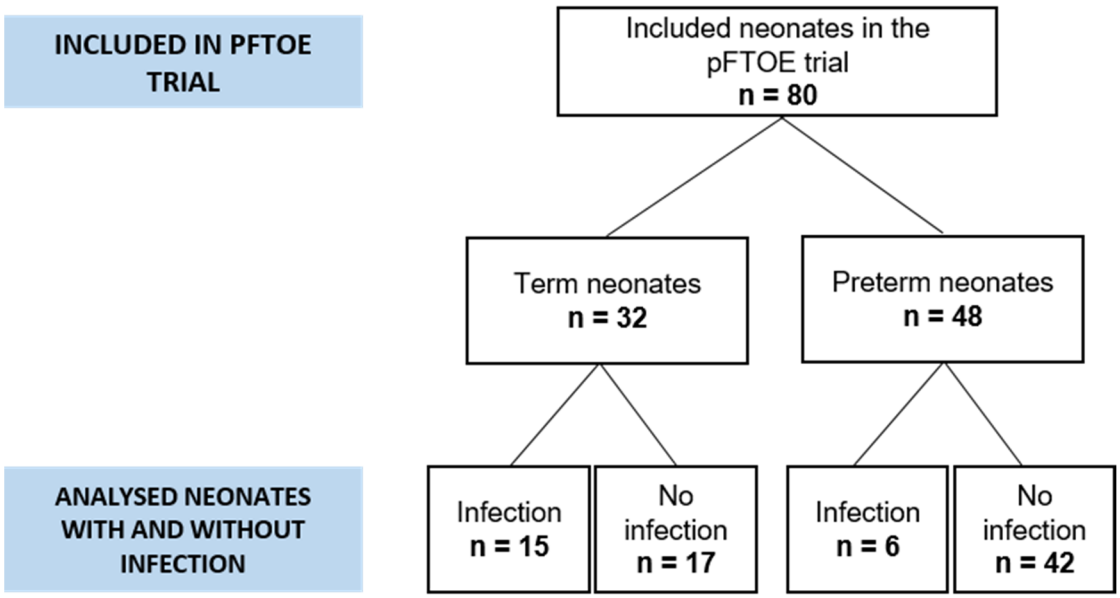

Figure 4. Flow chart of included neonates in the pFTOE trial.

4.1. Term neonates

4.1.1. Demographic data and clinical parameters of term neonates with and without infection

A total of 32 term neonates were included: 15 term neonates were stratified to the infection group as infection was confirmed through elevated laboratory findings. 17 term neonates were stratified to the no-infection group. Demographic data of term neonates with and without infection were displayed in **Table 1**. A statistically significant difference was observed in the diameter of the arm with 3.4 ±0.6 cm and 3.3 ±0.5 cm in the infection and no-infection group, respectively. Nevertheless, this difference can be assumed to be without clinical relevance.

Table 1. Demographic data of included term neonates with and without infection. Data are presented as n (%), mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Infection group n = 15	*No-infection group n = 17*	*p-value*
Gestational age (weeks)	39.6 ±1.7	38.5 ±1.4	0.059
Birth weight (g)	3543 ±615	3221 ±592	0.078
Female sex n (%)	8 (53.3)	7 (41.2)	0.492
Apgar 1	6 (5; 9)	8 (6; 9)	0.279
Apgar 5	8 (6; 9)	9 (8; 9)	0.435
Apgar 10	9 (9; 10)	9 (9; 10)	0.649
Umbilical artery pH	7.25 ±0.10	7.24 ±0.87	0.474
Maternal CRP (mg/L)	3.6 (0.8; 74.4)	3.6 (0.7; 4.9)	0.493
Maternal leukocytes (/µl)	10770 ±5736	11090 ±4043	0.715
Maternal fever during birth n (%)	2 (13.3)	0 (0.0)	0.120
Diameter of the arm (cm)	3.4 ±0.6	3.3 ±0.5	0.017*
Thickness of subcutaneous fat (cm)	0.54 ±0.20	0.49 ±0.13	0.054
Circumference of the arm (cm)	10.8 ±1.4	10.4 ±1.3	0.196

Abbreviations: cm = centimeter, CRP = C reactive protein, g = grams, l = liter, mg = milligram, pH = potential of Hydrogen, µg = microgram

There was no difference in mode of delivery between term neonates of the infection group and the no-infection group. The mode of delivery was in the infection group spontaneous (n=3; 20.0%), primary Caesarean section (n=7; 46.7%) and secondary Caesarean section (n=5; 33.3%), and in the no-infection group spontaneous (n=2; 11.8%), primary Caesarean section (n=14; 82.4%) and secondary Caesarean section (n=1; 5.9%). The overall p-value in mode of delivery between the infection and no-infection group was 0.078, with also no significant difference between each of the delivery modes (spontaneous and primary Caesarean section p=0.340; spontaneous and secondary Caesarean section p=0.545; primary and secondary Caesarean section p=0.060).

Concerning maternal anaesthesia (none, spinal anaesthesia, general anaesthesia) during labour, there was also no difference between the infection and no-infection group (p=0.409).

In none of the term neonates in the infection group and in two of the no-infection group (13.3%)(p=0.120), mothers received antenatal steroid for inducing fetal lung maturation, when they were at risk for giving preterm birth.
There were no differences in signs for maternal perinatal infection (leukocytes, CRP, maternal fever) between the infection and no-infection group (**Table 1**). Consequently, there were no difference in the rate of maternal antenatal antibiotic therapy between the two groups. In the infection group two mothers (13.3%) and in the no-infection group no mother (0.0%) (p=0.120) received antibiotics before birth as a consequence of risk for infection. However, immediately before Caesarean section, all mothers received ampicillin/sulbactam, as a single-shot therapy.

4.1.2. Primary and secondary outcome variables in term neonates with and without infection

In term neonates with and without infection, no differences in pFTOE within the first six hours after birth were observed (p = 0.293). Furthermore, no differences were detected in pTOI (p = 0.396), cTOI (p = 0.916), cFTOE (p = 0.374) or the ratio of cTOI/pTOI (p = 0.539) and cFTOE/pFTOE (p = 0.466).
Peripheral and cerebral NIRS reapplications were performed in median 165 (128; 318) minutes and 140 (75; 201) minutes after birth in the infection and no-infection group, respectively. Primary outcome parameter (pFTOE) and secondary outcome parameters, including routine monitoring parameters during either peripheral or cerebral reapplications were compared between term neonates with and without infection (**Table 2**). Time for peripheral reapplications (pTOI) was 4.3 ±1.5 and 3.5 ±1.1 seconds in the infection group and no-infection group, respectively (p=0.655). Time for cerebral reapplications (cTOI) was 3.8 ±0.8 and 3.9 ±0.8 seconds in the infection group and no-infection group, respectively (p=0.699). Mean overall HR during pTOI and cTOI measurements was 141 ±11 bpm in the infection group and 132 ±18 bpm in the no-infection group. Mean SABP calculated out of measurements before and after reapplications was 63 ±7 mmHg in the infection group and 60 ±9 mmHg in the no-infection group. Mean DABP calculated out of measurements before and after reapplications was 37 ±6 mmHg in the infection group and 34 ±8 mmHg in the no-infection group.

Table 2. Primary and secondary outcome variables in term neonates with and without infection. Data are presented as mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Infection group n = 15	*No-infection group n = 17*	*p-value*
Age at measurement (min)	165 (128; 318)	140 (75; 201)	0.720
Provided FiO_2	0.27 (0.21; 0.33)	0.22 (0.21; 0.30)	0.256
Peripheral NIRS measurements			
Mean pTOI (%)	72.2 ±4.3	72.3 ±3.1	0.396
Mean PI during pTOI	1.84 ±0.51	3.21 ±2.99	0.473
Mean HR during pTOI (bpm)	144 ±16	133 ±15	0.497
Mean SpO_2 during pTOI (%)	94 ±3	95 ±3	0.736
Mean pFTOE	0.229 ±0.064	0.235 ±0.032	0.293
Cerebral NIRS measurements			
Mean cTOI (%)	72.6 ±5.7	72.1 ±3.2	0.916
Mean PI during cTOI	1.55 ±0.33	2.59 ±1.92	0.357
Mean HR during cTOI (bpm)	145 ±13	132 ±16	0.082
Mean SpO_2 during cTOI (%)	92 ±3	94 ±3	0.135
Mean cFTOE	0.207 ±0.074	0.236 ±0.020	0.374
Cerebral/peripheral ratio			
cTOI/pTOI	1.01 ±0.12	0.99 ±0.07	0.539
cFTOE/pFTOE	1.04 ±0.67	1.02 ±0.21	0.466
Blood pressure and cardiac output			
MABP before measurements (mmHg)	44 (38; 52)	38 (33; 43)	0.225

SABP before measurements (mmHg)	62 ±7	60 ±9	0.475
DABP before measurements (mmHg)	36 ±7	34 ±8	0.338
MABP after measurements (mmHg)	45 (36; 57)	38 (34; 49)	0.219
SABP after measurements (mmHg)	63 ±7	60 ±10	0.340
DABP after measurements (mmHg)	39 ±9	35 ±9	0.218
COest.	33.58 (27.35; 56.36)	35.01 (23.92; 60.14)	0.865
COest./adj. (l/min)	0.33 (0.27; 0.56)	0.35 (0.24; 0.60)	0.865
CO (ml/kg/min)	92.47 (69.98; 125.54)	101.18 (69.55; 248.09)	0.637
	Temperature, capillary refill time		
Temperature peripheral before measurements (°C)	36.9 ±0.6	37.0 ±0.3	0.360
Temperature peripheral after measurements (°C)	37.1 ±0.4	37.1 ±0.3	0.455
Rectal temperature (°C)	37.3 ±0.4	37.2 ±0.2	0.075
Central capillary refill time (s)	1.72 (1.01; 3.01)	2.33 (1.01; 2.84)	0.791

Abbreviations: °C = degree Celsius, bpm = beats per minute, cFTOE= cerebral fractional tissue oxygen extraction, CO = cardiac output, COest = estimated cardiac output, COest/adj. = estimated/adjusted cardiac output, cTOI = cerebral tissue oxygenation index, DABP = diastolic blood pressure, FiO2 = fraction of inspired oxygen, HR = heart rate, kg = kilogram, l = liter, MABP = mean arterial blood pressure, min = minutes, ml = millilitre, mmHg = milimeter of mercury, NIRS = near-infrared spectroscopy, pFTOE = peripheral fractional tissue oxygen extraction, PI = perfusion index, pTOI = peripheral tissue oxygenation index, s= seconds, SABP = systolic blood pressure, SpO_2 = arterial oxygen saturation

4.1.3. Respiratory support and respiratory distress symptoms in term neonates with and without infection

In the infection group, four (26.7%) term neonates had no respiratory support, five (33.3%) term neonates received nasal high flow therapy and in six (40.0%) term neonates, nasal continuous positive airway pressure (NCPAP) was applied. In the no-infection group, four

(23.5%) term neonates had no respiratory support, six (35.3%) term neonates received nasal high flow therapy and in seven (41.2%) term neonates, NCPAP were applied. There was no overall difference in respiratory support between the infection and no-infection group (p=0.979). Comparing subgroups of respiratory support (no respiratory support, nasal high flow therapy and NCPAP), there was also no difference between individual respiratory support methods (none and nasal high flow therapy p=1.000; none and NCPAP p=1.000; nasal high flow therapy and NCPAP p=1.000). None of the included term neonates in the present study needed intubation. Collected data of respiratory distress symptoms include grunting, retractions, nasal flaring and tachypnoea. Combination of respiratory distress symptoms were documented in term neonates of both groups. Data are presented in **Table 3**. In all term neonates, respiratory rate was above normal levels and was therefore described as tachypnoea.

Table 3. Respiratory distress symptoms in term neonates with and without infection. Data are presented as n (%) or mean ±SD.

	Infection group n = 15	*No-infection group n = 17*	*p-value*
Grunting	7 (46.7%)	11 (64.7%)	0.305
Retractions	6 (40.0%)	10 (58.8%)	0.288
Nasal flaring	7 (46.7%)	9 (52.9%)	0.723
Respiratory rate (/min)	86 ±15	78 ±13	0.956

Abbreviations: min = minutes

4.1.4. Blood gas analysis and laboratory findings in term neonates with and without infection

In all term neonates, blood samples to rule out a potential infection were performed either within the first 24 hours after birth or between 24 and 48 hours after birth. In the infection group, in 13 term neonates (87.0%) infection was proven by laboratory parameters in the first blood sample, within the first 24 hours after birth. In two term neonates, no signs of infection were observed within the first 24 hours after birth, however, between 24 and 48 hours after birth. Blood culture results showed in none of the included term neonates of both groups a germ detection. All taken blood cultures were done within the first 24 hours. In the infection group, however, in 14 (93.3%) term neonates and in the no-infection group, in 15 (88.2%) term neonates, no blood culture results were available (p=0.621). Antibiotic therapy was provided

for 5.9 ±1.9 days and for 3.5 ±1.7 days (p=0.040*), in the infection group and no-infection group, respectively. Blood gas analysis and laboratory parameters are displayed in **Table 4**.

Table 4. Blood gas analysis and laboratory parameters in term neonates with and without infection. Data are presented as mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Infection group n = 15	*No-infection group n = 17*	*p-value*
Blood gas analysis			
Time between blood sample and NIRS (min)	54 ±46	59 ±46	0.741
pH	7.34 ±0.05	7.31 ±0.06	0.174
pCO_2 (mmHg)	48.0 ±9.0	52.2 ±9.8	0.218
pO_2 (mmHg)	41.3 ±6.8	44.0 ±6.6	0.263
HCO3 (mmol/L)	23.4 ±1.2	23.6 ±1.6	0.627
BE (mmol/L)	-0.23 ±2,0	0.13 ±2.08	0.624
Lactate (mmol/L)	2.18 ±1.36	1.43 ±0.48	0.041*
Blood sample within the first 24 hours after birth			
Age at first laboratory testing (hours)	21.8 ±5.0	21.4 ±2.4	0.760
CRP (mg/L)	10.8 (4.9; 48.6)	2.4 (0.6; 10.0)	<0.001*
Leukocytes (/µl)	28163 ±18002	18456 ±3612	0.068
IT-ratio	0.10 ±0.14	0.02 ±0.02	0.049*
Blood sample between 24 and 48 hours after birth			
Age at second laboratory testing (hours)	42.2 ±7.6	48.9 ±12.5	0.172
CRP (mg/L)	13.7 (1.2; 31.8)	3.6 (0.6; 7.0)	0.021*
Leukocytes (/µl)	16342 ±5844	11807 ±4897	0.068
IT-ratio	0.02 ±0.03	0.04 ±0.04	0.523

Abbreviations: BE = base excess, CRP = C-reactive protein, HCO3- = bicarbonate, IT-ratio = immature-to-total neutrophil ratio, l = liter, mg = milligram, min = minutes, mmHg = milimeter of mercury, mmol = millimole, NIRS = near-infrared spectroscopy, pCO_2 = partial pressure of carbon dioxide, pH = potential of Hydrogen, pO_2 = partial pressure of oxygen, µg = microgram

4.2. Preterm neonates

4.2.1. Demographic data and clinical parameters of preterm neonates with and without infection

A total of 48 preterm neonates were included: 6 preterm neonates were stratified to the infection group as infection was confirmed through elevated laboratory findings. 42 preterm neonates were stratified to the no-infection group. Demographic data of preterm neonates with and without infection are displayed in **Table 5**. A statistically significant difference was observed in Apgar score in minute five with 8 (8; 9) and 9 (7; 10) in the infection and no-infection group, respectively.

Table 5. Demographic data of included preterm neonates with and without infection. Data are presented as n(%), mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Infection group n = 6	*No-infection group n = 42*	*p-value*
Gestational age (weeks)	34.8 ±1.7	34.3 ±1.6	0.462
Birth weight (g)	2476 ±720	2284 ±474	0.390
Female sex n (%)	1 (16.7)	17 (40.5)	0.260
Apgar 1	8 (8; 9)	8 (5; 9)	0.473
Apgar 5	8 (8;9)	9 (7; 10)	0.019*
Apgar 10	9 (8; 10)	9 (8; 10)	0.099
Umbilical artery pH	7.31 (7.26; 7.34)	7.33 (7.12; 7.42)	0.452
Maternal CRP (mg/L)	6.3 (0.6; 11.5)	3.5 (0.6; 61.4)	0.452
Maternal leukocytes (/µl)	13167 ±4638	10082 ±3517	0.212
Maternal fever during birth n (%)	0 (0.0)	2 (4.8)	0.585
Diameter of the arm (cm)	2.9 ±0.4	2.7 ±0.4	0.625
Thickness of subcutaneous fat (cm)	0.30 (0.29; 0.46)	0.35 (0.23; 0.54)	0.756
Circumference of the arm (cm)	8.9 ±1.4	8.9 ±0.8	0.330

Abbreviations: cm = centimeter, CRP = C reactive protein, g = grams, l = liter, mg = milligram, pH = potential of Hydrogen, µg = microgram

There was no difference in mode of delivery between preterm neonates of the infection group and the no-infection group. The mode of delivery was in the infection group spontaneous (n=1; 16.7%), primary Caesarean section (n=5; 83.3%) and secondary Caesarean section (n=0; 0.0%), and in the no-infection group spontaneous (n=11; 26.2%), primary Caesarean section (n=20; 47.6%) and secondary Caesarean section (n=11; 26.2%). The overall p-value in mode of delivery between the infection and no-infection group was 0.218, with also no significant difference between each of the delivery modes (spontaneous and primary Caesarean section p=0.641; spontaneous and secondary Caesarean section p=1.000; primary and secondary Caesarean section p=0.295).

Concerning maternal anaesthesia (none, spinal anaesthesia, general anaesthesia) during labour, there was also no difference between the infection and no-infection group (p=0.925).

In five (83.3%) preterm neonates in the infection group and in 29 (69.0%) of the no-infection group (p=0.471), mothers received antenatal steroid for inducing fetal lung maturation, when they were at risk for giving preterm birth.

There were no differences in signs for maternal perinatal infection (leukocytes, CRP, maternal fever) between the infection and no-infection group (Table 5). Consequently, there were no difference in the rate of maternal antenatal antibiotic therapy between the two groups. In the infection group one mother (16.7%) and in the no-infection group four mothers (9.5%) (p=0.592) received antibiotics before birth as a consequence of risk for infection. However, immediately before Caesarean section, all mothers received ampicillin/sulbactam, as a single-shot therapy.

Causes for preterm birth were in the infection group planned Caesarean section in cases of multiple births (n=3; 50%), cervical insufficiency (n=2; 33%) and preterm labour (n=1; 17%). Causes for preterm birth were in the no-infection group premature rupture of the membranes (n=12; 26%), amniotic fluid infection (n=4; 10%), gestosis (n=10; 24%), cervical insufficiency (n=2; 5%), threatened rupture of the uterus (n=3; 7%), intrauterine growth restriction (n=2; 5%), pathological CTG (n=3; 7%), pathological findings of the placenta (n=8; 19%), multiple birth (n=2; 5%) and others (n=6; 14%).

4.2.2. Primary and secondary outcome variables in preterm neonates with and without infection

In preterm neonates with and without infection, no differences in pFTOE within the first six hours after birth were observed (p = 0.306). Furthermore, no differences were detected in pTOI

(p = 0.491), cTOI (p = 0.322), cFTOE (p = 0.478) or the ratio of cTOI/pTOI (p = 0.441) and cFTOE/pFTOE (p = 0.685).

Peripheral and cerebral NIRS reapplications were performed in mean 207 ±52 minutes and 151 ±67 minutes after birth in the infection and no-infection group, respectively. Primary outcome parameter (pFTOE) and secondary outcome parameters, including routine monitoring parameters during either peripheral or cerebral reapplications were compared between preterm neonates with and without infection (**Table 6**). Time for peripheral reapplications (pTOI) was 3.0 (3.0; 5.0) and 3.0 (2.5; 6.0) seconds in the infection group and no-infection group, respectively (p=0.945). Time for cerebral reapplications (cTOI) was 5.0 (4.0; 5.0) and 4.0 (1.5; 7.0) seconds in the infection group and no-infection group, respectively (p=0.448). Median overall HR during pTOI and cTOI measurements was 148 (131; 173) bpm in the infection group and 145 (116; 191) bpm in the no-infection group. Mean SABP calculated out of measurements before and after reapplications was 57 (55; 72) mmHg in the infection group and 58 (41; 74) mmHg in the no-infection group. Mean DABP calculated out of measurements before and after reapplications was 32 (32; 44) mmHg in the infection group and 31 (22; 45) mmHg in the no-infection group.

Table 6. Primary and secondary outcome variables in preterm neonates with and without infection. Data are presented as mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Infection group n = 6	*No-infection group n = 42*	*p-value*
Age at measurement (min)	207 ±52	151 ±67	0.079
Provided FiO_2	0.21 (0.21; 0.27)	0.21 (0.21; 0.40)	0.488
	Peripheral NIRS measurements		
Mean pTOI (%)	73.6 ±5.3	73.0 ±4.3	0.491
Mean PI during pTOI	2.26 (1.48; 2.70)	1.54 (0.68; 6.94)	0.312
Mean HR during pTOI (bpm)	159 ±18	147 ±15	0.862
Mean SpO_2 during pTOI (%)	96 ±4	94 ±3	0.414
Mean pFTOE	0.235 ±0.050	0.224 ±0.051	0.306

	Cerebral NIRS measurements		
Mean cTOI (%)	70.9 ±6.0	73.1 ±6.0	0.322
Mean PI during cTOI	2.46 (1.42; 3.14)	1.53 (0.45; 3.48)	0.129
Mean HR during cTOI (bpm)	160 ±14	145 ±14	0.540
Mean SpO_2 during cTOI (%)	95 (93; 100)	95 (88; 100)	0.883
Mean cFTOE	0.260 ±0.333	0.222 ±0.064	0.478
	Cerebral/peripheral ratio		
cTOI/pTOI	0.96 ±0.04	1.01 ±0.11	0.441
cFTOE/pFTOE	1.02 (0.98; 1.38)	0.99 (0.39; 2.74)	0.685
	Blood pressure and cardiac output		
MABP before measurements (mmHg)	42 (40; 47)	39 (31; 58)	0.254
SABP before measurements (mmHg)	58 (54; 67)	58 (36; 73)	0.913
DABP before measurements (mmHg)	33 (31; 51)	31 (21; 54)	0.357
MABP after measurements (mmHg)	38 (38; 53)	40 (31; 49)	0.628
SABP after measurements (mmHg)	56 (55; 76)	58 (46; 74)	0.756
DABP after measurements (mmHg)	32 (31; 40)	31 (22; 40)	0.177
COest.	45.03 (19.13; 45.84)	42.56 (17.02; 63.29)	0.513
COest./adj. (l/min)	0.45 (0.19; 0.45)	0.42 (0.17; 0.63)	0.513
CO (ml/kg/min)	176.69 (52.03; 266.48)	187.28 (84.67; 318.94)	0.640
	Temperature, capillary refill time		
Temperature peripheral before measurements (°C)	37.5 (36.7; 37.5)	37.0 (35.7; 37.8)	0.229

Temperature peripheral after measurements (°C)	37.5 (36.8; 37.6)	37.0 (36.1; 37.9)	0.187
Rectal temperature (°C)	37.1 ±0.3	37.1 ±0.4	0.808
Central capillary refill time (s)	3.47 ±0.22	2.47 ±0.79	0.056

Abbreviations: °C = degree Celsius, bpm = beats per minute, cFTOE= cerebral fractional tissue oxygen extraction, CO = cardiac output, COest = estimated cardiac output, COest/adj. = estimated/adjusted cardiac output, cTOI = cerebral tissue oxygenation index, DABP = diastolic blood pressure, FiO2 = fraction of inspired oxygen, HR = heart rate, kg = kilogram, l = liter, MABP = mean arterial blood pressure, min = minutes, ml = millilitre, mmHg = milimeter of mercury, NIRS = near-infrared spectroscopy, pFTOE = peripheral fractional tissue oxygen extraction, PI = perfusion index, pTOI = peripheral tissue oxygenation index, s= seconds, SABP = systolic blood pressure, SpO_2 = arterial oxygen saturation

4.2.3. Respiratory support and respiratory distress symptoms in preterm neonates with and without infection

In the infection group, all included preterm neonates had respiratory support, one (16.7%) preterm neonate received nasal high flow therapy and in five (83.3%) preterm neonates, NCPAP were applied. In the no-infection group, 12 (28.6%) preterm neonates had no respiratory support, eight (19.0%) preterm neonates received nasal high flow therapy and in 22 (52.4%) preterm neonates, NCPAP were applied. There was no overall difference in respiratory support between the infection and no-infection group (p=0.269).

Comparing subgroups of respiratory support (no respiratory support, nasal high flow therapy and NCPAP), there was also no difference between individual respiratory support methods (none and nasal high flow therapy p=0.429; none and NCPAP p=0.299; nasal high flow therapy and NCPAP p=1.000). None of the included preterm neonates in the present study needed intubation.

Collected data of respiratory distress symptoms include grunting, retractions, nasal flaring and tachypnoea. Combination of respiratory distress symptoms were documented in preterm neonates of both groups. Data are presented in **Table 7**. In all preterm neonates, respiratory rate was above normal levels and was therefore described as tachypnoea.

Table 7. Respiratory distress symptoms in preterm neonates with and without infection. Data are presented as n (%) or mean ±SD.

	Infection group n = 6	*No-infection group n = 42*	*p-value*
Grunting	3 (50.0%)	22 (52.4%)	0.913
Retractions	5 (83.3%)	24 (57.1%)	0.220
Nasal flaring	1 (16.7%)	11 (26.2%)	0.641
Respiratory rate (/min)	63 ±20	77 ±20	0.230

Abbreviations: min = minutes

4.2.4. Blood gas analysis and laboratory findings in preterm neonates with and without infection

In all preterm neonates, blood samples to rule out a potential infection were performed either within the first 24 hours after birth or between 24 and 48 hours after birth. In the infection group, in all six preterm neonates (100.0%) infection was proven by laboratory parameters in the first blood sample, within the first 24 hours after birth. Blood culture results showed in none of the included preterm neonates of both groups a germ detection. All taken blood cultures were done within the first 24 hours. In the infection group, however, in four (66.7%) preterm neonates and in the no-infection group, in 27 (64.3%) preterm neonates, no blood culture results were available (p=0.909). Antibiotic therapy was provided for 6.0 (5.0; 7.0) days and for 2.0 (2.0; 13.0) days (p=0.001*), in the infection group and no-infection group, respectively. Blood gas analysis and laboratory parameters are displayed in Table 8.

Table 8. Blood gas analysis and laboratory parameters in preterm neonates with and without infection. Data are presented as mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Infection group n = 6	*No-infection group n = 42*	*p-value*
Blood gas analysis			
Time between blood sample and NIRS (min)	49 ±51	33 ±22	0.385
pH	7.36 ±0.07	7.28 ±0.07	0.403

pCO_2 (mmHg)	49.3 ±12.9	57.3 ±11.3	0.377
pO_2 (mmHg)	45.8 ±9.3	42.7 ±8.5	0.234
HCO3 (mmol/L)	24.8 ±0.1	22.6 ±1.7	0.705
BE (mmol/L)	1.70 ±1.41	-0.36 ±1.93	0.837
Lactate (mmol/L)	1.75 ±0.35	1.33 ±0.56	0.582
Blood sample within the first 24 hours after birth			
Age at first laboratory testing (hours)	22.0 ±0.0	20.0 ±4.2	0.776
CRP (mg/L)	13.6 (12.8; 14.3)	1.2 (0.6; 7.6)	0.000*
Leukocytes (/µl)	17860 (16470; 19250)	15570 (7630; 28360)	0.358
IT-ratio	0.01 (0.00; 0.04)	0.03 (0.00; 0.16)	0.865
Blood sample between 24 and 48 hours after birth			
Age at second laboratory testing (hours)	44.0 ±1.4	44.0 ±4.2	0.539
CRP (mg/L)	13.2 (5.6; 20.8)	0.7 (0.6; 4.0)	0.001*
Leukocytes (/µl)	14230 (10960; 17500)	10650 (5750; 25460)	0.263
IT-ratio	0.01 (0.01; 0.01)	0.02 (0.00; 0.10)	0.711

Abbreviations: BE = base excess, CRP = C-reactive protein, HCO3- = bicarbonate, IT-ratio = immature-to-total neutrophil ratio, l = liter, mg = milligram, min = minutes, mmHg = milimeter of mercury, mmol = millimole, NIRS = near-infrared spectroscopy, pCO_2 = partial pressure of carbon dioxide, pH = potential of Hydrogen, pO_2 = partial pressure of oxygen, µg = microgram

4.3. Comparison of term and preterm neonates with infection

4.3.1. Comparison in demographic data und clinical parameters between term and preterm neonates with infection

A total of 21 neonates with infection, confirmed through elevated laboratory findings, were included: 15 term neonates and 6 preterm neonates. Demographic data of term and preterm neonates with infection are displayed in **Table 9**. A statistically significant difference was observed in parameters which are dependent on gestational age: gestational age, birth weight, diameter of the arm, thickness of subcutaneous fat and circumference of the arm.

Table 9. Demographic data of included term and preterm neonates with infection. Data are presented as n (%), mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Term neonates n = 15	*Preterm neonates n = 6*	*p-value*
Gestational age (weeks)	39.6 ±1.8	34.8 ±1.7	<0.001*
Birth weight (g)	3543 ±615	2476 ±720	0.001*
Female sex n (%)	8 (53.3)	1 (16.7)	0.178
Apgar 1	6 (5; 9)	8 (8; 9)	0.570
Apgar 5	8 (6; 9)	8 (8; 9)	0.680
Apgar 10	9 (9; 10)	9 (8; 10)	0.113
Umbilical artery pH	7.25 ±0.10	7.31 ±0.03	0.127
Maternal CRP (mg/L)	3.6 (1.0; 74.0)	3.5 (0.6; 61.4)	0.414
Maternal leukocytes (/µl)	10770 ±5736	13167 ±4638	0.557
Maternal fever during birth n (%)	2 (13.3)	0 (0.0)	0.347
Diameter of the arm (cm)	3.4 ±0.6	2.9 ±0.4	0.004*
Thickness of subcutaneous fat (cm)	0.59 (0.25; 0.74)	0.30 (0.10)	0.032*
Circumference of the arm (cm)	10.8 ±1.4	8.9 ±1.4	0.010*

Abbreviations: cm = centimeter, CRP = C reactive protein, g = grams, l = liter, mg = milligram, pH = potential of Hydrogen, µg = microgram

4.3.2. Comparison of primary and secondary outcome variables between term and preterm neonates with infection

In term and preterm neonates with infection, no differences in pFTOE within the first six hours after birth were observed (p = 0.558). Furthermore, no differences were detected in pTOI (p = 0.704), cTOI (p = 0.162), cFTOE (p = 0.225) or the ratio of cTOI/pTOI (p = 0.132) and cFTOE/pFTOE (p = 0.436).

Peripheral and cerebral NIRS reapplications were performed in median 165 (128; 318) minutes and 191 (163; 302) minutes after birth in term neonates and in preterm neonates, respectively. Primary outcome parameter (pFTOE) and secondary outcome parameters, including routine monitoring parameters during either peripheral or cerebral reapplications were compared

between term and preterm neonates with infection (**Table 10**). Mean overall HR during pTOI and cTOI measurements was 141 ±12 bpm in term neonates with infection and 152 ±17 bpm in preterm neonates with infection. Mean SABP calculated out of measurements before and after reapplications was 63 (54; 73) mmHg in term neonates with infection and 57 (55; 72) mmHg in preterm neonates with infection. Mean DABP out of before and after measurements was 37 ±6 mmHg in term neonates with infection and 35 ±5 mmHg in preterm neonates with infection.

Table 10. Primary and secondary outcome variables in term and preterm neonates with infection. Data are presented as mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Term neonates n = 15	*Preterm neonates n = 6*	*p-value*
Age at measurement (min)	165 (128; 318)	191 (163; 302)	0.186
Provided FiO_2	0.27 (0.21; 0.33)	0.21 (0.21; 0.27)	0.184
Peripheral NIRS measurements			
Mean pTOI (%)	72.2 ±4.4	73.6 ±5.3	0.704
Mean PI during pTOI	1.86 (1.30; 2.72)	2.26 (1.48; 2.70)	0.517
Mean HR during pTOI (bpm)	144 ±16	159 ±18	0.226
Mean SpO_2 during pTOI (%)	94 ±3	96 ±3	0.629
Mean pFTOE	0.229 ±0.064	0.235 ±0.050	0.558
Cerebral NIRS measurements			
Mean cTOI (%)	72.6 ±5.7	70.9 ±6.0	0.162
Mean PI during cTOI	1.60 (1.07; 1.85)	2.46 (1.42; 3.14)	0.350
Mean HR during cTOI (bpm)	145 ±13	160 ±14	0.254
Mean SpO_2 during cTOI (%)	92 (87; 95)	95 (93; 100)	0.459
Mean cFTOE	0.207 ±0.074	0.260 ±0.033	0.225

	Cerebral/peripheral ratio		
cTOI/pTOI	1.01 ±0.12	0.96 ±0.04	0.132
cFTOE/pFTOE	0.96 (0.30; 2.30)	1.02 (0.98; 1.38)	0.436
	Blood pressure and cardiac output		
MABP before measurements (mmHg)	44 (38; 52)	42 (40; 47)	0.563
SABP before measurements (mmHg)	62 (51; 77)	58 (54; 67)	0.244
DABP before measurements (mmHg)	37 (23; 45)	33 (31; 51)	0.535
MABP after measurements (mmHg)	45 (36; 57)	38 (38; 53)	0.258
SABP after measurements (mmHg)	62 (50; 73)	56 (55; 76)	0.336
DABP after measurements (mmHg)	39 (24; 57)	32 (31; 40)	0.174
COest.	33.58 (27.35; 56.36)	45.03 (19.13; 45.84)	0.139
COest./adj. (l/min)	0.33 (0.27; 0.56)	0.45 (0.19; 0.45)	0.139
CO (ml/kg/min)	92.47 (69.98; 125.56)	176.69 (52.03; 266.48)	0.020*
	Temperature, capillary refill time		
Temperature peripheral before measurements (°C)	37.0 (35.9; 37.6)	37.5 (36.7; 37.5)	0.271
Temperature peripheral after measurements (°C)	37.1 ±0.4	37.3 ±0.4	0.622
Rectal temperature (°C)	37.3 ±0.4	37.1 ±0.3	0.315
Central capillary refill time (s)	1.72 (1.01; 3.01)	3.40 (3.30; 3.72)	0.049*

Abbreviations: °C = degree Celsius, bpm = beats per minute, cFTOE= cerebral fractional tissue oxygen extraction, CO = cardiac output, COest = estimated cardiac output, COest/adj. = estimated/adjusted cardiac output, cTOI = cerebral tissue oxygenation index, DABP = diastolic blood pressure, FiO2 = fraction of inspired oxygen, HR = heart rate, kg = kilogram, l = liter, MABP = mean arterial blood pressure, min = minutes, ml = millilitre, mmHg = milimeter of mercury, NIRS = near-infrared spectroscopy, pFTOE = peripheral fractional tissue oxygen extraction, PI = perfusion index, pTOI = peripheral tissue oxygenation index, s= seconds, SABP = systolic blood pressure, SpO_2 = arterial oxygen saturation

4.3.3. Comparison of blood gas analysis and laboratory parameters between term and preterm neonates with infection

Blood gas analysis and laboratory parameters of term and preterm neonates with infection are displayed in **Table 11**. No differences in CRP, leukocytes and IT-ratio were detected between groups.

Table 11. Blood gas analysis and laboratory parameters in term and preterm neonates with infection. Data are presented as mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Term neonates n = 15	*Preterm neonates n = 6*	*p-value*
Blood gas analysis			
Time between blood sample and NIRS (min)	54 ±46	49 ±51	0.907
pH	7.34 ±0.05	7.36 ±0.07	0.396
pCO_2 (mmHg)	48.0 ±9.0	49.3 ±12.9	0.690
pO_2 (mmHg)	41.3 ±6.8	45.8 ±9.3	0.177
HCO3 (mmol/L)	23.4 ±1.2	24.8 ±0.1	0.520
BE (mmol/L)	-0.23 ±2.00	1.70 ±1.41	0.614
Lactate (mmol/L)	2.18 ±1.36	1.75 ±0.35	0.240
Blood sample within the first 24 hours after birth			
Age at first laboratory testing (hours)	21.8 ±5.0	22.0 ±0.0	0.768
CRP (mg/L)	10.8 (4.9; 48.6)	13.6 (12.8; 14.3)	0.869
Leukocytes (/µl)	28163 ±18002	17860 ±1966	0.118
IT-ratio	0.07 (0.01; 0.43)	0.01 (0.00; 0.01)	0.065
Blood sample between 24 and 48 hours after birth			
Age at second laboratory testing (hours)	42.2 ±7.6	44.0 ±1.4	0.714
CRP (mg/L)	13.7 (1.2; 31.8)	13.2 (5.6; 20.8)	0.643
Leukocytes (/µl)	15090 (11000; 19150)	14230 (10960; 17500)	0.164

IT-ratio	0.02 (0.00; 0.07)	0.01 (0.01; 0.01)	0.866

Abbreviations: BE = base excess, CRP = C-reactive protein, HCO3- = bicarbonate, IT-ratio = immature-to-total neutrophil ratio, l = liter, mg = milligram, min = minutes, mmHg = milimeter of mercury, mmol = millimole, NIRS = near-infrared spectroscopy, pCO_2 = partial pressure of carbon dioxide, pH = potential of Hydrogen, pO_2 = partial pressure of oxygen, µg = microgram microgram

4.4. Comparison of term and preterm neonates without infection

4.4.1. Comparison of demographic data und clinical parameters between term and preterm neonates without infection

A total of 59 neonates without infection, ruled out by normal laboratory findings, were included: 17 term neonates and 42 preterm neonates. Demographic data of term and preterm neonates without infection are displayed in **Table 12**. A statistically significant difference was observed in parameters which are dependent on gestational age: gestational age, birth weight, diameter of the arm, thickness of subcutaneous fat and circumference of the arm.

Table 12. Demographic data of included term and preterm neonates without infection. Data are presented as n (%), mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Term neonates n = 17	*Preterm neonates n = 42*	*p-value*
Gestational age (weeks)	38.5 ±1.4	34.3 ±1.6	<0.001*
Birth weight (g)	3222 ±592	2284 ±474	<0.001*
Female sex n (%)	7 (41.2)	17 (40.5)	0.960
Apgar 1	8 (6; 9)	8 (5; 9)	0.956
Apgar 5	9 (8; 9)	9 (7; 10)	0.171
Apgar 10	9 (9; 10)	9 (8; 10)	0.518
Umbilical artery pH	7.28 (7.11; 7.32)	7.33 (7.12; 7.42)	0.234
Maternal CRP (mg/L)	4.2 (1.6; 4.9)	3.5 (0.6; 61.4)	0.535
Maternal leukocytes (/µl)	11090 ±4044	10081 ±3517	0.866
Maternal fever during birth n (%)	0 (0.0)	2 (4.8)	0.503
Diameter of the arm (cm)	3.2 (2.6; 3.8)	2.7 (2.0; 3.5)	<0.001*

Thickness of subcutaneous fat (cm)	0.49 ±0.13	0.35 ±0.08	<0.001*
Circumference of the arm (cm)	10.4 ±1.3	8.9 ±0.8	<0.001

Abbreviations: cm = centimeter, CRP = C reactive protein, g = grams, l = liter, mg = milligram, pH = potential of Hydrogen, µg = microgram

4.4.2. Comparison of primary and secondary outcome variables between term and preterm neonates without infection

In term and preterm neonates without infection, pFTOE showed a trend towards higher values in term neonates compared to preterm neonates without reaching significance (p=0.052). No differences were detected in pTOI (p = 0.116), cTOI (p = 0.067), or the ratio of cTOI/pTOI (p = 0.770) and cFTOE/pFTOE (p = 0.819). Mean cFTOE, however, was significantly higher in term neonates compared to preterm neonates (p=0.027*).

Peripheral and cerebral NIRS reapplications were performed in median 140 (75; 201) minutes and 148 (50; 318) minutes in term neonates and in preterm neonates, respectively. Primary outcome parameter (pFTOE) and secondary outcome parameters, including routine monitoring parameters during either peripheral or cerebral reapplications were compared between term and preterm neonates without infection (**Table 13**). A significant difference was observed in HR during peripheral and during cerebral NIRS measurements, with higher HR in preterm neonates. Mean overall HR during pTOI and cTOI measurements was 131 (91; 162) bpm in term neonates without infection and 145 (66; 191) bpm in preterm neonates without infection. Mean SABP calculated out of measurements before and after reapplications was 60 ±9 mmHg in term neonates without infection and 58 ±7 mmHg in preterm neonates without infection. Mean DABP calculated out of measurements before and after reapplications was 34 ±8 mmHg in term neonates without infection and 31 ±6 mmHg in preterm neonates without infection.

Table 13. Primary and secondary outcome variables in term and preterm neonates without infection. Data are presented as mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Term neonates n = 17	*Preterm neonates n = 42*	*p-value*
Age at measurement (min)	140 (75; 201)	148 (50; 318)	0.116
Provided FiO_2	0.22 (0.21; 0.30)	0.21 (0.21; 0.40)	0.492
	Peripheral NIRS measurements		
Mean pTOI (%)	72.3 ±3.2	73.0 ±4.3	0.116
Mean PI during pTOI	2.13 (0.88; 8.78)	1.54 (0.68; 6.94)	0.545
Mean HR during pTOI (bpm)	133 ±15	147 ±15	0.024*
Mean SpO_2 during pTOI (%)	95 ±3	94 ±3	0.387
Mean pFTOE	0.236 ±0.319	0.224 ±0.051	0.052
	Cerebral NIRS measurements		
Mean cTOI (%)	72.1 ±3.2	73.1 ±6.0	0.067
Mean PI during cTOI	1.89 (0.89; 5.92)	1.53 (0.45; 3.48)	0.490
Mean HR during cTOI (bpm)	132 ±16	145 ±14	0.012*
Mean SpO_2 during cTOI (%)	94 (91; 99)	95 (88; 100)	0.474
Mean cFTOE	0.236 ±0.020	0.222 ±0.064	0.027*
	Cerebral/peripheral ratio		
cTOI/pTOI	1.00 ±0.07	1.01 ±0.11	0.770
cFTOE/pFTOE	0.99 (0.77; 1.27)	0.99 (0.39; 2.74)	0.819
	Blood pressure and cardiac output		
MABP before measurements (mmHg)	38 (33; 43)	39 (31; 58)	0.609

SABP before measurements (mmHg)	60 ±9	57 ±8	0.232
DABP before measurements (mmHg)	34 ±8	32 ±7	0.382
MABP after measurements (mmHg)	38 (34; 49)	40 (31; 49)	0.287
SABP after measurements (mmHg)	60 ±10	58 ±7	0.354
DABP after measurements (mmHg)	35 ±9	31 ±5	0.112
COest.	35.01 (23.92; 60.14)	42.56 (17.02; 63.29)	0.034*
COest./adj. (l/min)	0.35 (0.24; 0.60)	0.42 (0.17; 0.63)	0.034*
CO (ml/kg/min)	101.18 (69.55; 248.09)	187.28 (84.67; 318.94)	<0.001*
	Temperature, capillary refill time		
Temperature peripheral before measurements (°C)	37.0 ±0.3	37.0 ±0.5	0.587
Temperature peripheral after measurements (°C)	37.1 ±0.3	37.0 ±0.4	0.864
Rectal temperature (°C)	37.2 ±0.2	37.1 ±0.4	0.759
Central capillary refill time (s)	2.33 (1.01; 2.84)	2.47 (1.02; 4.19)	0.808

Abbreviations: °C = degree Celsius, bpm = beats per minute, cFTOE= cerebral fractional tissue oxygen extraction, CO = cardiac output, COest = estimated cardiac output, COest/adj. = estimated/adjusted cardiac output, cTOI = cerebral tissue oxygenation index, DABP = diastolic blood pressure, FiO_2 = fraction of inspired oxygen, HR = heart rate, kg = kilogram, l = liter, MABP = mean arterial blood pressure, min = minutes, ml = millilitre, mmHg = milimeter of mercury, NIRS = near-infrared spectroscopy, pFTOE = peripheral fractional tissue oxygen extraction, PI = perfusion index, pTOI = peripheral tissue oxygenation index, s= seconds, SABP = systolic blood pressure, SpO_2 = arterial oxygen saturation

4.4.3. Comparison of blood gas analysis and laboratory parameters between term and preterm neonates without infection

Blood gas analysis and laboratory parameters of term and preterm neonates without infection are displayed in **Table 14**. Significant difference between the two groups was observed in HCO3 with 23.6 ±1.6 and 22.6 ±1.7 in term and preterm neonates, respectively. Further CRP was significantly higher in term neonates compared to preterm neonates within the first 24

hours and between 24 and 48 hours after birth. Nevertheless, all CRP values were within normal ranges.

Table 14. Blood gas analysis and laboratory parameters in term and preterm neonates without infection. Data are presented as mean ±SD and median (minimum; maximum), as appropriate. A p-value < 0.05 was statistically significant and marked with *.

	Term neonates *n = 17*	*Preterm neonates* *n = 42*	*p-value*
Blood gas analysis			
Time between blood sample and NIRS (min)	59 ±46	33 ±22	0.105
pH	7.31 ±0.06	7.28 ±0.70	0.287
pCO_2 (mmHg)	52.2 ±9.8	57.3 ±11.3	0.628
pO_2 (mmHg)	44.0 ±6.6	42.7 ±8.5	0.773
HCO3 (mmol/L)	23.6 ±1.6	22.6 ±1.7	0.045*
BE (mmol/L)	0.13 ±2.08	-0.36 ±1.93	0.281
Lactate (mmol/L)	1.43 ±0.48	1.33 ±0.56	0.302
Blood sample within the first 24 hours after birth			
Age at first laboratory testing (hours)	21.4 ±2.4	20.0 ±4.2	0.506
CRP (mg/L)	2.4 (0.6; 10.0)	1.2 (0.6; 7.6)	0.009*
Leukocytes (/µl)	18456 ±3612	15659 ±5760	0.002*
IT-ratio	0.01 (0.00; 0.09)	0.03 (0.00; 0.16)	0.759
Blood sample between 24 and 48 hours after birth			
Age at second laboratory testing (hours)	48.9 ±12.5	44.0 ±4.2	0.038*
CRP (mg/L)	3.6 (0.6; 7.0)	0.7 (0.6; 4.0)	0.023*
Leukocytes (/µl)	10670 (6480; 15440)	10650 (5750; 25460)	0.605
IT-ratio	0.01 (0.00; 0.10)	0.02 (0.00; 0.10)	0.327

Abbreviations: BE = base excess, CRP = C-reactive protein, HCO3- = bicarbonate, IT-ratio = immature-to-total neutrophil ratio, l = liter, mg = milligram, min = minutes, mmHg = milimeter of mercury, mmol = millimole, NIRS = near-infrared spectroscopy, pCO_2 = partial pressure of carbon dioxide, pH = potential of Hydrogen, pO_2 = partial pressure of oxygen, µg = microgram

5. Discussion

In the present prospective observational study no differences in pFTOE, cFTOE and cFTOE/pFTOE ratio were observed between neonates, in term as well as in preterm neonates, with and without infection. Further, no differences in routine vital parameters were observed.

5.1. Influencing factors of the primary outcome

Various factors could have a potential influence on the lacking difference between the infection and no-infection group.

5.1.1. Cardiocirculatory condition

In the present study no critically ill neonates, neither term nor preterm neonates, were included. None of the included neonates were in need for inotropes or vasopressors by having a MABP below the threshold. The median MABP was 44 mmHg and 38 mmHg in term neonates with infection and in term neonates without infection, respectively. In preterm neonates with infection, the median MABP was 42 mmHg comparing to 39 mmHg in preterm neonates without infection. Further, no difference in cardiac output was observed between term or preterm neonates with and without infection. Beside these parameters, indicating a stable cardiocirculatory condition, there was also no difference in need for respiratory support between neonates with and without infection. None of the included neonates, term as well as preterm neonate, were in need for any invasive respiratory support. These results are comparable to a study published by Pichler et al. (94) who investigated whether it is possible to reduce arterial hypotension in preterm neonates by using cerebral and peripheral NIRS monitoring with dedicated interventions. They included 49 preterm neonates in the NIRS group and 49 preterm neonates in the control group, however, they observed no statistically significant reduction in burden of arterial hypotension. The authors assumed that a possible explanation for these results might be that the included preterm neonates had only borderline hypotension. None of them were in need for catecholamines in both groups.
Taking the study by Pichler et al. (94) and our present study into account, it can be assumed that stable neonates with mild infection do not show disturbances of microcirculation detectable with peripheral muscle NIRS. With the present findings, however, it cannot be ruled out whether neonates with proven sepsis (by blood culture) or more significant laboratory findings

(CRP, leukocytes), or compromised neonates in later need of mechanical ventilation or catecholamines might have already incipient compromised peripheral microcirculation.

5.1.2. Laboratory parameters

Beside the stable cardiocirculatory condition, the included neonates in our present study, had only a mild elevation of CRP values. The maximum of CRP in term neonates with infection was 48.6 mg/l and in 20.8 mg/l in preterm neonates. A subanalysis of neonates with a CRP elevation above 20 mg/l might result in a difference in peripheral oxygenation and pFTOE between the two groups. In this study, however, the number of included neonates with a CRP elevation above 20mg/l in term and preterm neonates is too small. Pichler et al. (114) investigated peripheral muscle oxygenation and perfusion in neonates with elevated CRP values. They described significantly lower pTOI values in neonates with CRP elevation compared to those without. Further, pFOE, was higher in neonates with CRP elevation compared to the control group, without reaching significance. A possible explanation for differences in the study by Pichler et al. (114) and the missing difference between neonates with CRP elevation and those without of our present study might be the difference in CRP values between the studies. Mean CRP values after the first 24 hours after birth in the study of Pichler et al. in neonates with a mean gestational age of 37.7 weeks were in mean 25.6 mg/l, which is higher compared to the CRP values in the same postnatal time period of our present study with 13.7 mg/l in term neonates.

What is more, not all suspected infections are diagnosed by elevated laboratory parameters. In clinical routine, neonates may present themselves as “infected” without any elevation in CRP or leukocytes. It may be assumed that other biomarkers for the diagnosis of a neonatal sepsis might give additional information. In literature many different methods and laboratory testing are discussed, including molecular testing using polymerase chain reaction and deoxyribonucleic acid microarray (31)(32). Presepsin as a biomarker has gained interest in research of neonatal sepsis. The sensitivity of presepsin is high and can be compared to the sensitivity of CRP. It has been investigated that the sensitivity of presepsin is 94.1% and in cases of a positive blood culture, the sensitivity of presepsin was 100%. Further, the negative predictive value of presepsin has been described with 97.4% (32)(58)(129). Another study investigated the potential correlation between EOS and neutrophil-to-lymphocyte ratio and the platelet-to-lymphocyte ratio (130). In literature the ratio of neutrophil/lymphocyte has been described of having a high predictive value for bacterial infection. The ratio of platelets/lymphocytes has also been described as an inflammatory marker for sepsis. Can et

al. (130) observed a positive correlation between neutrophil-to-lymphocyte ratio and platelet-to-lymphocytes ratio in term neonates with EOS.
Taking these into account it may be possible that in our cohort, term and / or preterm neonates of the no-infection group, due to stratification based on CRP values, leukocytes and / or IT-ratio, might have had an infection, which was not diagnosed by routinely used laboratory parameters. This might have had a potential influence on our results.

5.1.3. Blood culture

As already assumed, neonates with a culture proven sepsis may be suspicious for more pronounced alterations in microcirculation (94). In our present study none of the included term and preterm neonates had a positive blood culture. The percentage of neonates with an available blood culture, in general, is quite low. A blood culture was performed in three term neonates (infection group n = 2; no-infection group n = 1); in preterm neonates in 17 out of 48 had an available blood culture (infection group n = 2; no-infection group n = 15). Several studies have described, that blood cultures with an amount of 1ml of blood, have a high detection rate of positive results (31)(32)(54)(131). Comparing this with blood culture samples with an amount of 0.5 ml of blood, the rate of false negative results in neonates with low levels of bacteremia are quite high. In clinical routine, however, the sampling of at least 0.5 ml to 1 ml is, especially in preterm neonates, not always feasible, which is displayed in our present results. Beside the amount of blood culture samples as a potential influencing factor on the results of blood cultures, maternal antibiotic therapy, may also influence the results (132).

5.1.4. Prenatal and peripartum exposure to antibiotics

Prenatal and peripartum exposure to antibiotics may also be associated with laboratory findings in neonates. The use of peripartum maternal antibiotic therapy influences the risk of occurrence of EOS (133). Conditions in which prenatal antibiotic therapy is recommended are: maternal positive smear for GBS, history of neonatal GBS infection and/or maternal fever during labour and, preterm premature rupture of membranes (133). As Caesarean section increases the risk for maternal infection in postpartum period, a single-shot antibiotic prophylaxis is recommended. The antibiotic prophylaxis should be administered 60 minutes before the Caesarean section if possible (134). Many different antibiotics are used in clinical routine before Caesarean section as prophylaxis, whereby the most common antibiotics are cefazolin, cefuroxime, ampicillin and ampicillin-sulbactam (134).

In our present study, there was no difference in prenatal maternal antibiotic therapy based on a suspicious maternal infection or infection of amniotic fluid. Further there were no differences in the mode of delivery between neonates with and without infection. Therefore, it can be assumed that neither prenatal maternal antibiotic therapy due to suspicious infection nor single shot peripartum maternal antibiotic therapy in cases of Caesarean section, may have had an influence on the results of our present study.

5.1.5. Sample size

A possible explanation for the lacking difference between neonates with and without infection might be that our present study was underpowered. Fifteen term neonates and six preterm neonates with infection compared to 17 term neonates and 42 preterm neonates without infection were included. It may be assumed, that, especially in preterm neonates, a larger number of included neonates would have been appropriate.

5.2. Peripheral muscle oxygenation

Peripheral and cerebral oxygenation can be influenced by various factors. These include composite of the tissue (subcutaneous fat, diameter of the calf), body temperature, capillary refill time, partial pressure of carbon dioxide (pCO_2), haemoglobin concentration, HR, SpO_2, MABP, pH of umbilical artery, tobacco exposure during pregnancy and ductus arteriosus.

5.2.1. Composite of the tissue

A multivariate analysis described the influence of subcutaneous fat and diameter of the measured arm or calf on pFTOE (108). The diameter of the calf in this multi-association analysis (3.1 ±0.4cm) is comparable to the diameter of the arm, measured in our present study (3.4 ± 0.6 in the infection group and 3.3 ±0.5 cm in the no-infection group). Further, the subcutaneous fat in our present study is higher than in the multi-association analysis by Pichler et al. (108), who showed that diameter and subcutaneous fat had a negative correlation with pFTOE. In our present study, a significant difference in diameter of the arm was observed between the infection and no-infection group, however, this difference seems to be not of clinical relevance, due to the low difference between the two groups.

5.2.2. Body temperature and capillary refill time

Central and peripheral body temperature have both a negative correlation with pFTOE values (108). Although in our present study there was no statistically significant difference in term and preterm neonates with and without infection. Preterm neonates of the infection group, however, showed higher temperature compared to the no-infection group. In literature, capillary refill time is described to have a weak correlation with pFTOE, which was explained by the observer variability (108). In our present study, capillary refill time was assessed only by one person. In term neonates of our cohort, there was no difference between the two groups. In preterm neonates, a trend toward longer capillary refill time was investigated without reaching significance. Further, as capillary refill time was described as an independent parameter, a potential influence on pFTOE is not very likely.

5.2.3. Haemoglobin and blood gases

The influence of blood gas results, including pCO_2 or haemoglobin concentration, on peripheral oxygenation and perfusion, has been investigated by Pichler et al. (108). They observed a weak negative correlation of pFTOE with haemoglobin concentration and a positive with pCO_2. The potential association of haemoglobin, in cases of anaemic preterm neonates and pFOE has also been observed by Wardle et al. (95). They observed higher pFOE values in preterm neonates with anaemia. pCO_2 has not only been described in context with peripheral NIRS measurements, but also with cerebral NIRS measurements during the first 15 minutes after birth. A positive correlation of cFTOE with pCO_2 was observed in preterm neonates, whilst in term neonates no correlation exist (76). Although, in our present study, no significant difference in pCO_2 was observed between the two groups in term and in preterm neonate. Term and preterm neonates of the no-infection group, however, showed slightly higher values of pCO_2 compared to the infection group.

5.2.4. Vital parameters

The influence of vital parameters on pFTOE was also investigated in the multi-association analysis by Pichler et al. (108). They observed a significant positive correlation of SpO_2 and pFTOE and a trend toward positive correlation has been seen between HR and MABP and pFTOE. In our present study, there were no significant differences in routine vital parameters between neonates with and without infection. However, a trend towards higher HR was seen

in term neonates with infection compared to term neonates without infection. As no significant differences were observed, we assume, that this result may not influence our primary outcome.

5.2.5. Cardiac output

Cardiac output provides additional information on cardio-circulation and cerebral autoregulation. The gold standard for measurements is echocardiography, followed by thermodiluation. Whereby, especially the latter is not feasible in clinical routine at the NICU. An alternative approach for obtaining information about CO is provided by the formula of Liljestrand and Zander, as described in the methods (127)(128). Pfurtscheller et al. observed a significant positive correlation between CO and $crSO_2$ and a significant negative correlation between CO and cFTOE in preterm neonates with respiratory support. In term neonates and in preterm neonates without respiratory support no correlation between CO and cerebral oxygenation was observed (127).
In our present study, in term and in preterm neonates, there was no difference in CO between neonates with and without infection. Due to the small sample sizes no correlation analyses were performed in our cohort between CO and cerebral oxygenation or peripheral oxygenation. A possible correlation between CO and peripheral oxygenation can only be assumed as no comparable data exist, however, as none of the included term or preterm neonates were in a status of severe disturbances in microcirculation, a correlation is not very likely. Further, the study by Pfurtscheller et al. (127) was performed during the first 15 minutes after birth which is in contrast to our present study.

5.2.6. Asphyxia

Tax et al. (90) described an influence of perinatal asphyxia, defined as umbilical cord pH ≤7.15 and Apgar 5 ≤ 6, on peripheral muscle oxygenation and perfusion. They described lower pTOI and pDO_2 values and higher pFOE values in neonates with perinatal asphyxia. Furthermore, pFOE correlated significantly negatively with umbilical cord pH. Based on these results, one of our exclusion criteria was severe asphyxia, defined as an umbilical cord pH lower than 7.00. In our present cohort, there were no differences in umbilical cord pH between term and preterm neonates. Lowest umbilical cord pH was described in one preterm neonate of the no-infection group with a value of 7.12.

5.2.7. Prenatal tobacco exposure

Maternal smoking during pregnancy and potential influence on peripheral muscle oxygenation and perfusion and cerebral oxygenation has already been studied (93)(135). Pichler et al. (93) observed in healthy term neonates differences in peripheral oxygenation and perfusion parameters obtained by NIRS measurements in combination with venous occlusion within the first two days after birth. They described lower pTOI and higher pFOE in neonates with prenatal tobacco exposure compared to neonates of mothers who did not smoke during pregnancy. Wolfsberger et al. (135) described differences in cerebral oxygenation during the first 15 minutes after birth in healthy term neonates in regard to prenatal tobacco exposure. Comparable to the results by Pichler et al. with peripheral muscle NIRS measurements, $crSO_2$ was significantly lower and cFTOE higher in neonates with prenatal tobacco exposure.
In our present study, no data of maternal smoking during pregnancy were available. Therefore, a potential influence of prenatal tobacco exposure on our results cannot be ruled out.

5.2.8. Patent ductus arteriosus

Another parameter having a potential influence on peripheral muscle oxygenation and perfusion is PDA. Mileder et al. (111) investigated in preterm neonates with open ductus arteriosus and in preterm neonates with a closed ductus arteriosus, differences in peripheral NIRS parameters during the first three days after birth. They described significantly higher pFOE values in preterm neonates with open ductus arteriosus. Further, they investigated a positive correlation between the diameter of the ductus arteriosus and pFOE. The potential influence of patent ductus arteriosus in our present study, especially, in preterm neonates cannot be ruled out, as no echocardiography was routinely performed. In our cohort the majority of included preterm neonates had a gestational age > 33 weeks of gestational age. In literature, it has been described that in about 10% of neonates born between 30 to 37 weeks ductus arteriosus remains open until 96 hours after birth (136). Therefore, it can be assumed that there is a significant amount of included preterm neonates in our present study with PDA.

5.3. Peripheral oxygenation in term neonates

5.3.1. Peripheral tissue oxygenation index

The observed pTOI of term neonates with infection (72.2 ±4.3%) in our present study, is higher compared to an already published study by Pichler et al. (114), investigating the difference in peripheral oxygenation and perfusion in neonates with and without an elevation of CRP. In this work by Pichler et al. (114) pTOI in neonates with elevated CRP values was 68.9 ±6.6 %. In contrast, pTOI of neonates without elevated CRP values was 72.9 ±3.8 %. pTOI in neonates with no elevated CRP were comparable between the study by Pichler et al. (114) and our present work (pTOI term neonates no-infection group 72.3 ±3.1%). A further discrepancy between the study of Pichler et al. (114) and our present study is the time period after birth, when NIRS measurements were performed (in mean 41 hours after birth (114) versus 165 minutes after birth in our present study), which might have an influence on pTOI. What is more, as mentioned before, mean CRP values after the first 24 hours after birth in the work by Pichler et al. were higher compared to the CRP values in the same postnatal time period of our present study. Further, pTOI values of term neonates with infection in our present study were similar to the control group of our study and the control group of the study by Pichler et al. (114). This lead to the assumption, that the term neonates of our present study may not represent a cohort of neonates with disturbances in microcirculation due to only mild infections with mild elevations of CRP. We can only speculate that neonates in unstable cardiocirculatory condition and with significant elevations in infectious parameters, might show lower pTOI values, than observed in our present study.

5.3.2. Peripheral fractional tissue oxygen extraction

pFTOE has been compared between term neonates with and without infection in our present study, however, no differences were observed. Until now, pFTOE has not been investigated in neonates with infection, therefore, values of our present study cannot be compared to already published values. However, pFOE has been compared between neonates with and without elevated CRP values (114). As mentioned above, peripheral NIRS measurements were performed in the already published study (114) in mean 41 hours after birth and in our present study 165 minutes after birth. In neonates with elevated CRP values, pFOE values were 0.30 and in the control group 0.28 (114). These values were higher compared to the mean pFTOE values of our present study: pFTOE 0.23 ±0.06 in term neonates with infection versus 0.24

±0.03 in term neonates without infection. The difference between pFTOE and pFOE is in accordance with the study published by Hoeller et al. (116). They described higher values of pFOE compared to pFTOE.
Further, normal values of pFTOE have been described for the first 24 hours after birth (119). pFTOE values in our present study of term neonates with and without infection can be compared to mean pFTOE presented in the study by Wolfsberger et al. of the first time period ("0-6h after birth"). The same observation can be done in preterm neonates: pFTOE of our present study in preterm neonates with and without infection were comparable to normal values of pFTOE of the first six hours (119). As pFTOE values of term and preterm neonates with and without infection fit to normal values of pFTOE in healthy neonates (119), it might be assumed that pFTOE values presented in our present study do not represent abnormal values, which would be assumed in situations of disturbances in microcirculation.

5.3.3. Ratio of cerebral and peripheral oxygenation

Two studies have already described the ratio of cTOI/pTOI in term and preterm neonates, however, up to now, the ratio of cTOI/pTOI has not been described in term neonates separately (120)(121). As no differences between cTOI/pTOI were observed between term and preterm neonates with and without infection in the present study, values of this ratio in term neonates can be assumed to be comparable to those of preterm neonates.

5.4. Peripheral oxygenation in preterm neonates

5.4.1. Peripheral tissue oxygenation index

pTOI values in preterm neonates of our present study (73.6% infection group; 73.0% no-infection group) were comparable to already published pTOI values during the first 24 hours after birth (110). In this study changes of peripheral muscle oxygenation and perfusion parameters have been observed during the first 24 hours after birth in stable preterm neonates (110). pTOI did not show any changes during the first 24 hours after birth with almost constant pTOI values between 69.6% and 73.0%. Pichler et al. (137) described normal values of peripheral muscle NIRS measurements in term neonates within the first week after birth. The first measurement was performed in mean 20.7 hours after birth and the second 82.9 hours after birth. pTOI showed a significant decrease over time with 67% at the first measurement and 61% at the second one. Taking this study and the work of Wolfsberger et al. (110) into

account it can be assumed that changes of pTOI towards lower values occur after the first 24 hours after birth.
Hoeller et al. (120) described pTOI values of preterm neonates during the first 24 hours after birth. These values were also comparable to our present study, showing pTOI of 73.3% at one hour after birth and 74.7% at six hours after birth. Furthermore, Pichler et al. (94) also described pTOI values in mean between 2.0 to 2.5 hours after birth in preterm neonates, showing comparable pTOI values to our present study. One difference between the study of Hoeller et al. (120), Pichler et al.(94) and Wolfsberger et al. (110) to our present study is that those already published studies used continuous peripheral muscle NIRS measurements wheras in the present study reapplications were performed.

5.4.2. Peripheral fractional tissue oxygen extraction

As mentioned above, pFTOE have not been observed in neonates with infection neither in term nor in preterm neonates. Former studies, however, have investigated pFTOE and pFOE values in preterm neonates in other different conditions.
Normal values of pFTOE in term and preterm neonates were provided for the first 24 hours after birth (119). Time periods of 6 hours during the first 24 hours after birth were defined and normal values were described for each time period: 0.264 (0.229-0.300), 0.228 (0.192-0.264), 0.237 (0.200-0.274) and 0.220 (0.186-0.254) in the first, second, third and fourth time period. Comparing the first “6 h time period” to the following time periods, no significant changes over time were observed neither in term neonates nor in preterm neonates.
Pichler et al. (108) described pFTOE and pFOE values in term and preterm neonates with a mean gestational age of 35.5 ±2.9 weeks, measured in mean 106 ±221 hours after birth. pFTOE, described in this study (108), was 0.26 ±0.67 and pFOE was 0.30 ±0.74. The pFTOE value was comparable to our present study (pFTOE 0.24 ±0.05).
Wolfsberger et al. (110) described the course of pFOE in stable preterm neonates with no need for respiratory support during the first 24 hours after birth. Comparing these values of the first six hours after birth (pFOE 0.35 [0.29-0.40]) to pFTOE in preterm neonates of the no-infection group of our present study, it can be observed that the values of our present study are lower (pFTOE = 0.23 ±0.05). This is again in accordance with the work by Hoeller et al. (116), showing higher pFOE values compared to pFTOE values.

5.4.3. Ratio of cerebral and peripheral oxygenation

The ratio of cTOI/pTOI has been observed by Hoeller et al. (120) in preterm neonates during the first 24 hours after birth. They described a mean value of cTOI/pTOI three hours after birth of 0.97 ±0.17. This value is comparable to the ratio of cTOI/pTOI of our present study in preterm neonates with values obtained between two to four hours after birth (cTOI/pTOI in preterm neonates with infection: 0.96 ±0.04 and in preterm neonates without infection: 1.01 ±0.11). In our present study, we also described cFTOE/pFTOE values. However, up to now, this ratio has not been described in any other study yet. Therefore, comparison with published values were not possible.

5.4.4. Cerebral tissue oxygenation index

As mentioned above, Hoeller et al. (120) described values of cTOI and pTOI in preterm neonates during the first 24 hours after birth. cTOI values during the first six hours after birth of the study by Hoeller et al. (cTOI 73.1% at one hour after birth to 70.5% at six hours after birth), were again comparable to our present study (70.9% in the infection group; 73.1% in the no-infection group).

5.5. Comparison term and preterm neonates

In our present study, no differences were observed between term and preterm neonates with and without infection. When comparing term to preterm neonates without signs of infection, differences in HR and cFTOE were investigated. In pFTOE no statistically significant differences, nevertheless, a trend towards higher values in term neonates were observed. This was also seen in cFTOE with significant higher values in term neonates compared to preterm neonates. In contrast to our results, however, Pichler et al. (108) described that higher gestational age was associated with lower pFTOE values, which was explained by differences in tissue composition.

Preterm neonates without infection showed significantly higher heart rate compared to term neonates of the no-infection group. Higher heart rate in preterm neonates can be explained by lower parasympathetic tone which further results in higher heart rate (138). As HR is part of the calculation of CO using the formula of Liljestrand and Zander (128), there were also significant differences in CO, with higher values in preterm neonates. This is in contrast to the work by Pfurtscheller et al. (127), who described lower CO values in preterm neonates

compared to term neonates. One explanation for this discrepancy might be the difference in postnatal age at time point of measurement. Pfurtscheller et al. observed CO values during immediate fetal-to-neonatal transition period when enormous changes in cardiovascular system occur. In contrast in our present study, the majority of included neonates obtained NIRS measurement about two hours after birth.
No further differences between the term and preterm neonates were observed.

6. Conclusion

In the present study, no difference in pFTOE, measured by peripheral NIRS measurements with reapplications, in neonates with and without infection within the first six hours after birth were observed, neither in term nor in preterm neonates. Further, there were also no significant differences in pTOI, cTOI, or cFTOE, comparing neonates with laboratory signs of infection to those without. Beside peripheral and cerebral NIRS monitoring parameters, no differences were observed between the two groups in routine vital parameters, including SpO_2, HR, MABP and capillary refill time. In our present study, none of the included neonates were severely sick with need for catecholamine therapy or invasive respiratory support.

Therefore, it can be concluded that in term and preterm neonates with mild infection without respiratory and cardiocirculatory impairment no differences in peripheral oxygenation measured with NIRS as a sign of disturbances of microcirculation can be observed. This raises the question, whether more pronounced difference of pFTOE and/or pTOI, cTOI and cFTOE as well as the ratio of cTOI/pTOI or cFTOE/pFTOE, may be observed in neonates, who might develop a more severe infection with a compromised cardiorespiratory situation. Although the aim of the present study was to detect neonates at early stages of disturbances in microcirculation.
As no differences were observed in our present study, further studies should be performed with larger sample size, as well as in neonates with more severe infection with higher elevation of laboratory parameters.
Further, it may be assumed that the combination of different parameters, including pFTOE, cFTOE, PI, HR, MABP and/or provided FiO_2, might help to detect early changes in microcirculation. The development of a score/a kaleidoscope will be part of further research.

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