Dissertation

Relationship between cerebral and peripheral oxygenation and perfusion in preterm neonates: Changes in neonates with infection during the first day after birth

Verhältnis der zerebralen und peripheren Oxygenierung und Perfusion bei Frühgeborenen: Veränderungen bei Infektion am ersten Lebenstag

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, May 2020 *Nina Maria Höller*

Disclosures

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“Cerebral and peripheral muscle oxygenation and perfusion: course in moderate and late preterm neonates during the first day after birth”

All co-authors have agreed to use the data of this publication for the thesis.

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List of Abbreviations

AHIP	Avoiding Hypotension In Preterm Neonates trial
BPD	Bronchopulmonary dysplasia
Bpm	Beats per minute
cFOE	Cerebral fractional oxygen extraction
(Pa) / (tc)CO_2	(Arterial partial pressure of) / (transcutaneous) Carbon dioxide
CPAP	Continuous positive airway pressure
CRF	Case report form
CRP	C-reactive protein
cTOI	Cerebral tissue oxygenation index
CtOx	Cytochrome oxidase
DFP	Differential pathlength factor
ECG	Electrocardiography
E. coli	Escherichia coli
EEG	Electroencephalography
ELBW	Extremely low birth weight
EOS	Early-onset sepsis
FIRS	Fetal inflammatory response syndrome
GA	Gestational age
GBS	Group B streptococcus
Hb	Deoxygenated haemoglobin
HbO_2	Oxygenated haemoglobin
HbT	Total haemoglobin
HR	Heart rate
IL-6	Interleukin 6
IL-8	Interleukin 8
INSURE	Intubation-Surfactant-Extubation manoeuvre
IVH	Intraventricular haemorrhage
LOS	Late-onset sepsis
MABP	Mean arterial blood pressure
Mb	Deoxygenated Myoglobin
MbO_2	Oxygenated Myoglobin
NICU	Neonatal intensive care unit
NIRS	Near-infrared spectroscopy

PCT	Procalcitonin
PDA	Patent ductus arteriosus
(P)PROM	(Premature) Preterm rupture of membranes
PPV	Positive pressure ventilation
pTOI	Peripheral muscle tissue oxygenation index
PVL	Periventricular leukomalacia
RDS	Respiratory distress syndrome
ROP	Retinopathy of prematurity
SAA	Serum amyloid A
SD	Standard deviation
SpO_2	Arterial oxygen saturation
SvO_2	Peripheral venous oxygen saturation
VO_2	Peripheral oxygen consumption

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Zusammenfassung

Einleitung: Die Früherkennung kardiozirkulatorischer Probleme im Rahmen einer Infektion ist vor allem in der Betreuung von Frühgeborenen schwierig. Ziel dieser Studie war die simultane Messung von zerebraler und peripher muskulärer Oxygenierung mittels Nah-infrarot Spektroskopie (NIRS) am ersten Lebenstag. In der Folge wurde untersucht, ob zwischen dem Verhältnis von zerebraler zu peripher muskulärer Oxygenierung und einer Entzündung / Infektion bei Frühgeborenen ein Zusammenhang besteht.

Studiendesign: Diese prospektive Beobachtungsstudie war Teil einer randomisierten, kontrollierten Studie namens „Avoiding Hypotension in Preterm Neonates (AHIP)“, welche an der neonatologischen Intensivstation der Medizinischen Universität Graz, Österreich, durchgeführt wurde. Frühgeborene unter der 37. Schwangerschaftswoche mit einem erhöhten Risiko an einer Infektion, aufgrund eines vorzeitigen Blasensprunges, eines mütterlichen Amnioninfektionssyndroms und / oder erhöhter mütterlicher Entzündungsparameter, zu erkranken, wurden eingeschlossen. Die eingeschlossenen Frühgeborenen wurden in eine Infektions- (Interleukin 6 >100pg/ml und / oder Leukozyten >34000/µl und / oder C-reaktives Protein >10mg/l) und eine gesunde Kontrollgruppe unterteilt. Zusätzlich zur Routineüberwachung (Herzfrequenz (HR), arterielle Sauerstoffsättigung (SpO_2) und mittlerer arterieller Blutdruck (MAP)), wurden bei jedem Frühgeborenen, beginnend innerhalb der ersten sechs Lebensstunden, über 24 Stunden kontinuierliche NIRS Messungen (NIRO-200NX, Hamamatsu Photonics, Hamamatsu City, Japan) der zerebralen (cTOI) und peripher muskulären Gewebeoxygenierungs-Indizes (pTOI) simultan durchgeführt. Um mögliche Unterschiede in den kontinuierlichen cTOI und pTOI-Messungen der beiden Gruppen zu erkennen, wurden für jede Stunde Mittelwerte für cTOI, pTOI und das Verhältnis von cTOI / pTOI berechnet und die Ergebnisse beider Gruppen verglichen. Weiters wurden in beiden Gruppen Korrelationen zwischen den cTOI / pTOI Verhältnissen und den erhobenen demographischen und Routineüberwachungsparametern berechnet.

Ergebnisse: 98 Frühgeborenen wurden gemessen und in die Studie eingeschlossen. 11 Frühgeborene wurden der Infektionsgruppe (Median 32,3 [28,7-34,0] Schwangerschaftswochen) und 87 der Kontrollgruppe (Median 33,1 [32,1-34,1] Schwangerschaftswochen; p=0,45) zugeordnet.

Der Mittelwert für das cTOI / pTOI Verhältnis während der 24-Stunden Messdauer war 0,96 ± 0,02 vs. 0,97 ± 0,04 (Kontrollgruppe vs. Infektionsgruppe; p=0,618), für cTOI 70,1 ± 1,4% vs. 71,2 ± 2,6% (p=0,079) und für pTOI 73,4 ± 0,9% vs. 73,6 ± 1,4% (p=0,564).

Sowohl die cTOI / pTOI Verhältnisse als auch die cTOI und pTOI Werte jeder einzelnen Stunde zeigten keinen signifikanten Unterschied zwischen den beiden Gruppen.

In der Kontrollgruppe konnte eine statistisch signifikante positive Korrelation zwischen dem cTOI / pTOI Verhältnis und der Herzfrequenz beobachtet werden. Diese Korrelation war in der Infektionsgruppe nicht nachweisbar.

Zusammenfassung: In der aktuellen Studie konnte kein signifikanter Unterschied zwischen den cTOI / pTOI Verhältnissen von Frühgeborenen mit einer Entzündung / Infektion und jenen von gesunden Frühgeborenen während des ersten Lebenstages festgestellt werden. Das unterschiedliche Verhalten des cTOI / pTOI Verhältnisses in Bezug auf die Herzfrequenz zeigt mögliche Interaktionen von Makro- (HR) und Mikrozirkulation (cTOI / pTOI Verhältnis) auf, weshalb die Bestimmung des cTOI / pTOI Verhältnisses vor allem bei schwer kranken Frühgeborenen zur frühzeitigen Erkennung kardiozirkulatorischer Probleme von großem Interesse sein könnte.

Abstract

Introduction: Early recognition of cardio-circulatory signs of inflammation especially in case of sepsis is difficult in neonates. The aim of the present study was to evaluate cerebral and peripheral muscle oxygenation measured simultaneously with near-infrared spectroscopy (NIRS) on the first day after birth and to investigate, if the ratio of cerebral to peripheral muscle oxygenation is associated with inflammation / infection in preterm neonates.

Study design: The present prospective observational study was part of the single centre randomized controlled trail 'Avoiding Hypotension in Preterm Neonates (AHIP)' performed at the Neonatal intensive care unit, Medical University of Graz, Austria. Preterm neonates <37 weeks of gestation with risk of infection due to premature rupture of membranes, maternal amnion infection syndrome and / or elevated maternal inflammation parameters were included into the study. Preterm neonates were divided into an infection (interleukin 6 >100pg/ml and / or leucocyte counts >34000/µl and / or C-reactive protein >10mg/l) and a healthy control group. In addition to routine monitoring (heart rate (HR), arterial oxygen saturation (SpO_2) and mean arterial blood pressure (MAP)) continuous NIRS measurements (NIRO-200NX, Hamamatsu Photonics, Hamamatsu City, Japan) of cerebral (cTOI) and peripheral muscle tissue oxygenation index (pTOI) were performed simultaneously in each neonate over 24 hours starting within six hours after birth. To analyse possible differences between groups out of the continuous cTOI and pTOI measurements mean values of cTOI, pTOI and cTOI / pTOI ratio were calculated for every hour and compared between groups. Furthermore cTOI / pTOI ratios were correlated with demographic and routine monitoring parameters in both groups.

Results: 98 preterm neonates were measured and included into the study. 11 neonates were included in the infection (median 32.3 [28.7-34.0] weeks of gestation) and 87 preterm neonates in the control group (median 33.1 [32.1-34.1] weeks of gestation; p=0.45).

Mean value of cTOI / pTOI ratio of the 24-hours measuring period was 0.96 ± 0.02 vs. 0.97 ± 0.04 (control group vs. infection group; p=0.618), of cTOI 70.1 ± 1.4% vs. 71.2 ± 2.6% (p=0.079) and of pTOI 73.4 ± 0.9% vs. 73.6 ± 1.4% (p=0.564).

cTOI / pTOI ratios as well as cTOI and pTOI values of each hour during the 24-hour measuring period showed no significant difference between the two groups.

In the control group there was a significant positive correlation of cTOI / pTOI ratio and HR, whereas in the infection group no significant correlation was observed.

Conclusion: In the present study preterm neonates with inflammation / infection showed no significant difference in cTOI / pTOI ratios compared to neonates without inflammation / infection on the first day after birth. Different behaviour of cTOI / pTOI ratios in regard to HR suggest differences in the interaction of macro-circulation (HR) and micro-circulation (cTOI / pTOI ratios) and therefore, cTOI / pTOI ratios might still be of interest in the diagnosis of early stages of cardio-circulatory disturbances in more severely sick neonates.

1 INTRODUCTION

1.1 Infection in the neonatal period

Neonates are at risk of infection due to genetic, epigenetic and environmental factors (1). While term neonates are vulnerable to intracellular pathogens, preterm neonates are more often infected by pyogenic bacteria (1). When a newborn child suddenly becomes critically ill, a sepsis has to be taken in consideration (2). The risk of a sepsis is increased in preterm neonates compared to term neonates (3).

1.1.1 Sepsis in neonates

In preterm neonates early-onset sepsis (EOS) occurs in the first three days after birth and is mainly caused by organisms transmitted vertically from the mother to the infant before or at the time of birth (4). Late-onset sepsis (LOS) on the other hand occurs between four to 120 days after birth and may be caused by pathogens acquired at delivery or during the course of hospital care (4).

1.1.1.1 Risk factors

Maternal risk factors for EOS are (5-7): dietary intake of contaminated food, procedures during pregnancy (cervical cerclage, amniocentesis), prolonged rupture of membranes, fever, vaginal colonization with group B streptococcus (GBS), GBS bacteriuria, history of a previous infant with GBS infection, adequacy of maternal immune response and chorioamnionitis.

Colonizers of the maternal genitourinary tract can lead to contamination of the amniotic fluid, placenta, cervix or vaginal canal causing EOS (5).

On the other hand there are various infant risk factors associated with EOS such as (5): prematurity, low birth weight, congenital anomalies, complicated or instrument-assisted delivery, low Apgar scores of ≤ 6 at 5 minutes and immaturity of premature neonatal immune system.

Additionally the following social and ethnic factors are also associated with higher risk of neonatal sepsis: poor or late prenatal care, low socioeconomic status of the mother, poor maternal nutrition, maternal substance abuse, male sex and African American mother (5,8).

1.1.1.2 Pathogens

The pathogens most frequently involved in EOS are GBS in term and Escherichia coli (E. coli) in preterm neonates (together approximately 70%). The remaining pathogens to consider are: other streptococci (Strep. viridans, Strep. pneumoniae), Staphylococcus aureus, Enterococcus spp., Enterobacter spp., Haemphilus influenzae and Listeria monocytogenes (9).

Since the introduction of intrapartum maternal prophylaxis for GBS the incidence of early-onset GBS disease has decreased, but remains still the leading cause of EOS (9).

Apart from bacterial sepsis, viruses such as herpes simplex, enteroviruses, parechoviruses, rubella virus, cytomegalovirus, human immunodeficiency virus, influenza virus, respiratory syncytial virus, adenoviruses, rhinoviruses, rotaviruses and also fungal pathogens can be associated with congenital infections (5,10-13).

1.1.1.3 Clinical signs

Clinical signs associated with sepsis are (1,2,14): feeding intolerance, apnoea, bradycardia, distended abdomen, prolonged capillary refill time, neurological symptoms or arterial hypotension. In the early stages of shock neonates often compensate for cardiovascular dysfunction and maintain a normal blood pressure. The presence of arterial hypotension is often a late finding (15,16). Early cardiovascular and circulatory signs of inflammation especially in cases of sepsis are often difficult to interpret in neonates. In sepsis, microvascular dysfunction occurs secondary to perfusion heterogeneity, arteriovenous shunting and impaired autoregulation (17,18). A correlation between microcirculatory abnormalities and organ dysfunction has already been shown (19). Conventional parameters of oxygenation and haemodynamic status may fail to detect microcirculatory dysfunction (20).

1.1.1.4 Diagnostics

Additionally various laboratory parameters are used for identification of infected neonates (14,21-24): differential blood count, C-reactive protein (CRP), serum amyloid A (SAA), procalcitonin (PCT), interleukin 6 (IL-6) and interleukin 8 (IL-8).

A complete sepsis workup consists additionally of a blood culture, lumbar puncture for cell count and culture, culture and Gram staining of tracheal aspirates immediately after

endotracheal tube placement, as well as chest radiographs when respiratory symptoms are present and specific viral studies in cases of suspected viral etiology of sepsis (5,8,25).

1.1.1.5 Treatment

In case of bacterial sepsis antimicrobials including beta-lactams (ampicillin, oxacillin and cefotaxime) and also extended-spectrum beta-lactams (piperacillin-tazobactam) as well as carbapenems are used for treatment (5). Aminoglycosides and glycopeptides can be necessary, but require therapeutic-drug monitoring to limit oto- and nephrotoxicity (26). Recommended empirical treatment for neonatal sepsis is the combination of ampicillin and gentamicin, as GBS and E.coli are the predominant pathogens (9). When suspecting a meningitis cefotaxime may be added (5).

Furthermore antiviral medications such as acyclovir, or ganciclovir as well as (liposomal) amphotericin B, fluconazole and echinocandins (caspofungin) as an antifungal medication can be necessary in case of viral or fungal infection (5).

In case of culture-proven sepsis the treatment duration varies from 10 to 14 days. The recommended duration of treatment for gram-negative meningitis is 21 days or more. If there are any complications (e.g. brain abscesses, osteomyelitis, endocarditis) antimicrobial treatment may be prolonged. In case of negative blood cultures and well appearing clinical status of the neonate empirical antimicrobial therapy should be stopped at 48 hours. Very often the etiologic agent cannot be identified in cultures, but the neonate shows a concerning clinical status an empirical 10-day course of antimicrobial therapy should be completed (8).

1.1.1.6 Morbidity and Mortality

Hypotension as well as respiratory distress, hyper- and hypoglycaemia, thrombocytopenia and disseminated intravascular coagulation are associated with sepsis. Furthermore preterm infants with EOS and exposure to intrauterine inflammation due to chorioamnionitis have an increased risk of development of bronchopulmonary dysplasia. Treatment of EOS with broad-spectrum antibiotics increases also the risk of candida infections, including invasive sepsis, meningitis or localised disease (5).

There is also an association between perinatal infection and brain injury, such as periventricular leukomalacia, neurodevelopmental delays, cerebral palsy (5).

Mortality risk increases with increasing degree of prematurity and associated morbidities. Due to compromised immunity especially very-low-birth-weight neonates are at greatest risk of infection. The mortality related to sepsis in these infants is up to 20% (5). Lim et al. showed that preterm infants with EOS had a much higher mortality rate than preterm infants with LOS (40% versus 4.7%) (27).

1.1.2 Preterm premature rupture of membranes (PPROM)

Preterm premature rupture of the membranes near the limit of fetal viability affects approximately 4 in 1000 pregnancies (28). Patkai et al. (29) found out that PPROM in less than 25 weeks of gestation is associated with increased neonatal mortality and morbidity. The gestational age at occurrence is a predictor for the fetal outcome (30). The following fetal and neonatal complications can occur (28,29,31): fetal loss, restriction deformities, pulmonary hypoplasia, respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), intraventricular haemorrhage (IVH), sepsis and all other complications accompanied with extreme prematurity of surviving infants. Risk factors for PPROM are (28,32,33): increasing age of the mother, primiparae, a history of cervical insufficiency, antepartum bleeding, multiple gestations, previous PROM or preterm labour, tobacco use, cervical cerclage, amniocentesis and especially intrauterine infection. An early antibiotic administration is associated with a prolongation of pregnancy and a reduction of clinical chorioamnionitis and neonatal sepsis (34). A rupture of membranes ≥ 18 hours prior delivery increases the risk of EOS to 0.8% (35).

1.1.3 Intrauterine Infection

Intrauterine infection can be classified as intra-amniotic (amniotic cavity) and extra-amniotic (chorioamniotic space) infection. The isolation of microorganisms is the gold standard for the diagnosis of an intrauterine infection. Usually the amniotic cavity is sterile (34).

An intra-amniotic infection is also called chorioamnionitis, which is an acute inflammation of the membranes and chorion of the placenta (36). It is a polymicrobial infection due to ascending genital microbes (37).

In cases of chorioamnionitis microorganisms, such as Ureaplasma urealyticum, Fusobacterium species, Mycoplasma hominis, Escherichia coli, Staphylococcus aureus, GBS can be found in the amniotic fluid (38,39). Risk factors associated with the development of chorioamnionitis are (36,40,41): low parity, spontaneous labour, prolonged labour, longer

duration of membrane rupture, multiple digital vaginal examinations, meconium-stained amniotic fluid, internal fetal or uterine monitoring, epidural anaesthesia, diminished host response (e.g. due to smoking, drug abuse, obesity, immunodeficiency states) and presence of genital tract micro-organisms. The ascending route is the most common pathway of an intrauterine infection (39). Haematogenous / transplacental passage and iatrogenic infection are less common (36). In approximately 25% of all preterm births intrauterine infection is present. The lower the gestational age at birth the higher the frequency of intra-amniotic infection (34). If the microorganisms gain access to the fetus before birth, they can cause a multi-systemic organ involvement and additionally increase the rate of perinatal morbidities (34). By inhaling or swallowing infected amniotic fluid a sepsis can already begin in utero. An important criterion for the diagnosis of a clinical chorioamnionitis is the presence of maternal fever over 38°C and at least two of the following criteria: maternal leucocytosis (>15000 cells/mm³), maternal (>100 beats/minute) or fetal tachycardia (>160 beats/minute), uterine tenderness and foul odour of the amniotic fluid (8).

A Chorioamnionitis is not only associated with postpartum maternal infections, but also with potentially devastating fetal complications (premature birth, neonatal sepsis or cerebral palsy). Fetal response to infection – FIRS ('Fetal Inflammatory Response Syndrome') – may aggravate these complications (36).
In women with PPROM the main preventative strategy is therefore the administration of antibiotics to reduce the risk of clinical chorioamnionitis and as a consequence improve neonatal outcome (36).

1.1.4 Current clinical routine monitoring

Beside the clinical evaluation, monitoring of standard haemodynamic parameters in paediatric and neonatal critical care consists of (42):

1.1.4.1 Invasive / non-invasive blood pressure measurement

For assessment of haemodynamic status in neonates blood pressure measurement is the most frequently used method (43,44). Blood pressure, which is determined by cardiac output and systemic vascular resistance, is not linearly related to systemic blood flow. Using blood pressure alone for diagnosis of low systemic blood flow may lead to under- or overtreatment of patients with risk of adverse effects and iatrogenic damage(45). The three most common definitions for neonatal hypotension are (45):

- A blood pressure below the tenth (or fifth) percentile of normative blood pressure values derived from a reference population with regard to gestational age, birth weight and postnatal age.
- The lower border of normal mean arterial blood pressure (MABP) equals the numeric value of gestational age (GA) in whole weeks.
- MABP values below 30 mmHg are defined as hypotension due to the fact that cerebral blood flow becomes pressure dependent at a MABP values around 30 mmHg.

1.1.4.2 Heart Rate (HR)

HR can be estimated by electrocardiography (ECG) and pulse oximetry.

ECG is an important tool of the bedside monitoring for neonates at neonatal intensive care units (NICU). It allows a quick, accurate and reliable assessment of heart rate. Furthermore arrhythmias can be detected (46).

Pulse oximetry devices estimate the absorption of two different wavelengths of light. The changes depend on the amount of blood in the tissue and the relative amounts of oxygenated and deoxygenated haemoglobin. Heart rate can be estimated by measuring the changes in light absorption that occur with pulsatile flow (47).

Ventricular output is determined by stroke volume and heart rate. Since stroke volume can change in the neonatal period a simple linear correlation between systemic blood flow and HR can be excluded. Furthermore, HR can be influenced by many factors (e.g. temperature, stress, pain, and medication) and therefore a single HR value poorly reflects systemic perfusion. Nevertheless, large changes in HR may indicate relevant changes in cardiac output (45).

1.1.4.3 Arterial oxygen saturation (SpO_2)

Pulse oximetry allows not only the estimation of heart rate, but also of the oxygen saturation of arterial blood by measuring the pulsatile changes in light transmission across a tissue bed (48). Technical limitations of pulse oximetry include movement, ambient light, poor perfusion, skin pigmentation and dyshaemoglobinaemia. False low SpO_2 values can be differentiated from true desaturations by inspecting the quality of the pulse waveform displayed (49).
In preterm neonates born less than 32 weeks SpO_2 should be targeted between 91% and 95% (48). In term neonates a saturation target of >95% is commonly adopted, since retinopathy of

prematurity (ROP) and chronic lung disease are uncommon in infants born after 37 weeks of gestation (49).

1.1.4.4 Carbon dioxide (CO_2)

Measurement of carbon dioxide CO_2 is a fundamental evaluation in a neonatal intensive care unit. Normal ranges of arterial partial pressure of CO_2 ($PaCO_2$) are described from 35 mmHg to 45 mmHg in healthy neonates (50). Episodes of hypocapnia ($PaCO_2$ <35 mmHg) are associated with higher risk of developing BPD, IVH, and cystic periventricular leukomalacia (PVL) in the preterm neonates (51,52).

To minimize iatrogenic lung disease, which can occur with mechanical ventilation, permissive hypercapnia is a widely used ventilation strategy. Brown et al. found out that mild hypercapnia ($PaCO_2$ 45–55 mmHg) appears to be safe, whereas moderate hypercapnia ($PaCO_2$ >55 mmHg) increases neurologic risk and provides only little pulmonary benefit (53). Both low and high CO_2 values might have detrimental effects on neonatal morbidity and mortality (50). The optimal CO_2 values in ventilated neonates have not been found yet (54). Especially preterm neonates are vulnerable to lung and brain impairment and therefore normocapnia might be essential (50).
The most accurate way to assess the amount of CO_2 is the arterial blood gas analysis. Trying to avoid blood sampling alternative tools have been developed to measure CO_2 such as end-tidal CO_2 and transcutaneous monitoring ($tcCO_2$) (50).

Furthermore, if the neonate needs intensive care and / or is intubated and mechanically ventilated measurements of arterial blood gases and electrolytes are performed regularly.

1.2 Near-Infrared Spectroscopy (NIRS)

NIRS enables non-invasive measurement of oxygenation in regions of interest e.g. cerebral, renal and “peripheral muscle” tissue. Since the first description of NIRS by Jöbsis (55) in 1977 an increasing number of cerebral NIRS studies have been published (56-60). NIRS is based on two fundamental facts: the relative transparency of biological tissue (e.g. neonatal head) to near infrared light (wavelength 700-1000nm) and the presence of chromophores (colour bearing compounds) in the biological tissue, which have oxygenation-dependent absorption properties (e.g. haemoglobin and cytochrome oxidase) in the near infrared region. NIRS enables therefore the non-invasive measurement of changes in the concentration of

oxygenated haemoglobin (HbO_2), deoxygenated haemoglobin (Hb) and cytochrome oxidase (CtOx) (60). A modification of the Beer-Lambert law provides the physical and mathematical basis for NIRS (60):

$$A = \alpha B d C + G$$

A is the attenuation measured in units of optical density. α is the specific absorption coefficient of the chromophore at a particular wavelength ($\mu molar^{-1} \cdot cm^{-1}$). B is the differential pathlength factor (DFP), which is known for several biologic tissues. d is the distance between NIRS optodes (cm). C is the concentration of the chromophore in the tissue (µmol/L) and G is an additive term, which represents the scattering loss of NIR light on its way through the tissue (60).

There are different devices, which use the following NIRS techniques (59,60):

a) “Intensity modulated” or “phase resolved” NIRS measures the amplitude and phase shift of light, when a certain frequency is used. This method enables the non-invasive continuous measurement of absolute values of HbO_2 and Hb.
b) “Time resolved” NIRS measures the time that a picosecond pulse of light needs to pass through a tissue. This method also enables the non-invasive continuous measurement of absolute values of HbO_2 and Hb. These devices measure the actual pathlength of light.
c) “Continuous wave” NIRS measures changes in the light intensity. Therefore, this method only enables non-invasive continuous measurement of changes in the concentration of HbO_2, Hb and CtOx.
d) “Continuous wave spatially resolved” NIRS, is also called “multidistance spectroscopy”. The light intensity is measured at several different source-detector distances. This method enables the non-invasive continuous measurement of the tissue oxygenation saturation, which represents the oxygen saturation in small vessels of the tissue and corresponds to $HbO_2 / (HbO_2+Hb)$.

The devices most widespread in intensive care are based on the “continuous wave spatially resolved” technique.

1.2.1 Cerebral NIRS measurements

Most clinical work especially in term and preterm neonates on cerebral oxygenation has been undertaken using “continuous wave spatially resolved technique” NIRS (57-65).

First studies tried to establish normative data but a common theme remained the improvement of inter-individual and intra-individual test to test variations (60,63). A method to increase precision of measurements is the performance of repeated measurements with reapplications (64,66).

Until now different studies investigated the influence of manoeuvres or interventions in the clinical routine on cerebral haemodynamics in neonates: Some studies in preterm and term infants were undertaken during ventilation, using positive pressure ventilation (PPV) (67) or nasal continuous positive airway pressure (CPAP) (68). Furthermore, changes of cerebral oxygenation during periodic breathing (69) and during episodes of apnoea in preterm infants (70-72) were investigated.

Recent studies focused on the changes of cerebral haemodynamics and perfusion during neonatal transition (73-81).

Another research field is cardiocirculation and cerebral NIRS. Aminophyllin and indomethacin have a significant effect on cerebral haemodynamics, whereas caffeine and ibuprofen have no or only small effects (58). Other studies concentrated on the effect of patent ductus arteriosus (82) or cord clamping (83,84) on cerebral haemodynamics. Suresh et al. (85) found in their study that electroencephalography (EEG) and cerebral fractional oxygen extraction (cFOE) remained normal at MABP levels above 23 mmHg, concluding that cerebral perfusion is probably maintained at MABP levels above 23 mmHg.

1.2.2 Peripheral muscle NIRS measurements

Studies of “peripheral muscle” NIRS measurements with and without venous or arterial occlusion were performed in adults (86-89) and in newborn infants (62,90-100). Occlusions are performed with a pneumatic cuff around the upper arm or thigh. In case of venous occlusions the used pressure is above the venous pressure and below the diastolic arterial pressure (i.e. 20-30 mmHg), which means that there is an undisturbed arterial inflow, but an interrupted venous outflow. As a consequence changes are due to an arterial inflow of HbO2 and Hb and due to oxygen consumption. In case of arterial occlusions the used pressure is above the systolic arterial pressure (i.e. 100 mmHg), which means that there is an interrupted arterial inflow, as well as an interrupted venous outflow. In this case the measured changes are only due to oxygen consumption.

The measurement of “peripheral muscle” oxygenation has the potential to become a novel method for non-invasive evaluation of oxygenation, especially during states of shock or even during “occult shock”, when other vital signs are still normal (86,87,101).

Quality criteria for peripheral muscle NIRS measurements have increased the reproducibility, showing a decrease in mean standard deviations and an increase in the proportion of the patient’s variance components with a concurrent decrease of the measurement error (97). Additionally monitoring, laboratory and demographic parameters potentially influencing peripheral oxygenation and circulation in neonates by using NIRS and the venous occlusion method were analysed (90).

Until now peripheral NIRS measurements in preterm and term neonates were used to analyse peripheral venous oxygen saturation (SvO_2) (98) and the influence of anaemia (94,102) on peripheral oxygenation and blood flow. Furthermore the effect of limb cooling (103) and change of the global metabolic rate on peripheral oxygen consumption (VO_2) (104) were analysed, as well as the differences between forearm and calf oxygenation (92) and the influence of in utero exposure to smoking on peripheral muscular oxygenation (93) during the first day after birth.

Concerning cardio-circulation peripheral NIRS has been shown to have encouraging efficacy in identifying extremely low birth weight (ELBW) infants, who were likely to benefit from early echocardiography and subsequent intervention to close a patent ductus arteriosus (PDA) (99). Recently, Mileder et al. (105) demonstrated the influence of a PDA on peripheral muscle oxygenation and perfusion in neonates. Concerning blood pressure it has been demonstrated that peripheral blood flow decreases at MABP levels below 33 mmHg (85). Wardle et al. (95) also described changes of peripheral-muscle oxygenation measured with NIRS in preterm neonates with hypotension.

Concerning inflammatory processes CRP elevation, as well as increased leukocyte counts caused an impaired peripheral oxygenation and perfusion when routine haemodynamic variables were still normal (15,106).

1.2.3 Combination of cerebral and peripheral muscle NIRS measurements

Until now there are only a few studies using simultaneous measurement of cerebral and peripheral muscle oxygenation und perfusion in preterm and term neonates. Grossauer et al. (107) used these simultaneous NIRS measurements to compare cerebral (cTOI) and

peripheral muscle tissue oxygenation index (pTOI) in healthy neonates. Furthermore, a cTOI / pTOI ratio of 1.14±0.14 for healthy preterm and term neonates within the first eight weeks after birth was described (107). Regarding regional cerebral and peripheral muscle oxygenation during immediate transition, cerebral tissue oxygenation reaches faster a plateau compared to peripheral muscle oxygenation (108,109). Thereafter a decrease of 6% in peripheral tissue oxygenation was observed during the first week after birth (91), whereas in cerebral tissue oxygenation an increase of 9% was observed during the first two days after birth (110). In a recent study we reported on c TOI / pTOI ratios for cardio-circulatory stable preterm neonates over a 24-hour period after birth, where we found significantly lower values from hour 5 to 15 compared to the first hours after birth (111).

2 OBJECTIVES

Early cardiovascular and circulatory signs of centralization and shock due to inflammation especially in cases of sepsis are difficult to interpret in neonates. Neonates often compensate for cardiovascular dysfunction and maintain a normal blood pressure in the early stages of shock. NIRS enables both measuring cerebral and peripheral tissue oxygenation and perfusion. Especially simultaneous monitoring of cerebral and peripheral muscle oxygenation might have a great potential for early recognition of cardio-circulatory disturbances due to infection / inflammation in the care of critically ill neonates, while other vital parameters are still in normal range.

The aim of the present study was therefore to investigate, if there is an association between the ratio of cerebral and peripheral muscle oxygenation and inflammation / infection in preterm neonates on the first day after birth using simultaneous NIRS measurements.

2.1 Hypotheses

2.1.1 Main hypothesis

We hypothesized that in preterm neonates with inflammation / infection the cTOI / pTOI ratio is increased due to impaired peripheral microcirculation and beginning centralisation.

2.1.2 Secondary hypotheses

a. The changes of the cTOI / pTOI ratio correlate with demographic and laboratory inflammatory parameters.
b. The changes of cTOI / pTOI ratio do not correlate with cardio-circulatory routine parameters.

2.2 Outcome parameters

2.2.1 Primary outcome parameters

- cTOI / pTOI ratio and single NIRS parameters (cTOI and pTOI) during the 24-hour measuring period

2.2.2 Secondary outcome parameters

- Cardio-circulatory and physiological parameters during NIRS measurements and correlation with cTOI / pTOI ratio:
  - Mean HR
  - Mean SpO_2
  - Mean MABP
  - Mean central and peripheral temperature
- Laboratory inflammatory parameters
- Cerebral injury by ultrasound
- Mortality

3 METHODS

In a single centre randomised controlled study called ‘Avoiding Hypotension in Preterm Neonates (AHIP; ClinicalTrials.gov identifier: NCT01910467) Pichler et al. used simultaneous cerebral and peripheral muscle NIRS measurements in combination with dedicated intervention guidelines to help avoiding arterial hypotension and catecholamine administration in preterm neonates, resulting in a non-significant reduction in burden of arterial hypotension (112). The present observational study was part of this single centre randomized controlled trail.

3.1 Patients

Preterm neonates (<37+0 weeks of gestation) born from October 2013 to December 2016 with risk for inflammation / infection born after preterm premature rupture of membranes, amnion infection syndrome or increased markers of maternal systemic inflammation (CRP / leucocyte counts), who were admitted to the NICU of the Department of Paediatrics and Adolescent Medicine, Medical University of Graz, were considered for inclusion in the trial.

Further inclusion criteria were:

- Decision to conduct full life support
- Parental written informed consent
- Age under six hours
- No need for catecholamines before initiation of NIRS measurements

Exclusion criteria was:

- Congenital malformations

The study was approved by the Regional Committee on Biomedical Research Ethics of the Medical University of Graz (EK number: 25-237 ex 12 / 13).

3.1.1 Data collection

At the beginning of each measurement the medical history during pregnancy and birth was collected. Demographic (date and time of birth, birth mode, gestational age, gender, starting time of measurements) and perinatal data (birth weight, length, circumference of the head, APGAR score, diameter and circumference of the right arm), as well as respiratory support, medication and main diagnoses were documented for each neonate.

Furthermore, the following laboratory parameters (collected during routine blood tests) were documented in each neonate:

- From the umbilical cord blood:
  - Umbilical artery pH
  - IL-6
  - Procalcitonin
  - Blood culture

- From peripheral blood:
    - Haemoglobin value on the first day
    - Leukocyte counts on the first and second day
    - CRP values on the first and second day

Cerebral ultrasound was performed in each neonate at the beginning and the end of NIRS measurements, as well as on day 4, day 7, day 14 and before discharge. Clinical follow-up was conducted until term age or until discharge, whatever occurred first. Any adverse reactions during measurements were documented.

For data collection of this study a case report form (CRF) was used (see appendix).

3.1.2 Study groups

For the present analyses we divided the preterm neonates into:

- **Infection group:** preterm neonates with laboratory signs of inflammation / infection, with at least one of the following signs
    - Umbilical cord IL-6 >100pg/ml (113)
    - Positive blood culture of the umbilical cord
    - Leucocyte counts >34000/µl (114)
    - CRP >10mg/l (15,115) on the first or second day after birth
- **Control group**: preterm neonates with no signs of inflammation / infection

3.2 NIRS measurements

3.2.1 NIRS device

The NIRO-200NX (Hamamatsu Photonics K.K., Hamamatsu City, Japan) measures and displays changes in the concentration of HbO_2 and Hb (Figure 1). Changes in the concentration of total haemoglobin (HbT) can be calculated from the sum of changes in HbO_2 and Hb. The spatially resolved method of the NIRO-200NX additionally enables non-invasive continuous measurement of TOI, using the following equation:

$$TOI\ (\%) = \Delta HbO_2 / (\Delta HbO_2 + \Delta Hb)$$

Figure 1: *NIRO-200NX Near-infrared oxygenation monitor (Hamamatsu Photonics K.K., Hamamatsu City, Japan) (116).*

3.2.2 Execution of NIRS measurements

NIRS measurements started within the first 6 hours after birth and lasted for 24 hours (24-30 hours after birth). The cerebral optodes were placed over the left forehead. The interoptode distance was 4.0 cm. The peripheral muscle optodes were placed over the right forearm with an interoptode distance of 3.0 cm in neonates >1500g and 2.0 cm in neonates <1500g (Figure 3). The cerebral NIRS optodes were repositioned at least every 6 hours to prevent skin lesions. The cerebral and the peripheral muscle oxygenation were measured continuously and displayed as TOI. The sample interval was 2/s.

3.3 Monitoring of vital parameters

NIRS measurements were combined with routine monitoring for 24 hours (Figures 2 and 3). Data were stored in a polygraphic system (alpha-trace digitalMM, B.E.S.T. Medical Systems, Vienna, Austria) for further analysis.

Routine Monitoring:

1. Pulse oximetry
2. Blood pressure measurement
3. Rectal and peripheral temperature

3.3.1 Pulse oximetry – HR and SpO_2

For pulse oximetry the IntelliVue MP50 monitor (Philips, The Netherlands) was used. The pulse oximetry sensor was applied on the right palm or wrist for monitoring of HR and preductal SpO_2 (Figure 3).

3.3.2 Blood pressure measurement – MABP

For measurement of MABP the IntelliVue MP50 monitor (Philips, The Netherlands) was used.

- Invasive blood pressure measurement:

If neonates had an intra-arterial line due to medical reasons continuous invasive blood pressure measurements were performed.

- Non-invasive blood pressure measurement:

In case of non-invasive blood pressure measurements the pneumatic cuff was placed around the left upper arm (Figure 3). These measurements were performed at least every 30 minutes.

3.3.3 Rectal and peripheral temperature

For temperature measurements the IntelliVue MP50 monitor (Philips, The Netherlands) was used. A rectal and a skin sensor were used for central and peripheral temperature measurements.

Figure 2 shows the application of NIRS optodes, pulse oximetry and pneumatic cuff.

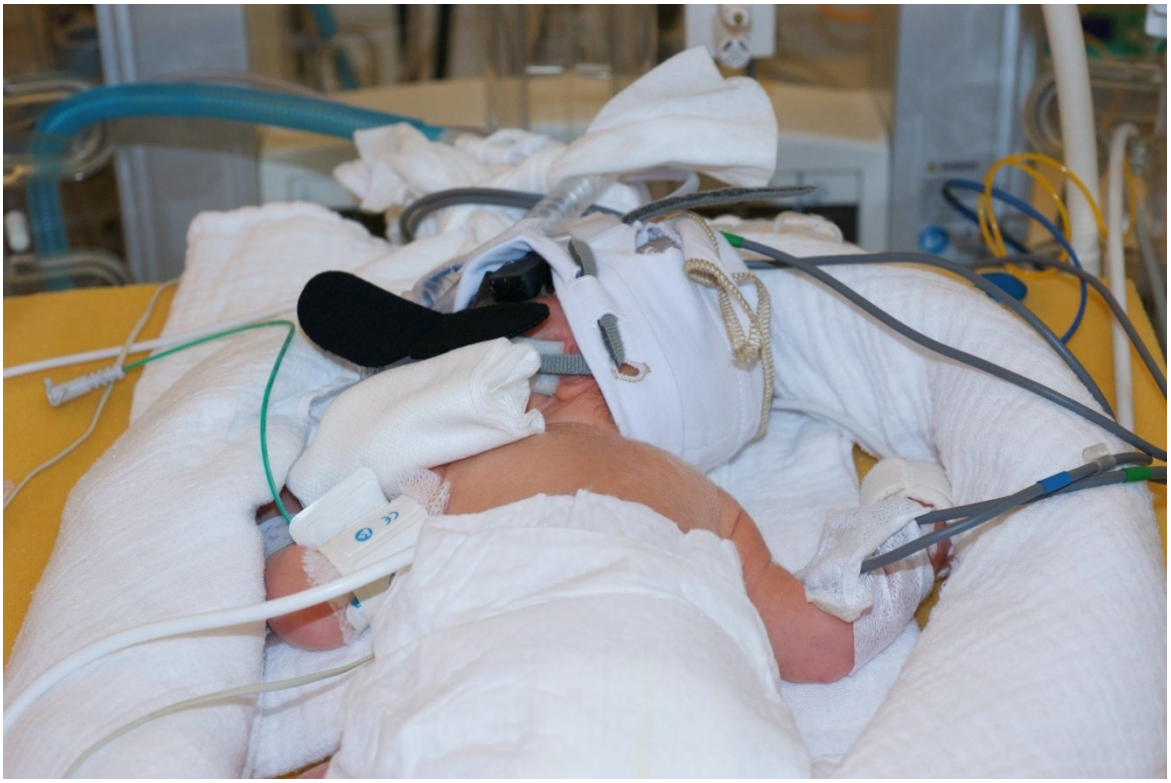

Figure 2: *Application of NIRS optodes, pulse oximetry and pneumatic cuff for blood pressure measurements in a neonate.*

Figure 3 displays the measuring station used for this study with ‘Giraffe’ incubator (GE Healthcare, United Kingdom), IntelliVue MP50 monitor, polygraphic system and NIRO-200NX.

Figure 3: *Measuring station with ‘Giraffe’ incubator (GE Healthcare, United Kingdom), IntelliVue MP50 monitor, polygraphic system, NIRO-200NX.*

3.4 Statistical analysis

In each neonate mean values of cTOI, pTOI, SpO_2, HR, MABP, rectal and peripheral temperature were calculated for every hour. Out of the mean values of each hour, cTOI / pTOI ratios were calculated. Furthermore, mean values of cTOI, pTOI, cTOI / pTOI ratio as well as mean values of SpO_2, HR, MABP, rectal and peripheral temperature for the 24-hour measuring period were calculated.

Data are presented as mean and standard deviation (SD) for normally distributed continuous variables and median and 25^{th} to 75^{th} percentile if the distribution was skewed.

Categorical demographic variables of the two groups were compared with the use of the chi-square test or Fisher’s exact test. Continuous variables of the two groups were compared using Student’s t-Test or Mann–Whitney-U test, as appropriate. Correlations between cTOI / pTOI ratio and cardio-circulatory / physiological parameters as well as laboratory inflammatory

parameters were shown using Pearson product-moment correlation or Spearman rank-order correlation, as appropriate.

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

4.1 Demographic and clinical parameters

A total number of 108 preterm neonates below 37+0 weeks of gestation with risk for an inflammation / infection were included in the AHIP trial. 10 (9.3%) preterm neonates had to be excluded due to delayed beginning of NIRS measurements > 6 hours after birth (n=6; 5.6%), use of catecholamines before start of NIRS measurements (n=2; 1.9%), missing NIRS data (n=1; 0.9%) and withdrawal of informed consent by the parents (n=1; 0.9%). No severe adverse reactions were observed during measurements. Out of the remaining 98 preterm neonates 11 (11.2%) were diagnosed with laboratory confirmed inflammation / infection (infection group) and compared to 87 preterm neonates (88.8%) without laboratory confirmed inflammation / infection (control group).

Figure 4 displays a flow diagram of the included neonates.

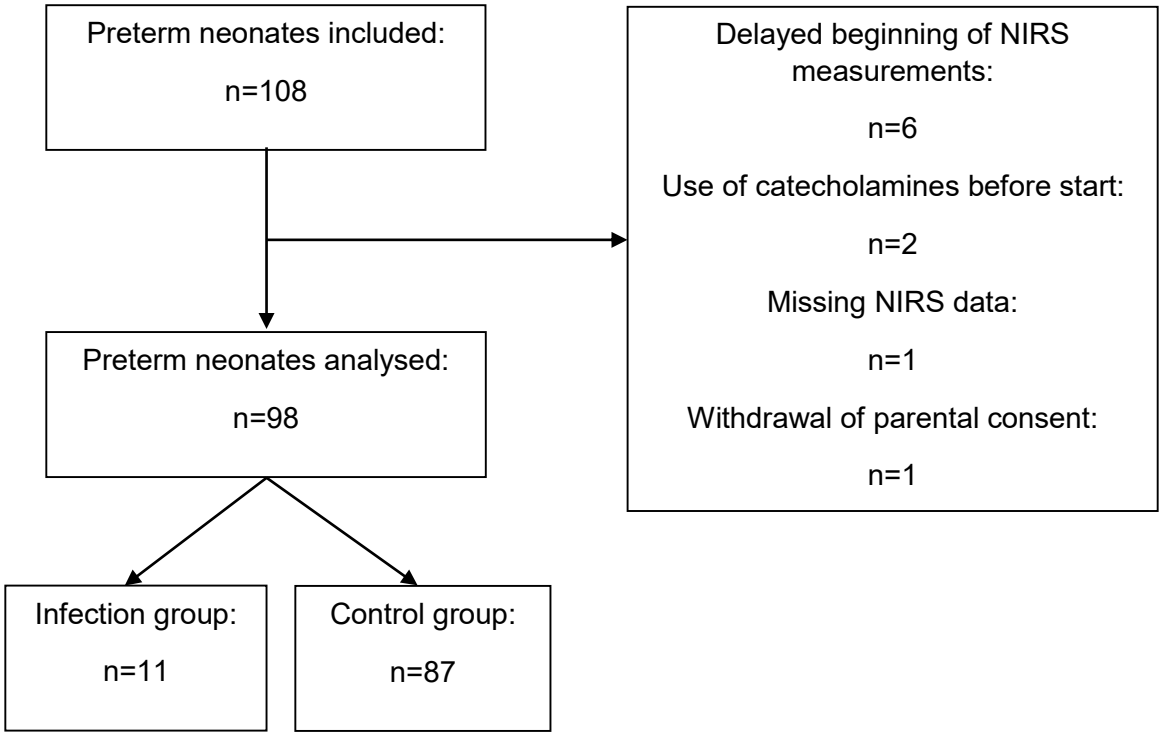

Figure 4: *Flow diagram demonstrating the number of included neonates and rationales for exclusion.*

In the control group 38 neonates (43.7%) were admitted to the NICU due to infant RDS, 28 neonates (32.2%) due to prematurity and 21 (24.1%) due to transient tachypnoea of the newborn.

Table 1 presents demographic data of patients in both groups.

	Control group	Infection group	p-value
Patients (n)	87 (88.8%)	11 (11.2%)	
Females / Males (n)	44 (50.6%) / 43 (49.4%)	5 (45.5%) / 6 (54.5%)	0.500
Caesarean section / Vaginal delivery (n)	65 (74.7%) / 22 (25.3%)	9 (81.8%) / 2 (18.2%)	0.463
Gestational age (weeks)	33.1 (32.1-34.1)	32.3 (28.7-34.0)	0.451
Postnatal age (h)	2.5 (1.5-4.0)	2.0 (1.5-3.5)	0.590
Birth weight (g)	1859.9 ± 488.0	1643.5 ± 624.5	0.183
Diameter of the upper arm (cm)	2.4 ± 0.4	2.2 ± 0.5	0.251
Thickness of subcutaneous fat on the upper arm, measured by ultrasound (cm)	0.3 ± 0.1	0.3 ± 0.1	0.634
Circumference of the upper arm (cm)	8.1 ± 1.2	8.2 ± 1.7	0.906

Table 1: *Demographic data of the control and the infection group. Continuous data are presented as mean ± SD or median (25th percentile to 75th percentile) based on distribution (*p<0.05).*

4.1.1 Distribution of gestational age

Figures 5 shows the distribution of the gestational age of the participating preterm neonates of both groups in weeks. In both groups most of the included preterm neonates were >32 weeks of gestation.

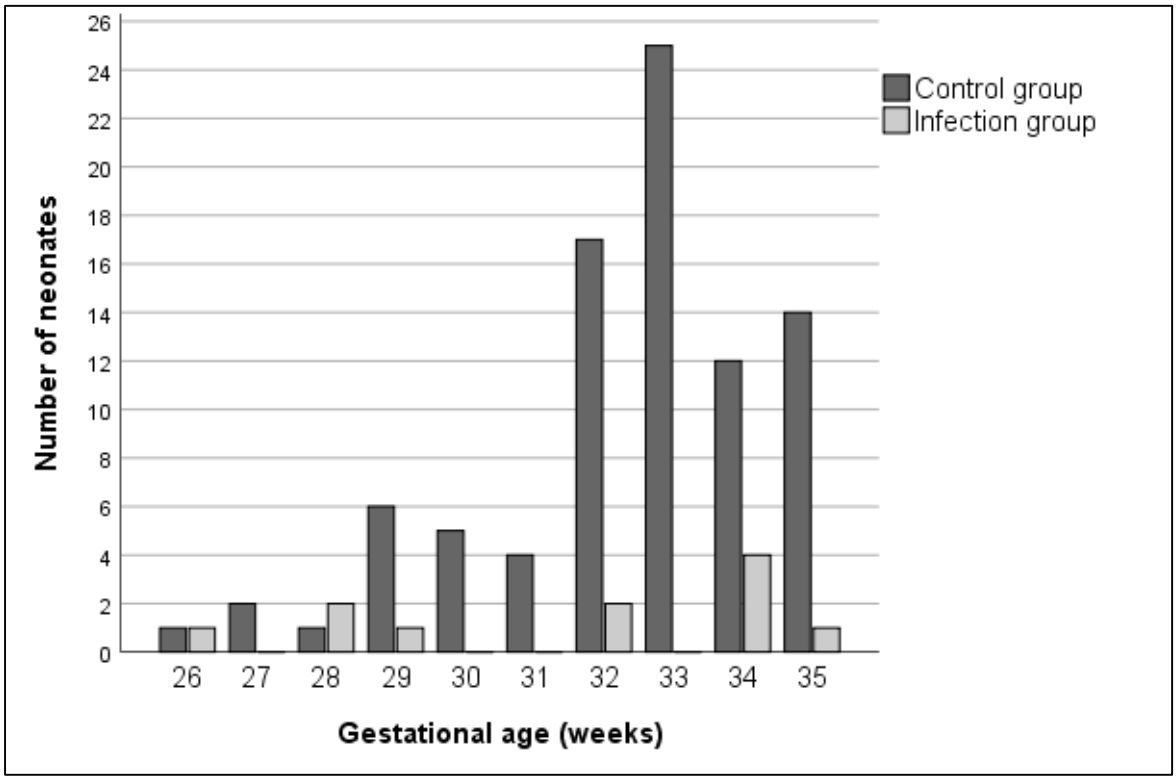

Figure 5: *Distribution of gestational age in weeks of the control and the infection group.*

4.1.2 Distribution of postnatal age

Figures 6 and 7 demonstrate the distribution of age at the beginning of measurements (postnatal age) of the participating neonates of both groups in hours. Median postnatal age of the control groups was a little bit lower than in the infection group (2.0 [1.5 - 4.0] vs. 2.5 [1.5 – 3.5] hours), without reaching significant difference (Table 1).

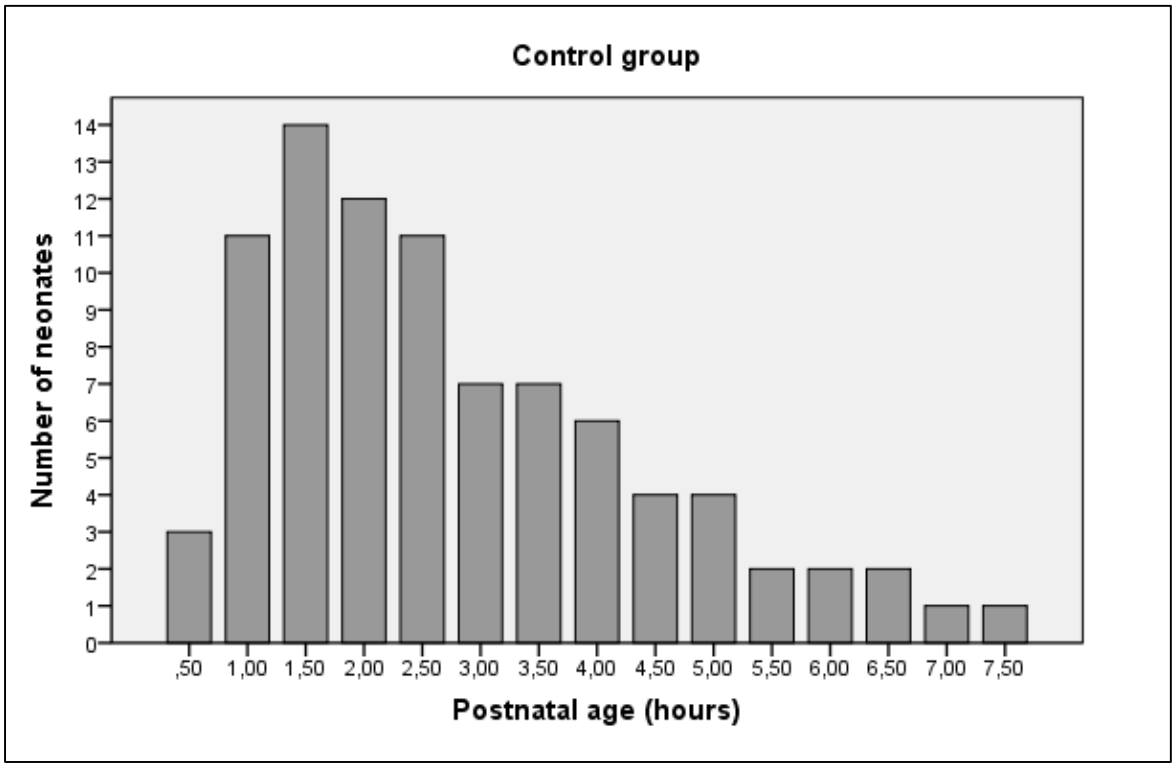

Figure 6: *Distribution of postnatal age in hours of the control group.*

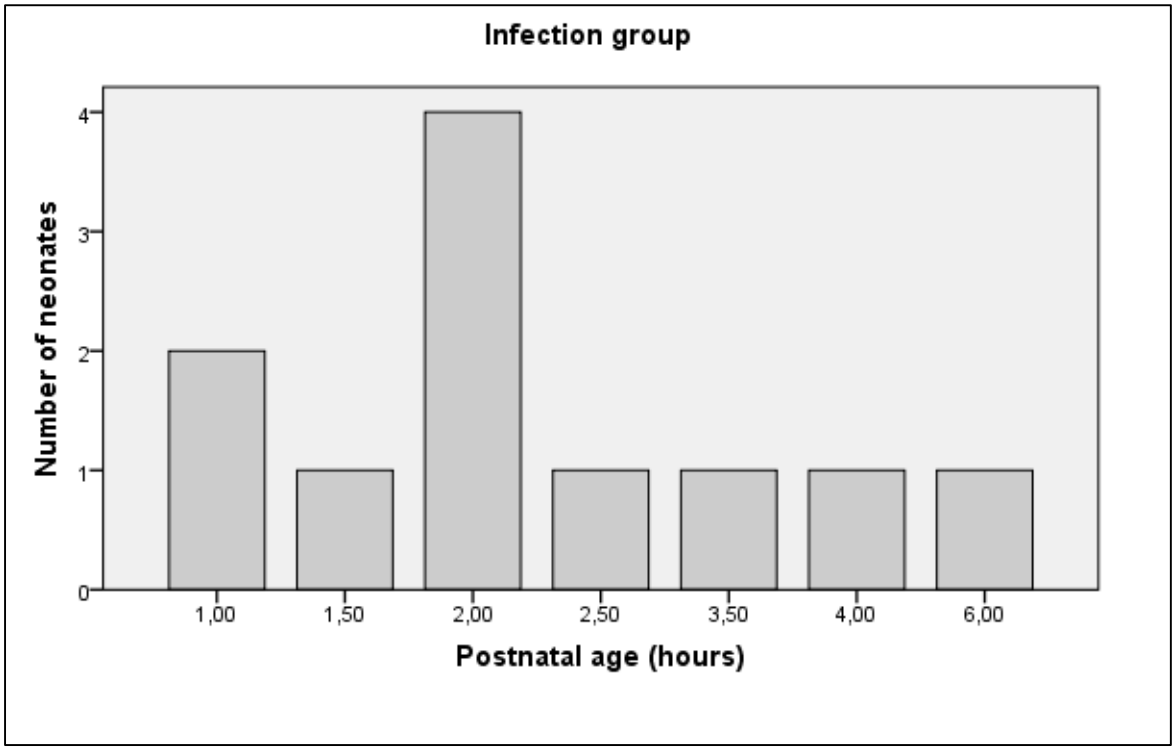

Figure 7: *Distribution of postnatal age in hours of the infection group.*

Table 2 presents the clinical parameters of patients in both groups.

	Control group	Infection group	p-value
Patients (n)	87 (88.8%)	11 (11.2%)	
Apgar 1 minute	8 (8-9)	8 (6-8)	**0.043***
Apgar 5 minutes	9 (8-10)	8 (7-9)	**0.010***
Apgar 10 minutes	9 (9-10)	9 (9-9)	0.065
Umbilical artery pH	7.30 ± 0.05	7.26 ± 0.04	**0.021***
Umbilical venous pH	7.35 ± 0.06	7.33 ± 0.07	0.371
Central capillary refill time at the beginning of measurement (s)	2.7 ± 0.6	2.6 ± 0.8	0.705
Central capillary refill time at the end of measurement (s)	2.7 ± 0.7	2.5 ± 1.0	0.457
Peripheral capillary refill time at the beginning of measurement (s)	2.6 ± 0.6	2.6 ± 0.6	0.903
Peripheral capillary refill time at the end of measurement (s)	2.6 ± 0.6	2.9 ± 0.7	0.247

Table 2: *Clinical parameters of the control and the infection group. Continuous data are presented as mean ± SD or median (25th percentile to 75th percentile) based on distribution (*p<0.05).*

4.1.3 Apgar scores

The Apgar scores at minutes 1 and 5 showed significantly lower values in the infection group compared to the control group. The Apgar scores at minute 10 did not differ between groups (Table 2).

Figure 8 - 10 show the comparison of Apgar scores at 1, 5 and 10 minutes between both groups.

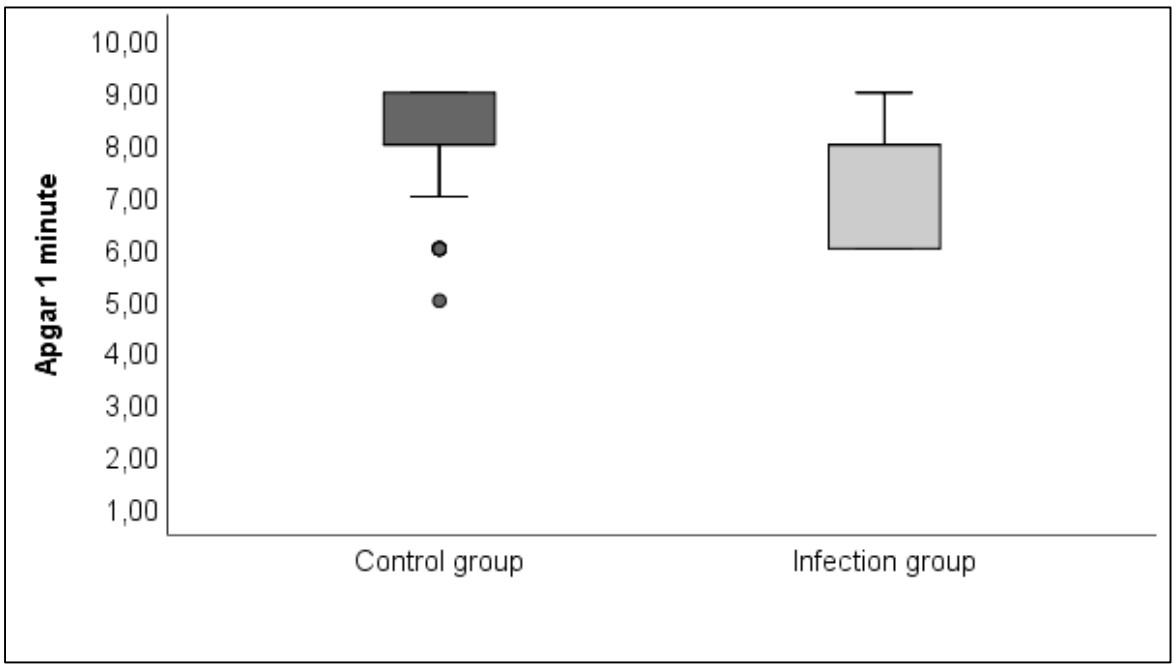

Figure 8: *Comparison of Apgar scores at 1 minute between both groups.*

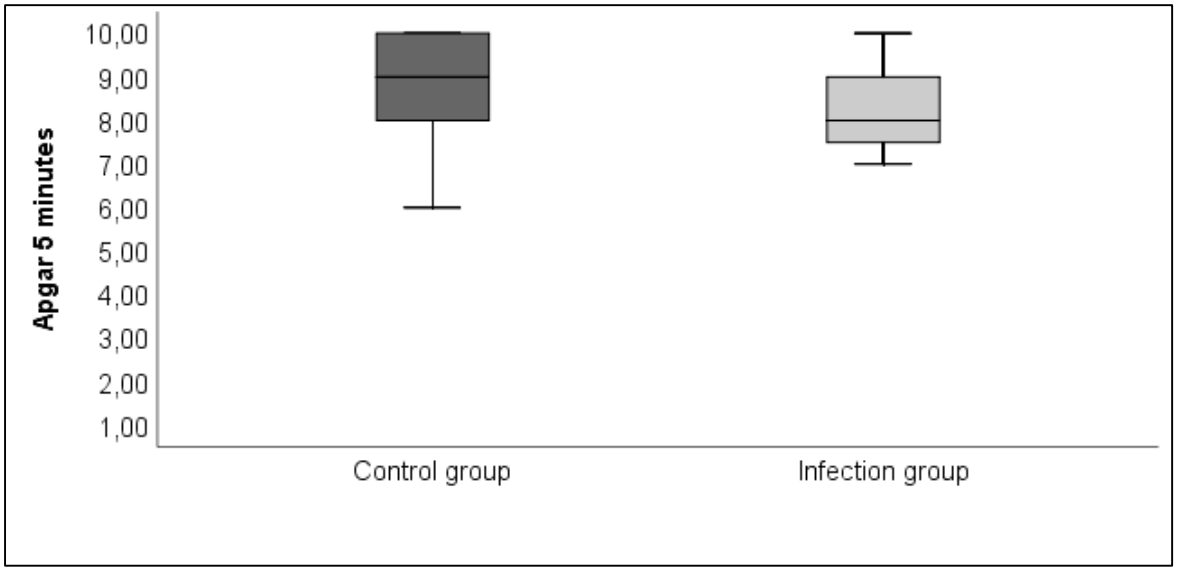

Figure 9: *Comparison of Apgar scores at 5 minutes between both groups.*

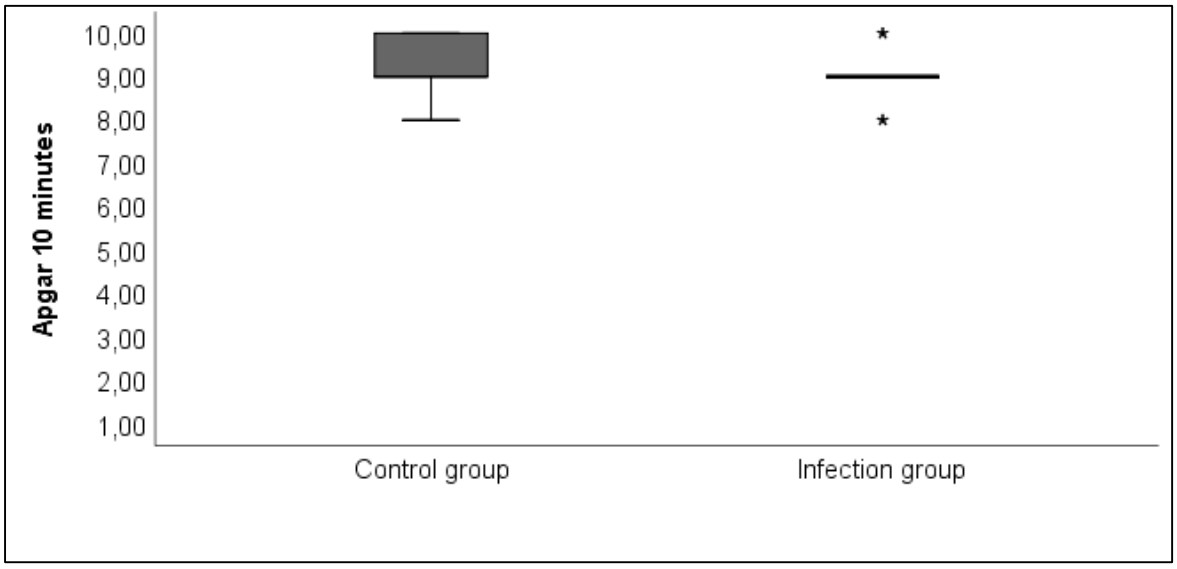

Figure 10: *Comparison of Apgar scores at 10 minutes between both groups.*

4.1.4 Umbilical pH

The umbilical artery pH showed significantly lower values in the infection group compared to the control group, whereas the umbilical venous pH did not differ between groups (Table 2).

Figure 11 and 12 demonstrates the comparison of the umbilical artery and venous pH between both groups.

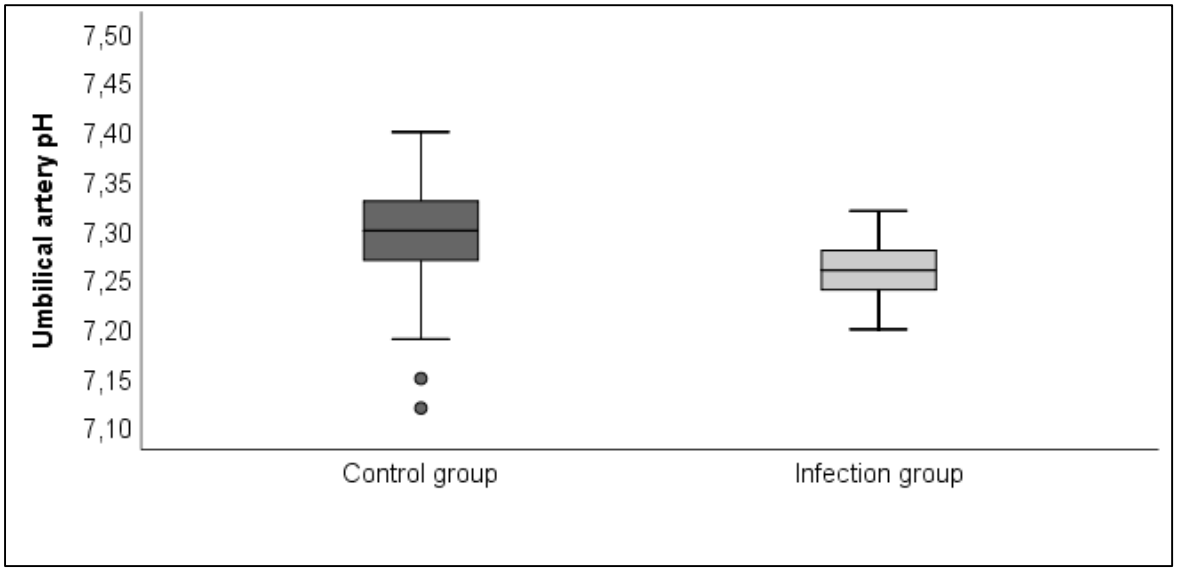

Figure 11: *Comparison of umbilical artery pH between both groups.*

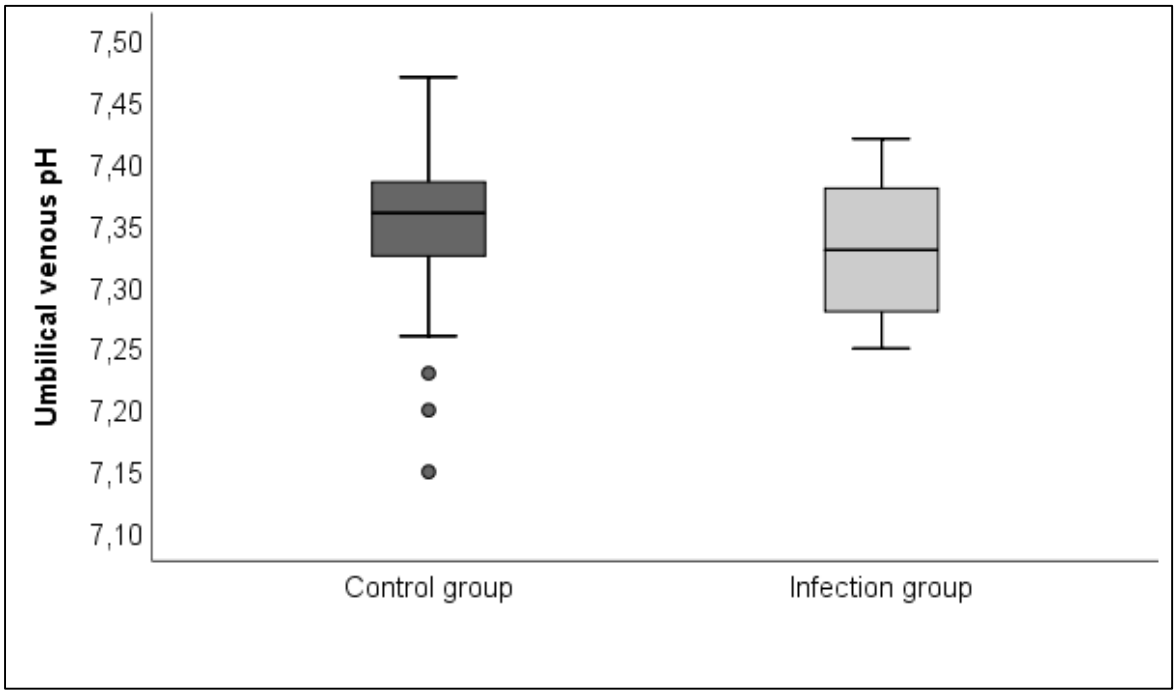

Figure 12: *Comparison of umbilical venous pH between both groups.*

4.1.5 Respiratory support

Table 3 gives information about the respiratory situation of the two groups. There was a significant difference concerning respiratory support between the two groups. In the infection group significantly more neonates needed respiratory support than in the control group.

	Control group (n=87)	Infection group (n=11)	p-value
Respiratory Support			**0.045**
– No respiratory support (n)	55 (63.2%)	3 (27.3%)	
– Non-invasive respiratory support (n)	28 (32.2%)	7 (63.6%)	
– Intubated (n)	4 (4.6%)	1 (9.1%)	

Table 3: *Respiratory situation of the control and the infection group (*p<0.05).*

Figure 13 shows the distribution of neonates without need for respiratory support or with need for non-invasive respiratory support via high flow nasal cannula or nasal CPAP or with need for intubation in both groups.

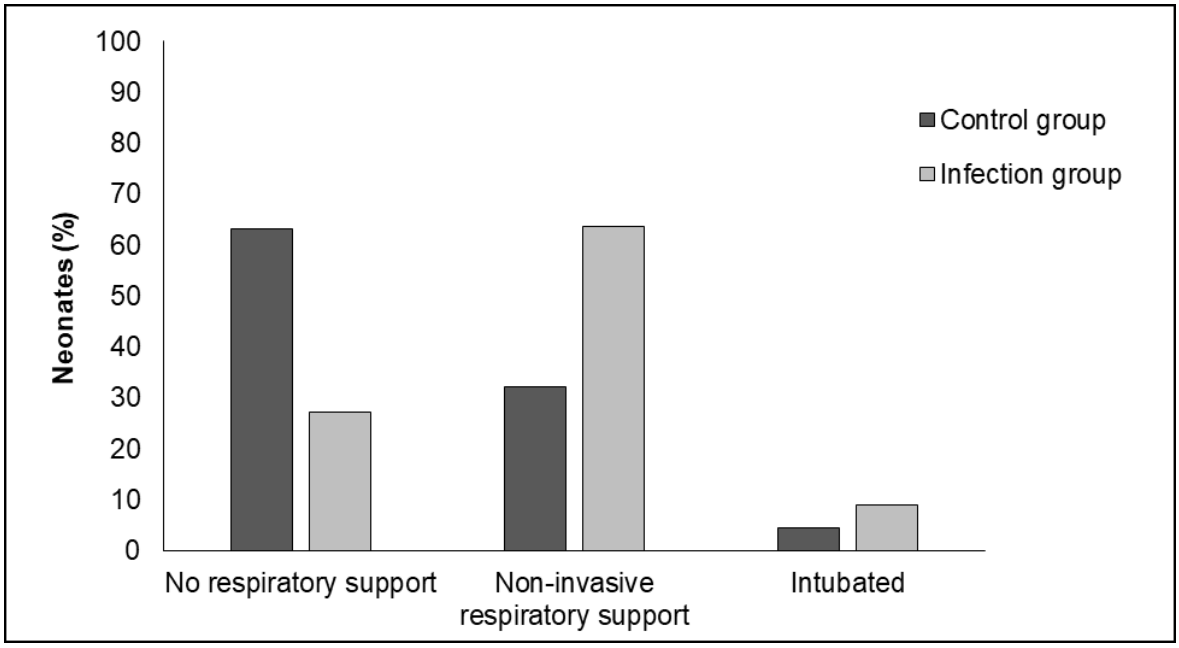

Figure 13: *Distribution of respiratory support in both groups.*

Figure 14 compares the need for surfactant administration during the transition period via INSURE (Intubation-Surfactant-Extubation) manoeuvre. The need for surfactant administration was significantly higher in the infection group compared to the control group (6 [54.5%] vs. 9 [10.3%]; p=0.001).

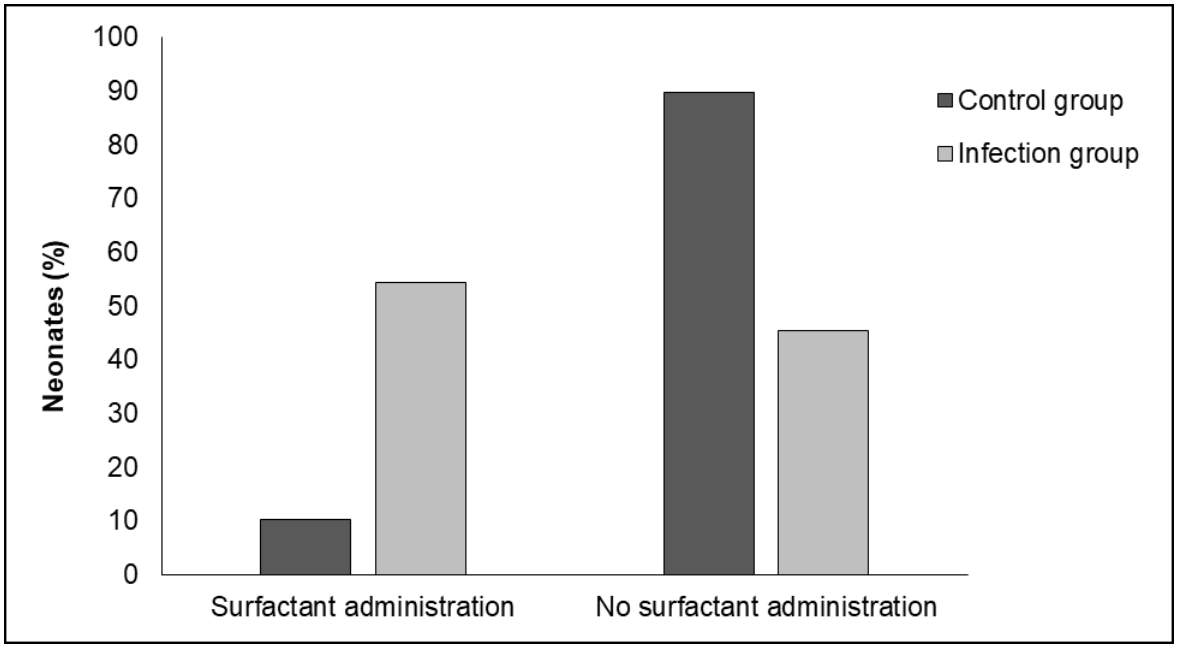

Figure 14: *Need for surfactant administration via INSURE manoeuvre in both groups.*

None of the participating neonates were resuscitated with chest compressions or received catecholamines. There were no significant differences in any other demographic or clinical parameters (Table 1 - 3).

4.2 Laboratory parameters

Blood samples were taken from the umbilical cord after birth and from the periphery on the first and second day after birth.

Due to stratification of the two groups there was a significant difference between both groups in regard to PCT from umbilical cord blood and CRP values from the first and second day after birth. Additionally, in the infection group two neonates had positive blood cultures (Table 4).

Table 4 presents laboratory parameters of neonates in both groups.

	Control group (n=87)	Infection group (n=11)	p-value
Haemoglobin (g/dL)	18.0 ± 3.0	18.2 ± 2.9	0.827
<u>Umbilical cord blood:</u>			
IL-6 (pg/mL)	6.7 (3.9-11.8)	8.7 (4.1-646.4)	0.168
PCT (ng/mL)	0.5 ± 2.7	0.8 ± 1.5	**0.041***
Positive blood culture / Negative blood culture (n)	0 (0%) / 58 (66.7%)	2 (18.2%) / 5 (45.5%)	**0.010***
<u>Blood sample of first day after birth:</u>			
Leucocyte count (/µL)	13938 ± 5928	15001 ± 11083	0.752
CRP (mg/L)	1.2 ± 1.3	31.7 ± 51.6	**<0.001***
<u>Blood sample of second day after birth:</u>			
Leucocyte count (/µL)	12509 ± 4709	15136 ± 9151	0.527
CRP (mg/L)	1.4 ± 1.2	25.7 ± 31.4	**<0.001***

Table 4: *Laboratory parameters of the control and the infection group. Continuous data are presented as mean ± SD or median (25th percentile to 75th percentile) based on distribution (*p<0.05).*

4.3 Primary outcome parameters

4.3.1 cTOI / pTOI ratio

The cTOI / pTOI ratio showed no significant difference between groups during the 24-hour measuring period (Table 5).

Hour	cTOI / pTOI ratio		p-value
	Control group	Infection group	
1	1.00 ± 0.15	1.03 ± 0.15	1.000
2	1.00 ± 0.14	1.00 ± 0.17	1.000
3	0.97 ± 0.17	0.96 ± 0.19	1.000
4	0.97 ± 0.17	0.99 ± 0.18	1.000
5	0.92 ± 0.14	1.05 ± 0.25	0.241
6	0.95 ± 0.16	0.93 ± 0.15	1.000
7	0.94 ± 0.16	1.00 ± 0.20	1.000
8	0.95 ± 0.17	0.91 ± 0.16	1.000
9	0.95 ± 0.21	0.99 ± 0.19	1.000
10	0.95 ± 0.16	0.94 ± 0.16	1.000
11	0.95 ± 0.18	0.92 ± 0.15	1.000
12	0.95 ± 0.16	0.96 ± 0.17	1.000
13	0.93 ± 0.16	0.94 ± 0.15	1.000
14	0.94 ± 0.16	1.03 ± 0.17	1.000
15	0.95 ± 0.18	0.97 ± 0.22	1.000
16	0.99 ± 0.17	0.98 ± 0.21	1.000
17	0.95 ± 0.17	0.96 ± 0.17	1.000
18	0.95 ± 0.16	0.93 ± 0.13	1.000
19	0.98 ± 0.16	0.94 ± 0.15	1.000
20	0.98 ± 0.14	0.95 ± 0.12	1.000
21	0.98 ± 0.18	0.98 ± 0.10	1.000
22	1.00 ± 0.17	0.94 ± 0.11	1.000
23	1.00 ± 0.15	1.01 ± 0.06	1.000
24	0.99 ± 0.19	1.03 ± 0.10	1.000

Table 5: *cTOI / pTOI ratios of the control and the infection group during the 24–hour measuring period. Data are presented as mean ± SD. There were no significant differences found.*

Figure 15 gives a graphical presentation of the cTOI / pTOI ratio changes over 24 hours.

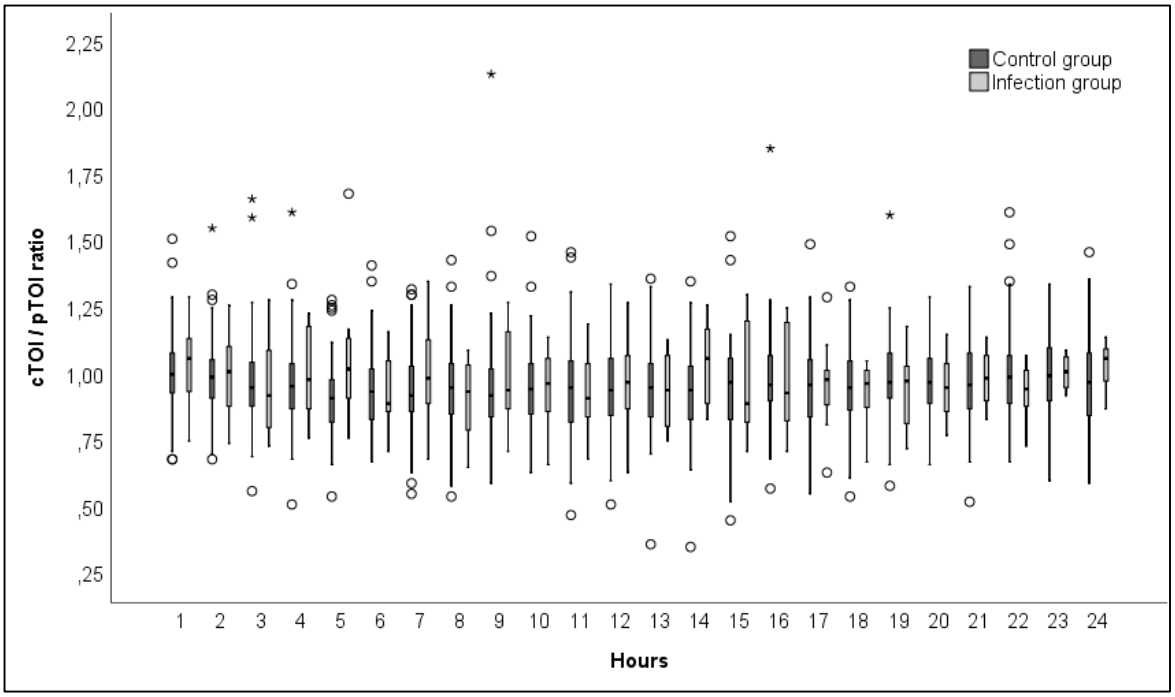

Figure 15: *cTOI / pTOI ratios of the control and the infection group during the 24–hour measuring period. There were no significant differences found.*

Mean values of cTOI / pTOI ratio (control group: 0.96 ± 0.02 vs. infection group: 0.97 ± 0.04; p=0.618) of the 24-hours measuring period did not differ between groups.

4.3.2 cTOI and pTOI

Regarding the single NIRS parameters cTOI and pTOI over the 24-hour measuring period, cTOI and pTOI did not show any significant difference between groups (Table 6).

Hour	cTOI (%)		p-value	pTOI (%)		p-value
	Control group	Infection group		Control group	Infection group	
1	73.1 ± 9.1	74.9 ± 9.8	1.000	73.3 ± 7.4	72.8 ± 5.9	1.000
2	73.0 ± 9.5	74.0 ± 12.7	1.000	73.8 ± 6.7	74.2 ± 6.0	1.000
3	71.4 ± 10.4	70.6 ± 13.7	1.000	74.2 ± 7.1	73.4 ± 4.7	1.000
4	71.0 ± 10.5	71.5 ± 10.3	1.000	73.8 ± 6.8	72.6 ± 5.7	1.000
5	68.7 ± 9.4	72.4 ± 9.1	1.000	75.4 ± 5.3*	70.4 ± 8.3	0.226
6	70.5 ± 10.2	69.4 ± 9.1	1.000	74.7 ± 6.1	74.6 ± 3.6	1.000
7	69.2 ± 10.3	68.6 ± 8.8	1.000	74.2 ± 6.1	69.6 ± 8.4	1.000
8	69.7 ± 10.4	67.5 ± 11.9	1.000	74.1 ± 6.6	74.6 ± 5.2	1.000
9	68.2 ± 10.3	73.7 ± 12.3	1.000	73.3 ± 7.9	74.8 ± 5.4	1.000
10	69.2 ± 10.2	68.9 ± 9.4	1.000	73.6 ± 7.8	73.9 ± 5.7	1.000
11	68.5 ± 11.3	67.2 ± 9.7	1.000	73.0 ± 8.1	73.4 ± 5.9	1.000
12	70.1 ± 11.5	69.3 ± 11.1	1.000	74.4 ± 7.2	72.6 ± 3.5	1.000
13	68.2 ± 11.0	70.3 ± 10.4	1.000	73.5 ± 7.5	75.0 ± 4.3	1.000
14	68.6 ± 11.7	75.2 ± 9.1	1.000	73.8 ± 7.9	73.5 ± 5.1	1.000
15	68.2 ± 10.8	71.0 ± 12.6	1.000	72.9 ± 8.3	74.0 ± 5.8	1.000
16	71.2 ± 9.6	71.5 ± 11.2	1.000	73.0 ± 7.7	73.6 ± 7.3	1.000
17	69.9 ± 11.1	69.9 ± 10.7	1.000	73.8 ± 7.0	73.2 ± 6.4	1.000
18	69.6 ± 11.0	69.9 ± 10.7	1.000	73.4 ± 6.7	75.2 ± 4.4	1.000
19	70.7 ± 10.2	69.9 ± 10.0	1.000	72.5 ± 7.6	74.6 ± 5.7	1.000
20	70.5 ± 10.1	69.5 ± 8.6	1.000	72.6 ± 7.4	73.1 ± 5.7	1.000
21	70.1 ± 10.5	70.8 ± 8.4	1.000	72.2 ± 8.6	72.5 ± 5.0	1.000
22	71.1 ± 8.9	70.6 ± 7.6	1.000	71.7 ± 7.3	75.9 ± 4.2	1.000
23	71.7 ± 10.0	75.2 ± 7.4	1.000	72.3 ± 5.8	74.2 ± 4.9	1.000
24	70.1 ± 10.3	76.9 ± 9.8	1.000	71.9 ± 7.1	74.6 ± 4.1	1.000

Table 6: *cTOI and pTOI values of the control and the infection group during the 24–hour measuring period. Data are presented as mean ± SD. There were no significant differences found.*

Figure 16 and 17 give a graphical presentation of the cTOI and pTOI changes over 24 hours.

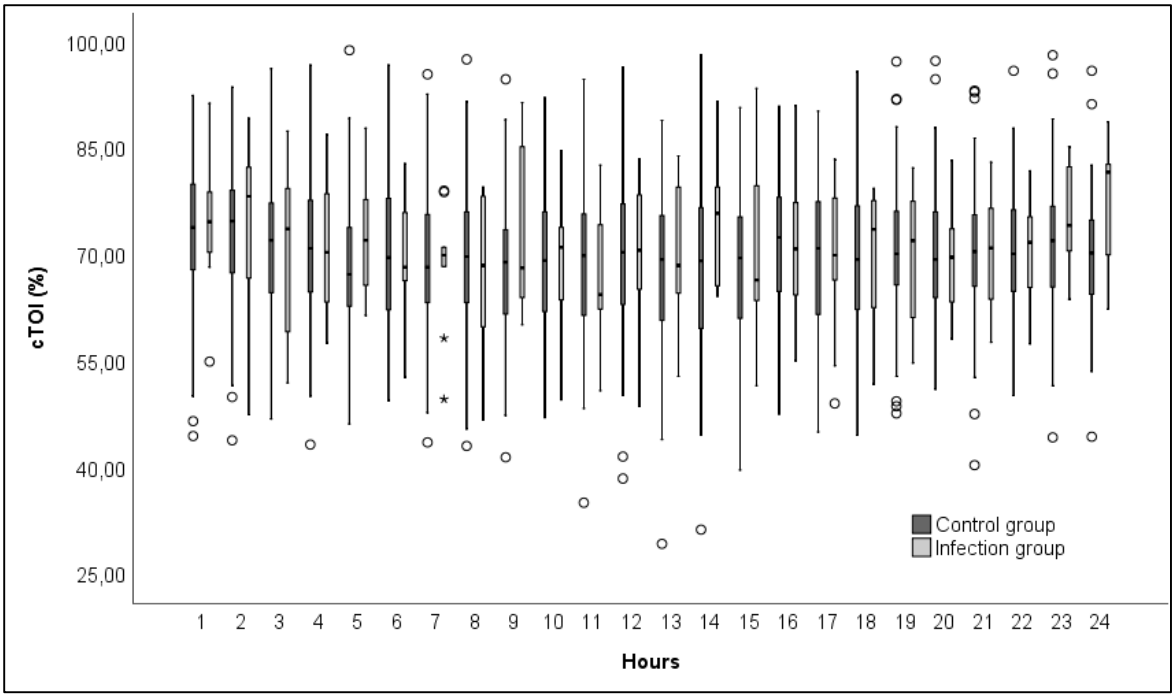

Figure 16: *cTOI values of the control and the infection group during the 24-hour measuring period. There were no significant differences found.*

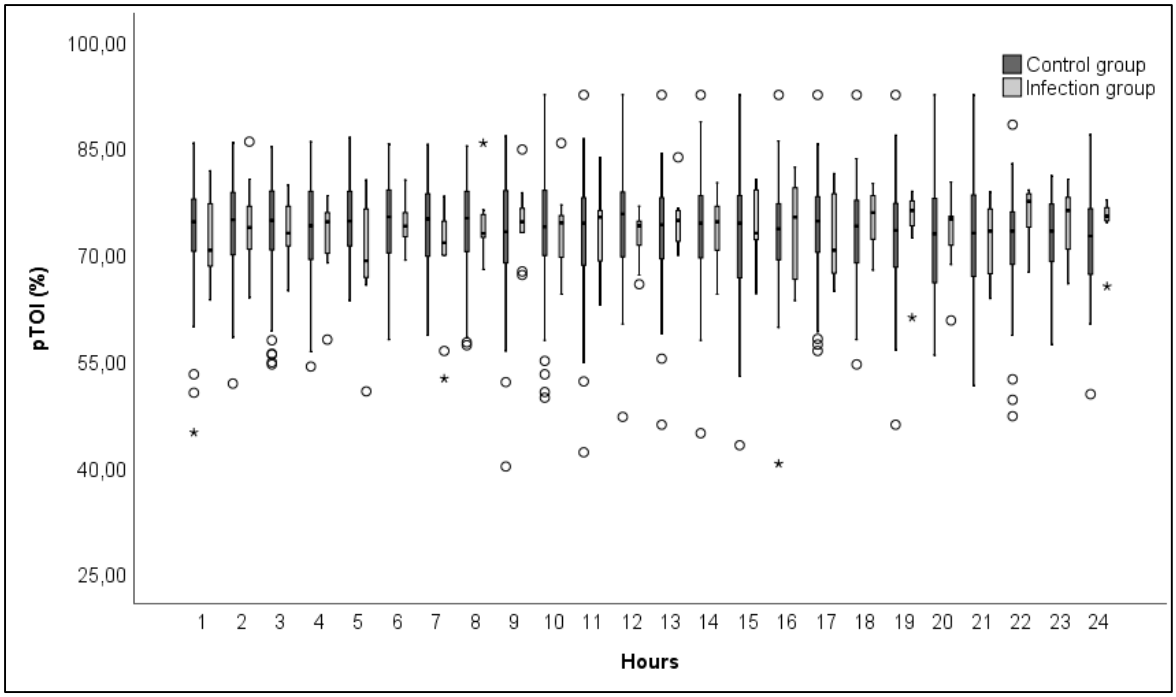

Figure 17: *pTOI values of the control and the infection group during the 24–hour measuring period. There were no significant differences found.*

Mean values of cTOI (control group: 70.1 ± 1.4% vs. infection group: 71.2 ± 2.6%; p=0.079) and pTOI (73.4 ± 0.9% vs. 73.6 ± 1.4%; p=0.564) of the 24-hours measuring period did not differ between groups.

4.4 Secondary outcome parameters

4.4.1 HR

HR values over the 24-hour measuring period did not show any significant difference between groups (Table 7).

Hour	HR (bpm)		p-value
	Control group	Infection group	
1	148 ± 13	155 ± 15	1.000
2	147 ± 13	155 ± 16	1.000
3	145 ± 14	148 ± 15	1.000
4	143 ± 13	147 ± 12	1.000
5	143 ± 12	146 ± 9	1.000
6	142 ± 13	147 ± 7	1.000
7	141 ± 12	142 ± 10	1.000
8	141 ± 11	143 ± 12	1.000
9	143 ± 12	144 ± 10	1.000
10	144 ± 12	141 ± 12	1.000
11	142 ± 12	141 ± 12	1.000
12	141 ± 12	141 ± 13	1.000
13	140 ± 12	142 ± 14	1.000
14	142 ± 12	144 ± 15	1.000
15	141 ± 12	143 ± 15	1.000
16	140 ± 12	144 ± 13	1.000
17	141 ± 12	147 ± 11	1.000
18	141 ± 12	144 ± 9	1.000
19	141 ± 10	143 ± 13	1.000
20	142 ± 11	143 ± 11	1.000
21	141 ± 11	145 ± 10	1.000
22	141 ± 11	145 ± 12	1.000
23	141 ± 11	147 ± 15	1.000
24	142 ± 12	145 ± 12	1.000

Table 7: *HR values of the control and the infection group during the 24–hour measuring period. Data are presented as mean ± SD. There were no significant differences found.*

Figure 18 gives a graphical presentation of the HR changes over 24 hours.

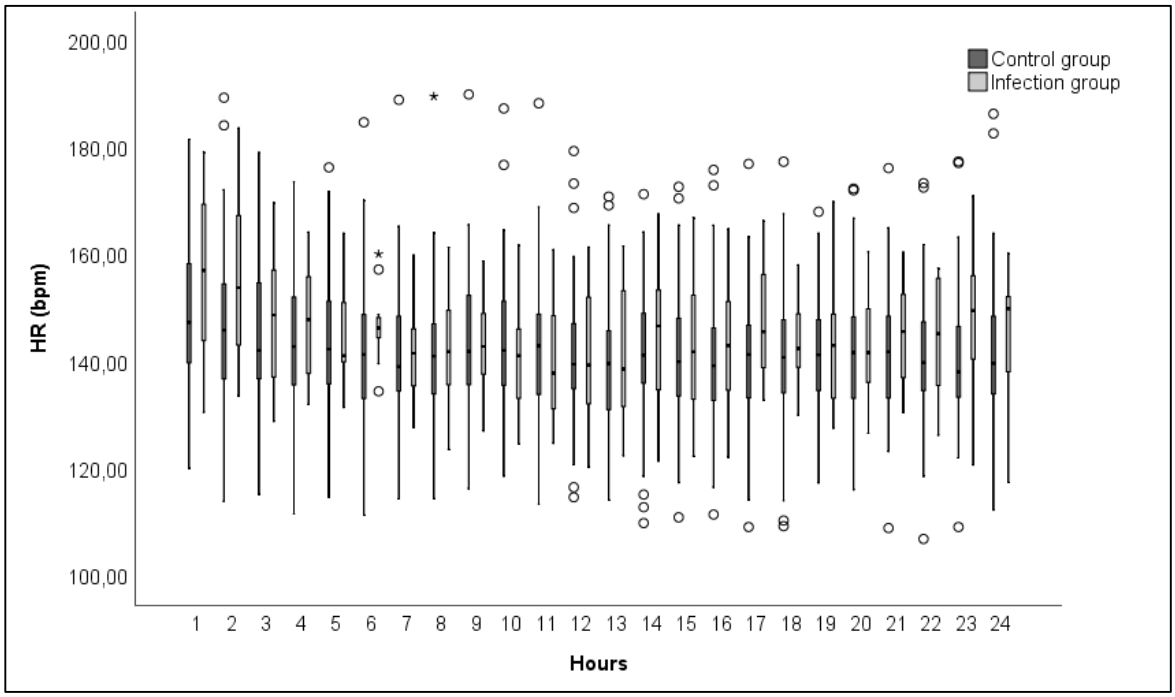

Figure 18: *HR values of the control and the infection group during the 24–hour measuring period. There were no significant differences found.*

Regarding the mean value of HR during 24 hours, the value of the infection group was significantly higher compared to the control group (145 ± 4 bpm vs. 142 ± 2 bpm; $p<0.001$).

4.4.2 SpO_2

Over the 24-hour measuring period there were no significant differences in SpO_2 values of the infection group compared the control group (Table 8).

Hour	SpO_2 (%)		p-value
	Control group	Infection group	
1	96 ± 3	94 ± 4	0.750
2	96 ± 2	94 ± 4	1.000
3	96 ± 2	94 ± 4	1.000
4	96 ± 2	95 ± 3	1.000
5	96 ± 3	95 ± 3	0.960
6	96 ± 3	95 ± 3	1.000
7	96 ± 2	95 ± 3	1.000
8	96 ± 3	95 ± 3	1.000
9	96 ± 3	96 ± 3	1.000
10	96 ± 3	96 ± 3	1.000
11	96 ± 3	96 ± 3	1.000
12	96 ± 3	96 ± 3	1.000
13	96 ± 3	95 ± 3	1.000
14	96 ± 3	96 ± 3	1.000
15	96 ± 3	96 ± 3	1.000
16	96 ± 2	95 ± 4	1.000
17	96 ± 3	95 ± 3	1.000
18	96 ± 2	95 ± 4	1.000
19	96 ± 3	95 ± 3	1.000
20	96 ± 3	95 ± 3	1.000
21	96 ± 2	96 ± 3	1.000
22	96 ± 3	95 ± 3	1.000
23	96 ± 3	95 ± 3	1.000
24	96 ± 3	95 ± 3	1.000

Table 8: *SpO_2 values of the control and the infection group during the 24–hour measuring period. Data are presented as mean ± SD. There were no significant differences found.*

Figure 19 gives a graphical presentation of the SpO_2 changes over 24 hours.

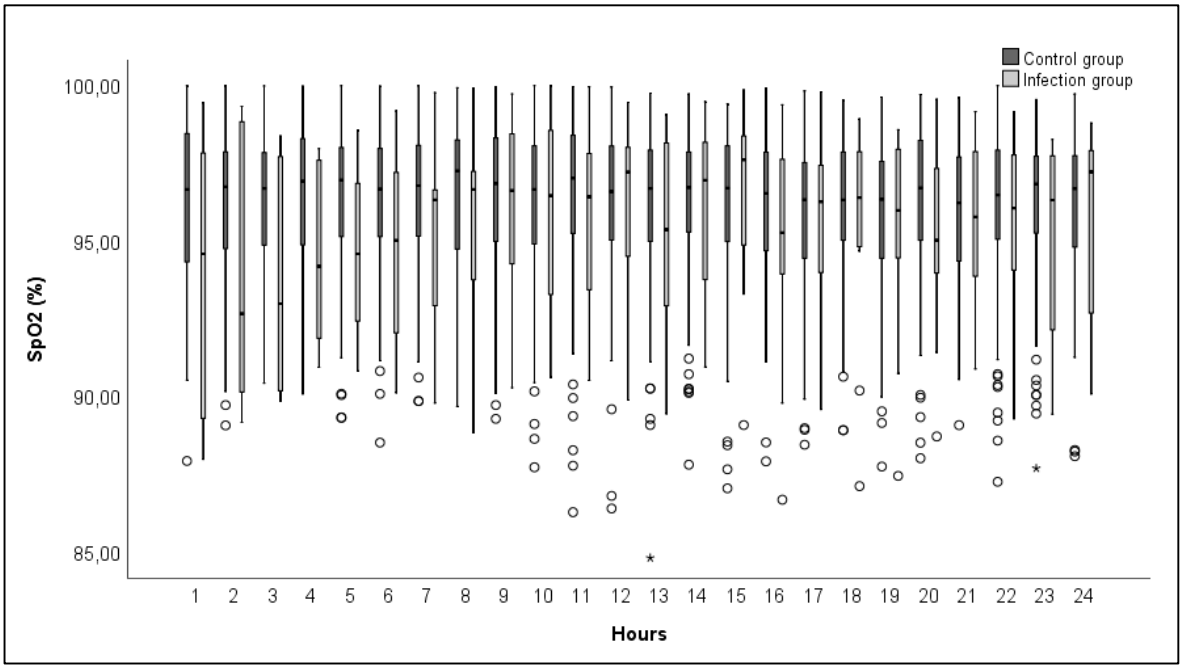

Figure 19: *SpO_2 values of the control and the infection group during the 24–hour measuring period. There were no significant differences found.*

Mean SpO_2 during 24 hours was significantly lower in the infection group compared to the controls (95 ± 1% vs. 96 ± 0%; $p<0.001$).

4.4.3 MABP

MABP values did not show any significant difference between both groups over the 24-hour measuring period (Table 9).

Hour	MABP (mmHg)		p-value
	Control group	Infection group	
1	38.9 ± 6.1	40.6 ± 7.7	1.000
2	39.6 ± 6.4	40.8 ± 5.1	1.000
3	39.8 ± 6.6	38.6 ± 3.9	1.000
4	40.8 ± 7.3	41.4 ± 3.4	1.000
5	41.8 ± 6.2	42.5 ± 4.8	1.000
6	42.5 ± 7.5	46.3 ± 4.5	0.720
7	43.4 ± 6.8	44.2 ± 5.7	1.000
8	43.6 ± 7.7	44.0 ± 8.6	1.000
9	44.2 ± 6.8	42.9 ± 6.2	1.000
10	44.2 ± 7.3	46.7 ± 9.6	1.000
11	44.8 ± 7.5	43.5 ± 6.2	1.000
12	44.8 ± 7.2	45.1 ± 5.5	1.000
13	44.0 ± 6.0	44.8 ± 5.9	1.000
14	44.8 ± 7.5	46.5 ± 8.1	1.000
15	45.4 ± 6.4	48.6 ± 10.6	1.000
16	44.6 ± 6.6	45.6 ± 7.0	1.000
17	45.2 ± 7.7	46.8 ± 8.9	1.000
18	45.1 ± 7.2	45.1 ± 8.2	1.000
19	44.4 ± 6.6	46.1 ± 6.7	1.000
20	44.8 ± 6.7	45.8 ± 8.4	1.000
21	44.5 ± 6.1	46.3 ± 9.8	1.000
22	44.0 ± 6.3	44.3 ± 7.6	1.000
23	45.3 ± 7.5	43.6 ± 5.5	1.000
24	43.3 ± 7.2	42.1 ± 6.6	1.000

Table 9: *MABP values of the control and the infection group during the 24–hour measuring period. Data are presented as mean ± SD. There were no significant differences found.*

Figure 20 gives a graphical presentation of the MABP changes over 24 hours.

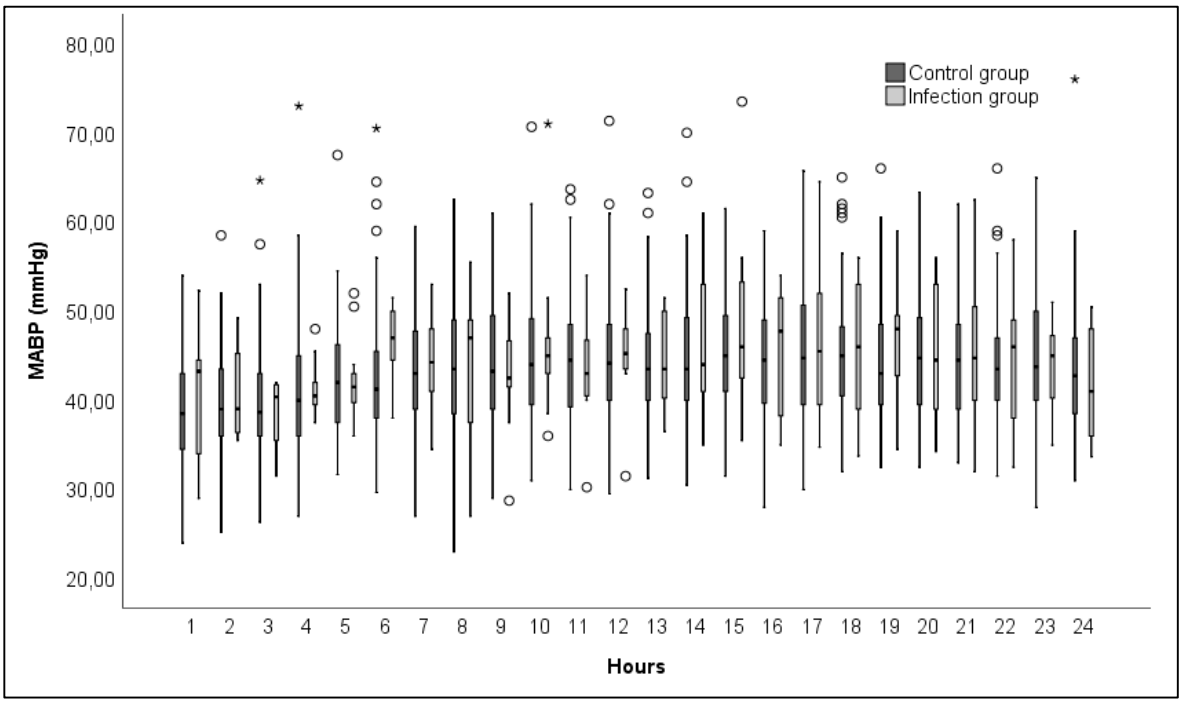

Figure 20: *MABP values of the control and the infection group during the 24–hour measuring period. There were no significant differences found.*

Mean MABP did not differ between groups during 24 hours (control group: 43.5 ± 1.9mmHg vs. infection group: 44.1 ± 2.5mmHg; p=0.208).

4.4.4 Rectal temperature

Rectal temperature values did not show any significant difference between the infection and the control group over the 24-hour measuring period (Table 10).

Hour	Rectal temperature (°C)		p-value
	Control group	Infection group	
1	37.0 ± 0.4	37.5 ± 0.3	0.887
2	37.1 ± 0.3	37.3 ± 0.1	1.000
3	37.1 ± 0.3	36.9 ± 0.5	1.000
4	37.0 ± 0.3	-	-
5	37.3 ± 0.3	37.2 ± 0.5	1.000
6	37.1 ± 0.4	-	-
7	37.1 ± 0.3	36.9 ± 0.2	1.000
8	37.1 ± 0.3	36.9 ± 0.1	1.000
9	37.1 ± 0.3	37.1 ± 0.5	1.000
10	37.0 ± 0.3	37.2 ± 0.0	1.000
11	37.2 ± 0.4	37.2 ± 0.3	1.000
12	37.0 ± 0.3	-	-
13	37.1 ± 0.3	37.0 ± 0.1	1.000
14	37.2 ± 0.4	-	-
15	37.1 ± 0.3	37.0 ± 0.3	1.000
16	37.1 ± 0.2	37.0 ± 0.1	1.000
17	37.1 ± 0.2	-	-
18	37.1 ± 0.4	37.5 ± 0.7	1.000
19	37.1 ± 0.4	37.3 ± 0.4	1.000
20	37.1 ± 0.3	-	-
21	37.2 ± 0.3	36.9 ± 0.2	1.000
22	37.1 ± 0.2	37.1 ± 0.5	1.000
23	37.0 ± 0.2	37.2 ± 0.1	1.000
24	37.1 ± 0.2	-	-

Table 10: *Rectal temperature values of the control and the infection group during the 24–hour measuring period. Data are presented as mean ± SD. There were no significant differences found.*

Figure 21 gives a graphical presentation of the rectal temperature changes over 24 hours.

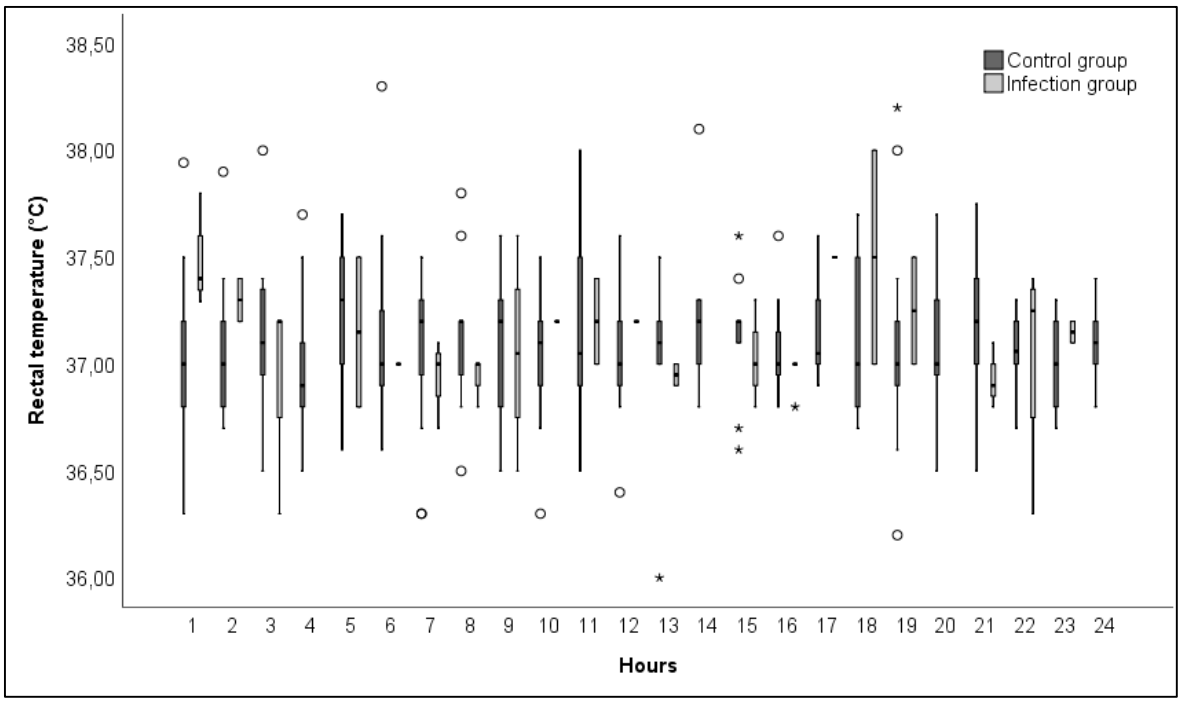

Figure 21: *Rectal temperature values of the control and the infection group during the 24–hour measuring period. There were no significant differences found.*

Mean rectal temperature did not differ between groups during 24 hours (control group: 37.1 ± 0.1°C vs. infection group: 37.2 ± 0.2°C; p=0.460).

4.4.5 Peripheral temperature

Peripheral temperature values over the 24-hour measuring period did not show any significant difference between groups (Table 11).

Hour	Peripheral temperature (°C)		p-value
	Control group	Infection group	
1	37.1 ± 0.4	37.2 ± 0.4	1.000
2	37.1 ± 0.3	37.3 ± 0.1	1.000
3	37.2 ± 0.3	36.9 ± 0.8	1.000
4	37.1 ± 0.3	-	-
5	37.2 ± 0.3	36.9 ± 0.6	1.000
6	37.1 ± 0.5	-	-
7	37.0 ± 0.7	37.0 ± 0.6	1.000
8	37.2 ± 0.3	-	-
9	37.1 ± 0.4	36.7 ± 0.3	1.000
10	37.0 ± 0.4	37.3 ± 0.2	1.000
11	37.2 ± 0.4	37.2 ± 0.4	1.000
12	37.1 ± 0.3	-	-
13	37.1 ± 0.2	37.4 ± 0.5	1.000
14	37.0 ± 0.3	-	-
15	37.1 ± 0.2	36.8 ± 0.0	1.000
16	37.0 ± 0.3	37.2 ± 0.2	1.000
17	37.2 ± 0.3	37.0 ± 0.0	1.000
18	37.1 ± 0.4	36.8 ± 0.1	1.000
19	37.0 ± 0.3	37.0 ± 0.4	1.000
20	37.1 ± 0.3	-	-
21	37.1 ± 0.3	-	-
22	36.9 ± 0.4	37.1 ± 0.3	1.000
23	37.1 ± 0.3	-	-
24	37.1 ± 0.2	37.3 ± 0.4	1.000

Table 11: *Peripheral temperature values of the control and the infection group during the 24–hour measuring period. Data are presented as mean ± SD. There were no significant differences found.*

Figure 22 gives a graphical presentation of the peripheral temperature changes over 24 hours.

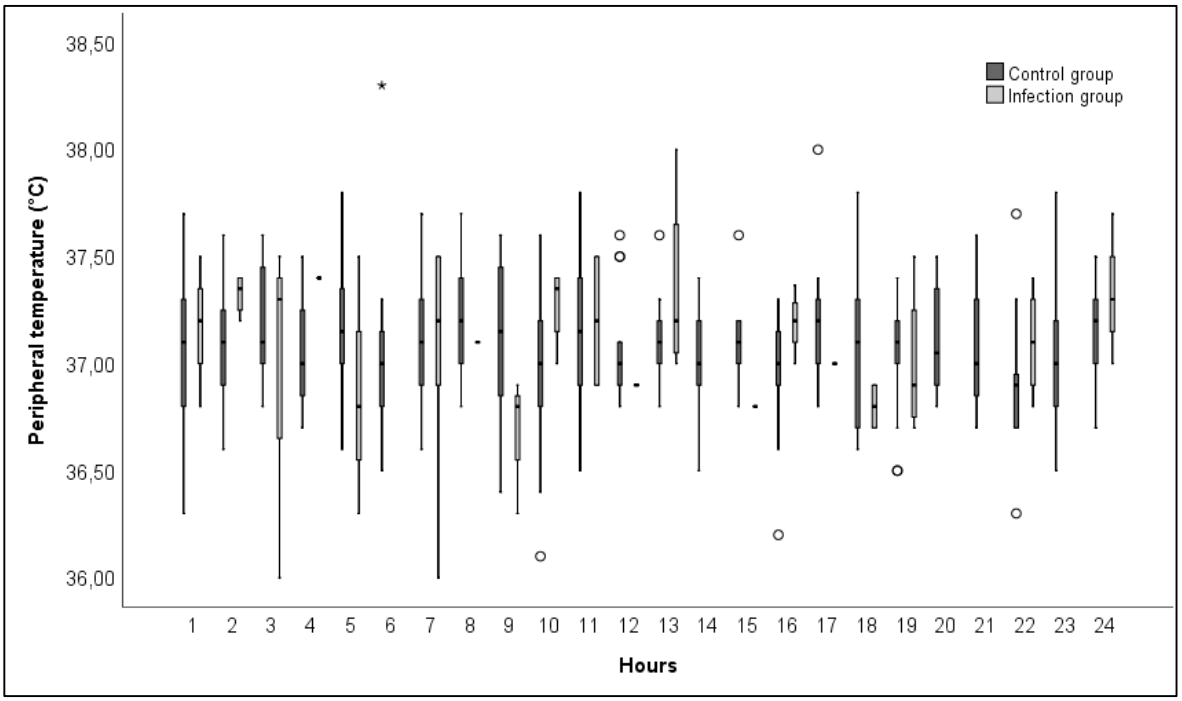

Figure 22: *Peripheral temperature values of the control and the infection group during the 24–hour measuring period. There were no significant differences found.*

Mean peripheral temperature did not differ between groups during 24 hours (control group: 37.1 ± 0.1°C vs. infection group: 37.0 ± 0.2°C; p=0.929).

4.4.6 Correlation between cardio-circulatory / physiological parameters and cTOI / pTOI ratio

4.4.6.1 HR

In the control group HR increased significantly with increasing cTOI / pTOI ratio (r=0.25; p=0.022) (Figure 23).

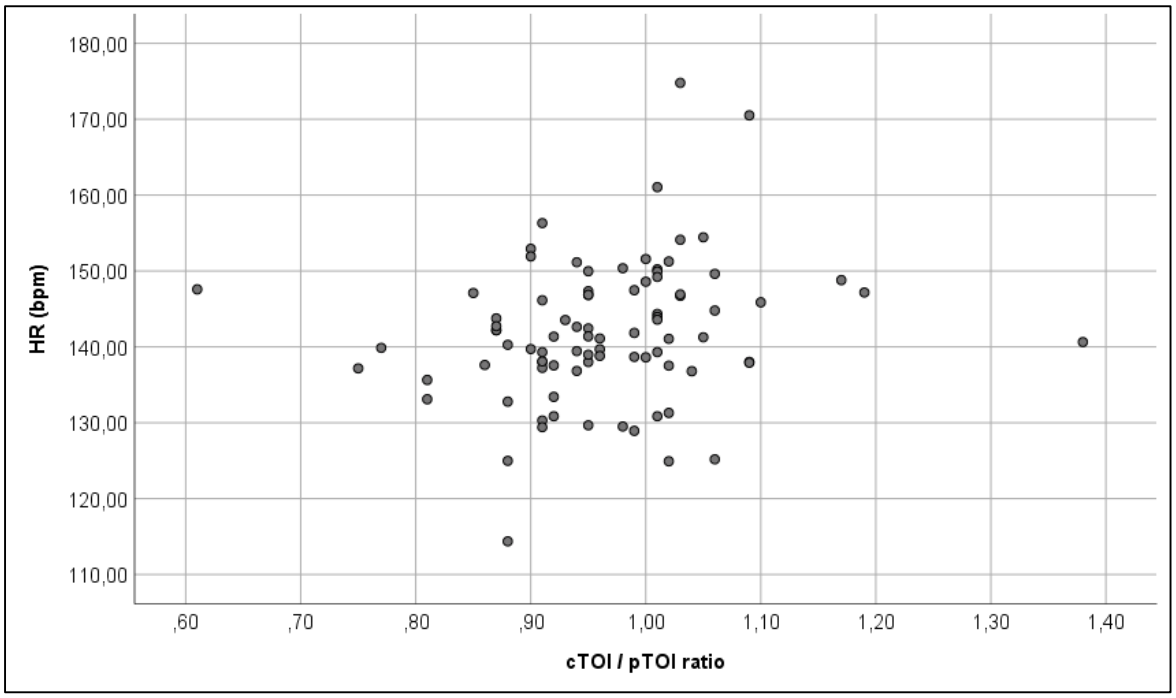

Figure 23: *Correlation between HR and cTOI / pTOI ratio of the control group (r=0.25; p=0.022).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and HR (Figure 24).

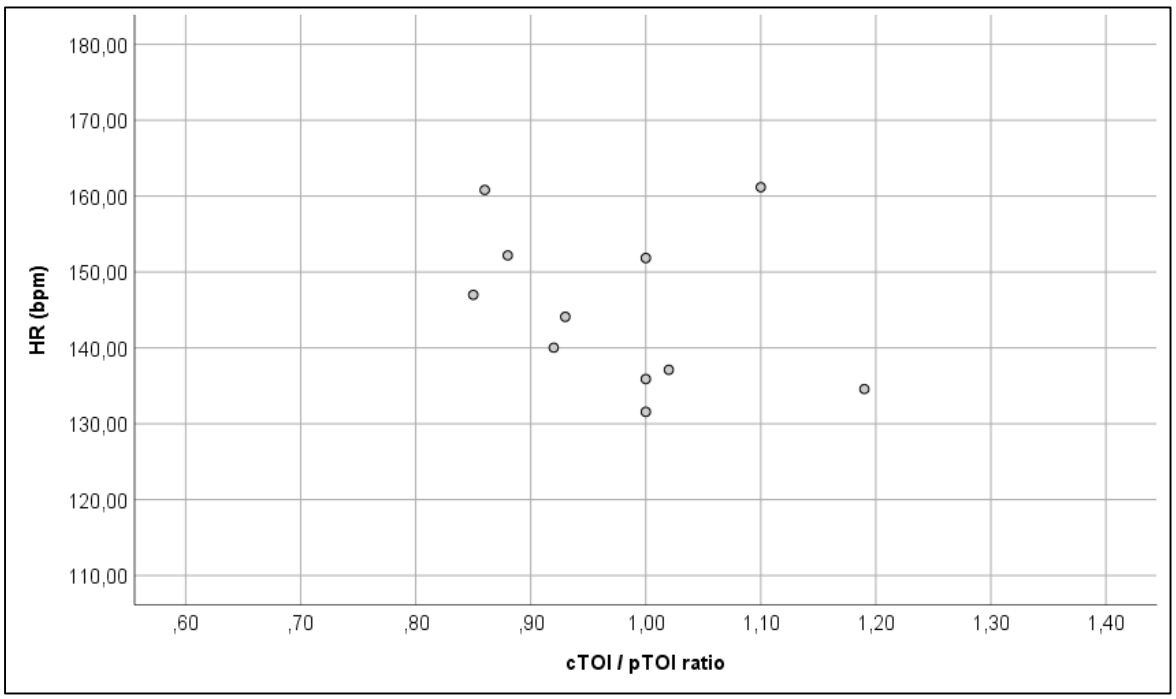

Figure 24: *Correlation between HR and cTOI / pTOI ratio of the infection group (r= -0.37; p=0.267).*

4.4.6.2 SpO_2

In the control group there was no significant correlation between cTOI / pTOI ratio and SpO_2 (Figure 25).

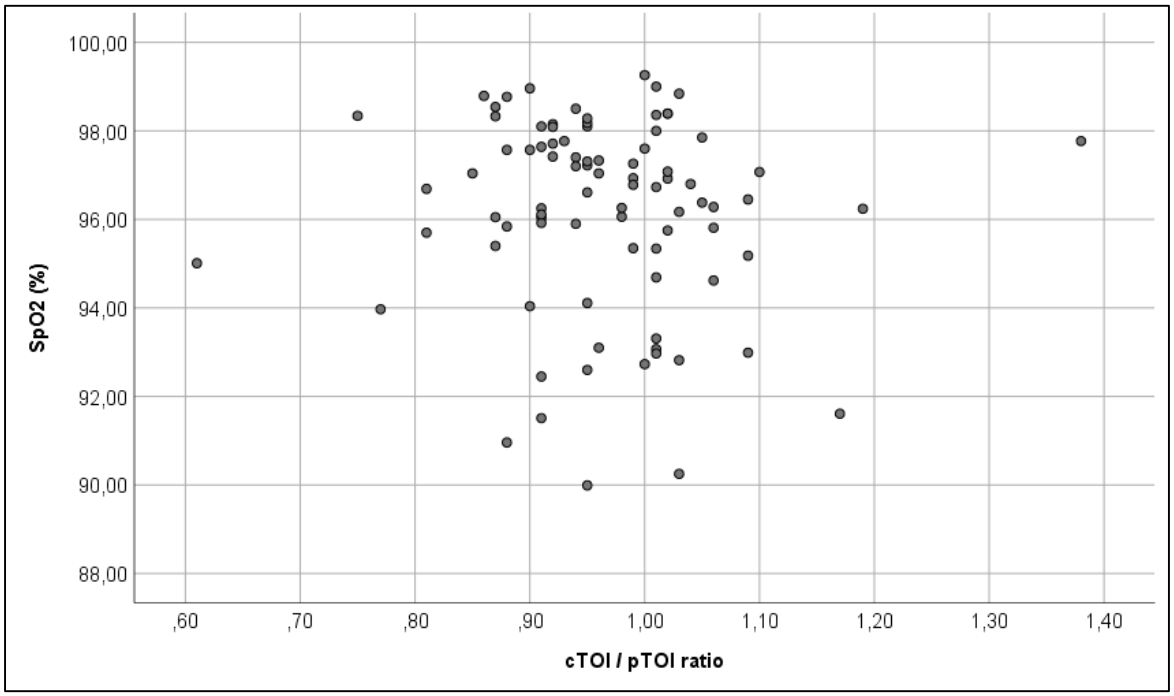

Figure 25: *Correlation between SpO_2 and cTOI / pTOI ratio of the control group (r= -0.12; p=0.251).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and SpO_2 (Figure 26).

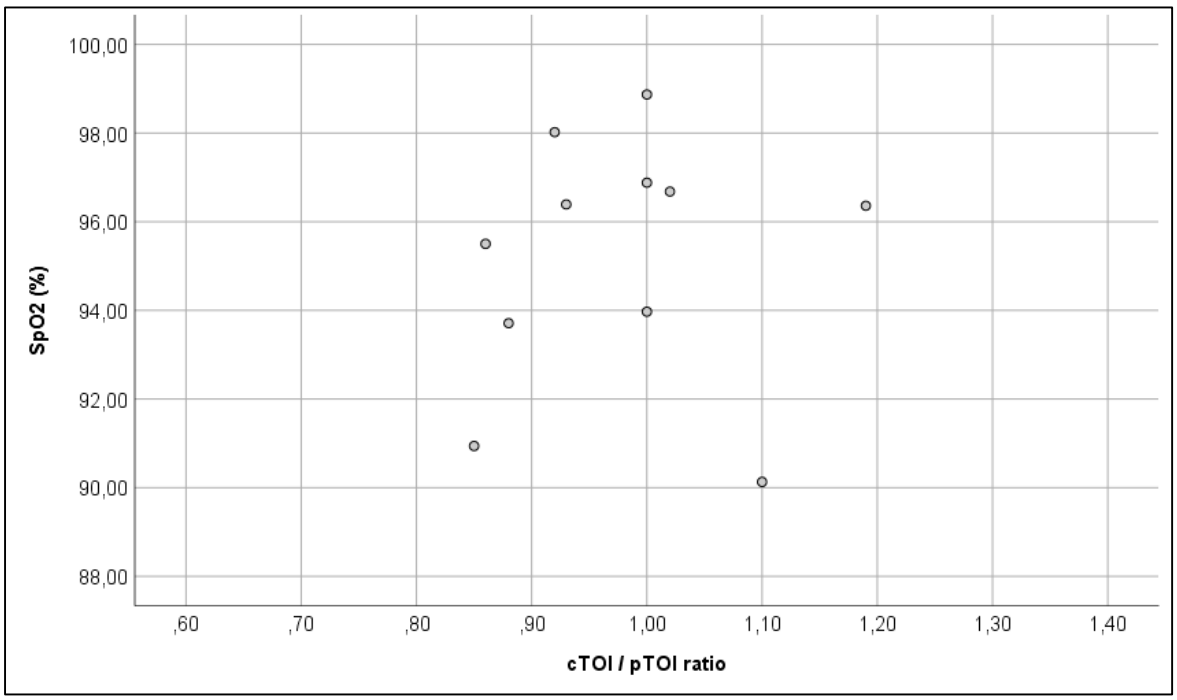

Figure 26: *Correlation between SpO_2 and cTOI / pTOI ratio of the infection group (r=0.15; p=0.667).*

4.4.6.3 MABP

In the control group there was no significant correlation between cTOI / pTOI ratio and MABP (Figure 27).

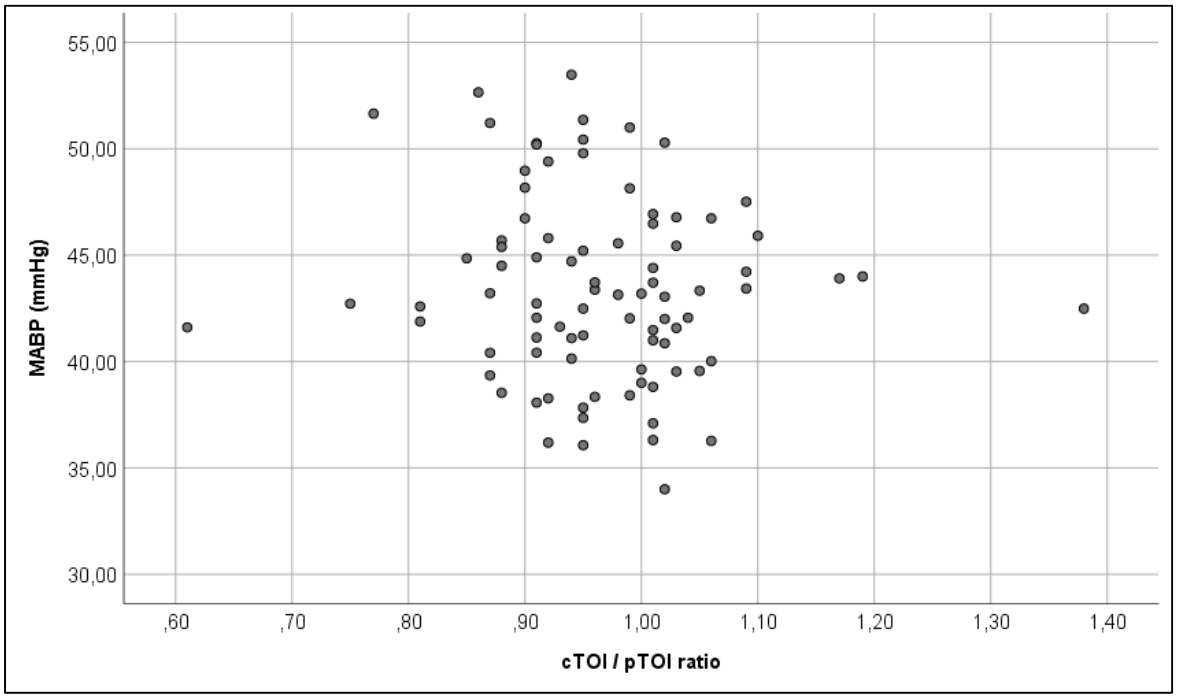

Figure 27: *Correlation between MABP and cTOI / pTOI ratio of the control group (r= -0.12; p=0.274).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and MABP (Figure 28).

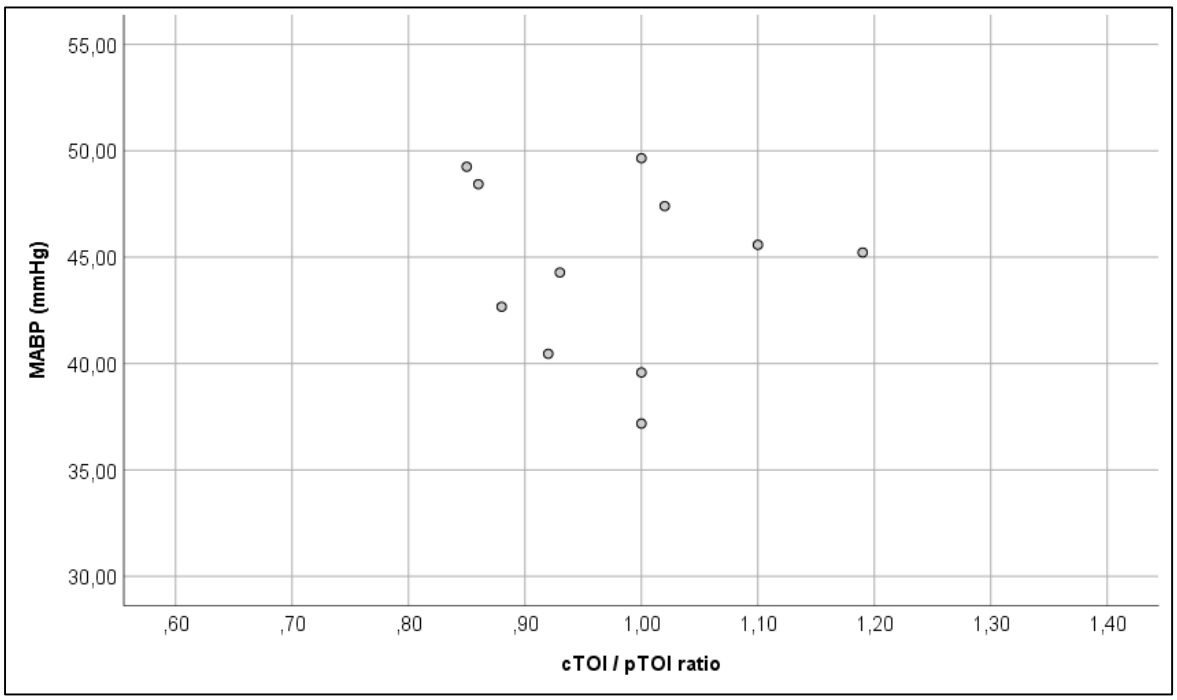

Figure 28: *Correlation between MABP and cTOI / pTOI ratio of the infection group (r= -0.12; p=0.727).*

4.4.6.4 Rectal temperature

In the control group there was no significant correlation between cTOI / pTOI ratio and rectal temperature (Figure 29).

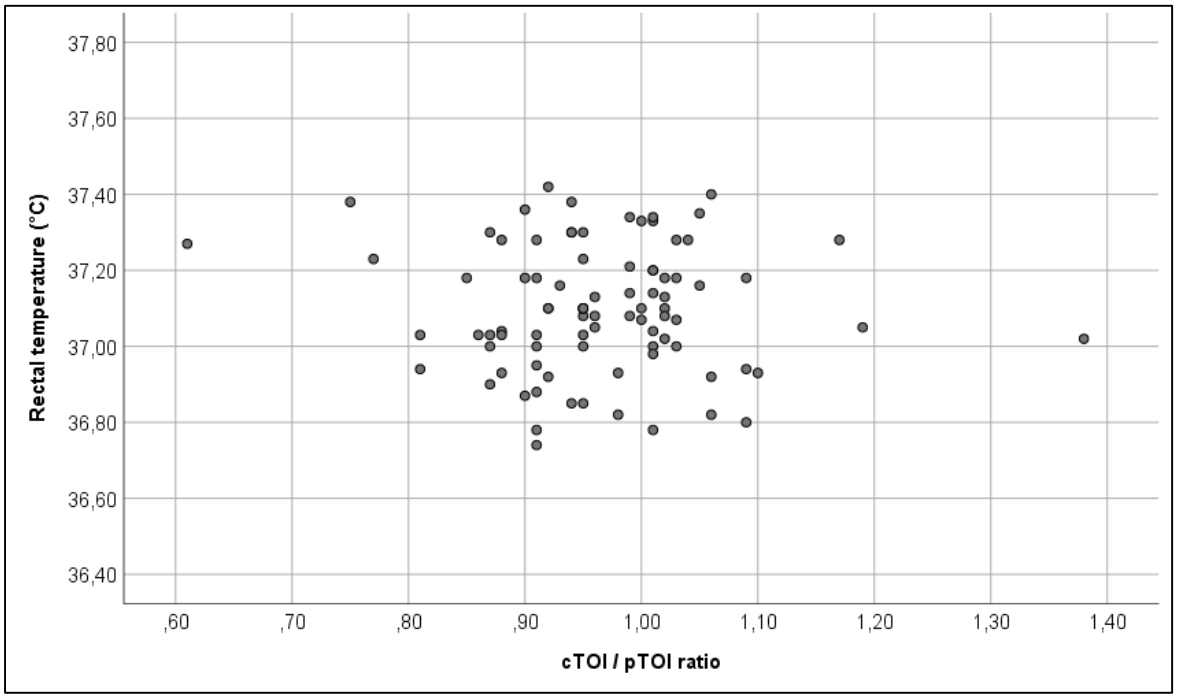

Figure 29: *Correlation between rectal temperature and cTOI / pTOI ratio of the control group (r=0.02; p=0.856).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and rectal temperature (Figure 30).

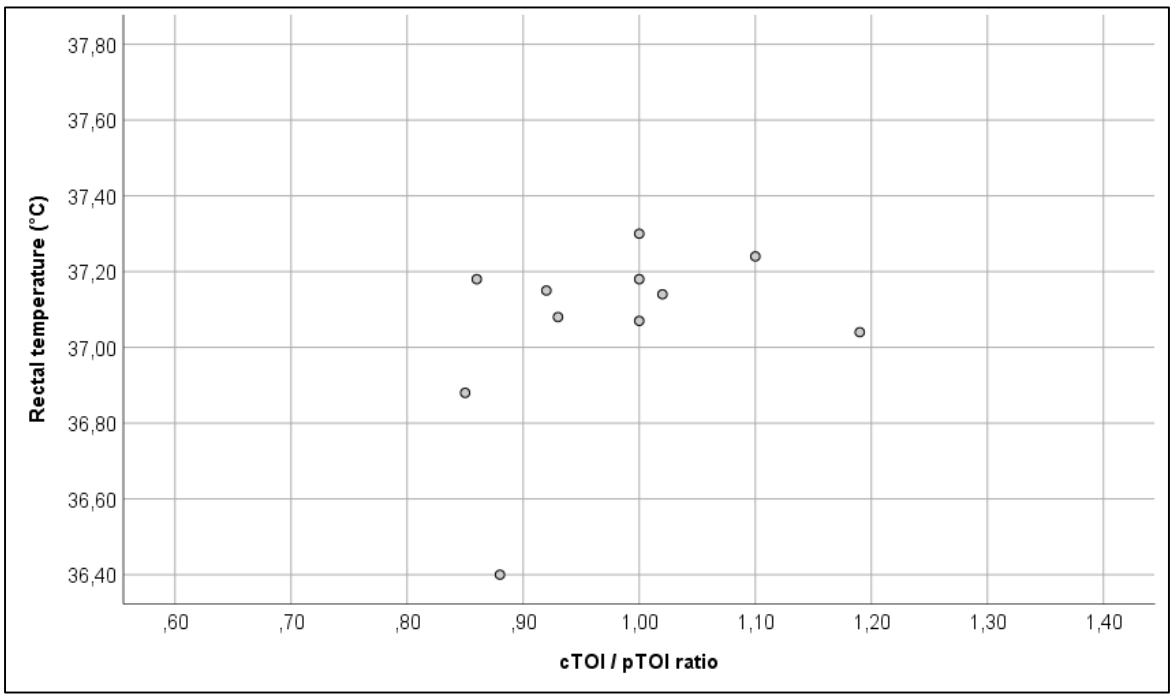

Figure 30: *Correlation between rectal temperature and cTOI / pTOI ratio of the infection group (r=0.28; p=0.404).*

4.4.6.5 Peripheral temperature

In the control group there was no significant correlation between cTOI / pTOI ratio and peripheral temperature (Figure 31).

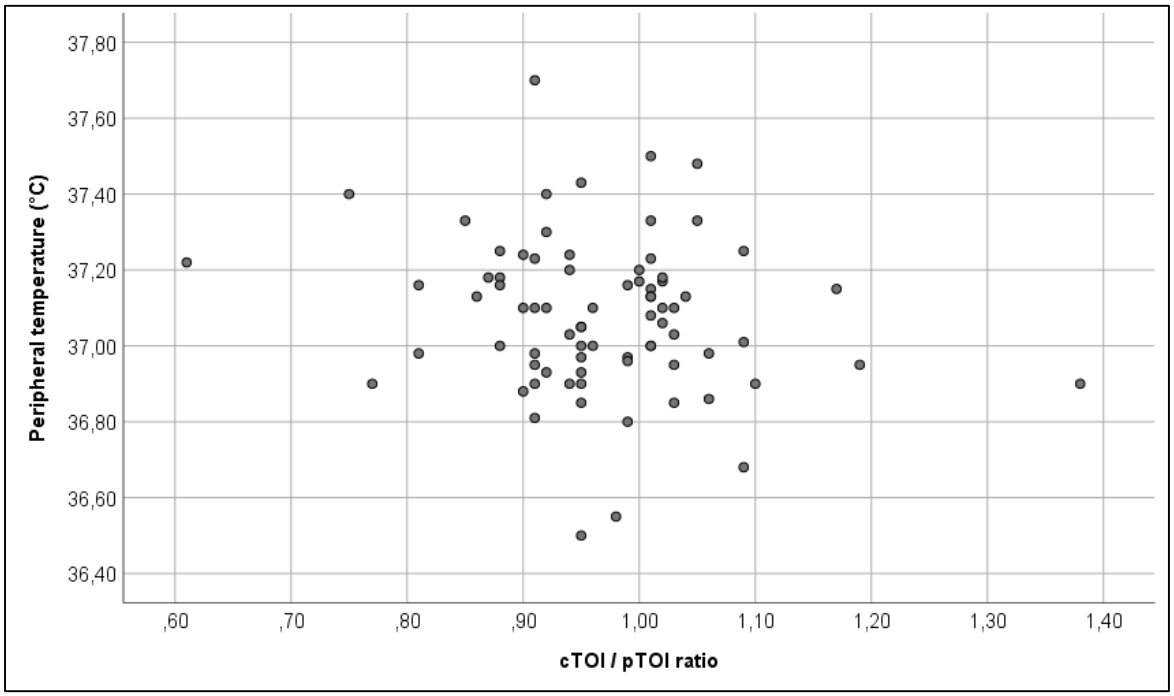

Figure 31: *Correlation between peripheral temperature and cTOI / pTOI ratio of the control group (r= -0.14; p=0.214).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and peripheral temperature (Figure 32).

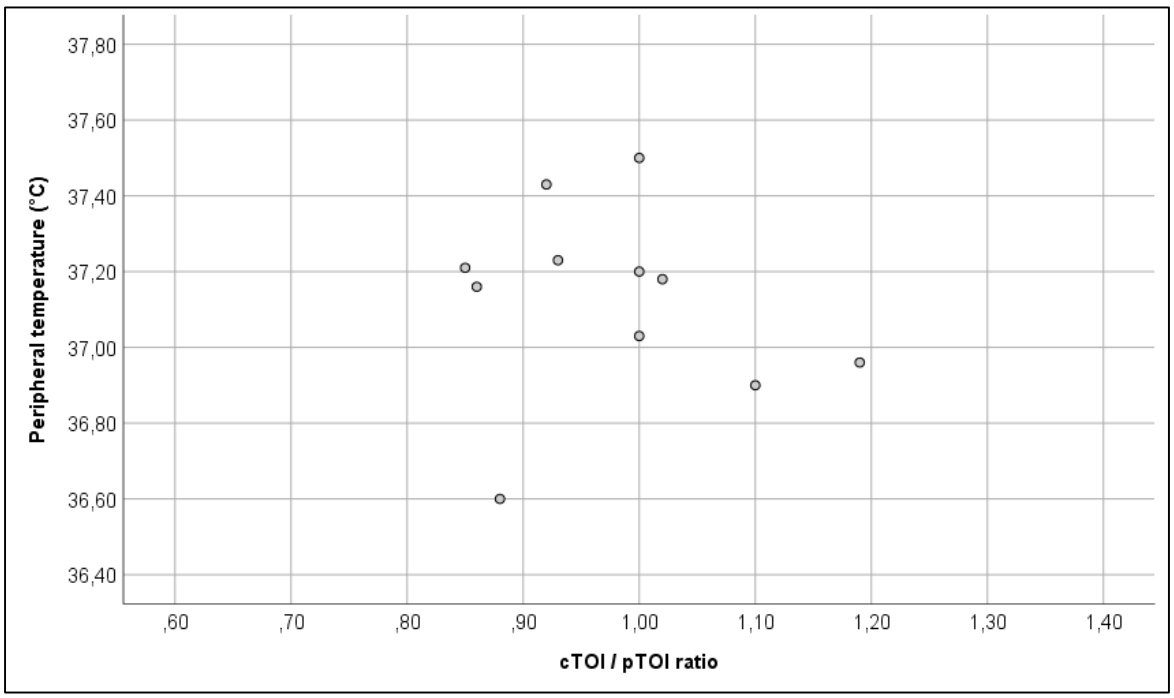

Figure 32: *Correlation between peripheral temperature and cTOI / pTOI ratio of the infection group (r= -0.27; p=0.429).*

4.4.7 Correlation between laboratory inflammatory parameters and cTOI / pTOI ratio

4.4.7.1 IL-6

In the control group there was no significant correlation between cTOI / pTOI ratio and IL-6 (Figure 33).

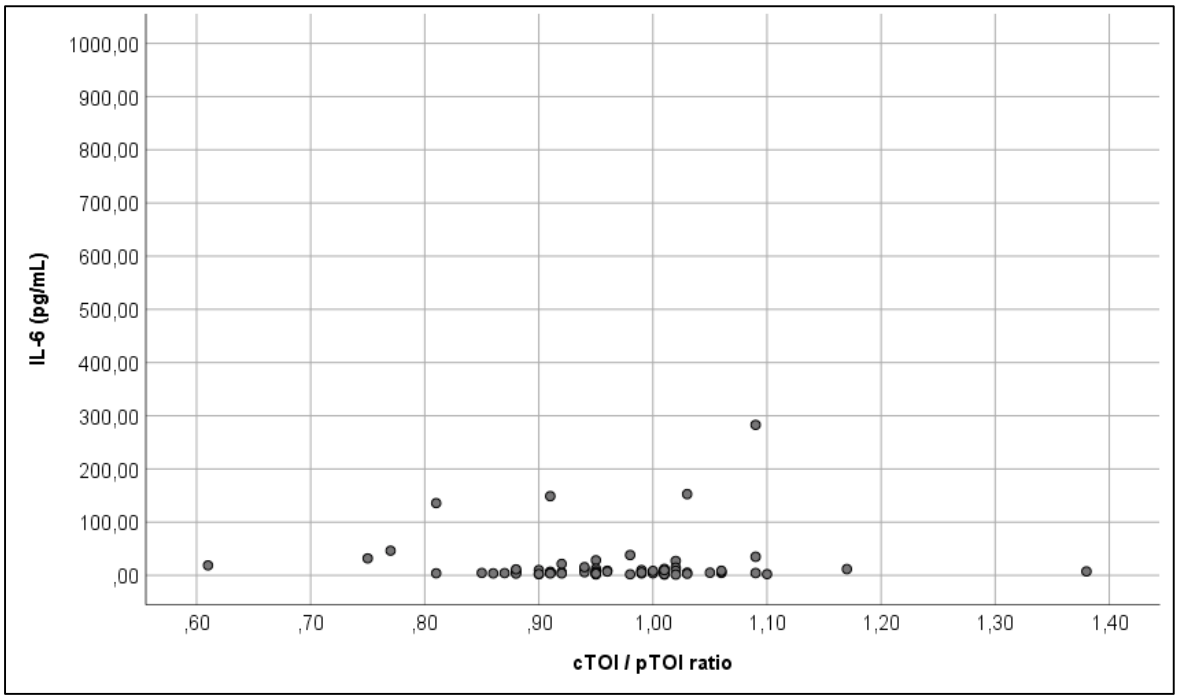

Figure 33: *Correlation between IL-6 and cTOI / pTOI ratio of the control group (r= 0.00; p=0.989).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and IL-6 (Figure 34).

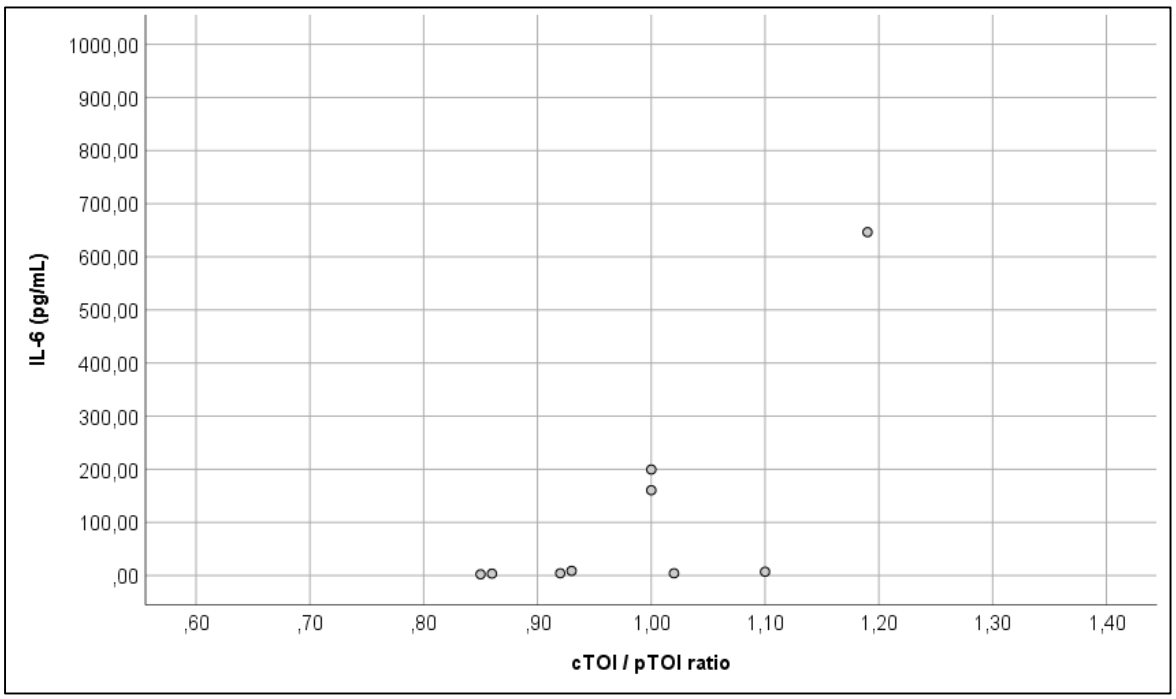

Figure 34: *Correlation between IL-6 and cTOI / pTOI ratio of the infection group (r= 0.38; p=0.253).*

4.4.7.2 PCT

In the control group there was no significant correlation between cTOI / pTOI ratio and PCT (Figure 35).

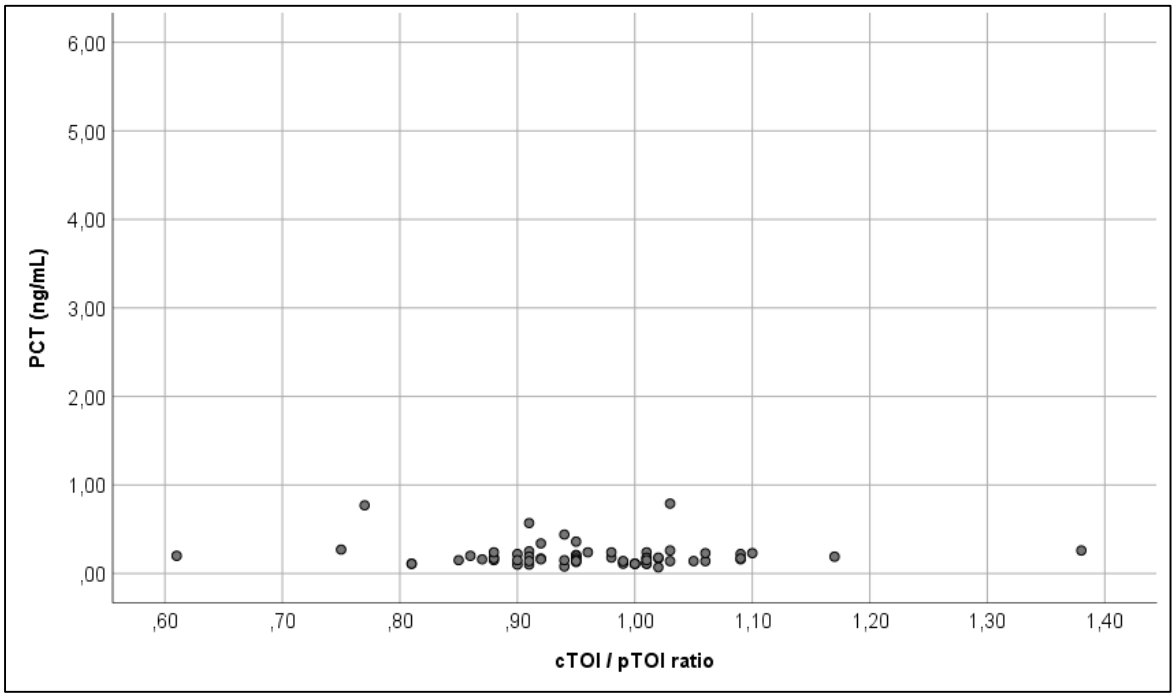

Figure 35: *Correlation between PCT and cTOI / pTOI ratio of the control group (r= -0.01; p=0.954).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and PCT (Figure 36).

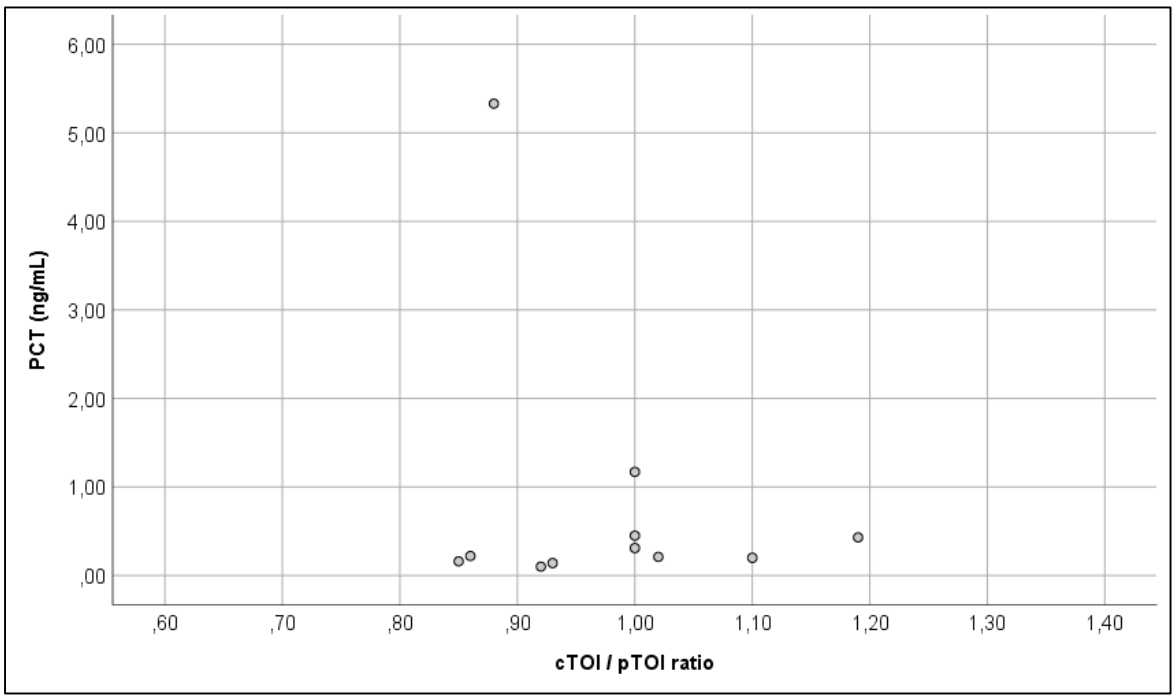

Figure 36: *Correlation between PCT and cTOI / pTOI ratio of the infection group (r=0.19; p=0.570).*

4.4.7.3 Leukocyte count of the first day after birth

In the control group there was no significant correlation between cTOI / pTOI ratio and the leukocyte count of the first day after birth (Figure 37).

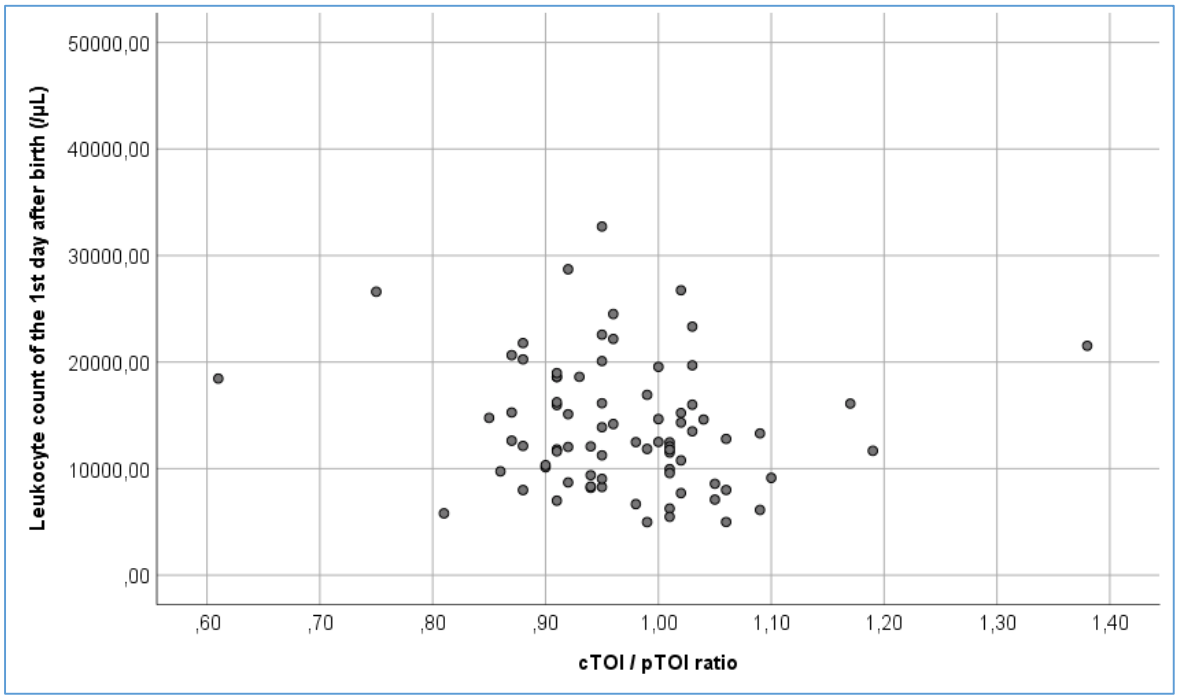

Figure 37: *Correlation between leukocyte count of the first day after birth and cTOI / pTOI ratio of the control group (r= -0.16; p=0.159).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and the leukocyte count of the first day after birth (Figure 38).

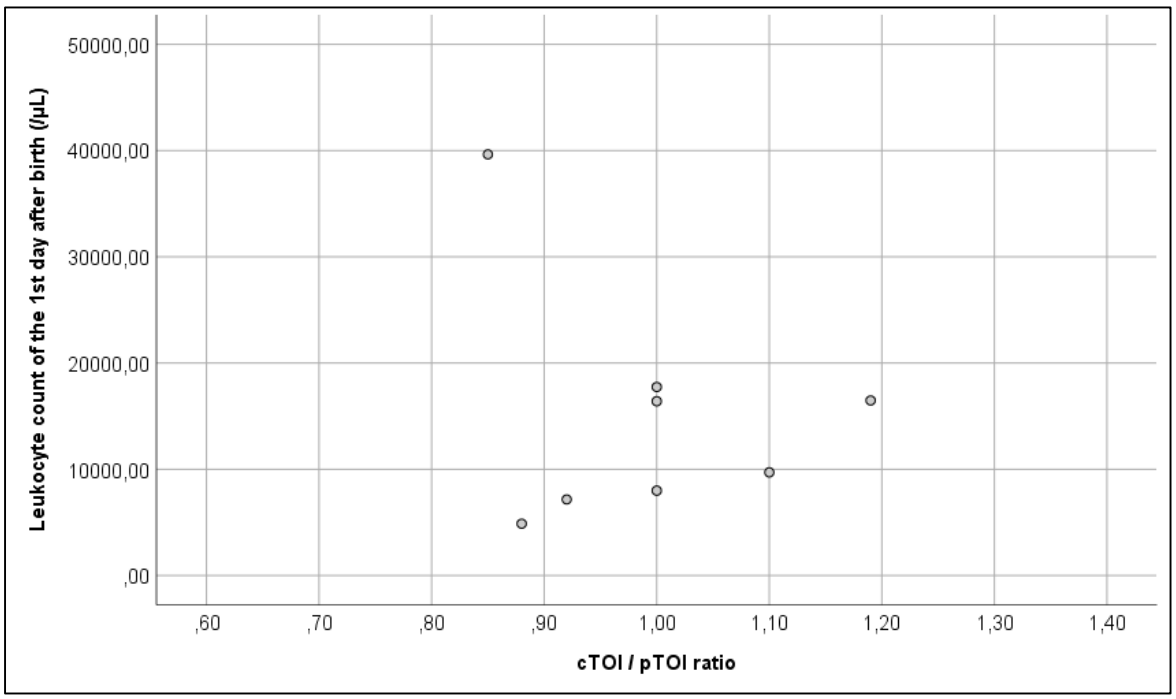

Figure 38: *Correlation between leukocyte count of the first day after birth and cTOI / pTOI ratio of the infection group (r=0.12; p=0.774).*

4.4.7.4 Leukocyte count of the second day after birth

In the control group there was no significant correlation between cTOI / pTOI ratio and the leukocyte count of the second day after birth (Figure 39).

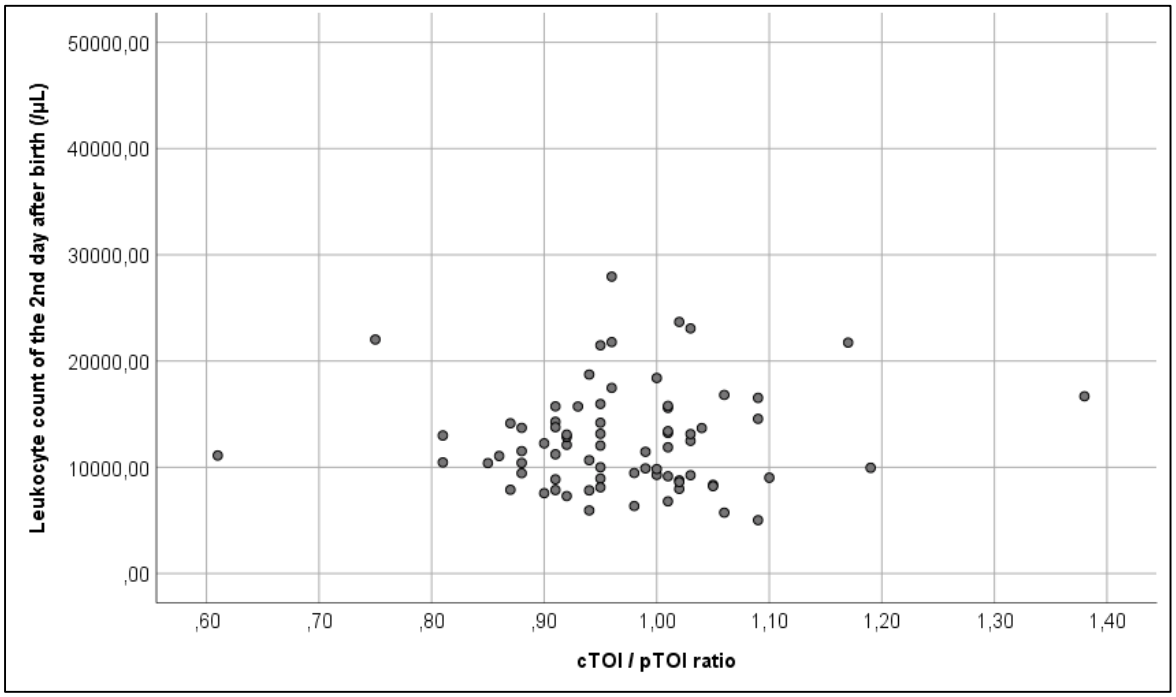

Figure 39: *Correlation between leukocyte count of the second day after birth and cTOI / pTOI ratio of the control group (r=0.03; p=0.809).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and the leukocyte count of the second day after birth (Figure 40).

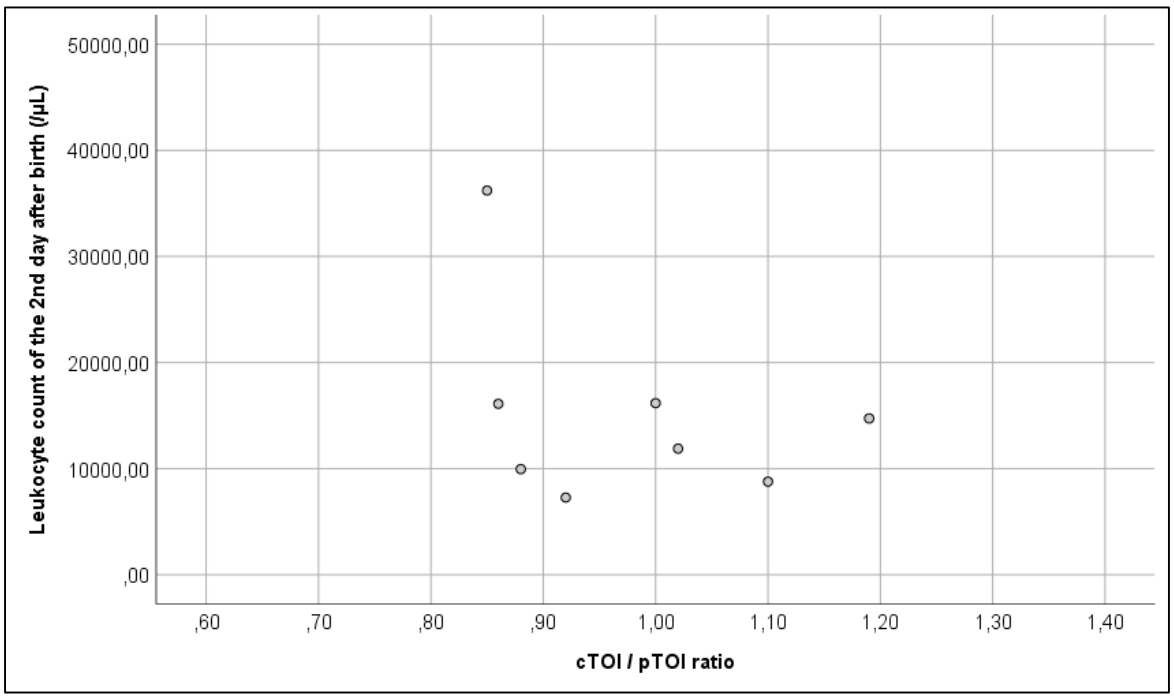

Figure 40: *Correlation between leukocyte count of the second day after birth and cTOI / pTOI ratio of the infection group (r= -0.38; p=0.352).*

4.4.7.5 CRP of the first day after birth

In the control group there was no significant correlation between cTOI / pTOI ratio and CRP of the first day after birth (Figure 41).

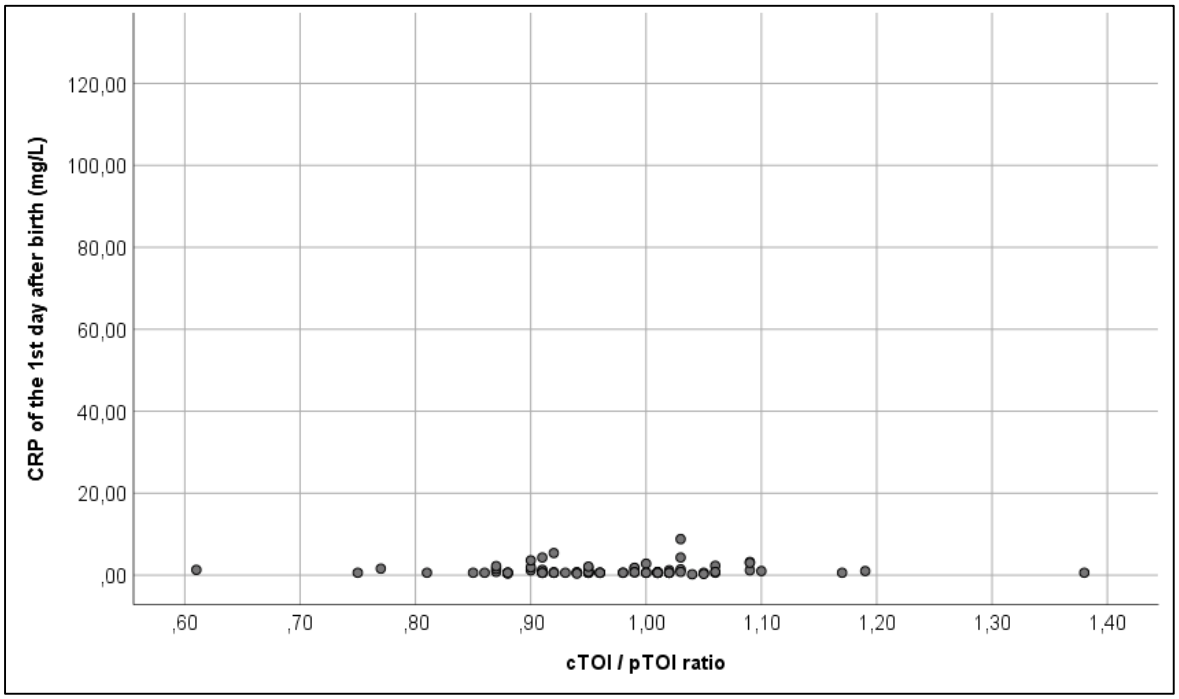

Figure 41: *Correlation between CRP of the first day after birth and cTOI / pTOI ratio of the control group (r=0.02; p=0.835).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and CRP of the first day after birth (Figure 42).

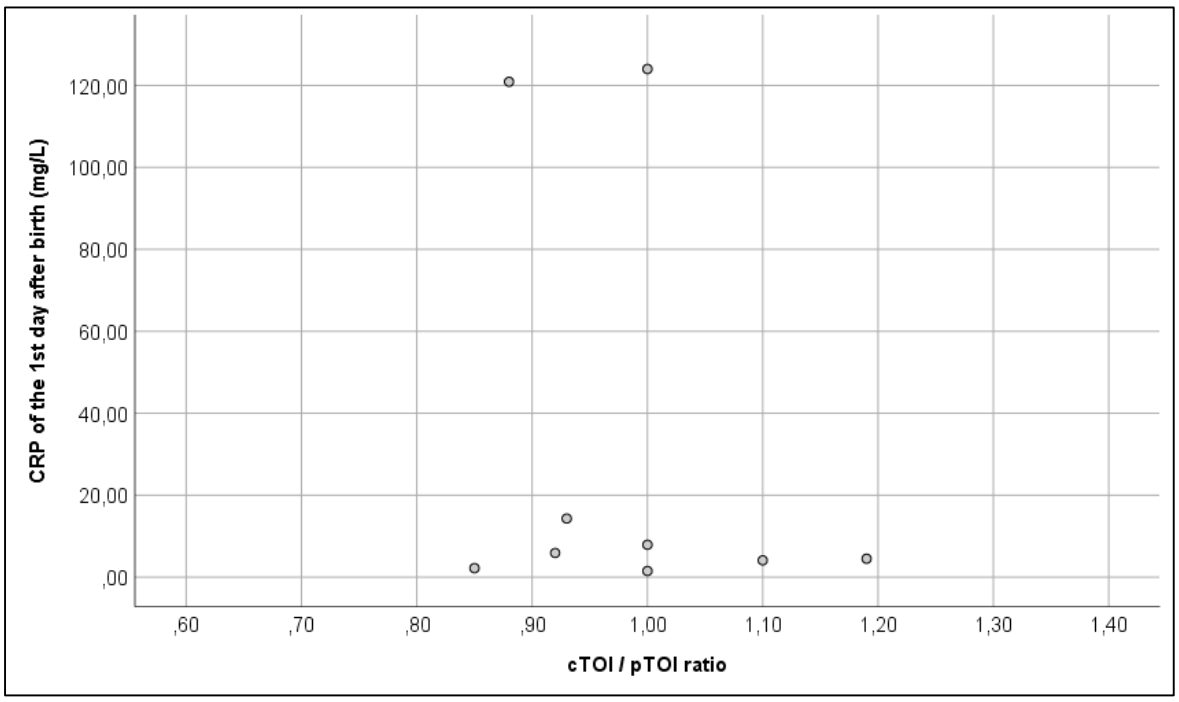

Figure 42: *Correlation between CRP of the first day after birth and cTOI / pTOI ratio of the infection group (r= -0.14; p=0.728).*

4.4.7.6 CRP of the second day after birth

Only CRP of the second day after birth of the control group showed a significant increase with increasing cTOI / pTOI ratio (r=0.24; p=0.035) (Figure 43).

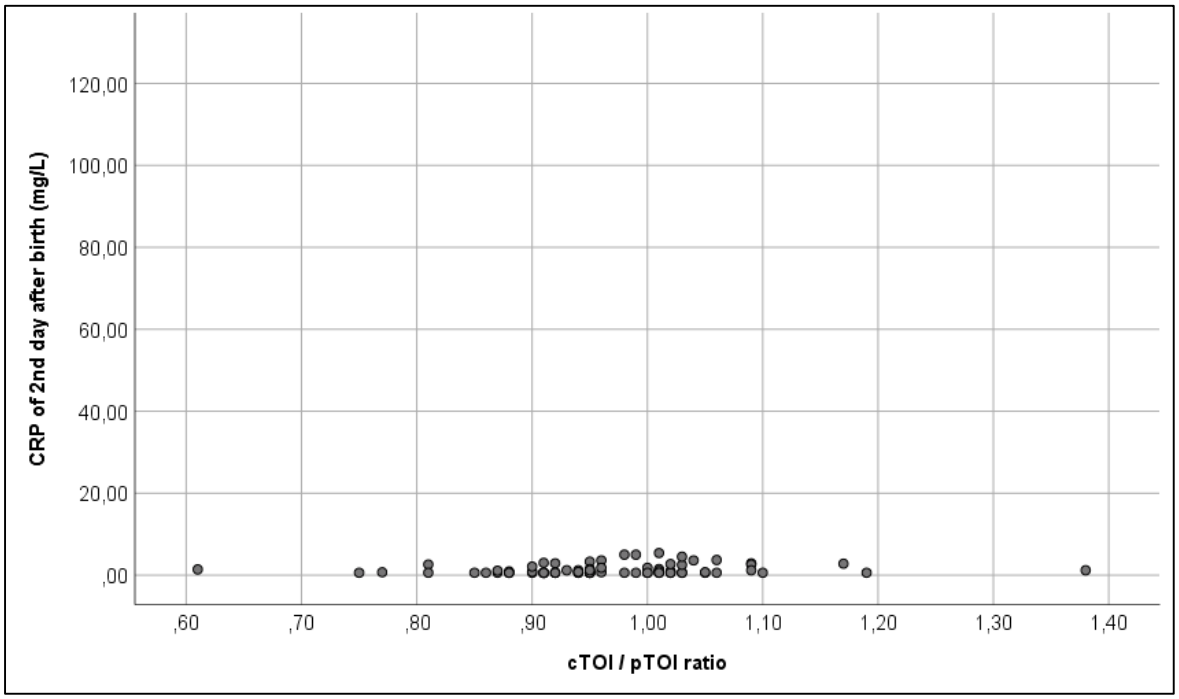

Figure 43: *Correlation between CRP of the second day after birth and cTOI / pTOI ratio of the control group (r=0.24; p=0.035).*

In the infection group there was no significant correlation between cTOI / pTOI ratio and CRP of the second day after birth (Figure 44).

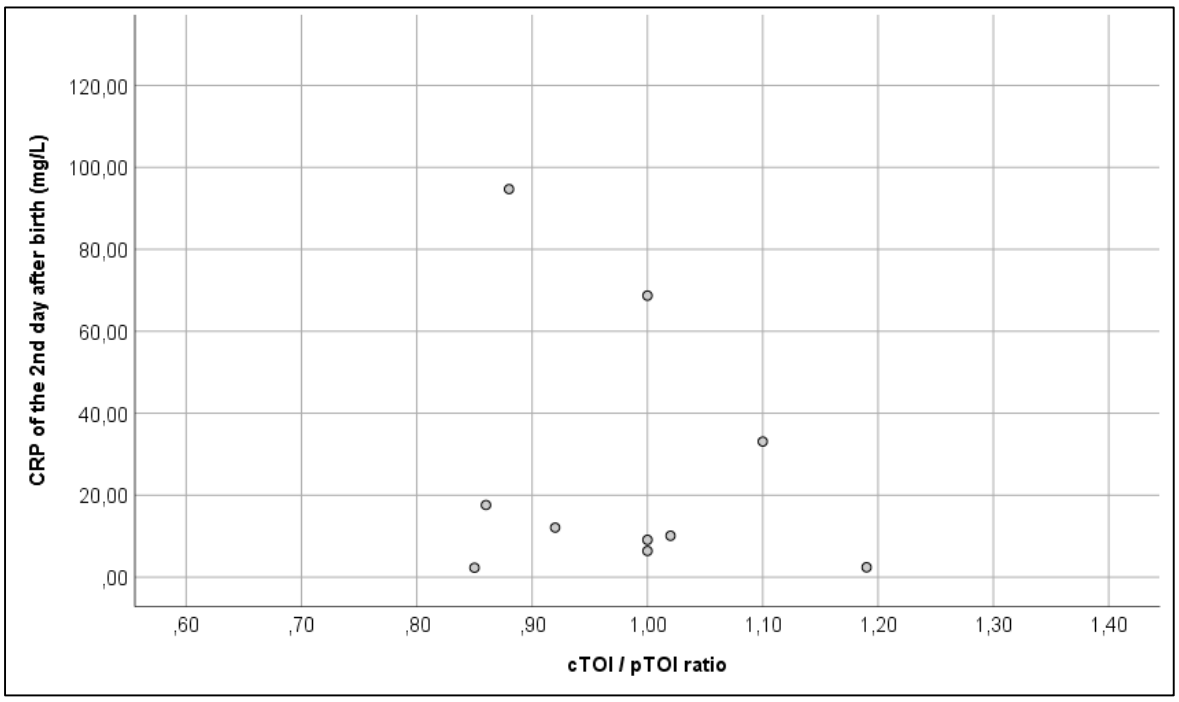

Figure 44: *Correlation between CRP of the second day after birth and cTOI / pTOI ratio of the infection group (r= -0.07; p=0.853).*

4.4.8 Cerebral injury by ultrasound

In the infection group there were no cerebral injuries. In the control group four neonates (4.6%) had a unilateral IVH grade I and one neonate (1.1%) suffered from bilateral PVL grade I.

4.4.9 Mortality

None of the included neonates died.

5 DISCUSSION

In the present study there is no significant difference in cTOI / pTOI ratio as well as cTOI and pTOI between neonates with infection / inflammation and a healthy control group during the first day after birth (Tables 5 and 6). Reasons for the missing statistical significance might be the low number of neonates in the infection group combined with a lack of severely sick

neonates (only 2 neonates in the infection group had positive blood cultures from the umbilical cord blood) in this group and also missing data during measurements.

Regarding mean cTOI and pTOI values over the 24-hour measuring period, mean pTOI values (control group: 73.4 ± 0.9% vs. infection group: 73.6 ± 1.4%; p=0.564) are similar compared to mean cTOI values (control group: 70.1 ± 1.4% vs. infection group: 71.2 ± 2.6%; p=0.079) in both groups. At first sight these results seem to be in contrast to the findings of Grossauer et al. (107), where the cTOI values in term and preterm neonates measured within the first eight weeks after birth were higher than the pTOI values (70.1 ± 6.7% vs. 62.1 ± 5.7%). As a consequence, mean cTOI / pTOI ratio in the present study (control group: 0.96 ± 0.02 vs. infection group 0.97 ± 0.04; p=0.618) is also lower compared to the cTOI / pTOI ratio of 1.14 ± 0.14 described by Grossauer et al. (107) However, differences between the two studies can be explained by the different postnatal age of included patients and the physiological decrease of pTOI from 67% to 61% in healthy term neonates during the first week after birth (91) and an increase of cTOI from 57% to 76.1% in premature infants during the first three days after birth (110).

Regarding secondary outcome parameters, neonates in the infection group have significantly lower Apgar scores at minute 1 and 5 as well as lower umbilical artery pH values as expected (Table 2, Figures 8, 9, 11). Furthermore, neonates in the infection group need more respiratory support (Table 3, Figure 13) and additionally have significantly more surfactant administrations compared to the controls (Figure 14).

Due to stratification of the two groups laboratory parameters show significant differences in PCT und CRP values on the first and second day after birth, whereas leukocyte counts do not differ between groups (Table 4). In the infection group only two neonates have positive blood cultures. Reason for this low number of positive blood cultures might be false negative results due to the fact that in most neonates the acquired amount of millilitre needed for blood culture analysis was not achieved.

Concerning cardio-circulatory parameters, HR values in each hour do not show any significant difference during the 24-hour measuring period between groups (Table 7). But mean HR value over the 24-hour measuring period shows significant higher values in the infection group compared to the control group (145 ± 4 bpm vs. 142 ± 2 bpm; p<0.001), whereby the difference is only 3 bpm, which represents a questionable clinical relevance. In the literature 150-160 bpm have been defined as sign of sepsis (117,118). Our mean HR value is below this limit, but single HR values of the infection group over the 24-hour measuring period partly reach this

limit (Table 7). Altogether the infection group shows a trend towards higher HR values compared to the control group.

SpO_2 values in each hour during the 24-hour measuring period do not show any significant difference between the two groups (Table 8). Regarding mean SpO_2 over the 24-hour measuring period, this value is significantly lower in the infection group compared to the controls (95 ± 1% vs. 96 ± 0%; $p<0.001$). Pichler et al. (15) described similar results for SpO_2 comparing term and preterm neonates with and without CRP elevation (95.4 ± 2.8% vs. 96.9 ± 2.7; $p=0.053$) during the first week of life.
MABP values in each hour during the 24-hour measuring period as well as mean MABP value over 24 hours also show no significant difference between both groups (Table 9). A reason for the missing difference between groups concerning MABP might be the fact that none of the included neonates in both groups had severe arterial hypotension or needed treatment with catecholamines. Regarding Figure 5 another reason might be that mainly moderate or late preterm neonates with compensated cardio-circulatory conditions were included.

At last, regarding the values of rectal and peripheral temperature in each hour during the 24-hour measuring period, there is again no significant difference between both groups (Table 10 and 11). Concerning the mean value of rectal and peripheral temperature over the 24-hour measuring period there is also no significant difference between groups. Thus, temperature seems to be a weak predictor of impaired (micro-)circulation as a result of inflammatory processes during the first day after birth.

When regarding the correlations between routine monitoring / laboratory inflammatory parameters and the cTOI / pTOI ratio in both groups, no significant correlations can be found (Figures 24 – 42, 44), except for HR and CRP of the second day after birth of the control group (Figures 23 and 43). Both HR and CRP show a significant increase with increasing cTOI / pTOI ratio. These findings indicate changed interactions of macro- and microcirculation in case of inflammation / sepsis. An increase in HR, as a marker of macro-circulation, as well as an increase in CRP, are typical changes during inflammatory processes, but are poor parameters for diagnosing early stages of cardio-circulatory impairment. Increasing cTOI / pTOI ratios on the other hand might serve as an early predictive marker of disturbances in microcirculation. A reason for the missing correlations between cardio-circulatory / physiological / laboratory inflammatory parameters and the cTOI / pTOI ratio of the infection group might be the low number of neonates in this group.

6 LIMITATIONS

One of the main limitations of this study is the low number of neonates that developed an infection. Additionally, only 2 neonates of the infection group have positive blood cultures from the umbilical cord blood and as a consequence have been diagnosed as sepsis. None of the included neonates developed severe septic shock or a cardio-circulatory unstable condition, where more pronounced early signs of impaired peripheral muscle and cerebral oxygenation could have been observed. Furthermore, when looking at the raw data, there are some missing data over the 24-hour measuring period in both groups, mainly due to loose of optical contact because of sensor displacement.

7 CONCLUSION

In the present study preterm neonates with inflammation / infection show no significant difference in cTOI / pTOI ratios compared to neonates without inflammation / infection measured simultaneously by cerebral and peripheral muscle NIRS on the first day after birth. One of the main reasons for the missing statistical significance between groups might be the low number of neonates in the infection group combined with a lack of severely sick neonates.

Therefore, continuous and simultaneous NIRS measurements of cerebral and peripheral muscle oxygenation in preterm neonates might still have the potential to detect early stages of cardio-circulatory disturbances in neonates with more severe diseases. This is supported by the finding in the present study that the groups differed in the behaviour of cTOI / pTOI ratios in regard to HR, suggesting differences in how macro-circulation (HR) affects and interacts with micro-circulation (cTOI / pTOI ratios).

Further studies with focus on more severely sick neonates with distinct clinical and laboratory-confirmed infection / sepsis might therefore be of great interest.

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9 APPENDIX

CRF Version 1.1., 23.01.2013

Avoiding hypotension in preterm neonates (AHIP)

Patienten-Nummer:
Untersuchungsdatum:

MUTTER:
Schwangerschaft Besonderheiten:

VBS: ….h pp	AIS: ja/nein	CRP:	Leukozyten:	

GEBURT:
Besonderheiten:

Apgar:	NapH:			
Gestationsalter:	Geburtsgewicht:	Länge:	KU:	Geschlecht:
Armumfang:	Armdurchmesser:			

LABOR:

Nabelschnur:	Il6:	PCT:	Kultur:
1. Lebenstag:	Leukozyten:	CRP:	Kultur:
2. Lebenstag:	Leukozyten:	CRP:	Kultur:

POSTPARTAL:
Infektion/Sepsis: ja/nein
Diagnosen:

NIRS MESSUNGEN:
Auffälligkeiten:

INTERVENTIONEN:

Echokardiographiebefund (1):	Echokardiographiebefund (2):	Echokardiographiebefund (3):
Füllung:	Füllung:	Füllung:
Funktion:	Funktion:	Funktion:
Ductus arteriosus:	Ductus arteriosus:	Ductus arteriosus:
Sonstige Auffälligkeiten:	Sonstige Auffälligkeiten:	Sonstige Auffälligkeiten:
Behandlung (1):	Behandlung(2):	Behandlung (3):

ZEREBRALE SONOGRAPHIE:
1.LT: unauffällig ja/nein - wenn nein Befund:
2.LT: unauffällig ja/nein - wenn nein Befund:
4.LT: unauffällig ja/nein - wenn nein Befund:
7.LT: unauffällig ja/nein - wenn nein Befund:
14.LT: unauffällig ja/nein - wenn nein Befund:
Entl.: unauffällig ja/nein - wenn nein Befund: