

**Critically ill children and
the microcirculation**
Go with the flow?

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**Critically Ill Children and the Microcirculation
Go with the Flow?**

**Kritisch zieke kinderen en de microcirculatie
Met de stroom mee?**

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PART I

INTRODUCTION

Chapter 1

Hemodynamic monitoring in critically ill children

Adapted from:

Biomarkers and clinical tools in critically ill children:
are we heading toward tailored drug therapy?

Erik A.B. Buijs, Alexandra J.M. Zwiers, Erwin Ista, Dick Tibboel, Saskia N.
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Introduction

Around 5,000 children between the age of 1 day and 18 years are admitted to one of the eight dedicated pediatric ICUs in the Netherlands [1]. They form a heterogeneous group: 45-55% present with acute, severe pathology and are deemed as critically ill [1]. The term critical illness denominates life-threatening disease, typically due to the severe dysfunction of the cardiovascular system and/or the respiratory system.

Cardiorespiratory dysfunction may result in a mismatch between oxygen consumption (VO_2) and oxygen delivery (DO_2) [2]. Figure 1 shows the equation that describes the VO_2 - DO_2 balance, in which CaO_2 and CvO_2 stand for arterial and mixed venous blood oxygen content, respectively [3]. For Q , the classical view is that it represents cardiac output. However it is more pragmatic when Q represents blood flow, for instance in case the VO_2 - DO_2 balance in a single organ is to be estimated [3, 4]. As can be deduced from the VO_2 - DO_2 equation, blood flow is key for preserving the VO_2 - DO_2 balance: it is both a prerequisite for DO_2 and a component in VO_2 . Also, blood flow can serve as a physiological compensatory mechanism once VO_2 - DO_2 mismatching is imminent [2]. If blood flow dysfunctions considerably and persistently, a cascade of events will follow that includes cellular dysfunction, organ dysfunction, (multiple) organ failure, and, ultimately, death of the child [5]. Hence, blood flow is one of the essential determinants for cellular homeostasis.

$$\frac{VO_2 : DO_2}{Q * (CaO_2 - CvO_2) : Q * (CaO_2)}$$

Figure 1. The equation describing the balance between oxygen consumption (VO_2) and oxygen delivery (DO_2). CaO_2 represents arterial blood oxygen content and CvO_2 represents venous blood oxygen content. The most feasible interpretation of Q is blood flow.

The macrocirculation and the microcirculation

Blood flow is regulated at three different levels within the circulation: the systemic circulation –i.e. towards and away from organs–, the regional circulation –i.e. between and within organs–, and tissue circulation –i.e. within organs at cellular level– [6]. Blood flow at the central and regional levels is also referred to as the macrocirculation; blood flow at tissue level as the microcirculation.

The macrocirculation encompasses the heart and all the blood vessels with a diameter $>100\mu\text{m}$ [7]. Its main function is to assure blood flow at the systemic and the regional level. Also, the macrocirculation should ensure blood supply to the microcirculation: macrocirculatory driving pressure –which is determined by cardiac output– generates microcirculatory blood flow. In clinical practice, inotropic, lusitropic, and chronotropic

agents are often primarily administered to improve blood flow at macrocirculatory level. By doing so, it is anticipated that DO_2 will enhance as well. Dopamine, for example, increases the strength of myocardial contraction –i.e. inotropic action– as well as the contraction rate –i.e. chronotropic action–, whilst milrinone affects the ability of the myocardium to relax –i.e. lusitropic action– [8].

The microcirculation consists of three entities: arterioles, capillaries, and venules [7]. This is where gases, nutrients, water, hormones, drugs, and waste products are exchanged between the blood and the tissue cells [9]. Adequate microcirculation is pivotal for normal cellular function and, therefore, also for organ function [7]. Whilst microcirculatory functioning relies heavily on the macrocirculation, the reverse is also true: the microcirculation determines partly macrocirculatory functioning. For instance, already in the 1960s Guyton described that three factors govern the regulation of cardiac output: 1) the function of the heart itself, 2) the resistance to blood flow through the peripheral tissue circulation, and 3) the degree of filling of the circulatory system [10, 11]. The latter is determined by the microcirculation as well given its function as a volume reservoir for blood [12]. So, cardiac output –and therefore macrocirculatory function– is to a great extent influenced by the microcirculation [10].

Thus, the macrocirculation and the microcirculation form a physiologically complex, dynamic entity in which the microcirculation is dependent on macrocirculation and vice versa. When VO_2 - DO_2 mismatching is impending, macrocirculatory function is, amongst others, maintained initially by two mechanisms: the regulation of vascular resistance to preserve arterial blood pressure and the enhancement of venous return / cardiac preload through redistribution of blood [10, 11]. Unfortunately, this initial response is a temporary compromise because it goes at the expense of microcirculatory reduction in the non-vital and, to a lesser extent, the vital organs [12]. Ultimately, in the face of severe disease, the microcirculation must be restored in order to maintain the VO_2 - DO_2 balance.

Moreover, research in critically ill adults with distributive or cardiogenic shock indicated that the microcirculation can be affected independently from the macrocirculation [13, 14]. Likewise, restoring the macrocirculation in adults with shock does not always imply that the microcirculation is restored as well [15].

Hence, it is advocated that both the macrocirculation and the microcirculation should be monitored and treated if necessary. This concept is increasingly supported by studies in adults using goal-directed therapy with endpoints such as arterial lactate [16].

Circulatory monitoring in the neonatal and pediatric ICU

Neonatal and pediatric ICUs offer advanced treatment and 24-hour monitoring. The purpose of monitoring is two-fold: to assess the nature, severity, and progression of disease –i.e. patient status monitoring– and to determine the type, timing, dose, and

effectiveness of treatment –i.e. therapeutic monitoring– [17]. Adequate monitoring is a prerequisite for adequate treatment.

Macrocirculatory monitoring, and in particular cardiac output monitoring, is one of the keystones of hemodynamic monitoring [18, 19]. In this respect, circulatory monitoring represents the cornerstone of intensive care management in critically ill children [20]. In adults, the pulmonary artery catheter is deemed the gold standard of invasive macrocirculatory monitoring [21]. Its true value is, however, increasingly debated and it is argued that it should be used only during specific disease conditions [21]. In critically ill children, the use of the pulmonary artery catheter is less feasible. Cardiac output monitoring in children in general is, among other things, hindered by technical and size constraints, potential toxicity of indicators, risk of fluid overload, difficulties in vascular access, intra-cardiac and extra-cardiac blood shunting [22]. The potential techniques for macrocirculatory monitoring in children have recently been reviewed by Lemson and De Boode and are outside the scope of this thesis [23, 24].

In clinical practice, much of the medical decision making in the neonatal and pediatric ICUs is based upon the macrocirculatory parameters arterial blood pressure, heart rate, and cardiac ultrasound. It is, however, suggested that these are poor representatives of microcirculatory functioning [20]. Therefore, estimates such as pH, base excess, and lactate are measured. However, these parameters are rather microcirculatory derivatives than that they directly represent the actual microcirculation. In a conceptual framework in which the microcirculation is the central component, neonatal and pediatric intensivists have to rely on either “upstream” or “downstream” markers in order to estimate microcirculatory blood flow [15, 25]. Figure 2 visualizes this concept and shows that few methods that are clinically available to study the actual microcirculation, which is the critical intermediary in neonatal and pediatric patients.



Figure 2. Conceptual framework of circulatory monitoring in the neonatal and pediatric intensive care indicating the importance of the microcirculation together with the few, routinely applied techniques for actual microcirculatory monitoring in clinical practice.

Non-invasive techniques for microcirculatory monitoring in the neonatal and pediatric ICU

Of the several non-invasive techniques to monitor the microcirculation, only refill time (CRT) is incorporated in routine clinical care and used on a regular basis in neonatal and

pediatric ICUs. CRT is the time in seconds required for the skin to turn from white to pink after release of external pressure to the capillary bed [26]. Age-related variation has been reported [27]. In children, CRT is assumed to be normal if it is below 2-3 seconds [26-28]. Arteriolar resistance, blood viscosity, and ambient temperature can all influence CRT [27, 29]. Inter-rater variability and location of the CRT measurement further hamper its reliability as marker for microcirculation [30-32]. However, CRT measurements are easily applicable, non-invasive, quick, and inexpensive [33]. Given that few alternatives are clinically available, studying the validity of CRT as a marker for the microcirculation in children is to be encouraged.

Another clinically available measure is the so-called core-toe temperature gradient. This measure assumes that during critical illness the redistribution of blood away from the extremities and towards vital organs changes the temperature of the extremities. In critically ill pediatric patients with cardiogenic or distributive shock, the core-toe temperature gradient correlates to CRT but its predictive value for poor outcome is low [34]. An argument favoring its importance is that the core-toe temperature gradient correlates poorly with macrocirculatory function in post-cardiac surgery children [35, 36]. Hence, it could provide novel information on the patient's status.

The peripheral perfusion index (PPI) is another non-invasive parameter that has been suggested as a microcirculatory marker in critically ill patients. PPI is derived from the photoelectric plethysmographic signal of pulse oximetry [37]. This signal comprises an arterial, pulsatile component and a non-pulsatile component. By computing the ratio between the pulsatile component and the non-pulsatile component, the PPI can be calculated [37]. Recently, PPI has been suggested to predict mortality in septic adults [38]. Also, PPI predicts central hypotension in adults receiving lower body negative pressure [39]. For newborns, the median (IQR) value for PPI is reported to be 1.70 (1.18-2.50) [40]. A PPI below 1.24 predicts increased disease severity better than SpO₂ and pulse rate [41]. Serial PPI measurements have been suggested as predictor for disease severity in neonates [41]. A great advantage of PPI measurements is that these are also feasible in preterms and very-low birth weight neonates [42-45]. As an alternative to pulse oximetry, near infrared spectroscopy –which is normally used for measuring tissue oxygenation– can also be used to assess peripheral perfusion [46, 47]. PPI measured by pulse oximetry and blood flow measured by near infrared spectroscopy correlate reasonably well in healthy neonates [48].

Another technique that might be valuable for studying the microcirculation in children is contrast-enhanced ultrasonography (CEUS) [49]. CEUS uses the routine ultrasound technique in combination with the infusion of artificial microbubble-based contrast agents which are small enough to pass capillaries [49]. No extravasation occurs however [49]. During the continuous infusion of the contrast agent, microbubble destruction can be obtained by applying ultrasound pulses at a so-called high mechanical index [50]. Observation of the

subsequent refilling with new contrast agent provides an image that includes microcirculatory replenishment, for instance when the renal cortex or the myocardium is visualized [51]. Thus, perfusion in single organs can be assessed. It should be stated, however, that this technique cannot measure blood flow in individual vessels and that values that are obtained represent the aggregate flow in vessels of variable size [52]. The same is true for other, relatively novel devices such as Laser Doppler flowmetry and Speckle imaging [52]. To date, the feasibility, validity, and side-effects of CEUS have been sparsely evaluated in children [53].

Next to CRT, PPI, and CEUS, the imaging modalities Orthogonal Polarization Spectral imaging (OPS) and Sidestream Dark Field imaging (SDF) have been developed for monitoring the microcirculation [54, 55]. Both are video microscopy devices emitting light at a wave length that is at the isobestic point of both oxy- hemoglobin and deoxy-hemoglobin [54, 55]. So, OPS and SDF can visualize erythrocytes irrespective of their oxygenation status. Also, OPS and SDF each combine microscopic and photographic features. In this way, video clips are generated that show the perfusion within the microvasculature. These clips are analyzed off-line using dedicated software [54, 56]. The main outcome parameters comprise vessel density and blood flow velocity [57]. SDF is a second generation video microscopy device and was found technologically superior to OPS. The OPS and/or SDF measurements were compared against intra-vital microscopy, against nailfold capillaroscopy, and against each other in animals and adults. Both devices proved usable at a wide range of hematocrit levels [54, 55]. OPS and SDF have been used in critically ill adults to obtain sublingual microcirculatory imaging data in various types of critical illness and during several therapeutic interventions [15, 58].

OPS and SDF are relatively new in the neonatal and pediatric ICU [20]. In term neonates and older children, OPS and SDF data are most often obtained in the buccal mucosa whilst in preterm neonates microcirculatory imaging is most often applied on the skin of the inner-upper arm near the axilla. Our research group previously applied OPS in critically ill children [59-61]. During respiratory failure inhaled nitric oxide improved the microcirculation whereas macrocirculatory parameters were unaltered [59]. In fourteen children with therapy-resistant respiratory failure, the microcirculation as assessed by OPS improved after extracorporeal membrane oxygenation [60]. The microcirculation observed by OPS in septic shock patients was a better predictor for survival than PRISM-II [61]. OPS was also used successfully in preterm neonates [62-67]. The studies in children will be reviewed in greater detail in the general discussion of this thesis.

“Downstream” monitoring

Researchers have used various approaches in their search for “downstream” microcirculatory biomarkers or, in other words, surrogate markers for Q in the DO_2 - VO_2 equation. For instance, an organ-oriented approach resulted in the identification of troponin and

brain natriuretic peptide as markers of cardiac stress or cardiac disease in children [68]. By using a disease-oriented approach, a set of serum protein markers has been identified that predicts mortality in children with septic shock [69, 70]. In clinical practice, pH, pCO₂, base excess, and lactate often serve as generalized markers, i.e. not specifically representing one organ system or one type of disease.

In this thesis, the focus will be on arterial lactate. Lactate is one of the normal end products of carbohydrate metabolism; the other main product is acetyl-CoA [71]. In normal aerobic conditions, lactate is constantly being produced during glycolysis from pyruvate by lactate dehydrogenase [72]. As a result, the reference range in Erasmus MC-Sophia for arterial lactate concentration in children is 0.5 to 2.0 mmol/L⁻¹. Once formed, most of the lactate is converted back to pyruvate to serve again as energy substrate or to serve as precursor for gluconeogenesis [72]. These three processes together, –i.e. glycolysis (lactate production), oxidation (lactate exchange), and gluconeogenesis (lactate use)– have been termed the lactate shuttle [72]. The lactate concentration rises only when production enhances and/or metabolism declines [73].

The classical view is that during critical illness anaerobic conditions shift the balance between acetyl-CoA production and lactate production towards the latter [72, 74]. Thus, lactate is often used as a “downstream” marker for tissue hypoxia [74]. The blood lactate concentration can, however, also increase during aerobic conditions induced by critical illness [72, 74]. The aerobic conditions that have been identified and that might be relevant for children include: a) liver dysfunction resulting in reduced lactate clearance; b) enhanced glycolysis –e.g. in cytokines or due to hyperglycemia– that exceeds the oxidative capacity of mitochondria resulting in increased lactate production; c) increased catecholamine levels causing increased activity of the Na⁺K⁺-adenosine triphosphatase membrane ion pump which drives cellular glucose uptake; d) alkalosis causing increased cellular efflux of lactate; e) mitochondrial dysfunction; and f) drug infusion or intoxication –e.g. epinephrine, nucleosidic reverse transcriptase inhibitors, methanol– [72, 74].

So, during critical illness both aerobic and anaerobic conditions are present that can increase blood lactate concentration. The benefit that lactate has over macrocirculatory parameters such as systemic blood pressure, is that it also conveys information on tissue perfusion. Therefore, in this thesis lactate is regarded as a “downstream” microcirculatory marker.

The more severely ill the child, the more lactate is produced [72, 74, 75]. Approximately thirty prospective or retrospective studies have focused on lactate as the primary parameter of interest and its relation to outcome in critically ill children [76-105]. Children with congenital heart defects have been studied most often, but there are also reports on children with septic shock, traumatic brain injury, acute severe asthma, mitochondrial disease, and therapy resistant cardiorespiratory failure requiring extracorporeal mem-

brane oxygenation (ECMO) [76-105]. The majority of studies concluded that increased lactate concentration –i.e. hyperlactatemia– is associated with poor outcome.

Research in adults, however, showed that the value of lactate as a predictor for outcome depends on the diagnosis at admission [106]. Depending on the type or the severity of illness, microvascular perfusion might be suboptimal or even absent. As a result, lactate is produced intra-cellularly, yet only moderately released to the circulation. Hence, the arterial blood lactate concentration remains false negatively low. A lactate washout phenomenon has been described in children with congenital cardiac defects who require cardiopulmonary bypass [95]. Another explanation as to how the predictive value of lactate might differ between patient groups with a different type of disease or a different severity of illness, is the fact that energy metabolism is required for lactate production. In children with severe and extensive tissue necrosis with complete isolation of the systemic circulation, energy metabolism is diminished or absent. Consequently, less lactate is formed. In other words, lactate might increase during critical illness of moderate severity whilst it stabilizes or even decreases during end-stage critical illness or during therapy. Co-morbidity such as liver dysfunction can be relevant as well [72].

Furthermore, intrinsic differences for both lactate production and lactate metabolism have been described between healthy children and adults as well as between healthy newborns and older children. For instance, at equal levels of exercise the blood lactate concentration is lower in children than in adults [107]. Likewise, lactate levels of pre-pubertal children are lower than those of post-pubertal children [108-110]. Other differences in lactate kinetics constitute amongst others that lactate is more rapidly released and cleared from the blood in younger children [108, 110, 111]. Furthermore, animal models show that lactate remains an important energy substrate early after birth in both cerebral and cardiac tissue. The neonatal heart, for instance, retains an enhanced capacity for anaerobic energy production after birth. Throughout maturation, however, lactate as an energy substrate diminishes loses importance [112-114]. This is likely to affect lactate kinetics. To complicate matters further, there are also extrinsic age-related differences in lactate concentration. A clear example is the peri-partum period: the lactate concentration in fetal scalp blood is often higher than 2 mmol/L⁻¹ in newborns with 5-minute-APGAR-scores exceeding 7 [115].

In summary, lactate concentration and its association with outcome is dependent on type of disease, severity of disease, co-morbidity, and age, among other things. Hence, results obtained in pediatric patients might not be relevant for neonatal patients. Moreover, the results obtained in separate disease-specific pediatric patient groups might not be similar.

In children with primary respiratory failure, the value of lactate as a predictor for outcome has been studied sparsely. Moreover, previous research has largely focused on the cross-sectional measurement of lactate. Lactate on admission and peak lac-

tate concentration have been reported by many to be good predictors for outcome. However, the clinical applicability of these parameters is poor: peak lactate can only be determined retrospectively and lactate on admission does not allow for follow-up –e.g. to assess therapeutic efficacy. Given that the lactate concentration can vary over time –e.g. due to changes in disease severity, co-morbidity, and age– it seems more plausible to develop so-called dynamic lactate indices which incorporate duration or trend over time of lactate derangement. Therefore, the focus of the lactate studies presented in this thesis will be on dynamic lactate indices as predictor for outcome in neonatal or pediatric patients with primary respiratory failure.

Aims and outline of this thesis

The aims of this thesis are:

- to study whether the microcirculation, as assessed by OPS imaging, SDF imaging and/or arterial lactate, is altered during critical illness
- to evaluate if any of these microcirculatory alterations normalize over time with therapeutic intervention
- to assess whether microcirculatory alterations are related to outcome.

With the exception of chapter 2, each original study that is presented in this thesis focuses on one of three critically ill patient groups:

- children with therapy-resistant primary respiratory failure requiring extracorporeal membrane oxygenation
- children with congenital diaphragmatic hernia who require catecholaminergic treatment
- children with return of spontaneous circulation after cardiac arrest who require therapeutic hypothermia

Part II describes the results that were obtained using the non-invasive microcirculatory imaging techniques OPS or, in particular, SDF. Both are relatively new video microscopy techniques in the neonatal and pediatric intensive care setting. First, in **chapter two**, the feasibility and reproducibility of microcirculatory measurements with SDF in the buccal region and the cutaneous inner-upper arm region of healthy, term neonates are described. In **chapter three** and **chapter four** the evolution of the microcirculation observed by either OPS or SDF is discussed before, during, and after start of extracorporeal membrane oxygenation (ECMO) treatment together with its relation with ECMO modality (venoarterial vs. venovenous). **Chapter five** reviews the current knowledge of cardiovascular catecholamine receptor distribution and function in children. Hereafter, the microcirculatory effect of catecholaminergic treatment in children with congenital

diaphragmatic hernia is discussed in **chapter six**. **Chapter seven** reports our microcirculatory findings with SDF in post-cardiac arrest children receiving therapeutic hypothermia.

Arterial lactate – dynamic lactate indices in particular, but cross-sectional lactate measurements as well– is the main topic in part III. Dynamic lactate indices incorporate duration or trend over time of lactate derangement and are a relatively sparsely studied in the neonatal and pediatric intensive care setting. Given that lactate can be affected by both hypoxic and non-hypoxic factors, we regard arterial lactate as a “downstream” microcirculatory marker. **Chapter eight** discusses whether or not static and/or dynamic measures for arterial lactate can be used to predict the need for ECMO in patients with congenital diaphragmatic hernia, while **chapter nine** focuses on the value of lactate for predicting mortality in patients who received ECMO.

Part IV discusses the results and implications of this thesis in a broader perspective (**chapter ten**). A summary is provided in **chapter eleven**.

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PART II

NON-INVASIVE MICROCIRCULATORY IMAGING

Chapter 2

Reproducibility of microvascular vessel density assessment in Sidestream Dark Field (SDF) derived images of healthy term newborns

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ABSTRACT

INTRODUCTION: Earlier studies of the microcirculatory status in (preterm) neonates have semi-quantitatively assessed the vessel density of the buccal and the cutaneous inner-upper arm microcirculation. However reproducibility of the vessel density analysis has never been validated in this patient group. This study aims to determine the reproducibility of the vessel density assessment in the buccal and cutaneous area in one-day-old term newborns and to investigate if the vessel density analysis of clips of both areas shows any correlation.

METHODS: This single-center, prospective observational study was performed at the Obstetrics and Gynecology department at a level III university children's hospital. All healthy, term infants younger than 24 hours were eligible for inclusion. Buccal and cutaneous microcirculation was measured using Sidestream Dark Field (SDF) imaging. The images were assessed independently for vessel density by two investigators. Reproducibility was evaluated by determining the intra-class correlation coefficient (ICC) and by performing Bland-Altman analysis. Paired T-test and Pearson correlations were performed for assessing if the vessel density analysis of the buccal and the cutaneous clips shows any correlation.

RESULTS: Twenty-eight healthy term newborns were included. Results of the vessel density assessment showed good reproducibility in the buccal area: ICC's for total and perfused vessel density were 0.93 (CI_{95%} 0.88-0.97) and 0.93 (CI_{95%} 0.85-0.97) with a near zero bias and acceptable limits of agreement in the Bland-Altman analysis. In contrast, the reproducibility of vessel density assessment for the cutaneous microcirculation turned out to be poor: ICC's for total and perfused vessel density were 0.31 (CI_{95%} 0.00-0.70) and 0.37 (CI_{95%} 0.00-0.74) with large biases (3.09 and 2.53, respectively) in the Bland-Altman analysis. There was no significant correlation between buccal and cutaneous vessel density.

CONCLUSIONS: The evaluation of the buccal vessel density in SDF-derived images in term newborns younger than 24 hours is highly reproducible whereas the evaluation of the cutaneous images is not. Also, buccal microcirculatory density does not correlate with cutaneous microcirculatory density.

INTRODUCTION

Orthogonal Polarization Spectral (OPS) imaging and its technically superior successor Sidestream Dark Field (SDF) imaging are non-invasive methods for directly visualizing the microcirculation that can be used at the patient's bedside. Changes in the microcirculation can be seen in critically ill patients even when clinical parameters that represent the systemic blood flow are within the normal range [1]. The degree of microcirculatory distress can be used as an indicator for disease severity and has been proven to predict poor outcome in adults and children [1-4]. The non-invasiveness of SDF imaging allows investigation of the microcirculation in body surfaces with a thin cover of epithelium, even during severe critical illness.

SDF illuminates the tissue of interest using light at a wave length that is at the isobestic point of both oxy- and deoxy-hemoglobin (530-548 nm). The scattered light is reflected by the background and absorbed by hemoglobin regardless of the oxygenations status. The images are created by projecting the reflected light [5, 6]. OPS and SDF data are analyzed offline for total vessel density (TVD), and perfused vessel density (PVD). These analyses are semi-quantitative and thus subject to inter-observer variability. In adults it is routine to perform microcirculatory measurements in the sublingual area. However, in children –neonates in particular– sublingual measurements are not feasible. Instead, microcirculatory measurements are performed in the buccal mucosa [3, 4, 7]. Size-constraints do not allow for buccal measurements in preterm neonates. Hence, transcutaneous inner-upper arm measurements near the axilla are performed [8-11].

OPS imaging has already been used to investigate microcirculatory alterations in neonates. Buccal microcirculatory improvement –defined as PVD– was observed in term neonates with respiratory failure after inhaled nitric oxide and extracorporeal membrane oxygenation [12, 13]. Weidlich et al. found that the cutaneous microcirculation –defined as PVD– decreased one day prior to infection in preterm infants [11]. Hiedl et al. showed that the cutaneous PVD decreased in premature neonates with a significant persistent ductus arteriosus [9].

Even though microcirculatory vessel density has been assessed in term and preterm neonates previously, the reproducibility of these measurements was never tested in this specific patient group. Also, to date it is unknown if microcirculatory clips of both areas are comparable to one another. Disparity between the two areas would affect the interpretation of research results, amongst other things.

Therefore, the aims of this observational study are: a) to evaluate the reproducibility of the semi-quantitative analysis of buccal and cutaneous microcirculatory measurements in one-day old term newborns, and b) to investigate if the vessel density analysis of the buccal and the cutaneous SDF clips shows any correlation.

METHODS

This single-center prospective observational study was performed between April and May 2013 at the Obstetrics and Gynecology department of the Erasmus MC – Sophia, a level III university children's hospital. Approval for this study was granted by the medical ethical review board of this hospital. All healthy term newborns younger than 24 hours were eligible for participation in the study. Exclusion criteria were gestational age below 36 weeks or above 42 weeks, age over 24 hours, any known congenital, hematologic or cardiorespiratory disorder and the absence of written parental informed consent. All neonates were born from mothers with a maternal indication or maternal request for hospital delivery.

Data collection

The buccal and cutaneous microcirculation was visualized using SDF imaging (Microscan™; Microvision Medical, Amsterdam, The Netherlands) according to the guidelines for optimal image acquisition [14]. Two examples of microcirculatory imaging in CDH patients are shown in figure 1. All video sequences were recorded on DV-tape (Sony DSR-20P digital video recorder), and 5-second clips (avi-format) were digitized and stored. Blinded, randomized video sequences were analyzed offline using dedicated software (Automated Vascular Analysis 3.0, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands). Both the horizontal and the vertical pitch were calibrated using the SDF calibration files. From every participant three clips were recorded from both the buccal and the cutaneous sites. Before measuring the buccal microcirculation saliva was removed using gauze and the lens of the device was covered with a disposable sterile cap. To avoid pressure artefacts, we adhered to the standard

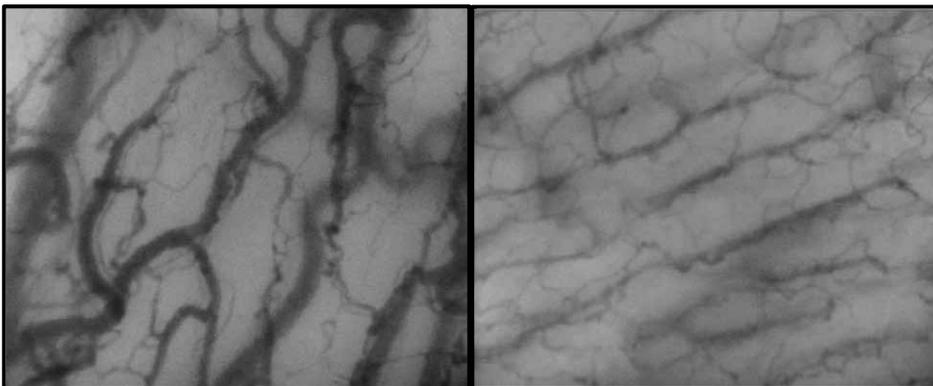


Figure 1. Two stills from video clips showing the buccal microcirculation (left pane) and the cutaneous inner-upper arm microcirculation (right pane) that were obtained with Sidestream Dark Field imaging in a healthy newborn aged < 24 hours.

operating procedure as published by Trzeciak et al. [15]. Hereafter, the cutaneous microcirculation was measured on the inner-upper arm near the axilla. This site contains little lanugo and is less prone to movement artifacts caused by breathing [16]. A drop of oil was used to assure better contact of the probe with the surface. Clips that did not meet the criteria for image quality were not included in the study [14]. For every clip, the TVD was estimated according to guidelines by counting the number of blood vessel crossing three equidistant horizontal and three equidistant vertical lines drawn on the screen and then dividing this number by the total length of the lines [14]. By only including the perfused vessels the PVD was determined as well.

Analyses of the clips were independently performed by two authors (VvdB and HvE). They were instructed by another author (EB), an experienced user of the SDF technique and AVA software. The training phase included independent analysis of five random and seven buccal clips. Thereafter both investigators compared and discussed results in order to create a consensus in scoring. Finally, all clips were analyzed. For every clip, the total TVD and the total PVD were calculated. During the analyses, the investigators were blinded for the order of the images.

Next to the microcirculatory data, demographic data were collected: Information on the gestational age, birth weight, Apgar scores and mode of delivery of the investigated subjects were gathered from the medical files.

Statistical analysis

For each patient the TVD and PVD of three images per site were averaged. If one or two clips were discarded due to poor image quality, the mean was calculated over the remaining clips. The inter-observer variability was determined by calculating the differences from the means of both authors and presenting these in a Bland Altman plot with 95% limits of agreement [17]. Also two-way mixed intra-class correlation coefficient (ICC) for inter-observer variability were calculated and presented with 95% confidence intervals (CI_{95}) [18]. This coefficient can vary between 0-1.0 and is considered very good when >0.81 , good when between 0.61-0.80, fair to moderate between 0.21-0.60 and poor below 0.20 [19]. Paired t-test and Pearson correlations were performed for both authors individually to assess any correlation between the vessel density analyses of the buccal and cutaneous SDF clips. Results were considered significant when $p < 0.05$. Demographic data was displayed with mean (SD) for parametric parameters and median (range) for non-parametric parameters. Statistical analysis was performed using SPSS 21 (IBM Corp., Armonk, New York) and GraphPad Prism 5 (GraphPad Software Inc., La Jolla, California).

RESULTS

A total of 28 healthy term newborns were included. Eighty-four buccal and 84 cutaneous clips were recorded of whom 14 buccal clips and 19 cutaneous clips were excluded due to poor quality. Three newborns were excluded for analyses of cutaneous inter-observer variability as all three cutaneous clips did not meet quality criteria. Demographic data and the means of the results of the assessment of the vessel density from both authors are presented in table 1 and 2.

Table 1. Baseline patient characteristics

	Healthy term neonates N = 28
Male gender ^a	13 (46)
Gestational age (weeks) ^b	39+3 (36+5 to 41+5)
Birth weight (grams) ^c	3393 (535)
Caesarian section as mode of delivery ^a	12 (39)
Temperature (°C) ^c	37.0 (0.3)
Apgar 1 minute ^b	9 (6 to 9)
Apgar 5 minute ^b	10 (8 to 10)

^aDiscrete data are presented as number and percentage, ^bcontinuous data are presented as median and range, ^ccontinuous data are presented as mean and standard deviation.

Table 2. The microcirculatory parameters of the buccal and cutaneous microcirculation as assessed by Sidestream Dark Field imaging in healthy newborns aged < 24 hours.

	Buccal microcirculation N = 28		Cutaneous microcirculation N = 25	
	Observer 1	Observer 2	Observer 1	Observer 2
Crossings small vessels ^a	42 (14)	40 (13)	76 (8)	51 (7)
Crossing non-small vessels ^a	30 (6)	32 (6)	8 (4)	17 (4)
Total TVD ^a	13.7 (2.4)	13.8 (2.8)	15.7 (1.6)	12.7 (1.2)
Total PVD ^a	13.6 (2.4)	13.6 (2.7)	15.0 (1.5)	12.4 (1.2)

^aData are presented as mean and standard deviation. Observer 1: HvE, observer 2: VvdB. PVD: perfused vessel density, TVD: total vessel density.

Inter-observer variability

Table 3 depicts the ICCs for inter-observer variability in both the buccal and cutaneous microcirculatory parameters. The buccal ICC for total PVD and TVD were 0.93 (0.85-0.97) and 0.93 (0.88-0.97) while the cutaneous ICC for total PVD and TVD were 0.37 (0-0.74) and 0.31 (0-0.70), respectively. Bland-Altman plots presenting the differences from the mean between the

Table 3. The intra-class correlation coefficient for inter-observer variability in the buccal and cutaneous microcirculatory data as assessed by Sidestream Dark Field imaging in healthy newborns aged < 24 hours.

	Buccal microcirculation N = 28	Cutaneous microcirculation N = 25
	ICC (CI _{95%})	ICC (CI _{95%})
Crossings small vessels ^a	0.95 (0.88-0.97)	0.13 (0-0.44)
Crossing non-small vessels ^a	0.70 (0.36-0.86)	0.27 (0-0.64)
Total TVD ^a	0.93 (0.88-0.97)	0.31 (0-0.70)
Total PVD ^a	0.93 (0.85-0.97)	0.37 (0-0.74)

^aData are presented as mean and standard deviation. Observer 1: HvE, observer 2: VvdB. ICC: intra-class correlation coefficient, PVD: perfused vessel density, TVD: total vessel density.

Table 4. Summary of the Bland-Altman plots showing the agreement between the analyses of two observers (HvE and VvdB) for the buccal and cutaneous microcirculatory data as assessed by Sidestream Dark Field imaging in healthy newborns aged < 24 hours

	Buccal microcirculation N = 28	Cutaneous microcirculation N = 25
	Bias (95% limits of agreement)	Bias (95% limits of agreement)
Crossings small vessels ^a	1.5 (-10.4 – 13.4)	25.3 (-10.0 – 40.7)
Crossing non-small vessels ^a	-2.0 (-12.6 – 8.8)	-8.9 (-16.81 – -1.1)
Total TVD ^a	-0.094 (-2.8 – 2.6)	3.1 (0.6 – 5.6)
Total PVD ^a	-0.005 (-2.6 – 2.6)	2.5 (0.2 – 4.9)

^aData are presented as mean agreement and 95% limits of agreement. PVD: perfused vessel density, TVD: total vessel density.

analyses of both authors of the buccal and that of the cutaneous vessel density were created. An overview of biases and 95% limits of agreement are presented in table 4 and figure 2.

Relationship between the buccal and cutaneous microcirculation

For both authors the paired t-test and Spearman correlation showed no significant relationship between the analysis of the buccal and the cutaneous SDF clips. Bland-Altman analyses and ICC calculations were not performed due to the lack of any correlation.

DISCUSSION

This is the first study to evaluate the reproducibility of the analysis of microcirculatory data obtained in one-day-old neonates. It shows that for the buccal measurements the reproducibility is high while the reproducibility of the transcutaneous measurements is moderate to poor. Also, our study shows that the cutaneous and buccal obtained SDF clips show no correlation between one another.

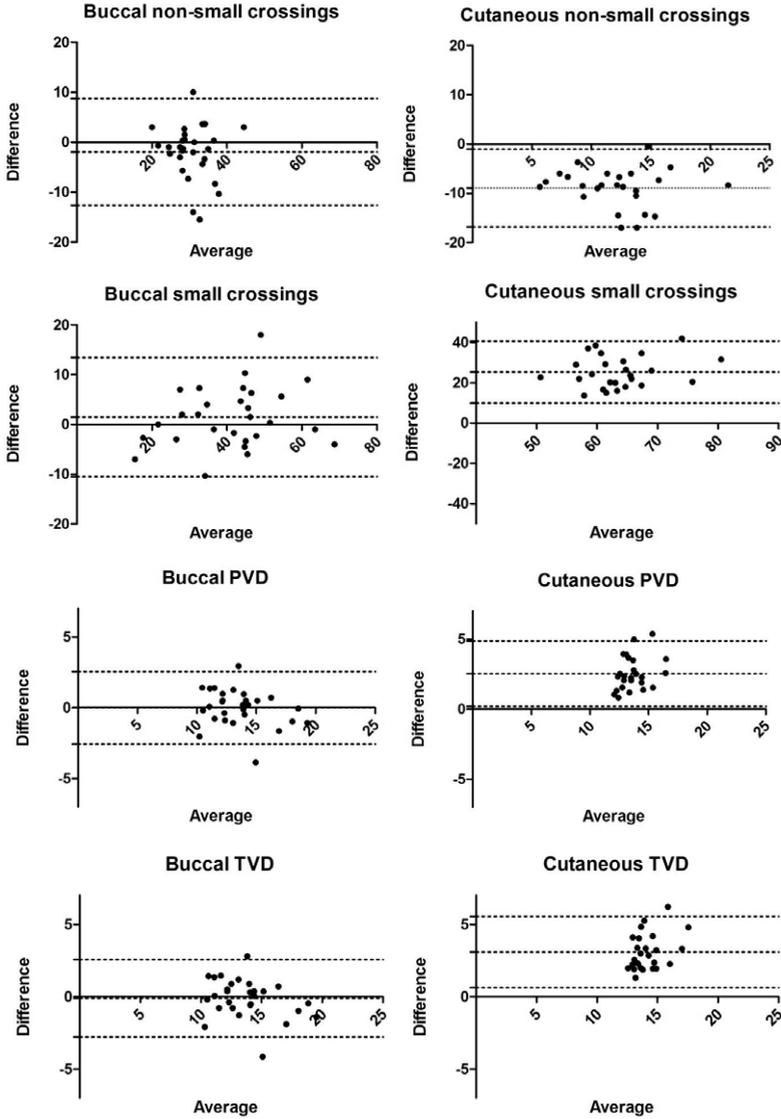


Figure 2. The Bland-Altman plots showing the agreement between the analyses of two observers (HvE and VvdB) for the buccal and cutaneous microcirculatory data as assessed by Sidestream Dark Field imaging in healthy newborns aged < 24 hours.

SDF images are rapidly and non-invasively obtained which makes it a promising technique for increasing our knowledge of microcirculatory alterations in pre-term or term infants. Furthermore it could help us to gain insight in cardiovascular adaptation phase after birth and pathophysiologic changes in times of illness. Also, microcirculatory imaging data might serve as a predictor for disease such as sepsis or as a marker

for therapeutic efficacy. However, the quantification of the recorded images has to be reproducible. Our results validate the use of the TVD and PVD as a method of assessment of SDF clips in the buccal area in one-day old term infants.

The SDF microcirculatory clips of the skin were of lower imaging quality in comparison to the buccal clips. This might be the main reason for the difference in reproducibility of the buccal and cutaneous TVD and PVD. In our opinion the skin of term infants is not thin enough to obtain high quality recordings with SDF imaging, although the skin of the term infant is still developing and made up of a disorderly capillary network in which the capillaries are more horizontal orientated and more superficial than in the skin of adults [20]. However, applying the extra pressure that is required “to look past” the epithelial cells could cause pressure artifacts and could therefore negatively affect the reliability of the microcirculatory imaging data. The assessment of vessel crossings and perfusion become more arbitrary in the case of low quality microcirculatory clips and distinguishing between individual vessel structures becomes harder. Another cause for the lower quality of the cutaneous clips could be that the non-sedated and incapacitated newborns were often awake and agitated after obtaining the buccal measurements. This could have led to, for instance, more extensive movement artifacts. In our study we observed a clear difference in interpretation of the cutaneous clips between the two investigators leading to consequently poorer inter-observer scores. Such a difference in interpretation was not seen in the analysis of the buccal clips.

To our knowledge this is the first study reporting on inter-observer reproducibility for the TVD and PVD in SDF clips using Bland-Altman plots and ICC in this patient group. Previous studies in healthy adults as well as critically ill adult patients have already established good inter-observer variability in the sublingual area [2, 21, 22]. A study by Buijs et al. reported ICC's ranging from 0.565 to 0.869 for microcirculatory assessment of buccal SDF clips in pediatric patients after cardiac arrest [4]. However when inter-observer agreement is presented as ICC only, all information of the relation between the variability and the mean is lost which makes this method inferior to a Bland-Altman plot. Moreover, no studies have reported on the inter-observer variability of the analysis of cutaneous SDF clips. Our study showed excellent ICCs and near zero bias for the analysis of the buccal measurement. For both the TVD as the PVD 64.3% of all measurements had a difference between the two raters that was less than one. In contrary, the cutaneous measurements had poor ICC and a large bias and more scattering of the differences.

Even though the results of analysis of cutaneous SDF clips in healthy term infants using TVD and PVD are poorly reproducible, measuring the microcirculation of the skin might still be feasible in preterm infants. It can be argued that because of the even less far developed and thinner skin of the premature infants, the quality of the clips and thus the reproducibility of the analysis would drastically improve. This would be highly desirable given that for this specific patient group the buccal measurements are impossible

due to the size of the probe. Several studies have already been performed in this group using OPS and SDF and have reported the microcirculatory status by presenting the cutaneous TVD and PVD [8-11, 16].

The limitations of the OPS device –a first generation device– and the SDF device –a second generation device– include the use of a relatively low-resolution analogue camera technology and lenses with relatively limited optical properties. These limitations can possibly explain the limited image quality found in the current study in the neonatal cutaneous microcirculation. Recently, a third generation device was introduced based on Incident Dark Field (IDF) imaging which incorporates improved optical lenses coupled to and a high-resolution computer-controlled imaging sensor [23, 24]. With the anticipated ability of automatic image analysis it is expected that this new generation handheld video microscope may overcome the limitations of SDF imaging that we have identified in the current study.

Finally, our results show that the buccal microcirculatory vessel density does not correlate to the cutaneous microcirculatory vessel density in healthy term newborns. This should be taken into account when interpreting neonatal microcirculatory studies and this disparity should be investigated in greater detail in both healthy and critically ill children.

Limitations

Several limitations should be acknowledged in the current study. Most importantly, the feasibility of SDF imaging is highest in capacitated and/or sedated subjects [25]. We included healthy neonates who could not be instructed and who were awake. Secondly, we did not analyze the clips for microvascular flow index (MFI; a score for the blood flow velocity) because MFI is developed in particular for critically ill patients and not for healthy subjects. Future validation studies should include a heterogeneous patient cohort that also includes critically ill and preterm patients. Thirdly, seven buccal clips, out of a total of 70, were extensively discussed in order to create consensus before starting the final analysis. These clips were included in the study and could have been recognized thereby heightening the change of low inter-observer bias. Nevertheless, after excluding these clips the respective ICCs for TVD and PVD are 0.92 and 0.93. Finally, it is recommended by De Backer et al. to regularly review the clips with several researchers in order to prevent drift in analyses [14]. In our study, we have only reviewed a total of 13 clips to create a consensus. However, in our view it is unlikely that the difference in the reproducibility of the buccal and the cutaneous scores were caused by a shift in analyses as this would imply subtle differences that worsen over time. Instead, our results showed a rather substantial and abrupt difference in reproducibility between the buccal and cutaneous TVD and PVD.

CONCLUSION

The semi-quantitative analysis of microcirculatory SDF clips is highly reproducible for buccal TVD and PVD in term newborns aged younger than 24 hours. In contrast, the reproducibility of the assessment of the cutaneous microcirculation in this specific patient group is poor. Also, there is no correlation between the buccal and cutaneous microcirculatory vessel density. Future studies results take these results into account.

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Chapter 3

The microcirculation is unchanged in neonates with severe respiratory failure after the initiation of ECMO treatment

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ABSTRACT

PURPOSE: Venoarterial extracorporeal membrane oxygenation (VA-ECMO) is known to improve cardiorespiratory function and outcome in neonates with severe respiratory failure. We tested the hypothesis that VA-ECMO therapy improves the microcirculation in neonates with severe respiratory failure.

METHODS: This single-center prospective observational pilot study took place in an intensive care unit of a level III university children's hospital. Twenty-one-term neonates, who received VA-ECMO treatment, were included. The microcirculation was assessed in the buccal mucosa, using Orthogonal Polarization Spectral imaging, within 24 hours before (T1) and within the first 24 hours after initiation of ECMO treatment (T2). Data were compared to data of a ventilated control group (N = 7).

RESULTS: At baseline (T1), median functional capillary density (FCD), microvascular flow index (MFI), and heterogeneity index (HI) did not differ between the ECMO group and the control group. At T2 the median FCD was lower in the control group (median [range]: 2.4 [1.4–4.2] versus 4.3 [2.8–7.4] cm/cm²; P value <0.001). For MFI and HI there were no differences at T2 between the two groups.

CONCLUSION: The perfusion of the microcirculation does not change after initiation of VA-ECMO treatment in neonates with severe respiratory failure.

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) is a cardiopulmonary bypass technique used as life support in selected newborns and children with acute reversible cardiorespiratory failure when conventional management is not successful [1, 2]. Worldwide, over 24,000 neonates have been treated with ECMO for respiratory problems [1-3].

ECMO therapy gives time to restore normal pulmonary oxygenation in neonates with severe respiratory failure who do not respond to maximal conventional therapy and is regarded as a bridge to recovery [1, 2, 4]. The institution of venoarterial ECMO (VA-ECMO) partly takes over oxygenation, and carbon dioxide removal and thereby allows ventilator settings to be reduced and restores circulation [4].

The institution of an ECMO circuit in neonates results in an expansion of the circulating volume by approximately factor 2.5. In VA-ECMO, the heart is bypassed and flow in the systemic circulation is generated mostly by the ECMO pump, producing nonpulsatile flow. Especially during high ECMO flow rate (120–200 mL/kg/min), this results in disturbance of the physiologic blood flow, which can be represented by a flattening of the arterial pulse waves on invasive blood pressure monitoring [4, 5].

In neonatal patients with severe respiratory failure, who meet the criteria for ECMO treatment [4], the circulation and oxygenation are severely compromised. Reflecting this condition, these patients' microcirculatory parameters are significantly reduced before VA-ECMO [6]. At the time when the patient no longer needs ECMO, the microcirculatory parameters are improved, correlating well with an improvement in clinical condition [6]. After VA-ECMO initiation, circulation and oxygenation generally improve rapidly and patients show a decrease in the need for vasoactive medication. Direct effects of artificial, nonpulsatile ECMO flow on the microcirculation are still not completely understood.

Based on clinical observations and the instant decrease of need for vasoactive medication after the start of ECMO therapy, we hypothesize that microcirculatory alterations observed in neonates with severe respiratory failure improve with the initiation of ECMO therapy.

MATERIALS AND METHODS

Patients.

Neonatal patients (aged ≤ 28 days) admitted to our intensive care unit and treated with VA-ECMO were enrolled in this study. Patients were treated with ECMO, according to our unit specific policy. Patients suffering from congenital heart disease were excluded. In accordance with the guidelines of the medical ethical review board of our hospital, informed consent was waived when standard therapy is monitored by noninvasive techniques.

Patients in the study group had severe cardiorespiratory failure and hypoxemia despite adequate conventional treatments such as mechanical ventilation, sedation, muscle paralysis, vasoactive drugs, and nitric oxide inhalation. All patients met the

established entry criteria for ECMO [4]. Starting ECMO treatment in a newborn implies a massive increase of the circulating volume (the priming volume of the used system is ± 350 mL, which is about 1.5 times the circulating volume of a newborn baby). The ECMO system was primed with a combination of Ringer's lactate, packed red blood cells and albumen. Bicarbonate and calcium were added based on bloodgas analysis of the priming fluid. Initially the aimed ECMO flow rate was 150–200 mL/kg/min and after 24 hours weaning of the flow was started under guidance of changes in arterial pO₂ and signs of pulmonary hypertension.

In addition to the microvascular measurements, patient's demographic and clinical parameters, such as gender, birth weight, gestational age, postnatal age, diagnosis, ECMO flow, heart rate, blood pressure, mean arterial blood pressure, body temperature, administered medication, hemoglobin, and hematocrit levels were recorded. Data were compared to data of control subjects, with severe respiratory failure, who did not receive ECMO treatment. In the control group, patients were measured several consecutive days after admission. The first two measurements on consecutive days were taken to serve as control for T1 and T2 and to evaluate the changes without ECMO treatment.

Procedures.

The microcirculation was assessed within 24 hours before start of ECMO (T1) and within 24 hours after start of ECMO (T2). OPS imaging [7] was used to visualize the microvascular network of the buccal mucosa. The measurements were done with a CYTOSCAN E-II Backfocustype device (Cytometrics, Philadelphia, PA, USA), using the 5x objective.

Before the measurements, saliva was gently removed with gauze. The lens of the OPS-imaging device was covered with a disposable sterile cap and was applied to the buccal mucosa without pressure, as described before [6]. Images from 3 different regions were obtained and stored on digital videotapes, using a Sony DSR-20P digital video recorder. Segments of 5 seconds were selected and captured in AVI (audio video interleave) format. Video segments that did not meet quality criteria were discarded [6, 8]. For every measurement, the functional capillary density (FCD), microvascular flow index (MFI), and heterogeneity index (HI) of the different video segments were averaged. If only one segment met the quality criteria, this score was taken. (This was the case for 2 ECMO patients at T2 and 1 control patient at T1).

Microcirculatory Analysis.

Quantification of the images was performed as described previously [6, 7]. To investigate vessel density, the images were analyzed with the Capiscope software program (version 3.7.1.0, KK Technology 1993–2000). For the FCD calculation, the analyst is required to trace out the path of the moving red blood cells within the capillaries (vessels, smaller than 10 μm). A functional capillary is defined as a capillary that has at least one red blood

cell moving through it, during the observation period. Dividing the length of the perfused capillaries by the area gives the functional capillary density value expressed in cm/cm².

The flow pattern was studied using the MFI, and the HI [8]. For MFI the predominant type of flow for small, medium, and large vessels in every quadrant of the images was determined, as described before by Boerma et al. [9]. For every measurement, the scores for the different video segments were averaged. If only one segment met the quality criteria, this score was taken. HI was calculated as the highest site flow velocity minus the lowest site flow velocity, divided by the mean flow velocity of all sites per measurement [8].

Statistical Analysis.

The data were analyzed using SPSS 17.0. Continuous data are presented as median and range, discrete data as number and percentage. The intergroup differences at T1 were assessed using the Mann Whitney test. Changes over time were assessed using analysis of covariance (ANCOVA) with the T2 measurement as outcome variable, the groups as factor, and the T1 measurement as covariate. In this way, differences at T2 are corrected for the baseline measurements. The level of significance was set at $P < 0.05$.

RESULTS

During the study period, 31 VA-ECMO patients were eligible for inclusion. Twenty-one patients were included in the study. Four patients were missed for inclusion due to logistic reasons (a researcher was not contacted in time or no investigator or camera available). Six patients were excluded because their video segments did not meet the quality criteria [6]. The excluded ECMO patients did not differ from the included ECMO patient group for gestational age, postnatal age, diagnosis, duration of ECMO treatment,

Table 1. Demographic data.

	ECMO N = 21	Controls N = 7
Gestational age [weeks]	39.0 (34.4-42.5)	38.1 (38.0-39.3)
Birth weight [kilograms]	3.1 (2.3-5.1)	3.0 (3.0-3.8)
Gender [males] (%)	12 (57)	4 (57)
Diagnosis [n] (%)		
CDH	10 (48)	7 (100)
MAS	5 (24)	
PPHN	5 (24)	
CCAM	1 (5)	
Survival [n] (%)	18 (86)	7 (100)

Continuous data are presented as medians and range, discrete data as number and percentage. CDH: congenital diaphragmatic hernia, MAS: meconium aspiration syndrome, PPHN: persistent pulmonary hypertension of the neonate, CCAM: congenital cystic adenomatoid malformation.

Table 2. Macrocirculatory data.

	T1 ECMO	T2 ECMO	T1 Controls	T2 Controls	P-value at baseline*	P-value over time†
	N = 21	N = 21	N = 7	N = 7		
Age [days]	1 (0-12)	1 (0-12)	1 (0-6)	1 (0-7)	0.694	NA
Time to or from start ECMO [hours]	2 (0.5-24)	2 (0.5-24)	-	-	NA	NA
Time to or from ICU admission [hours]	2.5 (0.3-55.4)	6.4 (2.3-82.7)	12.4 (1.0-145.3)	33.5 (17.9-173.5)	NA	NA
Time between SDF measurements [hours]	4.0 (1.3-39.2)		26.8 (13.0-32.3)		0.005	
Heart rate [beats/min]	180 (120-220)	150 (106-198)	138 (113-191)	129 (110-160)	0.046	0.387
Mean blood pressure [mmHg]	49 (29-77)	49 (35-86)	44 (32-60)	52 (41-63)	0.264	0.727
Pulse pressure [mmHg]	19 (10-40)	10 (0-33)	25 (12-36)	24 (15-32)	0.559	< 0.001
Vasopressor score	40 (0-140)	10 (0-108)	15 (0-75)	19 (0-66)	0.410	0.136
Dopamine [mcg/kg/min]	10 (0-20)	0 (0-20)	10 (0-21)	16 (0-21)	NA	NA
Dobutamine [mcg/kg/min]	10 (0-20)	5 (0-20)	10 (0-20)	5 (0-20)	NA	NA
Norepinephrine [mcg/kg/min]	0.1 (0.0-1.0)	0.0 (0.0-0.9)	0.0 (0.0-0.4)	0.0 (0.0-0.3)	NA	NA
Mean airway pressure [cm H ₂ O]	18 (12-27)	11 (7-21)	14 (9-16)	13 (8-16)	0.019	0.357
Inhaled nitric oxide [ppm]	20 (0-40)	0 (0-0)	0 (0-19)	0 (0-20)	0.012	0.002
Oxygenation index	31 (5-94)	2 (1-21)	5 (3-13)	3 (0-7)	0.004	0.520
PELOD	20 (11-31)	-	11 (11-20)	-	0.006	-
Hemoglobin [mmol/l]	9.2 (6.9-12.6)	8.7 (6.7-12.0)	8.7 (7.4-11.0)	8.5 (7.8-10.8)	0.336	0.978
Hematocrit [l/l]	0.45 (0.32-0.62)	0.41 (0.31-0.56)	0.43 (0.38-0.53)	0.40 (0.34-0.53)	0.514	0.384
Fluid amount administered [mL/kg]	-	-	63 (10-145)	54 (26-112)	NA	NA
Fluid balance [mL/kg]	-	-	33 (6-139)	26 (-25 - 56)	NA	NA
Temperature [degrees Celsius]	37.4 (34.4-38.6)	36.9 (35.9-38.4)	37.3 (36.7-38.4)	36.8 (36.5-37.3)	NA	NA
ECMO flow [mL/kg/min]	-	140 (110-210)	-	-	NA	NA

Data are presented as median and range. * Inter-group differences at T1 were assessed using Mann-Whitney test. † For the time dependent variables differences at T2 were assessed using ANCOVA with the baseline measurement as covariate. NA: not assessed, -: not relevant. ECMO: extracorporeal membrane oxygenation, ICU: intensive care unit, PELOD: pediatric logistic organ dysfunction.

or mortality. In the control group, four patients were missed for inclusion and seven patients had to be excluded due to insufficient quality of the images. Demographic data are presented in Table 1, clinical data in Table 2, and microcirculatory data obtained by SDF are presented in Table 3.

Table 3. Microcirculatory values.

	T1 ECMO N = 21	T2 ECMO N = 21	T1 Controls N = 7	T2 Controls N = 7	P-value at baseline*	P-value over time†
FCD [cm/cm ²]	4.5 (2.4-7.7)	4.3 (2.8-7.4)	5.0 (1.8-7.2)	2.4 (1.4-4.2)	0.811	< 0.001
MFI Large	2.76 (2.50-3.00)	2.88 (2.34-3.00)	2.92 (2.50-3.00)	3.00 (2.63-3.00)	0.266	0.367
MFI Medium	2.67 (2.13-3.00)	2.75 (2.13-3.00)	2.75 (2.38-3.00)	2.81 (2.50-3.00)	0.254	0.411
MFI Small	2.75 (2.06-3.00)	2.75 (2.08-3.00)	2.88 (2.44-3.00)	2.90 (2.63-3.00)	0.574	0.090
HI Large	0.10 (0.00-0.30)	0.09 (0.00-0.40)	0.09 (0.00-0.29)	0.00 (0.00-0.26)	0.951	0.2406
HI Medium	0.14 (0.00-0.60)	0.11 (0.00-0.35)	0.10 (0.00-0.51)	0.00 (0.00-0.27)	0.736	0.2421
HI Small	0.18 (0.00-0.73)	0.09 (0.00-0.37)	0.09 (0.00-0.40)	0.00 (0.00-0.17)	0.579	0.0971

Data are presented as median and range. * Inter-group differences at T1 were assessed using Mann-Whitney test. † For the time dependent variables, differences at T2 were assessed using ANCOVA with the baseline measurement as covariate. FCD: functional capillary density, MFI: microvascular flow index, HI: heterogeneity index

At baseline (T1), median FCD did not differ between the ECMO group and the control group (median [range]: 4.5 [2.4–7.7] versus 5.0 [1.8–7.2] cm/cm², P value = 0.811) (Figure 1). ANCOVA analysis indicated that at T2 the median FCD was 1.9 cm/cm² lower in the control group than it was in the ECMO group (median [range]: 2.4 [1.4–4.2] versus 4.3 [2.8–7.4] cm/cm²; P value <0.001). For MFI and HI, there was neither a difference at T1 nor a difference at T2 between the two groups (see Table 3 for absolute MFI values and HI values per vessel type as well as the associated P values).

At baseline, the disease severity indices oxygenation index (median [range]: 31 [5–94] versus 5 [3–13]; P value = 0.004) and the PELOD score (median [range]: 20 [11–31] versus 11 [11–20]; P value = 0.006) were more unfavourable for the ECMO patients than for the control patients. The heart rate was higher in the ECMO patients (median [range]: 180 [120–220] versus 138 [113–191] bpm; P value = 0.046), whereas the mean arterial blood pressure and the pulse pressure did not differ. The need for vasoactive medication as indicated by the vasopressor score did not differ between the two groups at T1. Mean airway pressure (median [range]: 18 [12–27] versus 14 [9–16] cm H₂O; P value = 0.019) and the median dosage of inhaled nitric oxide (median [range]: 20 [0–40] versus 0 [0–19] ppm; P value = 0.012) were both higher in the ECMO patients than in the control patients.

At T2, ANCOVA analysis indicated that there was no difference in OI between the ECMO group and the control group. The heart rate and the mean arterial blood pressure did not differ. Pulse pressure was lower in the ECMO patients than in the control patients

(median [range]: 10 [0–33] versus 24 [15–32]; P value <0.001). The vasopressor score did not differ at T2, nor did the mean airway pressure. Regarding the dosage of inhaled nitric oxide, ANCOVA analysis indicated that the need for more inhaled nitric oxide in the ECMO patients at T1 had disappeared at T2.

All patients in the control group survived. Three patients in the ECMO-treated group (2 diagnosed with CDH, 1 with CCAM) did not survive, due to recurrent and therapy resistant pulmonary hypertension. Subanalysis showed that neither FCD nor MFI, nor HI differed between the ECMO survivors and the ECMO nonsurvivors at T1 and at T2.

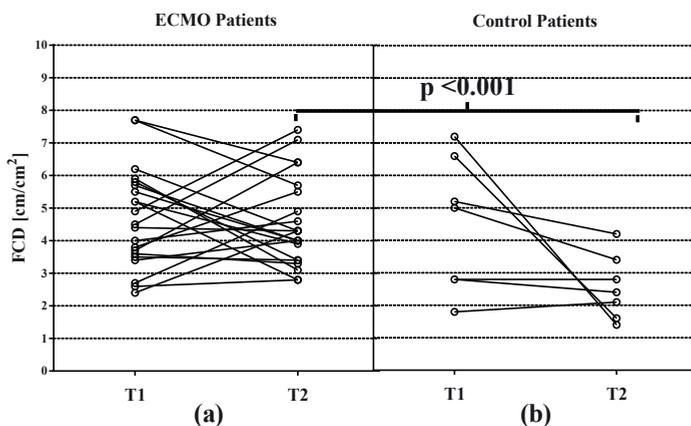


Figure 1. Diagram showing the functional capillary density (FCD). (a): ECMO patients, (b): ventilated control patients. No difference in median FCD was seen at T1 between the two groups: 4.5 cm/cm² (range 2.4–7.7) versus 5.0 cm/cm² (range 1.8–7.2), P value = 0.811. At T2, FCD was higher in ECMO group than in the control group: 4.3 cm/cm² (range 2.8–7.7) versus 2.4 cm/cm² (range 1.4–4.2), P value <0.001 .

DISCUSSION

The main finding of this study was that there was no change in microcirculatory parameters after the start of VA-ECMO therapy in patients with severe respiratory failure. In both the ECMO and the control group, the FCD at T1 was significantly lower than FCD values of neonates without any respiratory or cardiovascular problems (who served as a control group in a previous study [6]). The FCD in those patients was 8.1 cm/cm² (range, 6.6–9.4). MFI values in both study groups were relatively high and HI values relatively low, in contrast to observations in patients with sepsis. There was no difference in MFI and HI between the two groups at T1 and T2. Deterioration of the FCD was observed in patients with severe respiratory failure, who did not receive ECMO treatment. Despite the fact that patients in the ECMO group were more severely ill, in comparison to the patients

in the ventilated control group (Oxygenation Index and PELOD score in ECMO group significantly higher), ECMO succeeded to better microcirculatory support compared to solely conservative treatment with mechanical ventilation and pharmacologic support.

Thus, ECMO seems to prevent a further deterioration of microcirculatory perfusion. The start of ECMO instigates an instant improvement in oxygenation, which makes vasopressors and the use of high mean airway pressures instantly redundant. No correlation between the vasopressor score or the main airway pressure and FCD was found.

Deterioration of microvascular perfusion in patients in the ventilated control group was not correlated with mortality. This is in contrast with observations in patients with severe sepsis [10-12]. The underlying pathophysiology in patients in our study is different from sepsis. Therefore, data from patients with sepsis cannot be extrapolated to this patient group. Both patient groups revealed a relatively normal flow pattern and selectively affected vessel density. At this stage, it is not clear if this could be explained by their specific hemodynamic pattern. Patients in this study suffered from hypoxic respiratory failure, mainly due to failure of adequate feto-neonatal transition of the circulation. Typically, these patients display a hemodynamic pattern with persistent pulmonary hypertension of the neonate (PPHN), which is clinically characterized by a persistent high pulmonary vascular resistance and an abnormal vascular response, leading to worsening of gas exchange and shunting (intracardiac, extracardial, and intrapulmonary) and right ventricular failure. PPHN occurs as a primary disease or in association with abnormal lung development, for example, in congenital diaphragmatic hernia and is a critical determinant of morbidity and mortality [13].

All patients had pulmonary hypertension, assessed by echocardiography and differences in the pre- and postductal oxygen saturation (due to shunting through persistent fetal pathways such as the ductus arteriosus). This can compromise the pulmonary venous return and preload of the left ventricle and, therefore, influence global hemodynamics. No measures of cardiac output (CO) were available in this study, so this cannot be verified.

During cardiopulmonary bypass (CPB) in adults, microcirculatory alterations have been described before [14-17]. We found one report on microcirculatory alterations during CPB in neonates where OPS was used, which shows a reduction in vessel density during CPB [18].

The circulatory volume increases by about 150%, when a newborn is attached to an ECMO circuit. Therefore, it is necessary that the system is primed with blood products. The addition of these products is titrated against normal values for the age. Thus, with ECMO, blood is transfused, which could improve the microcirculation [19]. However, there was no increment in the hemoglobin level, to support this. With the attachment of the system, a large amount of fluid is administered, which could influence the perfusion of the microcirculation [20]. Due to the relatively large amount of circulating volume in

the system, it is difficult to comment on volume expansion in the patient in absolute numbers. During cannulation and shortly afterwards extra fluid was administered on discretion of the treating physician, based on clinical judgment and following standard unit policies and procedures.

Disturbance of physiologic flow also triggers the catecholamine system leading to vasoconstriction and altered tissue perfusion [21]. Although the mechanism behind this is not completely understood, Agati et al. [22-24] reported that in cardiac patients on CPB nonpulsatile flow seemed to affect the microcirculation and organ perfusion in a more negative way than pulsatile flow did. No correlation between ECMO flow and FCD was seen in our study.

All in all, the initiation of ECMO therapy instigates many changes in the homeostasis of the critically ill patient. It is difficult to unravel the complex processes that take place and to assess separate factors, in order to understand the effect of the different components of the treatment. Nowadays, the importance of microcirculatory improvement is recognized [25, 26]. With this paper, we have shown that the current way of using ECMO treatment stabilizes the microcirculation, but does not restore microvascular density. More research is needed to explore the different factors that have influence on the microcirculation. In addition, follow-up investigations of the microcirculation are necessary as well as comparison of survivors and nonsurvivors within the group that received ECMO treatment. In this way, the prognostic value of microcirculatory parameters can be determined.

There were some limitations to our study. First, the lack of CO measurements limits the possibility to relate microvascular observations to global hemodynamics. Changes in CO could possibly play a role in the decrease of FCD between T1 and T2 in the control group. In children, mixed venous saturation and cardiac output are not routinely measured. A prerequisite for adequate CO monitoring is a tool that is accurate, is easy to use, and has an acceptable risk benefit profile. These three factors have constituted the major hurdle to bedside pediatric cardiac output measurement to date [27]. The reliability of echocardiography evaluation of cardiac output in children is debatable because even in the hands of experienced operators the inter- and intraindividual variation is large [28].

Second, the control group consisted entirely of patients with CDH, while the ECMO group also contained patients with severe respiratory failure and pulmonary hypertension due to other causes. Patients with CDH suffer from a specific hemodynamic pattern, based on a structural congenital abnormality [13]. This could possibly have different implications on the development of global hemodynamics and the microcirculation.

Unfortunately, the exact amounts of priming fluids and fluids, given during or shortly after the cannulation procedure prior to T2, are not well documented. In addition, 12 of the 21 ECMO patients were first measured within 2 hours of IC admission. In these patients, no reliable data on the amount of fluid administration prior to admission

was available. Therefore, we are unable to provide reliable data for fluid balance, fluid amount, and type of fluids administered for ECMO patients in this study.

In this pilot study, the microcirculation was assessed before and after the start of ECMO; therefore, long-term effects of ECMO could not be evaluated. In addition, the median time interval for the subsequent SDF measurements in the ECMO group was shorter than that of the control group. The earlier microcirculatory evaluation in the ECMO group might be of influence on our results.

Finally, this study is observational and not randomized controlled, which skews outcome data. If children in the control group had disposed progressive respiratory and/or circulatory failure, they would have received ECMO treatment. From an ethical perspective, randomization for this type of treatments is unacceptable.

CONCLUSION

The perfusion of the microcirculation does not change after initiation of VA-ECMO treatment in neonates with severe respiratory failure.

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Chapter 4

The microcirculation in children with primary respiratory disease requiring venoarterial or venovenous extracorporeal membrane oxygenation: a prospective cohort study

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ABSTRACT

OBJECTIVES: Venoarterial extracorporeal membrane oxygenation (VA) restores the microcirculation in children, but the timing of microcirculatory improvement is unknown. Moreover, while the use of venovenous ECMO (VV) increases relative to VA, macrocirculatory failure is generally less severe prior to VV and physiologic, pulsatile blood flow is maintained during VV. The purpose of this study is to evaluate if the evolution of the microcirculation differs between VV and VA patients over time; to determine when the microcirculation improves during ECMO; and to study if failure to correct microcirculatory impairment is related to poor outcome.

DESIGN: Prospective, observational cohort study.

SETTING: Level III intensive care unit (ICU), one of two designated ECMO centers in the Netherlands.

PATIENTS: Thirty-one VA and 17 VV patients with primary respiratory failure requiring ECMO support between May 2008 and February 2012

MEASUREMENTS AND MAIN RESULTS: The buccal microcirculation was measured non-invasively using Sidestream Dark Field imaging together with macrocirculatory, respiratory, and biochemical parameters. Data were obtained before and after cannulation, on day two and day three of ECMO support, and before and after decannulation. The microcirculation did not differ over time between the VA and VV patients. The microcirculation was impaired before cannulation, and failed to improve immediately after cannulation, in contrast to the macrocirculatory, respiratory, and biochemical parameters. The microcirculation did not improve until day 2 of ECMO support. Improvement was sustained up to after decannulation, also in VV. There was no association between microcirculatory impairment and mortality.

CONCLUSIONS: The microcirculation does not differ between VA and VV and is impaired prior to cannulation, also in the patients who receive VV. While macrocirculatory, respiratory, and biochemical parameters improve immediately after start of ECMO, microcirculatory improvement is delayed with approximately 1 day. Both VA and VV restore the microcirculation.

INTRODUCTION

Children with refractory primary respiratory disease are candidates for extracorporeal membrane oxygenation (ECMO) [1]. In most ECMO centers, the current viewpoint is that children with primary respiratory disease are, in principle, treated with venovenous (VV) ECMO [2, 3]. Venoarterial (VA) ECMO becomes an option only when contra-indications for VV are known a priori –including severe circulatory failure–, or when venovenous is attempted without success. Worldwide, VV is increasingly used relative to VV [3-5].

ECMO should provide a bridge to recovery while perfusion and oxygenation are restored at both the systemic –macrocirculatory– and the tissue –microcirculatory– level [6]. The latter can now be monitored non-invasively at the patient’s bedside [7]. Using this approach, our group has shown that microcirculation improved in children who were successfully weaned from VA support [8]. In children with congenital diaphragmatic hernia (CDH), the microcirculation was more severely affected in those who ultimately required VA than in those who did not [9]. Furthermore, in critically ill children who did not receive ECMO, microcirculatory deterioration was associated with mortality [10]. Microcirculatory monitoring might be particularly valuable in children because the possibilities for invasive hemodynamic monitoring are limited in this age group [11].

The time window of microcirculatory improvement during VA has not been investigated. Moreover, despite its increased use, the microcirculation has never been studied in VV patients. In VV patients macrocirculatory failure tends to be less severe than in VA patients [1, 2]. VV recipients also experience less pronounced inflammation after cannulation than VA recipients [12, 13]. Furthermore, unlike in VA, physiologic, pulsatile flow is maintained during VV and microemboli are unlikely to enter the systemic circulation [1, 3]. All of these factors have been associated with microcirculatory deterioration, although none have been studied in the context of ECMO [7, 14, 15].

Therefore, this study has three aims: to evaluate if the evolution of the microcirculation in the VV group differs from that of the VA group before, during, or after ECMO support; to determine the timing of microcirculatory improvement during ECMO; and to study if microcirculatory impairment is related to poor outcome. The hypothesis was that the microcirculation would be less severely deteriorated prior to ECMO support in the VV recipients than in VA recipients, that the microcirculation would not recover immediately after starting ECMO, and that failure to correct microcirculatory impairments would be associated with poor outcome.

MATERIALS AND METHODS

Study design and Setting:

For this prospective observational cohort study were included patients admitted to the level III intensive care unit (ICU) of Erasmus MC-Sophia Children’s Hospital, one of

two designated ECMO centers in the Netherlands. The medical ethical review board approved the study and parental informed consent was obtained prior to the start of microcirculatory data collection.

Patients:

Eligible for inclusion were all children –i.e., age 0 to 16 years at ICU admission – with refractory, primary respiratory disease necessitating ECMO support between May 2008 and February 2012. Patients with primary cardiac disease were excluded for two reasons: to minimize the heterogeneity in the patient groups and to prevent bias since pre-ECMO data was anticipated to be missing, as many of these children receive ECMO after failing to wean from cardiopulmonary bypass. For the same reason, the patients receiving rapid-response ECMO –i.e., cannulation within 2 hours after emergency department arrival for cardiopulmonary resuscitation– and the patients cannulated at other hospitals were not eligible for inclusion. Also excluded were all of the patients receiving a VA system not equipped with the standard roller pump, patients for whom consent was either not obtained or withdrawn, and patients with less than two microcirculatory measurements due to logistic reasons. When patients received multiple ECMO runs, only data related to the first ECMO run were included (n=3).

Data collection:

Data were obtained within 12 hours before (T0; baseline) and after cannulation (T1), at 24 ± 12 hours (T2) and 48 ± 12 hours (T3) after cannulation, before ECMO decannulation (T4), and within 48 hours after decannulation (T5). The primary endpoint was survival at ICU discharge.

Non-invasive microcirculatory imaging

The microcirculation was measured using Sidestream Dark Field imaging (Microscan BV, MicroVision Medical, Amsterdam, the Netherlands) at three different buccal sites according to the guidelines for optimal image acquisition [16, 17]. To avoid pressure artifacts continuous flow in the greater microvessels was assured. All of the video sequences were recorded on DV-tape, and 5-second clips (avi-format) were digitized and stored. Blinded, randomized video sequences were analyzed offline using dedicated software (Automated Vascular Analysis 3.0, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands). Total vessel density (TVD), perfused vessel density (PVD), proportion of perfused vessels (PPV), microvascular flow index (MFI), and heterogeneity index (HI) were calculated for the small (S; $\varnothing \leq 10 \mu\text{m}$) and non-small vessels (NS; \varnothing between 10 and $100 \mu\text{m}$) [10, 18, 19]. To this end, a grid with three equidistant horizontal and three equidistant vertical lines was placed over the video sequence. The number of vessel crossings was determined together with the vessel-specific flow category

and the total grid length. The type of flow was scored as continuous (3), sluggish (2), intermittent (1), or absent (0). Vessels with intermittent or absent flow were categorized as non-perfused. TVD was calculated by the number of crossings divided by the grid length and PPV was calculated by the number of perfused crossings divided by the total number of crossings. PVD equaled the product of TVD and PPV. For determining MFI and HI each video sequence was divided into four equally sized quadrants. Per quadrant the predominant type of flow was scored. MFI represented the mean score of the predominant type of flow, and HI represented the difference between the highest quadrant and the lowest quadrant score, divided by the mean score of all of the quadrants for one measurement. For all of the other scores, the mean of the scores for the three video sequences per measurement was taken. Prior to the final analysis, inter-observer variability was determined for every microcirculatory parameter using 120 (38%) video sequences obtained for the current study (n=60) and for another study (n=60). The Spearman's rank correlation coefficient and the intra-class correlation coefficient (ICC) respectively ranged from 0.533-0.932 (mean $r=0.768$) and 0.565-0.869 (mean ICC=0,750).

Demographic and time-dependent parameters

Together with the microcirculatory measurements, patient demographics, disease severity measures, macrocirculatory parameters –i.e., heart rate (HR), mean arterial blood pressure (MABP), and vasopressor score (VP-score)–, respiratory parameters, and biochemical parameters were recorded. Relevant co-morbidities before and during ECMO were registered using definitions described previously [5, 20]. Renal failure was assessed only prior to cannulation, as every patient routinely received continuous hemofiltration during ECMO. Pulmonary hypertension was assumed to be present in the case of inhaled nitric oxide (iNO) therapy combined with either echocardiographic confirmation or with persistent pre-ductal to post-ductal saturation differences greater than 20% prior to commencement of iNO treatment. The oxygenation index (OI), VP-score, and the pediatric logistic organ dysfunction score (PELOD) were determined as described previously [21-23]. When the ECMO mode was converted within 24 hours after the initial cannulation, the timing of follow-up measurements was based on the date of conversion. The mode of ECMO support with the longest duration was scored.

Hospital treatment protocol:

After the initial stabilization, patients were treated according to institutional policy. Respiratory and circulatory management have been described previously, as have the patient selection criteria, the sedative and analgesic management of ECMO patients, the cannulation procedure, and the ECMO weaning procedure [3, 24]. The ECMO system was primed with a combination of Ringer's lactate, packed red blood cells, and albumen. Bicarbonate and calcium were added based on blood gas analyses of the priming fluid.

The biochemical properties of the primed ECMO circuit were checked and adjusted to age-appropriate normal values prior to cannulation. Initially, the desired ECMO flow rate aimed for was 120–150 mL/kg/min, and after 24 hours it was attempted to reduce the ECMO flow rate under the guidance of changes in arterial pO_2 and signs of pulmonary hypertension. VV was the preferred mode for patients with meconium aspiration syndrome and for patients with isolated primary respiratory failure –i.e., good myocardial function as assessed by cardiac echo and no severe circulatory failure as assessed by conventional parameters. VA was preferred in patients with CDH or isolated septic shock, and in patients with primary respiratory failure that were accompanied by poor myocardial function and/or circulatory failure. Both the timing of ECMO and type of ECMO modality were decided by the attending intensivist. The ECMO membrane and tubing were supplied by Medtronic (Medtronic Inc., Minneapolis, MN, USA); the ECMO roller pumps were provided by Stöckert Instrumente GmbH (Stöckert Instrumente GmbH, Munchen, Germany).

Statistical analysis:

Continuous data are reported as medians (IQR); discrete data as numbers (%). The descriptive and inferential statistics for the baseline characteristics were performed with non-parametric tests. For the microcirculatory parameters, analyses were done in two steps: First, the inter-group differences at baseline were assessed using non-parametric tests. To assess differences over time between VV and VA, mixed effects models were performed with the covariates time, group, and the interaction term. If necessary, the microcirculatory parameters were first transformed (log or power function). All of the models were assessed using likelihood tests, the goodness-of-fit statistic AIC, and the residual diagnostic plots. Second, to test for overall differences over time, mixed effects models were constructed with time as the single parameter. In the event that significant differences were found, sub-tests between the time points were performed that included correction for multiple testing. A sub-analysis was performed to assess the relationship between the microcirculation and mortality. All of the statistics were calculated using IBM SPSS statistics v20.0 (IBM Corp., Armonk, NY, USA) with the exception of the mixed effects models, which were created using R statistics 2.15.2. A p -value <0.050 was considered statistically significant.

RESULTS

Fifty-two of the 100 ECMO patients were excluded (Figure 1). Out of the 48 included patients, 31 (65%) received VA and 17 VV. The characteristics of the included patients did not differ from those of the excluded patients in terms of the number of non-survivors, the number of VA recipients, and the length of ECMO support. The excluded patients,

however, were older than the included patients (median age: 114 days vs. 6 days, $p=0.014$).

The baseline characteristics of the included patients are shown in Table 1. The VV and VA groups did not differ in median age at the time of cannulation, time between ICU admission and ECMO start, length of ECMO support, and length of ICU stay. Furthermore, in the VV group four (24%) patients died during their ICU stay. The causes of death included alveolar capillary dysplasia ($n=1$), pulmonary consolidation ($n=1$), sepsis-induced multiple organ failure ($n=1$), and persistent pulmonary hypertension ($n=1$) due to non-functioning Fontan circulation. Only the non-survivor with sepsis was successfully weaned from ECMO. In the VA group, 10 (32%) patients died. The causes of death were: recurrent, refractory pulmonary hypertension in CDH patients ($n=7$), hypoxic-ischemic brain injury due to hemorrhagic complications during ECMO ($n=1$), sepsis-induced multiple organ failure ($n=1$), and idiopathic persistent pulmonary hyper-

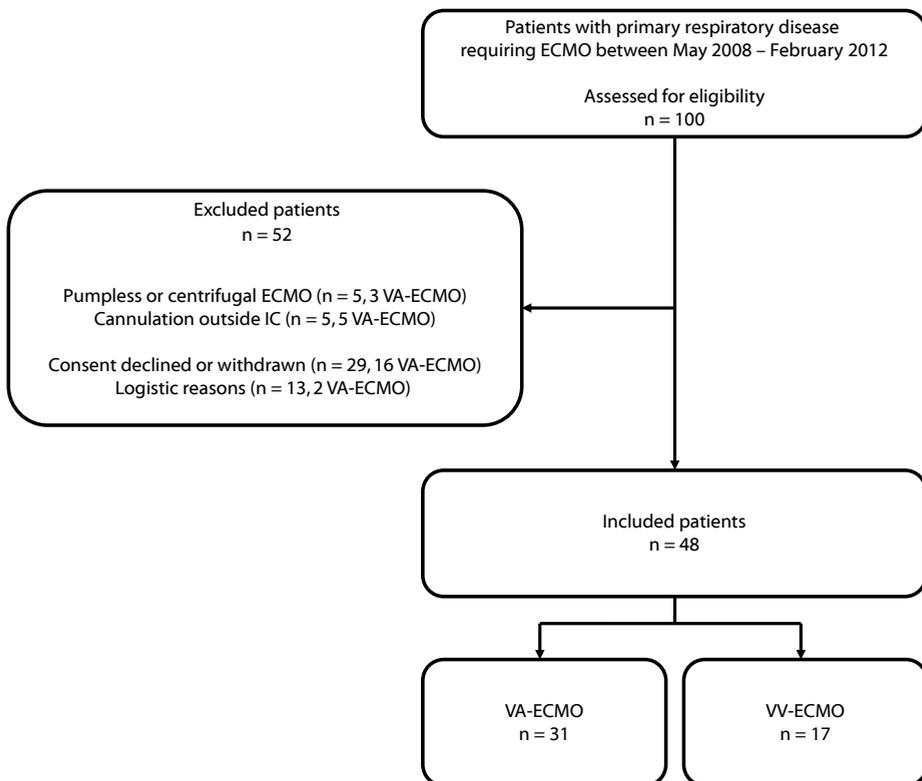


Figure 1. Flowchart for the patients with primary respiratory disease requiring extracorporeal membrane oxygenation who were assessed for inclusion in the study.

Table 1. The baseline patient characteristics for the patients who required venovenous and those who required venoarterial extracorporeal membrane oxygenation.

	VV N = 17	VA N = 31	p-value
Male gender, n (%)	8 (47%)	18 (58)	0.551
Age at ECMO start in d, median (IQR)	14 (8 18)	6 (6 4)	0.215
Neonatal patients at ECMO start, n (%)	9 (53)	23 (74)	0.201
Weight at ECMO start in kg, median (IQR)	4.3 (8.7)	3.2 (1.2)	0.011
Time ICU admission – ECMO start in d, median (IQR)	1 (2)	1 (5)	0.813
Time ICU admission – ECMO stop in d, median (IQR)	7 (8)	10 (11)	0.158
Time ECMO stop – discharge / death in d, median (IQR)	3 (21)	7 (64)	0.207
Conversion, n (%)	1 (6)	5 (16)	NA
Primary diagnosis at ECMO start, n (%)			
- MAS	5 (29)	1 (3)	
- CDH	0 (0)	15 (48)	
- Idiopathic PH	2 (12)	4 (13)	NA
- Respiratory disease; infectious	5 (29)	7 (23)	
- Respiratory disease; non-infectious	4 (24)	2 (7)	
- Septic shock	1 (6)	2 (7)	
Co-morbidity			
- Cardiac arrest	5 (29)	7 (23)	0.731
- Cardiac co-morbidity	2 (12)	4 (13)	1.000
- Hemorrhagic / coagulation disorder	9 (53)	21 (68)	0.361
- Malignancy	0 (0)	0 (0)	-
- Neurologic disease	2 (12)	7 (23)	0.460
- Organ transplantation	0 (0)	0 (0)	-
- Primary immunodeficiency	1 (6)	0 (0)	0.354
- Pulmonary hypertension	12 (71)	27 (87)	0.247
- Renal failure	2 (12)	4 (13)	1.000
Length of ECMO support in d, median (IQR)	5 (9)	7 (9)	0.207
Length of ICU stay in d, median (IQR)	19 (22)	23 (65)	0.419
Non-survival at ICU discharge, n (%)	4 (24)	10 (32)	0.741
Primary COD, n (%)			
- Respiratory	2 (50)	8 (80)	
- Septic shock	1 (25)	1 (10)	NA
- Cardiac	1 (25)	0 (0)	
- Neurologic	0 (0)	1 (10)	

Continuous data are presented as medians and interquartile range, discrete data as number and percentage. * Differences vs. VV at $p < 0.05$ using non-parametric tests. CDH: congenital diaphragmatic hernia, COD: cause of death, d: days, ECMO: extracorporeal membrane oxygenation, ICU: intensive care, kg: kilogrammes, MAS: meconium aspiration syndrome, NA: not assessed, PH: pulmonary hypertension, VA: venoarterial extracorporeal membrane oxygenation, VV: venovenous extracorporeal membrane oxygenation.

tension complicated by pneumonia (n=1). Eight of the non-survivors could be weaned from VA.

Macrocirculatory, respiratory, and biochemical parameters

Before cannulation, the median (IQR) VP-score was higher in the VA group than in the VV group (50 [49] vs. 28 [32]; p -value: 0.040). Also, HR and the MABP were both unfavorable in the VA group (HR: 162 [27] vs. 144 [34] bpm; p -value: 0.039, MABP: 49 [20] vs. 63 [27] mmHg; p -value: 0.026). In contrast, the disease measures OI and PELOD did not differ (OI: 29 [24] vs. 32 [18]; p -value: 0.675, PELOD: 20 [20] vs. 21 [20]; p -value: 0.332). None of the respiratory or biochemical parameters differed between the groups. The descriptive and inferential statistics for all of the macrocirculatory, respiratory, and biochemical parameters before, during, and after ECMO are presented in the supplements (supplementary Table 1).

After mixed effects models showed overall differences over time, sub-tests indicated that, in comparison to before cannulation, the macrocirculatory parameters HR and VP-score improved immediately after cannulation (Figure 2). The same held true for the respiratory parameters OI, iNO use, and arterial saturation, the biochemical parameters pO₂, pCO₂, and BE, and the disease severity score PELOD (Figure 2). Moreover, the improvement of all of these parameters was sustained until after decannulation (supplementary Table 1). At day 2 of ECMO support, pCRT and arterial lactate had improved as well.

The microcirculation: VV vs. VA

Table 2 depicts the median (IQR) values for the microcirculation the VV and VA groups before, during, and after ECMO support. Before cannulation, none of the microcirculatory parameters differed between the VA and VV group. Moreover, mixed effects models revealed that during and after ECMO support the microcirculation did not differ between the VA and VV group (Table 2 and supplementary Table 2).

The microcirculation: Evolution over time

A whole-group-analysis indicated that, in comparison with the values before cannulation, TVD NS, TVD S, PVD S, and HI NS did not change over time (Table 2). In contrast, PVD NS, PPV NS, PPV S, MFI NS, MFI S, and HI S improved during and after ECMO support. However, immediately after cannulation, when ECMO support was highest, none of these parameters had improved. Improvements occurred not until day 2 of ECMO and persisted until after decannulation (Figure 3).

A sub-analysis was performed to test whether one or more of the microcirculatory parameters were related to ICU mortality. Neither mixed effects modeling, nor Cox regression analysis indicated that the microcirculation differed between the survivors and

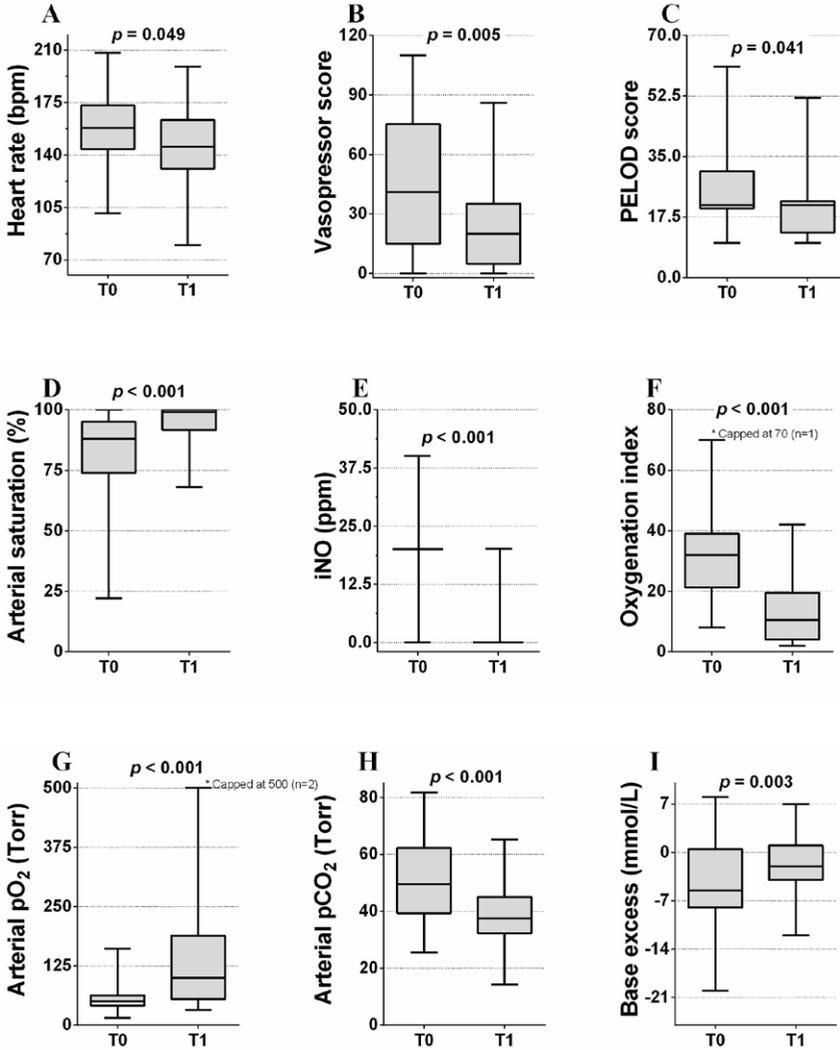


Figure 2. Boxplots showing the macrocirculatory parameters, respiratory parameters, biochemical parameters, and overall disease severity indices immediately before (T0) and after ECMO start (T1) in children with refractory, primary respiratory disease requiring extracorporeal membrane oxygenation (n=48). Most parameters increased immediately after ECMO start. A: heart rate in beats per minute, B: vasopressor score, C: pediatric logistic organ dysfunction score, D: arterial saturation in percentage, E: inhaled nitric oxide in parts per million, F: oxygenation index, G: arterial partial oxygen pressure in Torr, H: arterial partial carbon dioxide pressure in Torr, I: base excess in millimoles per liter. Differences vs. T0 were assessed with mixed effects models and sub-tests.

Table 2. The microcirculatory parameters over time of the patients with primary respiratory disease who required either venovenous (n = 17) or venoarterial (n = 31) extracorporeal membrane oxygenation.

	Before cannulation		After cannulation		ECMO day 2		ECMO day 3		Before decannulation		After decannulation	
	VV	VA	VV	VA	VV	VA	VV	VA	VV	VA	VV	VA
Time to ECMO start/stop in h	-1.6 (1.8)		+2.6 (2.6)		+23.7 (8.9)		+49.4 (9.1)		-9.2 (20.7)		+2.6 (13.1)	
TVD Non-small in n/mm	6.1 (1.7)	6.5 (1.8)	6.8 (2.3)	5.7 (1.4)	6.9 (1.3)	6.7 (1.6)	7.0 (0.8)	6.7 (2.5)	6.5 (1.4)	6.8 (2.1)	5.6 (2.0)	6.9 (1.3)
	6.3 (1.8)		5.8 (1.9)		6.7 (1.5)		6.9 (1.9)		6.7 (1.7)		6.6 (2.0)	
TVD Small in n/mm	10.5 (3.3)	11.9 (3.9)	9.7 (4.6)	11.4 (1.9)	10.6 (2.4)	10.1 (3.1)	11.0 (5.3)	10.8 (2.7)	11.3 (3.6)	11.8 (3.2)	10.0 (5.3)	11.5 (3.4)
	11.3 (4.0)		11.3 (1.9)		10.4 (2.7)		10.8 (3.3)		11.4 (3.1)		11.1 (2.0)	
PVD Non-small in n/mm	4.0 (2.0)	4.8 (2.8)	4.5 (3.0)	4.6 (1.8)	6.5 (1.4)	6.3 (1.9)	6.7 (1.4)	6.1 (2.5)	6.1 (1.1)	6.4 (2.2)	5.4 (1.3)	6.2 (2.3)
	4.7 (2.5)		4.6 (2.1)		6.4 (1.8)*		6.5 (1.8)*		6.3 (1.5)*		5.9 (1.9)*	
PVD Small in n/mm	9.4 (5.5)	9.6 (4.4)	9.0 (5.3)	10.0 (3.7)	10.0 (2.5)	9.7 (4.0)	10.7 (6.3)	10.3 (2.2)	10.5 (3.6)	10.7 (3.5)	9.8 (5.5)	10.7 (3.6)
	9.5 (4.5)		9.2 (4.2)		9.9 (2.9)		10.4 (3.8)		10.7 (3.6)		10.6 (4.7)	
PPV Non-small in %	87 (33)	80 (32)	90 (39)	87 (22)	92 (15)	94 (10)	96 (19)	95 (8)	92 (6)	96 (11)	94 (9)	92 (13)
	82 (31)		88 (22)		93 (11)*		96 (9)*		94 (9)*		93 (10)*	
PPV Small in %	85 (35)	85 (24)	94 (28)	91 (24)	97 (10)	98 (9)	98 (9)	98 (4)	95 (11)	99 (8)	97 (6)	96 (8)
	85 (30)		92 (25)		98 (9)*		98 (5)*		97 (9)*		97 (8)*	
MFI Non-small in au	2.62 (0.62)	2.79 (0.49)	3.00 (0.63)	2.92 (0.38)	2.95 (0.16)	3.00 (0.10)	3.00 (0.11)	3.00 (0.08)	2.92 (0.11)	3.00 (0.13)	3.00 (0.07)	2.92 (0.28)
	2.76 (0.52)		2.92 (0.38)		3.00 (0.13)*		3.00 (0.08)*		2.98 (0.13)*		2.95 (0.14)*	
MFI Small in au	2.50 (0.71)	2.58 (0.58)	3.00 (0.75)	2.67 (0.58)	3.00 (0.23)	3.00 (0.25)	3.00 (0.15)	3.00 (0.08)	2.83 (0.25)	3.00 (0.21)	3.00 (0.08)	2.92 (0.17)
	2.58 (0.58)		2.83 (0.60)		3.00 (0.25)*		3.00 (0.08)*		3.00 (0.25)*		3.00 (0.17)*	
HI Non-small in au	0.39 (1.26)	0.36 (0.08)	0.00 (0.40)	0.34 (0.38)	0.17 (0.35)	0.00 (0.35)	0.00 (0.35)	0.00 (0.34)	0.34 (0.18)	0.00 (0.35)	0.00 (0.34)	0.34 (0.38)
	0.36 (0.60)		0.34 (0.39)		0.00 (0.35)		0.00 (0.34)		0.17 (0.35)		0.34 (0.35)	
HI Small in au	0.43 (1.29)	0.39 (0.90)	0.00 (1.16)	0.38 (0.41)	0.00 (0.37)	0.00 (0.36)	0.00 (0.35)	0.00 (0.34)	0.35 (0.37)	0.00 (0.36)	0.00 (0.34)	0.34 (0.35)
	0.39 (0.93)		0.35 (0.44)		0.00 (0.36)*		0.00 (0.34)*		0.00 (0.36)*		0.00 (0.35)*	

The microcirculatory parameters are shown for non-small (NS; 10 μm ≤ Ø < 100 μm) and small (S; Ø < 10 μm) vessels and were collected before and after start of ECMO, at ECMO day 2 and ECMO day 3, and before and after stop of ECMO. Continuous variables are presented as median (IQR) for the VA and VV patients (top line) as well as for the total group (bottom line). † p < 0.050 vs. VV with non-parametric tests, * p < 0.050 vs. T0 with mixed models and sub-tests. au: arbitrary units, HI: heterogeneity index, MFI: microvascular flow index, n/mm: number per millimeter, PPV: proportion of perfused vessels, PVD: perfused vessel density, TVD: total vessel density, VA: venoarterial extracorporeal membrane oxygenation, VV: venovenous extracorporeal membrane oxygenation.

the non-survivors. The median (IQR) time between decannulation and ICU discharge for the whole ECMO group was 7 (25) days, and for the non-survivors only 5 (18) days. The microcirculation did neither differ between the patients who could (n=43) and could not (n=5) be weaned from ECMO.

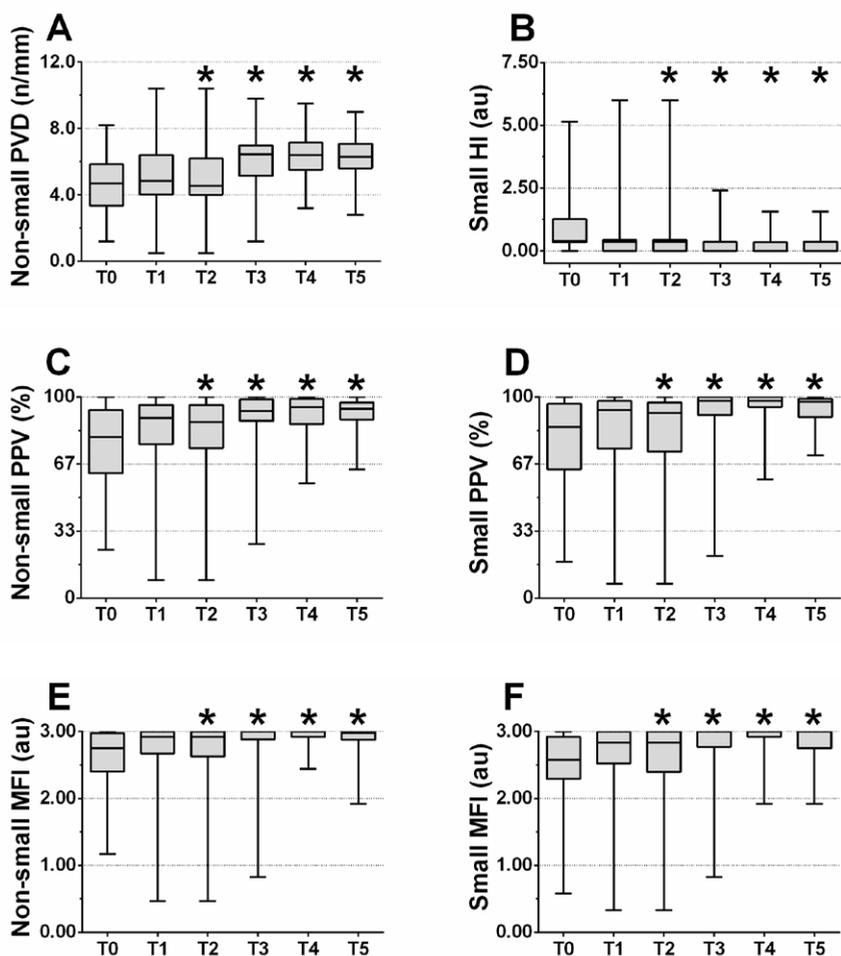


Figure 3. Boxplots showing the microcirculatory parameters for non-small (NS; $10\ \mu\text{m} \leq \text{Ø} < 100\ \mu\text{m}$) and small (S; $\text{Ø} < 10\ \mu\text{m}$) vessels that were collected before (T0) and after start of ECMO (T1), at ECMO day 2 (T2) and ECMO day 3 (T3), and before (T4) and after stop of ECMO (T5) in children with primary respiratory disease requiring extracorporeal membrane oxygenation (n=48). The microcirculation did not increase until after ECMO day 1. A: non-small perfused vessel density in crossing per millimetre, B: small heterogeneity index in arbitrary units, C: non-small proportion of perfused vessels in percentage, D: non-small microvascular flow index in arbitrary units, E: small proportion of perfused vessels in percentage, F: small microvascular flow index in arbitrary units. Differences at *p<0.050 vs. T0 with mixed models and sub-tests.

DISCUSSION

In this study, the microcirculation did not differ between patients diagnosed with primary respiratory failure receiving either VA or VV support. Both VA and VV restore the microcirculation. However, in contrast to the cardiorespiratory and biochemical parameters, the microcirculation is not improved immediately after the start of ECMO.

This is the first study to compare the microcirculation between VV and VA patients. Although macrocirculatory failure –i.e., HR, MABP, and VP-score– was less severe in the VV patients prior to ECMO, the microcirculation was impaired and did not differ from the microcirculation in VA patients. The microcirculatory impairment in the VV patients could be explained by the fact that a certain degree of macrocirculatory failure was also present prior to VV, given that 85% of the VV patients received vasopressive drug therapy –median (IQR) VP-score: 28 (32)–. Additionally, clinical studies have shown that the microcirculation is decreased in adults with hypoxemic hypoxia during acute respiratory distress and in adults exposed to hypobaric hypoxia [25, 26]. Yet another explanation for the microcirculatory deterioration in the VV patients could be that both hypoxia and critical illness in general can cause systemic inflammation [27, 28]. Systemic inflammation, in turn, has been associated with endothelial dysfunction and microcirculatory impairment [14].

For the VA patients, the microcirculatory impairment prior to ECMO start appears to be consistent with the findings of other, non-ECMO studies to the effect that the microcirculation is affected during shock –i.e., macrocirculatory failure [7, 10]. Moreover, an earlier clinical study in neonates reported that the microcirculation was higher after VA decannulation when compared to before VA cannulation [8]. Microcirculatory improvement during ECMO were also reported in six adults, but neither the timing nor the ECMO modality were specified [29]. The current study, which focused on a larger pediatric cohort and which used an imaging device technically superior to the one used previously in the pediatric studies –i.e., sidestream dark field imaging rather than orthogonal polarization spectral imaging–, thus supports the earlier findings for VA. In contrast to the earlier studies, but in line with the international consensus guidelines, the present study provides a description of the total microvascular density together with functional density, microvascular blood flow velocity, and heterogeneity [16]. We observed that total vessel density was relatively unaffected in ECMO children. Microcirculatory abnormalities were predominantly heterogeneous and became particularly apparent after discrimination between total and functional microcirculation. Similar findings have been reported in adults with hypovolemic, cardiogenic, or distributive shock [7, 30-32].

Moreover, not only VA improves the microcirculation. The current study shows for the first time that the microcirculation is restored after VV. This is important because VV is, relative to VA, nowadays more frequently used [3-5]. Also, because VV has advantages over VA such as sparing of the carotid and/or femoral artery, VV has been proposed

for indications that in the past were exclusively treated with VA [33, 34]. VV is the third intervention in critically ill children that is reported to be beneficial for the microcirculation – next to VA and iNO [35]. In the present study, approximately 80% of the patients received ECMO support despite iNO therapy. iNO non-responsiveness has been related previously to endothelial dysfunction and ECMO dependency [36, 37]. Our results suggest that VV or VA might serve as a rescue treatment for improving the microcirculation in critically ill children with primary respiratory disease who are unresponsive to iNO treatment.

Another important finding is that microcirculatory improvement occurred not until day 2 of ECMO support. Microcirculatory improvement coincided with improvements in arterial lactate and pCRT. In contrast, the rate of microcirculatory recovery did not keep pace with the rate of recovery of respiratory, macrocirculatory, and biochemical parameters after cannulation. It has been argued previously that macrocirculatory parameters might not always adequately reflect microcirculatory function during critical illness [7, 10]. Interestingly, Lam et al. reported that microcirculatory improvement was delayed by 24 hours in adults who received extracorporeal left ventricular support [38]. Another study in adults with end-stage heart failure or cardiogenic shock demonstrated an overall microcirculatory improvement that was highest after 24 hours of mechanical circulatory support with one of several types of devices [39]. In the current study we did not unravel which factors contributed to the delay in microcirculatory improvement. Candidate factors include: ongoing inflammation after cannulation [12, 13], an increment of circulating volume after cannulation [40], non-physiologic blood flow with loss or decline of pulsatility in the case of VA [15], and systemic micro-emboli in the case of VA .

The inflammatory response following cannulation has been characterized by others. Marked inflammation was reported to be present within minutes after cannulation and to persist for several days [12]. Interestingly, it was reported that inflammation subsided significantly after approximately 24 hours, the time after which we observed microcirculatory improvement [41, 42]. Furthermore, with the start of ECMO, the patient's circulating volume is increased by approximately a factor of 1.5. The biochemical properties of the primed ECMO circuit were checked and adjusted to age-appropriate normal values prior to cannulation. Hence, Hb and Ht did not change, unlike after conventional blood transfusion. A study in preterm infants showed a favorable effect of blood transfusion that is particularly pronounced after 24 hours, the time after which we observed microcirculatory improvement [40]. The non-physiologic blood flow with loss or decline of pulsatility during VA seems to exert relatively little influence on the microcirculation as it increased before ECMO blood flow settings were weaned. Future research should elucidate which factors in particular inhibit microcirculatory improvement during ECMO and whether the delay in microcirculatory improvement is adaptive or not.

In critically ill children not receiving ECMO, microcirculatory deterioration has been related to poor outcome [10]. Moreover, early microcirculatory resuscitation in adults was associated with improved survival [31, 32]. In the current study, we did not observe that microcirculatory deterioration was related to mortality. The lack of an association might be explained by the large time window between follow-up and outcome. For instance, the median (IQR) time window between decannulation and ICU discharge was 5 (18) days. We feel it is unlikely that the microcirculation is already impaired 5 days prior to the actual event. A sub-analysis that discriminated between the patients who could ($n=43$) and could not ($n=5$) be weaned from ECMO failed to produce different results. However, given in the small sample size of the latter group, the likelihood of obtaining statistically significant differences was low. Still, we did observe that the microcirculation followed the improvement of the routinely measured macrocirculatory parameters, respiratory parameters, biochemical parameters, and disease severity indices. Also, microcirculatory improvement coincided with improvements in arterial lactate and pCRT. For arterial lactate, similar findings were reported previously in adults with septic shock [43]. Yeh et al. reported that microcirculatory impairment preceded increments in arterial lactate concentration [44]. Therefore, we argue that non-invasive microcirculatory monitoring not only reflects the state of the microcirculation adequately, but also that it could be a valuable addition to the routine hemodynamic monitoring of ECMO patients. Unfortunately, given that the microcirculation did not differ over time between the VA and VV groups, microcirculatory monitoring will probably be less suited to select the correct modality for individual patients. The association between microcirculatory impairment and poor outcome in ECMO patients should be explored in sufficiently powered future studies.

Several limitations of this study should be addressed. Most importantly, in this observational study the CDH patients might be overrepresented, as our center is a nationwide referral center. Bias might have been introduced, as these patients all received VA in accordance with hospital protocol [3, 24]. Propensity matching or case-control matching would have been more ideal, but this would require a multicenter study. Second, data were collected during the first 3 days and on the final day of ECMO support whereas the duration of ECMO support varied between patients. The effect of, for instance, ECMO weaning could therefore not be taken into account. The median (IQR) length of ECMO support was 6 (9) days, however, and did not differ between VA and VV. Further, the relatively small sample size precluded statistical analysis that incorporates the effects of hypothetic confounders such as morbidity, co-morbidity, and age. A substantial number of patients had to be excluded due to declined parental consent or logistic reasons and, to decrease disease heterogeneity, patients with primary cardiac disease were excluded. Still, we did observe consistent, significant differences and the sample size is larger than any other study in this field. Results should be interpreted with caution and

are applicable only to children with primary respiratory disease. Also, the number of VV patients was approximately half of that of the VA patients. Consequently, the data might be skewed, which might have influenced the results. Previous research by our group has shown that a sample size of 15 ECMO patients is sufficient to obtain statistically significant differences [8].

CONCLUSIONS

Both VA and VV restore the microcirculation in patients diagnosed with primary respiratory failure. The evolution of the microcirculation over time does not differ between patients receiving either VA or VV support and the microcirculation is impaired prior to ECMO start, also in VV patients. While macrocirculatory, respiratory, and biochemical parameters improve immediately after ECMO support is provided, the microcirculation does not improve until after 1 day.

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Supplemental table 1. The macrocirculatory parameters, respiratory parameters, biochemical parameters, and disease severity indices of the patients with primary respiratory disease who required either venovenous (n = 17) or venoarterial (n = 31) extracorporeal membrane oxygenation.

	Before cannulation		After cannulation		ECMO day 2		ECMO day 3		Before decannulation		After decannulation	
	VV	VA	VV	VA	VV	VA	VV	VA	VV	VA	VV	VA
Oxygenation index	32 (18)	29 (24)	19 (15)†	7 (10)†	14 (16)	8 (5)	12 (13)†	6 (4)†	8 (9)	7 (4)	8 (7)	7 (12)
PELOD ^a	32 (18)	20 (20)	10 (16)*	13 (10)	9 (12)*	12 (10)	8 (6)*	12 (10)	7 (8)*	-	8 (8)*	-
Vasopressor score	21 (10)	50 (49)†	21 (10)	18 (31)	12 (9)*	10 (20)	11 (10)	0 (10)	-	-	-	-
Heart rate in bpm	28 (32)†	162 (27)†	24 (30)	20 (30)*	17 (31)	139 (22)	0 (19)	135 (25)	0 (15)	0 (21)	0 (20)	0 (19)
MABP in mmHg	41 (60)	49 (20)†	138 (41)	146 (33)*	10 (25)*	46 (14)	0 (12)*	58 (17)†	0 (16)*	141 (34)	144 (25)	144 (31)
ECMO blood flow ^b in ml/kg/m	158 (29)	60 (17)†	146 (33)*	51 (15)†	135 (24)*	50 (30)	131 (25)*	82 (49)†	138 (28)*	51 (12)	51 (9)	50 (17)
iNO in ppm	63 (27)†	52 (24)	54 (20)	118 (44)	48 (17)	83 (35)†	61 (20)	103 (29)	51 (18)	37 (46)†	-	-
Arterial saturation in %	-	20 (1)	97 (46)	109 (45)	106 (48)	0 (20)	97 (34)*	0 (0)	56 (65)*	0 (1)	0 (0)†	0 (20)†
SvO ₂ in %	20 (2)	90 (15)	4 (20)	100 (5)	0 (3)*	96 (5)	0 (0)*	99 (2)†	0 (4)*	95 (6)	97 (6)	97 (4)
pH	88 (21)	7.26 (0.16)	84 (19)	7.39 (0.09)	74 (14)	7.40 (0.10)	77 (9)	7.41 (0.08)	97 (6)*	66 (19)	-	-
pO ₂ in Torr	11.3 (4.0)	7.27 (0.17)	80 (15)	123 (180)	76 (35)	7.38 (0.10)	76 (9)	7.38 (0.08)	69 (15)*	7.31 (0.13)	7.35 (0.12)	7.34 (0.12)
	50 (40)	51 (22)	68 (95)	99 (134)*	82 (38)*	76 (35)	68 (22)†	98 (55)†	71 (31)	92 (53)	77 (58)	98 (45)
	82 (21)	89 (46)*	80 (110)	83 (82)	89 (46)*	83 (82)	89 (46)*	80 (45)*	90 (49)*			

Supplemental table 1. (continued)

	Before cannulation		After cannulation		ECMO day 2		ECMO day 3		Before decannulation		After decannulation	
	VV	VA	VV	VA	VV	VA	VV	VA	VV	VA	VV	VA
pCO ₂ in Torr	47 (33)	51 (22)	37 (16)	38 (13)	44 (8)	43 (11)	44 (8)	40 (9)	38 (9)	42 (14)	49 (12)	47 (17)
	50 (23)		38 (13)*		44 (8)*		41 (8)*		41 (10)*		48 (14)	
Base excess in mmol/L	-6 (13)	-5 (7)	-3 (7)	-2 (4)	1 (5)	2 (4)	1 (2)	2 (3)	0 (4)	0 (4)	-1 (8)	-1 (4)
	-6 (9)		-2 (5)*		2 (5)*		2 (2)*		0 (4)*		-1 (5)*	
Arterial lactate in mmol/L	2.9 (5.9)	2.6 (3.2)	4.4 (4.6)	3.2 (5.6)	2.1 (2.1)	1.8 (0.8)	1.5 (0.9)	1.5 (0.4)	1.3 (1.2)	1.0 (0.6)	0.8 (1.1)	1.2 (1.0)
	2.6 (4.0)		3.7 (4.0)		1.8 (0.8)*		1.5 (0.6)*		1.2 (0.8)*		1.0 (1.0)*	
Hb in mmol/L	7.3 (1.8)	7.4 (2.0)	7.5 (2.3)	7.8 (1.6)	7.0 (1.4)	7.3 (0.9)	6.9 (0.5)	7.3 (0.7)	7.0 (1.2)	6.9 (0.8)	6.5 (1.6)	6.7 (0.5)
	7.4 (2.0)		7.7 (1.7)		7.1 (1.0)		7.0 (0.7)		6.9 (1.0)		6.6 (0.8)*	
Ht in L/L	0.36 (0.08)	0.34 (0.08)	0.36 (0.08)	0.37 (0.05)	0.35 (0.07)	0.34 (0.04)	0.33 (0.02)	0.34 (0.04)	0.34 (0.05)	0.32 (0.04)	0.32 (0.04)	0.32 (0.05)
	0.34 (0.08)		0.37 (0.05)		0.34 (0.04)		0.33 (0.03)		0.33 (0.04)		0.32 (0.05)*	

Data were collected before and after start of ECMO, at ECMO day 2 and ECMO day 3, and before and after stop of ECMO. Continuous variables are presented as median (IQR) for the VA and VV patients (top line) as well as for the total group (bottom line). † p<0.050 vs. VV with non-parametric tests, * p<0.050 vs. TO with mixed models and sub-tests. bpm: beats per minute, ECMO: extracorporeal membrane oxygenation, h: hours, Hb: hemoglobin, Ht: hematocrit, iNO: inhaled nitric oxide, kg: kilograms, L: liters, m: minute, ml: milliliter, mmHg: millimeter of mercury, mmol: millimoles, ppm: parts per million, pCO₂: arterial partial carbon dioxide pressure, pCRT: peripheral capillary refill time, PELOD: pediatric logistic organ dysfunction score, pO₂: arterial partial oxygen pressure, SvO₂: mixed venous oxygen saturation, VA: venoarterial extracorporeal membrane oxygenation, VV: venovenous extracorporeal membrane oxygenation. †PELOD score was determined 0-24 hours before ECMO start as well as 0-24, 24-48, and 48-72 hours after ECMO start. †ECMO blood flow and SvO₂ were measured only during ECMO support with the data after cannulation as baseline.

Supplemental table 2. Output for the mixed effects models with the interaction term between time (T1-T5, see Figure 3) and group (venovenous [VV] or venoarterial [VA] extracorporeal membrane oxygenation) evaluating whether any of the microcirculatory parameters differed over time between the patients in the venovenous (n = 17) or venoarterial (n = 31) group.

Microcirculatory perfusion parameter	Mixed effects model parameter	Degrees of Freedom	F-value	p-value
TVD Non-small in n/mm	Intercept	185	254.58	
	Interaction	185	0.94	0.423
TVD Small in n/mm	Intercept	185	189.05	
	Interaction	185	2.08	0.105
PVD Non-small in n/mm	Intercept	185	87.26	
	Interaction	185	0.79	0.502
PVD Small in n/mm	Intercept	189	18.68	
	Interaction	189	0.70	0.482
PPV Non-small in %	Intercept	185	11.64	
	Interaction	185	0.24	0.866
PPV Small in %	Intercept	185	14.21	
	Interaction	185	0.34	0.799
MFI Non-small in au	Intercept	185	32.31	
	Interaction	185	0.25	0.860
MFI Small in au	Intercept	185	22.32	
	Interaction	185	0.61	0.607
HI Non-small in au	Intercept	189	-8.25	
	Interaction	189	-0.64	0.522
HI Small in au	Intercept	185	6.39	
	Interaction	185	1.64	0.182

The microcirculatory parameters are shown for non-small (NS; $10 \mu\text{m} \leq \varnothing < 100 \mu\text{m}$) and small (S; $\varnothing < 10 \mu\text{m}$) vessels and were collected before and after start of ECMO, at ECMO day 2 and ECMO day 3, and before and after stop of ECMO. au: arbitrary units, HI: heterogeneity index, MFI: microvascular flow index, n/mm: number per millimeter, PPV: proportion of perfused vessels, PVD: perfused vessel density, TVD: total vessel density, VA: venoarterial extracorporeal membrane oxygenation, VV: venovenous extracorporeal membrane oxygenation.

Chapter 5

Cardiovascular catecholamine receptors in children: their significance in cardiac disease

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Abstract

Adrenoceptors and dopamine receptors are grouped together under the name 'catecholamine receptors'. Catecholamines and catecholaminergic drugs act on catecholamine receptors located on or near the cardiovascular system. The physiological effects of catecholamine receptor stimulation are only partly understood. The catecholaminergic drugs used in critical care medicine today are not selective, or are, at best, in part selective for the various catecholamine receptor subtypes. Many patients, however, depend on them. A variety of animal models has been developed to unravel catecholamine distribution and function. However, the identification of species heterogeneity makes it imperative to determine catecholamine receptor distribution and function in humans. In addition, age-related alterations in catecholamine receptor distribution and function have been identified in human adults. This might have implications for our understanding of the effect of catecholamines in pediatric patients. This paper will focus on the pediatric population, and will review currently available *in vitro* data on the distribution and the function of catecholamine receptors in the cardiovascular system of fetuses and children. Discussed as well are relevant young animal models and *in vivo* hemodynamic effects of cardiotonic drugs acting on the catecholamine receptor in children requiring major cardiac surgery. Better understanding of these topics might provide clues for new, receptor subtype-selective, therapeutic approaches in newborns and children with cardiac disease.

Introduction

The sympathetic nervous system exerts action on the cardiovascular system via norepinephrine and epinephrine, which target α -adrenoceptors and β -adrenoceptors. Next to dopamine, norepinephrine and epinephrine are classified under the so-called biogenic catecholamines. Likewise, the α -adrenoceptors and β -adrenoceptors are grouped with the dopamine (DA-) receptors under the name 'catecholamine receptors'. Catecholamine receptors belong to the class of G-protein coupled receptors (GPCR). In general, these receptors couple to: 1) adenylyl cyclase (AC) and subsequent cyclic adenosine monophosphate formation, 2) phospholipase C followed by hydrolysis of phosphoinositides, and 3) ion channel activity.[1]

Nine genetically distinct adrenoceptor subtypes have been identified; α_{1A} , α_{1B} , α_{1D} , α_{2A} , α_{2B} , α_{2C} , β_1 , β_2 , and β_3 . [2, 3] In humans the adrenoceptors α_{1A} , α_{1B} and α_{1D} are encoded by three distinct genes located on the chromosomes 8, 5, and 20, respectively. α_{2A} , α_{2B} and α_{2C} are encoded by genes on chromosomes 10, 2, and 4; β_1 , β_2 , and β_3 by genes on chromosomes 10, 5 and 8. Five DA-adrenoceptor subtypes have been identified, categorized into two groups known as DA₁-like (DA₁ and DA₅; chromosome 5 and 4) and DA₂-like (DA₂, DA₃, and DA₇; chromosome 11, 3, and 11). All five are suggested to be present in the cardiovascular system of humans.[4-8]

Catecholamines and drugs with catecholamine-like properties act on catecholamine receptors located on or near the heart or blood vessels. The current paradigm is that these locations include the pre-synaptic and post-synaptic nerve terminals, the smooth muscle membrane, and the endothelium.[3, 9, 10]

The physiological effects of catecholamine receptor stimulation are only partly understood.[3] Classically α_1 -adrenoceptor and α_2 -adrenoceptor stimulation induces peripheral vasoconstriction, whereas α_2 -adrenoceptors are also involved in the central control of blood pressure. In addition, presynaptic α_2 -adrenoceptors on adrenergic nerve endings inhibit norepinephrine release. β_1 -adrenoceptor and β_2 -adrenoceptor stimulation has chronotropic and inotropic effects, while β_2 -adrenoceptors also induce vasodilation. The catecholamine agonists and/or antagonists used in critical care medicine today are not selective, or are, at best, in part selective for the various catecholamine receptor subtypes. Many patients, however, depend on them.

A variety of animal models has been developed to unravel the effects of catecholaminergic drugs. However, the distribution and function of the catecholamine receptors in the cardiovascular system in animals differs from that in human adults which makes it imperative to study catecholamine receptors in humans.[11-15] In addition, the distribution of adrenoceptor subtypes in human adults varies across distinctive cardiac tissues and vascular beds.[3, 16-19] Also, humans show age-related differences in adrenoceptor distribution and function.[20-22] This might have implications for our understanding of the effect of catecholamines in pediatric patients.

This review has three focal points: 1) Developmental aspects of catecholamine receptors in human, fetal cardiovascular tissue given their significance for extreme low birth weight infants. 2) Distribution and function of catecholamine receptors in the pediatric cardiovascular system. Here we restricted ourselves to *in vitro* studies as these enable the study of isolated tissues not subjected to confounding factors such as the baroreflex, pharmacokinetic differences, and shear stress. 3) *In vivo* hemodynamic effects of cardiotoxic drugs acting on the catecholamine receptor in children with congenital heart disease (CHD) requiring major cardiac surgery. Ultimately, better understanding of these topics might provide clues for new, receptor subtype-selective, therapeutic approaches and evidence-based pharmacotherapy in newborns and children with cardiac disease.

Catecholamine receptors in fetal cardiac tissue

Stimulation by epinephrine, norepinephrine, and isoprenaline increases the sino-atrial rate in human fetal hearts as early as the fifth week of gestation.[23, 24] The same agents induce AC-activity in fetal cardiac tissue aged 6-7 weeks.[25] The chronotropic response to epinephrine or isoprenaline has been reported to develop in a biphasic manner: the contraction rate and contraction force increase from the gestational weeks 5-10 but then hardly changes over the weeks 10-18.[23] However, contradictory findings have been reported.[24, 26, 27] After week 18 the inotropic response and the maximum chronotropic response become stronger as development continues.[23, 24, 26-28] β -adrenoceptors seem to be the predominant adrenoceptor subtype in fetal, cardiac tissue.[25, 27, 29] It remains unclear whether maturational effects are present at the post-receptor level as well.[25, 28]

Gennser et al. determined the norepinephrine concentration in atria and ventricles from fetuses aged of 9-11 weeks and fetuses aged 12 weeks.[30] Whereas the norepinephrine concentration of the older fetuses was evenly distributed over the atria and ventricles, the norepinephrine concentration in the ventricles of the younger fetuses was markedly lower. Neither age group showed differences between the left and right parts of the cardiac tissue. In another study, aortic tissue obtained from fetuses with a gestational age of 16-21 weeks contained high concentrations of norepinephrine and low concentrations of epinephrine.[31]

Others observed catecholamine-containing cells (CCCs) in the adventitia of fetal vascular tissue but not in the heart itself at weeks 8-9 of gestation.[32] At the weeks 17-18 the interatrial area contained CCCs, whereas fewer cells were seen around the large vessels. The ventricles did not contain CCCs. In all cases CCCs were found in association with extrinsic nerves.

It remains a matter of debate at which gestational age adrenergic nerves are present in human, fetal cardiac tissue. Papp reported the presence of adrenergic nervous cells at the gestational weeks 9-10; others reported the absence at the gestational weeks 17-18.

[23, 32, 33] It is stated that adrenergic nerves become functionally active approximately from the weeks 12-14 onwards.[34, 35] In contrast, Coltart et al. observed that contractile responses of cardiac tissue developed during the weeks 12-22 whereas no alterations in the electrophysiological recordings were seen.[26] This suggests the presence of autonomic adrenoceptors rather than neural adrenergic control. Bkaily et al. demonstrated that α_{1A} -adrenoceptors are physically and functionally present in myocytes of fetuses at the gestational age of 20 weeks.[36]

The literature search yielded neither reports on radioligand binding assays mapping out the total and the subtype-specific adrenoceptor distribution in fetal cardiac tissue, nor reports on the distribution and/or function of DA-adrenoceptors.

Catecholamine receptors in fetal vascular tissue

There are no reports on the distribution of catecholamine receptors in peripheral vascular tissue of human fetuses. To date, three reports focus on the function of catecholamine receptors in peripheral vascular tissue.[37-39] Various techniques were used such as organ chamber force contraction measurements, organ chamber perfusion pressure measurements and histochemistry.

First, the contractile response to epinephrine, norepinephrine, tyramine, and isoprenaline was studied in the isolated ductus arteriosus, the aorta, and the pulmonary trunk of human 10 to 24-week-old fetuses.[37] Isoprenaline was without effect, but epinephrine and norepinephrine both caused a dose-dependent vasoconstriction in the ductus. The vessel contraction force from the youngest fetuses was about one third of that of the oldest ones.

Second, the ductus arteriosus was studied together with the main, right and left pulmonary artery and the entire thoracic aorta obtained from 10 to 24-week-old fetuses.[38] The ductus contained abundant adrenergic fibers in between the smooth muscle bundles of the tunica media. Adrenergic nerves were predominantly present in the peripheral parts of the tunica media, but in the youngest fetuses only in the outermost parts of the tunica media. The descending aorta contained no nerve terminals. It was concluded that adrenoceptors are functionally active in the human ductus arteriosus, and that adrenoceptor function correlates with the presence of specific adrenergic nerve fibers in the smooth muscle layer of the media.

Third, Ehinger et al. studied the adrenergic innervation and the functional response to norepinephrine in the ductus venosus of 20 to 23-week-old fetuses.[39] An increasing amount of adrenergic nerves was observed towards the ductus venosus with a distinct accumulation of adrenergic nerves at the origin of the ductus venosus. Norepinephrine induced vasoconstriction in the ductus venosus which could be reversed by phenoxybenzamine.

Catecholamine receptors in cardiac tissue in children

A. Non-failing, structurally normal cardiac tissue

Most of the studies described in this review made use of structurally abnormal cardiac tissue. By using radioligand binding assays Sucharov et al. determined that the β_1 : β_2 -adrenoceptor density ratio is approximately 80:20 in non-failing, structurally normal left ventricles.[40] Berkenboom et al. used contraction force measurements to study adrenoceptor functionality in isolated coronary arteries obtained from children in irreversible neurological coma.[41, 42] Both α_1 -adrenoceptors and α_2 -adrenoceptors, and β_1 -adrenoceptors and β_2 -adrenoceptors were found present. Endogenous norepinephrine exerted its vasodilatory effects predominantly through β -adrenergic stimulation. [41] The authors also established that β -adrenoceptor function was independent from the endothelium.[42]

B. Effects of pathology on adrenoceptor density and/or gene expression

Total and subtype specific β -adrenoceptor density next to β_1 -mRNA and β_2 -mRNA gene expression was determined in explanted left ventricles obtained from children with idiopathic dilated cardiomyopathy (IDC) and in explanted left ventricles of children with non-failing hearts.[40] While total β -adrenoceptor density was decreased by 35% in the IDC patients, the β_1 : β_2 -ratio (80:20) remained unaltered, indicating a concomitant decrease in both β_1 -adrenoceptor and β_2 -adrenoceptor density. Conversely, only the β_2 -mRNA gene expression was lower in the IDC group than in the control group suggesting discordant β -adrenoceptor regulation.

Sun et al. studied β -adrenoceptor density in relation to disease severity in the right ventricular outflow tract (RVOT) of patients diagnosed with tetralogy of Fallot (TOF).[43] The total β -adrenoceptor density in the TOF patients without hypoxic spells was lower than in the TOF patients with hypoxic spells. In contrast, Brodde et al. observed a higher total β -adrenoceptor density in the RVOT of TOF patients with minimal right-to-left shunts (NYHA class I-II) than in the RVOT of the TOF patients with significant shunting (NYHA class III-IV).[44] The β_1 : β_2 -ratio did not differ in the more severely ill patients in both studies, and approximated the β_1 : β_2 -ratio found by others (71:29).[45]

Adrenoceptor distribution was also studied in atrial tissue: mean total β -adrenoceptor density is 68 femtomoles (–) - 125 I]-iodocyanopindolol / mg protein in right atrial appendages of patients with acyanotic CHD, the β_1 : β_2 -ratio is 68:32.[46] Others found a lower total β -adrenoceptor density in the more severely ill children.[47, 48] The decrease was attributed to a shift in adrenoceptor distribution; β_1 -adrenoceptor density decreased whereas β_2 -adrenoceptor density did not.

β_2 -gene expression in CHD infants with congestive heart failure (CHF) was lower than that in infants without CHF. [49] β_1 - gene expression and β_2 -gene expression concomi-

tantly decreased in CHD patients with more post-surgical complications resulting in a prolonged length of stay.[50]

Apart from the presence or absence of CHD, and next to disease severity, the type of CHD seems to be a determinant in the distribution of β -adrenoceptor subtypes as well.[51] Although in all cases β_1 -adrenoceptor downregulation occurs, additional β_2 -adrenoceptor downregulation was observed in newborns with congenital aortic valve stenosis and transposition of the great arteries.

Next to total β -adrenoceptor density, McGrath et al. also studied the effects of pathology on total α -adrenoceptor density.[52] Whereas α -adrenoceptor density was markedly higher in cyanotic TOF patients than in acyanotic control patients with perimembranous ventricular septal defect, there was no difference in β -adrenoceptor density. Surprisingly, in the TOF patients α -adrenoceptor or β -adrenoceptor density was not related to the degree of cyanosis (Table 1).

C. Effects of pathology on adrenoceptor function

Alterations in adrenoceptor density and adrenoceptor function might not be reciprocal to each other. For instance, Molenaar et al. concluded that although the β_2 -adrenoceptor density is low in ventricular tissue, β_2 -adrenoceptors are nearly as effective as β_1 -adrenoceptors in enhancing cardiac contractility and relaxation in cyanotic TOF patients.[45] The authors postulated that this may be so because β_2 -adrenoceptors selectively couple to the G_s -protein/AC-system. During disease, a shift in β -adrenoceptor subtype density might be counterbalanced by a shift in the effectiveness of the post-receptor pathway activity. Others observed that the β_1 -adrenoceptor induced AC-activity was higher in the RVOT of cyanotic TOF patients than in the RVOT of acyanotic TOF patients.[43] This indicates sensitization of the β_1 -adrenoceptor pathway. Minimal β_2 -adrenoceptor induced AC-activity was found.

Others studied relations between β -adrenoceptors, AC-activity, and contractile force induced by several catecholamines in right ventricular tissue obtained from TOF patients with heart failure corresponding to NYHA class II.[53] Considering that the other adrenoceptors were blocked, increases in contractile force induced by norepinephrine were closely associated with small increases of AC-activity mainly through β_1 -adrenoceptors. Interestingly, the AC-activity induced by dopamine was approximately 1/3 of that induced by epinephrine, norepinephrine, or isoprenaline.

In atrial tissue obtained from acyanotic children with CHF with moderate pressure and volume overload, β_2 -adrenoceptor coupling to AC-activity was markedly more efficient than coupling between β_1 -adrenoceptors and AC-activity.[51] A partial decoupling between β_2 -adrenoceptors and AC-activity occurred in the more severely ill patients.

Brodde et al. studied cardiac β -adrenoceptor function in atrial tissue obtained from children with acyanotic CHD.[46] The children's AC-activity could be induced by both ad-

Table 1. Effects of pathology on adrenoceptor density and/or gene expression in children with CHD

Type of tissue	Condition	Study population	Type of adrenoceptor	Conclusions	Reference
Left ventricle (nfs)	IDC	19 (7±1 yrs)*	β_1, β_2	Total, β_1 and β_2 decreased in IDC patients; β_2 - but not β_1 -gene expression decreased in IDC patients	Sucharov et al ⁴⁰
RVOT	TOF	29 (1-97 mos)†	β_1, β_2	Total, β_1 and β_2 increased in patients with hypoxic spells compared to patients without	Sun et al ⁴³
RVOT	TOF	16 (3 mos-15 yrs)†	β_1, β_2	Total, β_1 and β_2 decreased in NYHA III-IV compared to NYHA I-II	Brodde et al ⁴⁴
RVOT	TOF	27 (2.5-35 mos)†	β_1, β_2	$\beta_1:\beta_2$ - ratio \approx 71:29	Molenaar et al ⁴⁵
Right atrial appendage	CHD	28 (7 d-18 yrs)†	β_1, β_2	$\beta_1:\beta_2$ - ratio \approx 68:32	Brodde et al ⁴⁶
Right atrial appendage	CHD	26 (3 d-15.8 yrs)†	β_1, β_2	Total and β_1 but not β_2 decreased in the more severely ill	Kozlik et al ⁴⁷
Right atrial appendage	CHD	19 (-)	β_1, β_2	Total and β_1 but not β_2 decreased in the more severely ill; β -density alterations were independent of age	Kozlik et al ⁴⁸
Right atrium (nfs)	CHD	25 (-)	β_1, β_2	β_2 -Gene expression decreased in the more severely ill	Buchhorn et al ⁴⁹
Right atrium (nfs)	CHD	26 (14±4 mos)*	β_1, β_2	β_1 - and β_2 -gene expression concomitantly decreased in the more severely ill	Buchhorn et al ⁵⁰
Right atrial appendage	CHD	68 (3 d-15.8 yrs)†	β_1, β_2	Total and β_1 decreased in the more severely ill patients with CHD; additional β_2 -downregulation in CAVS and TGA	Kozlik et al ⁵¹
RVOT	TOF, pVSD	22 (11-240mos)†	α β	α But not β higher in cyanotic TOF than in acyanotic pVSD patients; no relation between α or β and degree of cyanosis	McGrath et al ⁵²

* Number of children (mean age \pm SD). † Number, age range. CAVS: congenital aortic valve stenosis, nfs: not further specified, pVSD: perimembranous ventricular septal defect, TGA: transposition of the great arteries.

renoceptor and non-adrenoceptor pathways. Reithmann et al. studied β -adrenoceptor induced AC-activity and non-adrenoceptor induced AC-activity in right atrial tissue of children with CHD of different severity.[54] Both were markedly decreased in the patients with more severe heart failure, indicating a post-receptor defect which subsequently was shown to originate from lower activity of the catalytic subunit of AC.

Others hypothesized that cardiopulmonary bypass (CPB) in children with acyanotic CHD would desensitize atrial β -adrenoceptors as CPB is associated with an increase in endogenous catecholamines.[55] While CPB affected neither total nor β -adrenoceptor subtype density, adrenoceptor induced AC-activity was lower after CPB than before, indicating a CPB induced uncoupling between β -adrenoceptors and AC-activity.

Borthne et al. studied the adrenergic regulation of myocardial contractile force in children with CHD.[56, 57] The relative functional role of each adrenoceptor subtype was determined by selectively blocking the adrenoceptor subtypes while measuring contraction force in isolated atrial tissue. Endogenous norepinephrine, for instance, caused a near maximal contraction in atrial tissue, which was induced by both β -adrenoceptors (77-86%) and α_1 -adrenoceptors (14-23%). The α_1 -adrenoceptor induced response in the patients with increased ventricular pressure load was higher than that in patients with normal ventricular pressure load. This indicates that in the more severely ill the α_1 -adrenoceptors seem to contribute substantially to the inotropic response.

In a third study by Borthne et al. examined the effects of endogenous norepinephrine release, graded by frequency variation of field stimulation, in atrial myocardial specimens of CHD children. [58] The authors concluded that at a low level of electrical stimulation, the inotropic response to endogenous norepinephrine is exerted to a relatively greater extent by the α_1 -adrenoceptor pathway.

Shavit et al. studied α -adrenergic responsiveness by stimulation of phenylephrine in the presence of propranolol, and β -adrenergic responsiveness by stimulation of isoprenaline in pediatric heart patients pre-surgically treated with β -blockers.[59] The authors postulated that treatment with selective α -agonists could increase contractility and cardiac output (Table 2).

D. Effects of therapeutically administered drugs and endogenous catecholamine plasma levels on adrenoceptor density and/or gene expression

Several authors report alterations in β -adrenoceptor density in relation to therapeutically administered catecholamines or elevated endogenous catecholamine plasma levels. Brodde et al. observed that total adrenoceptor density was higher in atrial and ventricular tissue obtained from TOF patients treated with the non-selective β -antagonist propranolol.[44] The β_1 : β_2 -ratio was unaltered indicating a concomitant increase in both β_1 - and β_2 -adrenoceptor density. This is in good agreement with the results reported by Kozlik et al., with the exception that Kozlik et al. reported greater increase in β_1 -adrenoceptor density than in β_2 -adrenoceptor density in the TOF patients receiving propranolol.[51] In the more severely ill CHD patients, the elevated endogenous plasma norepinephrine concentration correlated negatively with total and β_1 -adrenoceptor density.[47, 48, 51] As norepinephrine is a preferential agonist of β_1 -adrenoceptors, norepinephrine is probably, responsible for the shift of β -adrenoceptor subtype population. Others observed an increase in β_1 -adrenoceptor and β_2 -adrenoceptor gene expression in right atrial tissue obtained from TOF patients treated with propranolol.[50] In patients with CHF, propranolol treatment caused an upregulation of β_2 -adrenoceptor gene expression.[49]

Table 2. Effects of pathology on adrenoceptor function in children with CHD

Type of tissue	Condition	Study population	Type of adrenoceptor	Conclusions	Reference
RVOT	TOF	29 (1-97 mos) [*]	β_1, β_2	β -Mediated AC activity higher in patients with hypoxic spells; β_1 -coupling to AC activity; minimal β_2 -coupling to AC activity	Sun et al ⁴³
RVOT	TOF	27 (2.5-35 mos) [*]	β_1, β_2	β_1 and β_2 equally capable of enhancing contractile force and hastening of relaxation indicating selective coupling which compensate low β_2 -density	Molenaar et al ⁴⁵
Right atrial appendage	CHD	28 (7 d-18 yrs) [*]	β_1, β_2	AC activity can be induced by β and non-AR pathways	Brodde et al ⁴⁶
Right atrial appendage	CHD	68 (3 d-15.8 yrs) [*]	β_1, β_2	β_2 -Coupling to AC markedly more efficient than β_1 -coupling in less severely ill; β_2 -decoupling from AC in the more severely ill;	Kozlik et al ⁵¹
Right ventricle (nfs)	TOF	-	β_1, β_2	NE increases contractile force by β_1 -mediated AC activity; dopamine increases AC activity to a lesser extent than E, NE and isoprenaline	Kaumann et al ⁵³
Right atrial appendage	CHD	31 (4 d-13.9 yrs) [*]	β	β and AR-independent induced AC activity decreased in the more severely ill; post-AR defect is due to decrease in activity of the catalytic subunit of AC	Reithmann et al ⁵⁴
Right atrial appendage	CHD	12 (0.75-17 yrs) [*]	β_1, β_2	β -Induced AC activity decreased after CPB compared to before; E decreases AC activity	Schranz et al ⁵⁵
Right atrial appendage	CHD	30 (0.5-176 mos) [*]	α_1 β	Endogenous NE stimulates contraction predominantly by β but also by α_1	Borthne et al ⁵⁶
Right atrium (nfs), RVOT	CHD	13 (4-139mos) [*]	α_1 β	Endogenous NE stimulates contraction predominantly by β but also by α_1 ; relative α_1 -contribution to contraction increases in more severely ill	Borthne et al ⁵⁷
Right atrium (nfs)	CHD	21 (14-165 mos) [*]	α_1 β	High sympathetic activity stimulates contraction predominantly by β and relatively little by α_1 ; low sympathetic activity stimulates contraction by β and by α_1 to a relative greater extent	Borthne et al ⁵⁸
Right atrium (nfs)	CHD	5 (2-18 mos) [*]	α β	β -Stimulation by isoprenaline causes rapid and more pronounced contraction than α -stimulation by PE	Shavit et al ⁵⁹

* Number (age range). AC activity: adenylyl cyclase activity, AR: adrenoceptor, E: epinephrine, NE: norepinephrine, nfs: not further specified, PE: phenylephrine.

E. Effects of endogenous catecholamine plasma levels and therapeutically administered drugs on adrenoceptor function

Incidental data suggest that therapeutically administered catecholamines inhibit adrenoceptor function.[55, 57] In the face of β -adrenoceptor desensitization due to β -blockers, the α -adrenoceptor induced regulation of cardiac contractility might be increasingly important. Consequently α -adrenoceptors may be a potential therapeutic target. These can either be directly stimulated by α -agonists or indirectly by administering a β -blocking agent, thereby unmasking α -adrenoceptors. Berkenboom et al. studied α -adrenoceptor unmasking in coronary arteries of non-failing, structurally normal hearts: at equipotent doses, non-selective β -blockers and selective β_1 -blocking agents were equally capable of unmasking α -adrenoceptors.[41, 42]

Catecholamine receptors in cardiac tissue in young animals

Here we describe relevant young animal models with respect to heart failure or associated pathologic conditions. Apart from the acute effects, chronic exposure to α -adrenergic stimuli alters cardiac structure and function. These alterations resemble the features of cardiac hypertrophy and heart failure, and are therefore regarded as a useful model. In the neonatal rat α_{1A} -receptor stimulation induces biochemical, genetic, and morphological markers of ventricular hypertrophy.[60-63] Agonistic α_1 -adrenoceptor auto-antibodies, which originate during cardiac disease in rats, might exacerbate ventricular hypertrophy.[64] Importantly, α_{1B} -receptors may antagonize the hypertrophic actions of α_{1A} -receptor stimulation.[65, 66] Moreover, Rokosh et al. found that myocardial α_{1B} -mRNA and α_{1D} -mRNA levels were repressed after prolonged hypertrophic adrenergic stimuli whereas the α_{1A} -mRNA level was increased.[67] In the neonatal rat myocyte several adrenergic post-receptor pathways co-exist, all affecting cardiac hypertrophy (Figure 1).[68-79]

In neonatal rat myocytes cardiac hypertrophy is generally believed to exclusively involve α_1 -adrenergic signaling, whereas cardiac contractility is under both α -adrenergic and β -adrenergic control.[80] However, chronic β_1 -adrenergic stimulation also seems to contribute directly to ventricular hypertrophy.[81] Interestingly, following chronic exposure to norepinephrine, the expression of β_1 -adrenoceptors and β_2 -adrenoceptors was decreased in neonatal rat myocytes whereas β_3 -adrenoceptor expression was increased.[82] In contrast, others did not observe a decrease in total β -adrenoceptor density or β -adrenoceptor desensitization in chronically stimulated myocytes of neonatal rats, rabbits, and lambs.[83-85] In neonatal mouse myocytes, β -adrenergic stimulation caused cardiac hypertrophy rather than α -adrenergic stimulation.[86] After chronic β -adrenergic stimulation AC-activity increased in rabbits, whereas decoupling occurred in lambs.[84, 85] A model of neonatal myocytes overexpressing AC-type 6 and

Idiopathic dilated cardiomyopathy models in neonatal hamsters and turkeys have been engineered.[102-104] These hamsters showed decreased β -adrenoceptor density and α_1 -adrenoceptor density in ventricular myocardial tissue compared to controls.[102] Plasma norepinephrine, and epinephrine levels, but not dopamine levels were increased. β -blocking via low dose metoprolol increased β -adrenoceptor density to the level of non-cardiomyopathic controls, whereas α -adrenoceptor density remained decreased. Plasma catecholamine levels normalized as well. In a functional contraction study of genetically inbred cardiomyopathic hamsters, left ventricular tissue showed exaggerated contraction force, and delayed relaxation time.[103] Surprisingly, β -adrenoceptor density in the left ventricles of neonatal, cardiomyopathic turkeys was higher than that in healthy controls.[104] Binding affinity decreased however. β -blocking via low dose propranolol during the development of cardiomyopathy affected neither β -adrenoceptor density, nor β -adrenoceptor affinity, but did improve cardiac function. Agonistic β_1 -auto-antibodies obtained from sera of cardiomyopathy patients enhance the beating frequency of neonatal rat myocytes indicating a pathologic role of the immune system.[105, 106]

Teitel et al. developed a model of cyanotic heart disease in neonatal lambs which approximates the pathophysiological conditions in CHD infants. By decreasing blood flow in the pulmonary artery, thereby mimicking RVOT obstruction in combination with an atrial septosomy permitting right-to-left shunting, arterial oxygen saturation can be reduced to 60-75% for a longer time. Bernstein et al. and Doshi et al. used this model to study β -adrenoceptor density and AC-activity in myocardial tissue.[107-109] Compared to controls, β -adrenoceptor density (45%) and AC-activity (39%) decreased in the left ventricle, but remained unaltered in the right ventricle.[107] In a subsequent study reduced levels of β -adrenoceptor mRNA were observed in the hypoxemic lambs, indicating that downregulation was regulated at the transcriptional level.[109] The ventricular β_1 : β_2 -ratio did not differ between hypoxemic and control lambs. Also, the total β -adrenoceptor density as well as the β_1 : β_2 -ratio (~ 40:60) was unchanged in the right atrium.[108] In the hypoxemic lambs, the plasma epinephrine level, but not norepinephrine level was increased.[107]

***In vivo* studies of cardiogenic drugs in children**

For the human *in vitro* studies cardiovascular tissue was in most cases obtained from CHD patients requiring major cardiac surgery. This chapter focuses on *in vivo* data on the hemodynamic effects of cardiogenic drugs in CHD children requiring major cardiac surgery and CPB.

Li et al. studied the effects of dopamine cessation on hemodynamic status and oxygen transport in neonates after the Norwood procedure.[110] Dopamine cessation did not significantly change arterial pressure, pulmonary blood flow, systemic blood flow and

oxygen delivery (DO₂). It did decrease heart rate, rate-pressure product, and oxygen consumption (VO₂), resulting in a lower oxygen extraction ratio. The authors therefore conclude that dopamine has detrimental effects on the VO₂-DO₂ balance.

The cardiovascular effects of dobutamine in different dosages have been studied in children after surgical correction of various forms of CHD.[111] In 35% of the patients dobutamine infusion was stopped after both the cardiac index (CI) and the heart rate had increased to a critical level. The mean arterial pressure (MAP) went up as well, whereas the stroke volume or peripheral vascular resistance remained unaltered. The authors concluded that dobutamine is an effective inotropic agent in children, because it acts principally by stimulating myocardial β_1 -adrenoceptors, which produce a predominantly chronotropic effect without changes in systemic vascular resistance (SVR). A double-blinded crossover study evaluated the hemodynamic effects of either dopamine or dobutamine in children on high-dose inotropic support after surgical repair of either TOF or atrioventricular septal defect (AVSD).[112] Significant hemodynamics effects were not observed in the patients who additionally received a non-selective α -antagonist. Of the others, those who received dopamine had higher pulmonary artery pressure and a higher pulmonary vascular resistance index compared to those who received dobutamine. Therefore it was concluded that dobutamine and dopamine are equipotent inotropic agents in children and that dopamine causes pulmonary vasoconstriction. This effect was attributed to α -adrenergic adrenoceptors. Kwapisz et al. studied the effects of dopexamine and low-dose dobutamine on CI, MAP, and SVR in children undergoing elective, non-complex cardiac surgery.[113] Both drugs increased CI, but the children treated with dobutamine showed also higher MAP and SVR. Therefore, the authors concluded that dobutamine in low dosage seems to have an α -adrenergic effect in the peripheral resistance vessels.

The hemodynamic effects of dobutamine combined with phenoxybenzamine were compared to the hemodynamic effects of enoximone in children after open heart surgery for TOF or AVSD.[114] Enoximone is a selective phosphodiesterase (PDE) 3 inhibitor which increases intracellular cAMP. With the exception of an increased MAP in the dobutamine group, all other hemodynamic parameters of interest did not differ between the two groups. Therefore the combination of dobutamine and phenoxybenzamine has no advantages over enoximone to assist discontinuation of CPB and to maintain an acceptable hemodynamic state in the early post-operative period. In contrast, a combination of dopamine and nitroglycerin was found to be inferior to the PDE 3 inhibitor amrinone in patients with transposition of the great arteries during weaning of CPB.[115]

The hemodynamic and renal functions of low-dose fenoldopam in addition to standardized perioperative therapy were studied in CHD neonates undergoing major cardiac surgery.[116, 117] Fenoldopam is a peripheral DA₁-receptor agonist. Fenoldopam infusion was well tolerated and did neither negatively affect hemodynamics or vasopressor

support, nor increase renal function.[116] On the other hand, Costello et al., observed increased urine output and blood pressure next to a lesser need for vasopressor support in children after cardiac surgery requiring CPB.[117]

A recent landmark paper by Shekerdemian addresses both the unique pathophysiology of CHD patients and the therapeutic options in the circulatory management of children after surgery for CHD.[118] We recommend this as an excellent guide for daily clinical use of these drugs.

Conclusion

In vitro sensitivity to catecholamines is observed in cardiac tissue of human fetuses as early as the fifth gestational week. AC-activity is first seen at the gestational weeks 6-7. The chronotropic and inotropic responses to catecholamines increase thereafter as maturation progresses. Endogenous catecholamines seem to be present in or near cardiac tissue early during ontogeny. However, the sympathetic nervous system becomes active at a later stage. There are no published studies using adrenoceptor specific radioligands to study adrenoceptor distribution in fetal cardiac tissue, nor did we find reports evaluating the distribution or the function of DA-adrenoceptors in fetal cardiovascular tissue. On the basis of functional studies β -adrenoceptors seem to be predominantly present in fetal, cardiac tissue. α -adrenoceptors are present in the ductus arteriosus and the ductus venosus.

Catecholamine receptors in the postnatal period are predominantly studied in cardiac tissue of CHD children. Only three studies made use of non-failing cardiac tissue. β_1 -adrenoceptors are the predominant adrenoceptor subtype regarding both distribution and function in both structurally normal and abnormal cardiac tissue, but β_2 -, α_1 - and α_2 -adrenoceptors are also present and active. β -adrenoceptor density is affected by cardiac disease, type and severity of disease, and therapeutically administered catecholamines. This seems to resemble the findings in human adults. There is no conclusive evidence of a concomitant decrease in β_1 -adrenoceptors and β_2 -adrenoceptors in CHD. Moreover, decreased β -adrenoceptor density does not imply decreased cardiac function. Several reports suggest that post-receptor pathways might counteract the altered adrenoceptor distribution. In addition, α -adrenoceptors may become more functionally important in the face of β -adrenoceptor downregulation. β -blocking agents upregulate β -adrenoceptor density. Elevated plasma norepinephrine levels, indicating increased activity of the sympathetic nervous system, are related to decreased β -adrenoceptor density. Age-related effects on adrenoceptor density and function in the cardiovascular system have been sparsely studied.

Little is known of the distribution and function of catecholamine receptors in the isolated peripheral vasculature in children. Moreover, to the best of our knowledge there

are no *in vitro* studies evaluating the distribution or function of DA-adrenoceptors in the cardiovascular tissue of children. This is also true for young animal models.

There are however several useful neonatal or young animal models with respect to heart disease. In neonatal rat myocytes cardiac hypertrophy is associated with α_1 -adrenoceptor stimulation. This confirms the notion that α -adrenoceptors are involved in CHD in children. The animal models provide more insight in the role of the α_1 -adrenoceptor subtypes, as well as the α -adrenoceptor related post-receptor pathways associated with cardiac pathology. With regard to β -adrenoceptor distribution and functioning, the decrease in ventricular β -adrenoceptor density due to cardiomyopathy is remarkably similar between infants and neonatal hamsters. In CHD children, right myocardial tissue is used in almost all cases whereas in animal models, in most cases, isolated myocytes are used without differentiation between left or right to study β -adrenoceptors. A model of lambs with cyanotic heart disease shows neither alterations in β -adrenoceptor density nor function in the right ventricle. However in the left ventricle of these lambs, as well as in hypertrophied left ventricles in a hamster model, both β -adrenoceptor density and, in the case of the lambs, β -adrenoceptor function decreases. In the right atrium of lambs no alterations in β -adrenoceptor density are observed, whereas in CHD infants this does occur. In general, the animal models indeed elucidate the pivotal role of β -adrenoceptor distribution and post-receptor β -adrenoceptor functioning in cardiac disease such as cardiac hypertrophy. β -blocking agents increase β -adrenoceptor density in CHD infants. In cardiomyopathic hamsters, and possibly turkeys, β -blockers seem to upregulate β -adrenoceptor function.

Catecholamine receptor distribution and function in isolated tissues can be studied in various ways. Catecholamine receptor distribution is usually studied with radioligand binding assays; functional responsiveness with contraction force measurements. Correlating radioligand-binding data to functional data is difficult because alterations in density are not necessarily reflected in an altered functional response. It is also difficult to compare the maximum cardiac or vascular contractile, or relaxant responses as various end-points exist. Also, absolute numbers of adrenoceptors should be interpreted cautiously as methodologies differ. Furthermore, ethical restrictions and the low availability of human specimens hamper progression in this field, as is reflected by the few available studies and the small sample sizes. This is especially true for studies evaluating adrenoceptors in the peripheral vasculature and for DA-adrenoceptors in the cardiovascular system.

Because most *in vitro* studies use cardiac tissue obtained from children suffering from CHD for which major cardiac surgery and CPB is required, we reviewed the literature describing the hemodynamic effects of cardiotonic drugs in these children *in vivo*. While several alternatives are available, the classical compounds dopamine and dobutamine still seem to be the agents of first choice.

Future perspectives

There is a great need for further characterization. Further characterization of subtype specific catecholamine receptors in various cardiovascular tissues in children is warranted, particularly in relation to maturation. Thereafter, effects of pathology and effects of administered vasoactive agents on adrenoceptor density and function should be studied, particularly with respect to the peripheral vasculature in children. Despite species heterogeneity, animal models are valuable tools, especially as stepping stone to elucidate post-receptor pathways and to identify new therapeutic targets.

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Chapter 6

Increasing mean arterial blood pressure and heart rate with catecholaminergic drugs does not improve the microcirculation in children with congenital diaphragmatic hernia: a prospective cohort study

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ABSTRACT

OBJECTIVE: To study whether dopamine, norepinephrine, and epinephrine improve not only mean arterial blood pressure and heart rate, but also microcirculatory perfusion in children with congenital diaphragmatic hernia (CDH).

DESIGN: Prospective observational cohort study from November 2009 to July 2012

SETTING: Intensive care unit (ICU) of a level III university children's hospital

PATIENTS: Twenty-eight consecutive CDH newborns, of whom 7 did not receive any catecholaminergic support and 21 received dopamine as the drug of first choice. Fourteen of the latter also received either norepinephrine or epinephrine in addition to dopamine. Twenty-eight healthy neonates, matched for gestational age, postnatal age, and gender, served as controls.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: Data were obtained before and after dopamine start, and before and after norepinephrine or epinephrine start in case it was given. For the CDH without catecholaminergic support data were obtained on admission day 1 and 2, and for the controls on day 1 of life. The buccal microcirculation was studied using Sidestream Dark Field imaging. Also collected were macrocirculatory, respiratory, and biochemical parameters. Mean arterial blood pressure had improved after dopamine start, whereas the microcirculation had not. After the start of either norepinephrine or epinephrine, both blood pressure and heart rate had increased. However, the microcirculation failed to improve again. The microcirculation in the healthy controls was better than in the CDH patients with catecholaminergic support. After cut-off values for abnormal microcirculation had been defined, abnormal microcirculation after dopamine start predicted the need for additional catecholaminergic support (AUC: 0.74-0.88, sensitivity: 77-77%, specificity: 69-77%). Likewise, microcirculatory impairment was associated with the need for extracorporeal membrane oxygenation.

CONCLUSIONS: Catecholaminergic drug support with dopamine, norepinephrine and/or epinephrine improved macrocirculatory function, but did not improve the microcirculation in neonates with CDH. The microcirculation was not only impaired, but it also predicted poor outcome.

INTRODUCTION

During critical illness there is often a mismatch between oxygen consumption and oxygen delivery [1]. Adequate perfusion and oxygenation at both the systemic –macro-circulatory– and the tissue –microcirculatory– level is needed for preserving oxygen delivery [2]. For improving the macrocirculatory component, the catecholaminergic drugs dopamine, norepinephrine, and epinephrine are recommended [3].

In critically ill children the possibilities for invasive hemodynamic monitoring are limited [4]. Neonatal and pediatric intensivists rely on surrogate “upstream” –e.g. arterial blood pressure– or “downstream” –e.g. arterial lactate– markers to estimate oxygen delivery and to guide treatment [5]. Observing the microcirculation has been difficult until the fairly recent introduction of techniques to visualize microcirculatory perfusion non-invasively at the bedside [6]. These have revealed that macrocirculatory parameters do not always reflect the microcirculation adequately in children with sepsis [7, 8].

Moreover, in critically ill adults macrocirculatory correction with catecholamines was not always followed by microcirculatory correction [2]. For instance: norepinephrine-induced increments in arterial blood pressure were not accompanied by microcirculatory increments [9, 10]. More generally, early microcirculatory correction in adults with septic shock resulted in lower disease severity scores and lower mortality rate [11].

The *in vivo* microcirculatory effects of catecholaminergic drugs have not been studied in critically ill children [12]. Yet, the pathophysiology of cardiorespiratory dysfunction and/or the rationale for catecholaminergic support in adults is most often different from that in children. In the case of congenital diaphragmatic hernia (CDH), the cardiorespiratory dysfunction is caused predominantly by lung hypoplasia and by structural and functional abnormalities in the pulmonary vasculature [13, 14]. Disease severity varies highly between patients and varying levels of catecholaminergic support are needed to increase output of, predominantly, the right ventricle and to increase systemic pressure in an attempt to counterbalance right-to-left shunting [15]. As a group, however, the CDH patients are homogenic in terms of age, pathogenesis, and treatment, which has been standardized based on international consensus guidelines [15].

CDH thus comprises an *in vivo* model in which the effects of catecholaminergic drug support on the microcirculation can be studied in children. Hence, this study aimed to describe the microcirculatory effects of dopamine, norepinephrine, and epinephrine infused for improving cardiorespiratory function in CDH patients. Also, the aim was to study whether microcirculatory impairment predicted poor outcome. We hypothesized that dopamine, norepinephrine, and epinephrine will all improve the microcirculation and that microcirculatory impairment predicts poor outcome.

MATERIALS AND METHODS

Study design, Setting, and Patients:

This prospective observational cohort study included CDH neonates born between November 2009 and July 2012 and admitted to the intensive care unit (ICU) of a level III university children's hospital, one of two designated CDH centers in the Netherlands. The local medical ethical review board approved the study and parental informed consent was obtained. The exclusion criteria were denied or withdrawn informed consent, major congenital cardiac anomaly, extreme prematurity –i.e., gestational age <32.0 weeks–, and late ICU admission –i.e., >2 days postnatally. Excluded from statistical analysis were the patients in whom less than two microcirculatory measurements were done or for whom the protocol for catecholaminergic drug support was not followed. Neonates without cardiorespiratory disease were included as controls and were matched for gender, gestational age (\pm 1 week), and postnatal age (\pm 36 hours). These neonates were born from mothers with a maternal indication or maternal request for hospital delivery.

Hospital treatment protocol:

Treatment complied with the internationally standardized protocol of the CDH Euro Consortium [15]. In short, the preferred ventilation mode was high frequency oscillation ventilation. The ventilation strategy included permissive hypercarbia ($p\text{CO}_2$: 50-60 Torr) together with pursuance of pre-ductal saturation above 80%, pH above 7.20, and arterial lactate below 5 mmol/L. A pre-ductal and post-ductal difference up to 10% was accepted in case arterial lactate remained below 5 mmol/L. Cardiac ultrasound was performed to screen for structural and/or functional cardiac abnormalities. Pulmonary hypertension (PH) was diagnosed as present in case the pulmonary circulation pressure was either suprasystemic or 2/3 of the systemic pressure; in case a bidirectional or right-to-left shunt over the persisting ductus arteriosus was observed; and/or in case the tricuspid regurgitant jet velocity exceeded 2.8 m/s [16]. Persistent PH was countered with inhaled nitric oxide (iNO; 20 ppm) as the primary drug of choice [17].

Age-appropriate mean arterial blood pressure and heart rate were constantly pursued [18, 19]. When blood pressure was decreased, fluid boluses were initially given: maximally 3x 10-20 ml/kg NaCl 0.9%. If this was ineffective, local hospital protocol prescribed to first start dopamine (5-15 mcg/kg/min). If hypotension persisted in spite of dopamine infusion, either norepinephrine (0.05-0.30 mcg/kg/min) or epinephrine (0.02-0.22 mcg/kg/min) was additionally infused depending on myocardial function –i.e., epinephrine in case of myocardial dysfunction or right ventricular dilation. Both the decision to start treatment and the decision to deviate from protocol were left to the attending intensivist. Additional therapeutic details –including criteria for venoarterial extracorporeal membrane oxygenation (VA-ECMO) – are described elsewhere [15].

Data collection:

For the patients requiring catecholaminergic support in the form of dopamine (CDHvp+), data were obtained before (T1) and after (T2) dopamine start. For a sub-group of CDH patients who required additional catecholaminergic support (CDHvp+sub), data were also obtained before (T3) and after (T4) start of either norepinephrine or epinephrine. For the CDH patients not requiring catecholaminergic drug support (CDHvp- group), data were obtained within 4 hours of ICU admission (D1) and within 24 ± 6 hours of ICU admission (D2). For the healthy controls, data were obtained once within 24 ± 6 hours of birth (C1). The primary microcirculatory parameters were perfused vessel density (PVD) and microvascular flow index (MFI). The outcome parameters were need for additional catecholaminergic support and need for VA-ECMO as represented by the oxygenation index at the points of data collection and VA-ECMO receiver yes/no.

The microcirculation

The microcirculation was evaluated using Sidestream Dark Field imaging (SDF; MicroVision Medical, Amsterdam, the Netherlands) [20]. The microcirculation was measured at three sites in the buccal mucosa according to the guidelines for optimal image acquisition [21]. Two examples of microcirculatory imaging in CDH patients are available as supplemental digital content (Supplemental video clip 1 and Supplemental video clip 2). Continuous flow in the greater microvessels was assured to avoid pressure artefacts. All video sequences were recorded on DV-tape, and 5-second clips (avi-format) were digitized and stored. Blinded, randomized video sequences were analyzed offline using dedicated software (Automated Vascular Analysis 3.0, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands). Total vessel density (TVD), PVD, proportion of perfused vessels (PPV), MFI, and heterogeneity index (HI) were calculated for small (S; $\varnothing \leq 10 \mu\text{m}$) and non-small vessels (NS; \varnothing between 10 and $100 \mu\text{m}$) [7, 10, 22]. To this end, a grid with three equidistant horizontal and vertical lines was placed over each video sequence. Hereafter, the number of vessel crossings was determined together with the vessel-specific flow category and the total grid length. The type of flow was scored as continuous (3), sluggish (2), intermittent (1), or absent (0). Vessels with intermittent or absent flow were categorized as non-perfused. TVD was calculated by the number of crossings divided by the grid length, PPV by the number of perfused crossings divided by the total number of crossings. PVD equalled the product of TVD and PPV. For determining MFI and HI each video sequence was divided in four equally sized quadrants. Per quadrant the predominant type of flow was scored. MFI represented the mean score of the predominant type of flow, HI the difference between the highest quadrant and the lowest quadrant score that is then divided by the mean score of all quadrants for one measurement. For all other scores, the average of the three video sequences per measurement was taken. Prior to the final analysis, inter-observer variability was determined for all microcirculatory parameters using 120

(41%) video sequences obtained for the current (n=60) and for another study (n=60). The Spearman's rank correlation coefficient and the intra-class correlation coefficient (ICC) respectively ranged from 0.533-0.932 (mean $r=0.768$) and 0.565-0.869 (mean ICC=0,750).

Demographic and time-dependent parameters

Together with the microcirculatory measurements, data were collected on patient demographics, outcome –oxygenation index at the points of data collection, VA-ECMO received–, and disease severity –score for neonatal acute physiology II (SNAP II), vasopressor score, alveolar-arterial oxygen tension difference (AaDO₂). The vasopressor score, oxygenation index, AaDO₂, and SNAP II were determined as previously described [23-26]. Also obtained were macrocirculatory, respiratory, and biochemical parameters. The presence of PH within 24 hours after ICU admission was estimated by cardiac ultrasound using the definition that is described in the section hospital treatment protocol.

Statistical analysis:

Continuous data are presented as median (IQR); discrete data as number (%). Intra-group differences over time were assessed with the Wilcoxon signed ranks test. Inter-group differences with the healthy control group as reference were assessed with first the Kruskal Wallis test and thereafter with the Mann Whitney U test and Bonferroni correction if relevant. Mixed effects models were performed with the covariates time, group, and the interaction term to test for microcirculatory differences between the CDHvp- and the CDHvp+ group. For each microcirculatory parameter of patients in the CDHvp+ group, the Spearman's rank correlation coefficient was calculated between the change from baseline and the baseline values before the start of dopamine. Also, with the Mann Whitney U test it was tested whether the microcirculation at T1/D1 and T2/D2 differed between the patients that required additional vasopressor support and those that did not. For the microcirculatory parameters with significant differences, the area under the curve (AUC) of receiver operating characteristics was determined. Microcirculatory cut-off values were identified and sensitivity, specificity, positive predictive value, and negative predictive value were calculated. With the cut-off values, the association between the microcirculation and outcome was explored in greater detail. All statistics were done using IBM SPSS statistics v20.0 (IBM Corp., Armonk, NY, USA) except the mixed effects models which were created using R statistics 2.15.2. Figures 2-4 were created using Graphpad Prism (Graphpad Software Inc., La Jolla, CA, USA). A p-value <0.050 was considered statistically significant.

RESULTS

Sixteen (27%) out of the 59 eligible CDH patients were excluded a priori; in 10 cases due to denied consent (Figure 1). In addition, 15 (25%) patients were excluded from

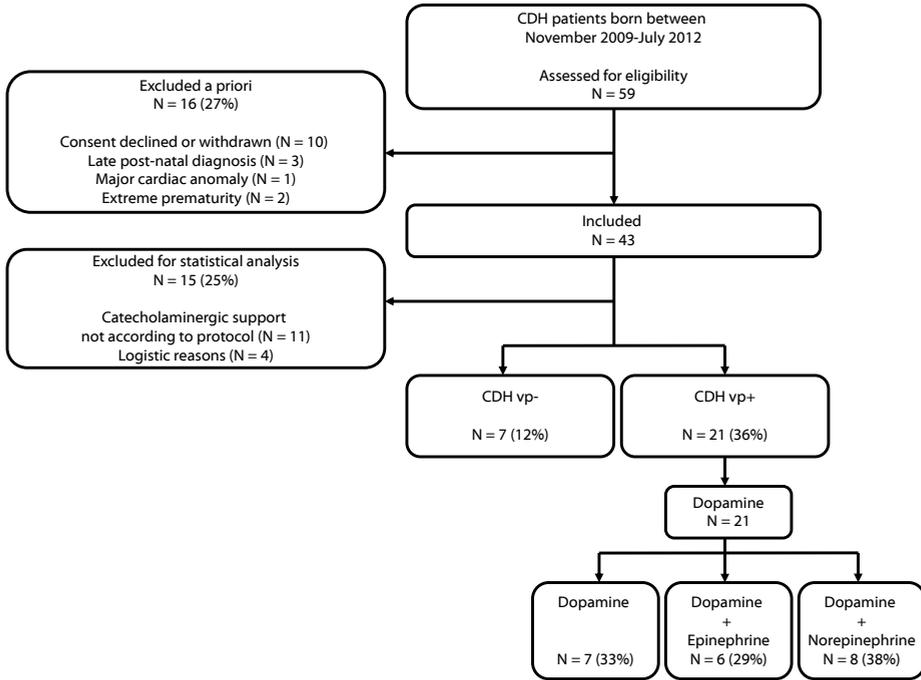


Figure 1. Flowchart for the patients with congenital diaphragmatic hernia who were assessed for inclusion in the study. CDHvp+ = CDH patients requiring catecholaminergic support in the form of dopamine, CDHvp- = CDH patients not requiring catecholaminergic drug support.

statistical analysis; in 11 cases due to deviation from the protocol for catecholaminergic drug support. The 28 included patients did not differ from those excluded with regards to gender, side of diaphragmatic defect, lung-to-head ratio, intra-thoracic liver position, VA-ECMO requirement, length of ICU stay, and survival at discharge.

Seven (25%) out of the 28 included patients required no catecholaminergic drug support at all (CDHvp-). Twenty-one patients first received catecholaminergic support in the form of dopamine (CDHvp+). In a sub-group of 14 (50%) patients (CDHvp+sub), dopamine support was insufficient for maintaining mean arterial blood pressure within age-appropriate range. Hence, these patients additionally received either norepinephrine (n=8) or epinephrine (n=6). This was started median (IQR) 4.4 (9.3) hours after dopamine start.

Table 1 shows the baseline characteristics for the CDHvp- group, the CDHvp+ group, the CDHvp+sub group, and the control group. Six (21%) CDH patients received VA-ECMO. One additional patient was refrained from VA-ECMO. After starting dopamine, therapy continuance was regarded to be futile in this non-survivor. In total, four (14%) patients did not survive to ICU discharge. They all died from therapy-resistant cardiopulmonary

Table 1. Baseline characteristics of the patients with congenital diaphragmatic hernia who received no catecholaminergic support, who received dopamine, and who received either norepinephrine or epinephrine in addition to dopamine

Variable	CDHvp- (n = 7)	CDHvp+ (n = 21)	CDHvp+sub ^a (n = 14)	Controls (n = 28)
Male gender, n (%)	5 (71)	10 (48)	8 (57)	15 (54)
Gestational age in wk, median (IQR)	38.6 (1.5)	38.3 (1.5)	38.3 (1.9)	39.4 (1.6)
Prematurity (32.0-37.0wk), n (%)	0 (0)	3 (14)	2 (14)	2 (7)
Postnatal age at admission in hr, median (IQR)	2 (14)	1 (1.3)	1 (1)	-
Postnatal age at first data collection in hr, median (IQR)	5 (14)	2 (6)	2 (4)	18 (15)
Birth weight in kg, median (IQR)	3.0 (0.7)	3.0 (0.4)	3.0 (0.3)	3.5 (0.7)
First recorded core body temperature in °C, median (IQR)	36.0 (1.3)	36.6 (1.0)	36.6 (1.1)	37.1 (0.3)
Length of ICU stay in d, median (IQR)	5 (2)	20 (21) ^b	24 (38) ^b	-
Outborn, n (%)	3 (43)	4 (19)	2 (14)	-
Right-sided diaphragmatic defect, n (%)	1 (14)	7 (33)	6 (43)	-
Intrathoracic liver position, n (%)	1 (14)	12 (57)	10 (71) ^b	-
Lung-to-head ratio, median (IQR)	2.1 (0.7)	1.7 (1.5)	1.7 (1.9)	-
Gestational age LHR determination in wk, median (IQR)	32.3 (1.6)	31.6 (2.1)	32.3 (2.3)	-
Fetal endotracheal occlusion therapy ^c , n (%)	0 (0)	1 (5)	1 (7)	-
Score for neonatal acute physiology II, median (IQR)	21 (13)	37 (27) ^b	39 (25) ^b	-
Fluid balance day 1 in mL/kg/d, median (IQR)	0.8 (1.0)	2.4 (1.9)	2.5 (2.1)	-
Fluid balance day 2 in mL/kg/d, median (IQR)	0.8 (1.6)	0.0 (1.7)	-0.1 (1.5)	-
VA-ECMO ^{c,d} , n (%)	0 (0)	6 (29)	6 (43)	0 (0)
Time admission-start VA-ECMO in d ^e , median (IQR)	-	3 (8)	3 (8)	-
Duration VA-ECMO support in d ^e , median (IQR)	-	7 (6)	7 (6)	-
Nonsurvival at discharge ^{c,e} , n (%)	0 (0)	4 (19)	3 (21)	0 (0)
Time admission-start dopamine in hr ^e , median (IQR)	-	2.0 (3.7)	1.9 (3.2)	-
Time admission-start norepi/epi in hr ^e , median (IQR)	-	-	8.8 (10.9)	-
Apgar 1 min, median (IQR)	7 (4)	6 (5)	5 (5)	9 (0)
Apgar 5 min, median (IQR)	9 (4)	7 (4)	7 (4)	10 (1)
Apgar 10 min, median (IQR)	9 (2)	7 (4)	7 (4)	-
Umbilical cord pH, median (IQR)	7.30 (0.13)	7.29 (0.06)	7.28 (0.09)	-
Umbilical cord pCO ₂ in Torr, median (IQR)	49 (29)	53 (12)	53 (12)	-
Umbilical cord base excess in mmol/L, median (IQR)	0 (6)	-1 (3)	-3 (4)	-

CDH = congenital diaphragmatic hernia, CDHvp- = CDH patients not requiring catecholaminergic drug support, CDHvp+ = CDH patients requiring catecholaminergic support in the form of dopamine, CDHvp+sub = CDH patients requiring either norepinephrine or epinephrine in addition to dopamine, IQR = interquartile range, VA-ECMO = venoarterial extracorporeal membrane oxygenation. ^aData of the patients in this group are also included in the dopamine group. ^b $p < 0.025$ versus CDHvp- using nonparametric overall and subtests with Bonferroni correction. ^cDifferences not assessed. ^dOne additional patient was excluded from extracorporeal membrane oxygenation. ^eOne additional nonsurvivor was excluded from norepinephrine/dopamine. Dashes indicate values not determined or not calculated. Patients in the CDHvp+ group and the CDHvp+sub group were more severely ill than the patients in the CDHvp- group. Categorical variables are presented as n (%) and continuous variables as median (IQR).

failure that resulted from pulmonary hypoplasia and PH. Cardiac ultrasound –performed within the first 24 hours of ICU admission– showed that 20 patients (CDHvp- group: n=4, CDHvp+ group: n=16) had PH (Supplemental Figure 1). The length of ICU stay was shorter for the CDHvp- group than for the CDHvp+ group and the CDHvp+sub group. Accordingly, the SNAP II was lower in the CDHvp- patients. A higher proportion of patients in the CDHvp+sub group was diagnosed with an intra-thoracic liver position. None of the CDHvp- patients were treated with VA-ECMO and all survived up to ICU discharge.

The control group consisted of 28 healthy neonates born from mothers with either a medical indication (n=24) or a maternal request for hospital delivery (n=4). The maternal medical indications included history of complicated pregnancy (n=4), premature rupture of membranes (n=4), endocrine disease (n=4), hematologic disease (n=4), peripartum pathology (n=4), psychiatric disease (n=2), and other (n=2).

1. Effects of dopamine infusion

In the 21 patients that first received dopamine –the CDHvp+ patients– the median (IQR) starting dose was 7 (5) mcg/kg/min. Dopamine was started a median 2.0 (3.7) hours after ICU admission. Five patients already received 20 ppm iNO prior to the start of dopamine. In two patients the microcirculatory measurement prior to dopamine start was missing, in one other the microcirculatory measurement after dopamine start was missing.

The macrocirculatory, respiratory, and biochemical parameters for the CDHvp+ and the CDHvp- groups are shown in Table 2. In the CDHvp+ group, the blood pressure was increased after dopamine and the heart rate tended to be increased (Figure 2). Apart from a marginal increase in core body temperature, none of the other parameters increased over time. In the CDHvp- group, none of the parameters increased from ICU admission day 1 and day 2.

Table 3 shows the microcirculatory parameters for the CDHvp+ group, the CDHvp- group, and the control group. All of the microcirculatory parameters failed to improve with the start of dopamine. For each microcirculatory parameter, however, there was a negative correlation between the change from baseline, when dopamine was given, and the baseline value before the start of dopamine (range of $r = -0.48$ to -0.79 ; $p < 0.050$). When compared to the control group, the microcirculatory parameters PVD NS, PPV NS, PPV S, MFI NS, MFI S, HI NS, and HI S were all lower before and after the start of dopamine (Table 3 and Figure 3). Mixed effects models indicated that only HI NS and PPV S differed between the CDHvp+ and the CDHvp- patients.

2. Effects of norepinephrine or epinephrine infusion

A subgroup of 14 (50%) patients required either norepinephrine (n=8) or epinephrine (n=6) in addition to dopamine support (the CDHvp+sub group). Norepinephrine was

started median (IQR) 11.6 (48.6) hours after ICU admission with a dose of 0.11 (0.30) mcg/kg/min. At that time, the median (IQR) dopamine dose was 10 (7) mcg/kg/min. Epinephrine was started 4.4 (6.0) hours after ICU admission with a dose of 0.05 (0.09) mcg/kg/min. The dopamine dose at that time was 10 (7) mcg/kg/min. Prior to the start

Table 2. Macrocirculatory, respiratory, and biochemical parameters of the patients with congenital diaphragmatic hernia who received no catecholaminergic support, who received dopamine, and who received either norepinephrine or epinephrine in addition to dopamine

Variable	CDHvp- D1 (n = 7)	CDHvp- D2 (n = 7)	CDHvp+ T1 (n = 19)	CDHvp+ T2 (n = 20)	CDHvp+sub T3 (n = 13)	CDHvp+sub T4 (n = 14)
Time data collection-start treatment in hr ^a , median (IQR)	-	-	-0.42 (1.5)	1.5 (1.4)	-0.9 (3.8)	1.8 (2.1)
Time data collection-ICU admission in hr ^a , median (IQR)	0.5 (2.4)	25.4 (5.1)	1.1 (2.5)	3.6 (3.8)	5.7 (9.7)	8.8 (14.7)
Dose in µg/kg/min ^a , median (IQR)	0 (0)	0 (0)	0 (0)	7 (5)	0 (0)	0.11 (0.21)
Heart rate in beats/min, median (IQR)	120 (28)	120 (26)	130 (30)	142 (37)	148 (28)	174 (38) ^b
Mean arterial blood pressure in mm Hg, median (IQR)	48 (21)	43 (10)	35 (5)	41 (14) ^b	39 (8)	45 (8) ^b
Vasopressor score ^a , median (IQR)	0 (0)	0 (0)	0 (0)	8 (5)	10 (6)	23 (15)
Abnormal peripheral capillary refill time (>3s), n (%)	2 (29)	0 (0)	7 (37)	8 (40)	4 (39)	6 (43)
Oxygenation index, median (IQR)	3 (14)	2 (2)	10 (14)	9 (20)	11 (21)	9 (26)
AaDO ₂ , median (IQR)	149 (328)	56 (58)	344 (444)	310 (394)	319 (440)	282 (466)
High frequency oscillation ^{a,c} , n (%)	2 (29)	1 (14)	9 (47)	9 (45)	6 (46)	7 (50)
Inhaled nitric oxide in ppm, median (IQR)	0 (0)	0 (0)	0 (20)	0 (8)	0 (20)	0 (20)
Post-ductal saturation in %, median (IQR)	96 (4)	98 (2)	95 (8)	97 (14)	91 (14)	94 (24)
Δ saturation in %, median (IQR)	2 (2)	2 (3)	4 (6)	2 (10)	3 (11)	2 (6)
Core body temperature in °C, median (IQR)	36.0 (1.3)	36.6 (0.6)	36.6 (0.9)	37.0 (0.8) ^b	37.0 (0.5)	37.2 (0.9)
pH, median (IQR)	7.41 (0.11)	7.34 (0.11)	7.25 (0.17)	7.27 (0.15)	7.28 (0.13)	7.27 (0.13)
pO ₂ in Torr, median (IQR)	154 (195)	102 (71)	121 (139)	100 (94)	66 (50)	73 (60)
pCO ₂ in Torr, median (IQR)	40 (14)	37 (8)	53 (29)	50 (22)	53 (24)	50 (28)
Base excess in mmol/L, median (IQR)	1 (-)	-3 (-)	-6 (8)	-6 (6)	-5 (4)	-5 (3)
Arterial lactate mmol/L, median (IQR)	2.3 (1.8)	1.2 (1.3)	1.9 (3.9)	2.2 (1.3)	1.9 (1.6)	1.4 (0.9)
Ht in L/L, median (IQR)	0.52 (0.11)	0.49 (0.12)	0.46 (0.13)	0.45 (0.09)	0.38 (0.10)	0.40 (0.12)
Hb in mmol/L, median (IQR)	10.4 (2.5)	9.6 (2.9)	9.8 (2.8)	9.3 (2.1)	8.7 (2.2)	9.7 (2.6)

CDH = congenital diaphragmatic hernia, CDHvp- = CDH patients not requiring catecholaminergic drug support, CDHvp+ = CDH patients requiring catecholaminergic support in the form of dopamine, CDHvp+sub = CDH patients requiring either norepinephrine or epinephrine in addition to dopamine, IQR = interquartile range, Δ saturation = difference between preductal and postductal arterial saturation. ^aNot assessed for differences.

^bIntragroup differences at $p < 0.05$ using nonparametric tests. ^cTen patients receiving dopamine and seven patients receiving norepinephrine/epinephrine were included in a trial randomizing between conventional mechanical ventilation and high-frequency oscillatory ventilation. Dashes indicate values not determined or not calculated. Although mean arterial blood pressure and/or heart rate increased after the start of dopamine and after the start of either norepinephrine or epinephrine, the respiratory and biochemical parameters did not differ over time. Data are shown for the CDHvp- group at day 1 (D1) and day 2 (D2) of admission, for the dopamine group before (T1) and after (T2) start of dopamine, and for the norepinephrine/epinephrine subgroup before (T3) and after (T4) start of norepinephrine. Categorical variables are depicted as n (%) and continuous variables as median (IQR).

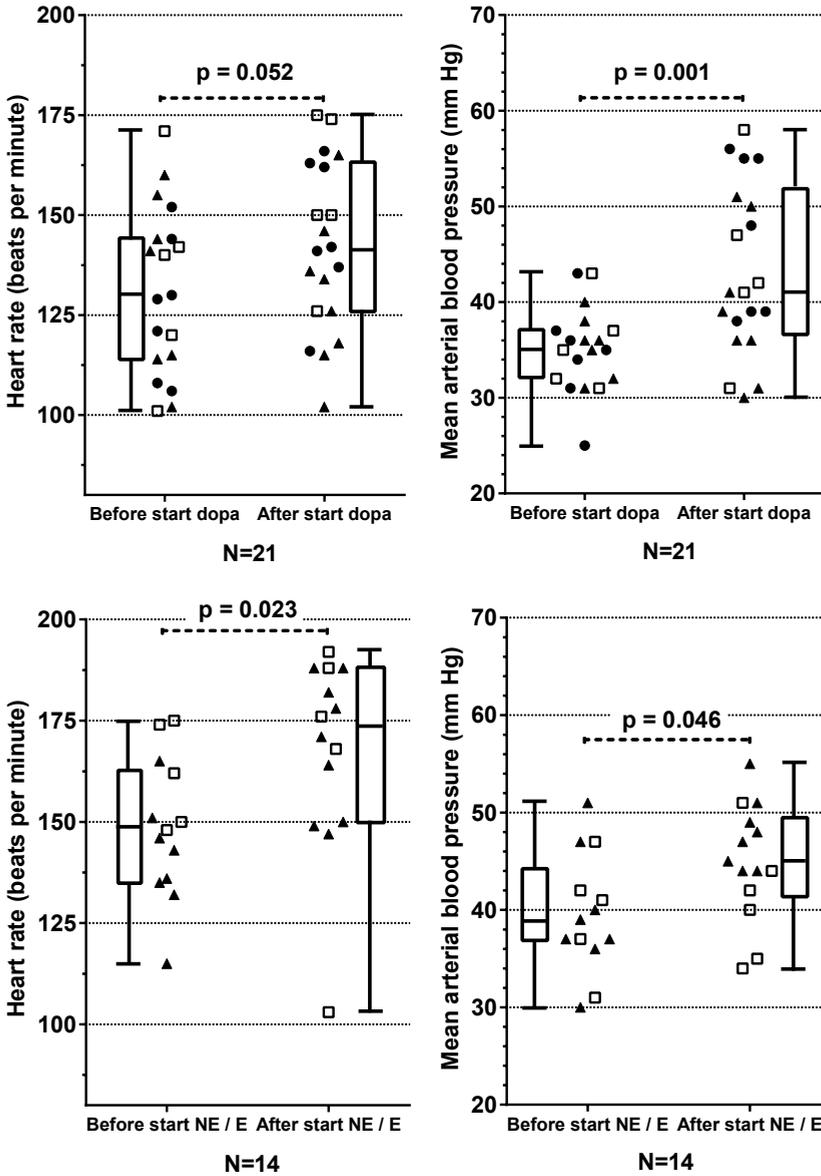


Figure 2. Scatter plots and box plots showing that the macrocirculatory variables heart rate (left panel) and/or mean arterial blood pressure (right panel) increased after the start of catecholaminergic support in patients with congenital diaphragmatic hernia who received dopamine (n = 21) and a subgroup of patients who received either norepinephrine (NE) or epinephrine (E) in addition to dopamine (n = 14). Circles represent the patients that did not require additional vasopressor support, triangles represent the patients receiving NE, and squares represent the patients receiving E. Intragroup differences were assessed using Wilcoxon signed ranks test.

of norepinephrine, one patient received 10 ppm iNO and one other 20 ppm iNO. Three patients received 20 ppm iNO prior to the start of epinephrine. In one patient, iNO (20 ppm) was started together with epinephrine.

Table 2 shows the macrocirculatory, respiratory, and biochemical parameters of the CDHvp+sub patients. Figure 2 (lower pane) shows that both the arterial blood pressure and the heart rate were increased after the start of either norepinephrine or epinephrine. In contrast, the microcirculatory parameters all failed to improve (Table 3). In comparison to the healthy neonates, the microcirculatory parameters PPV NS, PPV S, MFI S, and HI S were all lower in the CDHvp+sub group before and after start of either norepinephrine or epinephrine (Table 3 and Figure 4). None of the microcirculatory parameters were lower in the CDHvp+sub group than in the CDHvp- group.

Table 3. Microcirculatory parameters of the patients with congenital diaphragmatic hernia who received no catecholaminergic support, who received dopamine, and who received either norepinephrine or epinephrine in addition to dopamine.

	CDHvp- D1 (n = 7)	CDHvp- D2 (n = 7)	CDHvp+ T1 (n = 19)	CDHvp+ T2 (n = 20)	CDHvp+sub T3 (n = 13)	CDHvp+sub T4 (n = 14)	Controls C1 (n = 7)
TVD non-small in n/mm	7.2 (2.1)	7.9 (2.4)	7.9 (2.8)	6.7 (2.4)	6.9 (2.4)	7.0 (2.9)	7.5 (1.0)
PVD non-small in n/mm	6.8 (0.6)	7.0 (2.5)	6.0 (2.7) ^a	5.8 (2.2) ^a	5.9 (2.9)	5.6 (3.9)	6.9 (1.0)
PPV non-small in %	91 (19)	90 (9)	72 (20) ^a	84 (19) ^a	85 (29) ^a	87 (18) ^a	95 (5)
MFI non-small in au	3.00 (0.11)	3.00 (0.07)	2.78 (0.33) ^a	2.85 (0.36) ^a	2.83 (0.41) ^a	2.92 (0.23)	3.00 (0.06)
HI non-small in au	0.00 (0.35)	0.34 (0.35)	0.36 (0.05) ^{ab}	0.35 (0.38) ^{ab}	0.35 (0.39) ^a	0.34 (0.11)	0.00 (0.34)
TVD small in n/mm	12.8 (4.0)	12.2 (1.9)	10.5 (6.5)	10.7 (3.5)	12.0 (4.9)	13.0 (5.7)	11.8 (4.5)
PVD small in n/mm	12.5 (3.9)	11.6 (2.3)	8.8 (6.0)	9.3 (3.5)	9.6 (4.1)	10.5 (3.6)	11.7 (4.5)
PPV small in %	98 (4)	97 (3)	88 (29) ^{ab}	84 (19) ^{ab}	87 (28) ^a	92 (35) ^a	100 (1)
MFI small in au	3.00 (0.18)	3.00 (0.00)	2.67 (0.50) ^a	2.79 (0.41) ^a	2.88 (0.63) ^a	2.79 (0.58) ^a	3.00 (0.00)
HI small in au	0.34 (0.35)	0.00 (0.00)	0.36 (0.43) ^a	0.36 (0.40) ^a	0.35 (0.43) ^a	0.36 (0.42) ^a	0.00 (0.00)

CDH = congenital diaphragmatic hernia, CDHvp- = CDH patients not requiring catecholaminergic drug support, CDHvp+ = CDH patients requiring catecholaminergic support in the form of dopamine, CDHvp+sub = CDH patients requiring either norepinephrine or epinephrine in addition to dopamine, TVD = total vessel density, n/mm: number per millimeter, PVD = perfused vessel density, PPV = proportion of perfused vessels, MFI = microvascular flow index, au = arbitrary units, HI = heterogeneity index. ^aIntergroup differences (i.e., vs C1) at $p < 0.05$ using nonparametric tests at $p < 0.025$ using nonparametric overall and subtests with Bonferroni correction (no comparison with CDHvp-). ^bIntergroup differences (i.e., vs CDHvp-) at $p < 0.05$ using nonparametric tests at $p < 0.025$ using mixed effects models. Intragroup differences were assessed at $p < 0.05$ using nonparametric tests, however these were not present at $p < 0.025$ using mixed effects models. Also shown are the microcirculatory data of the healthy controls. Although the microcirculation was impaired in the CDH patients who received catecholaminergic drug support, catecholaminergic drug support failed to improve the microcirculation. The microcirculatory variables are shown for nonsmall ($10 \mu\text{m} \leq \text{Ø} < 100 \mu\text{m}$) and small ($\text{Ø} < 10 \mu\text{m}$) vessels. Data were collected at day 1 (D1) and day 2 (D2) of admission for the CDHvp- group, before (T1) and after (T2) start of dopamine for the CDHvp+ group, before (T3) and after (T4) start of either norepinephrine or epinephrine for the CDHvp+sub group, and once within 24 hr of birth for the control group. Continuous variables are represented as median (interquartile range).

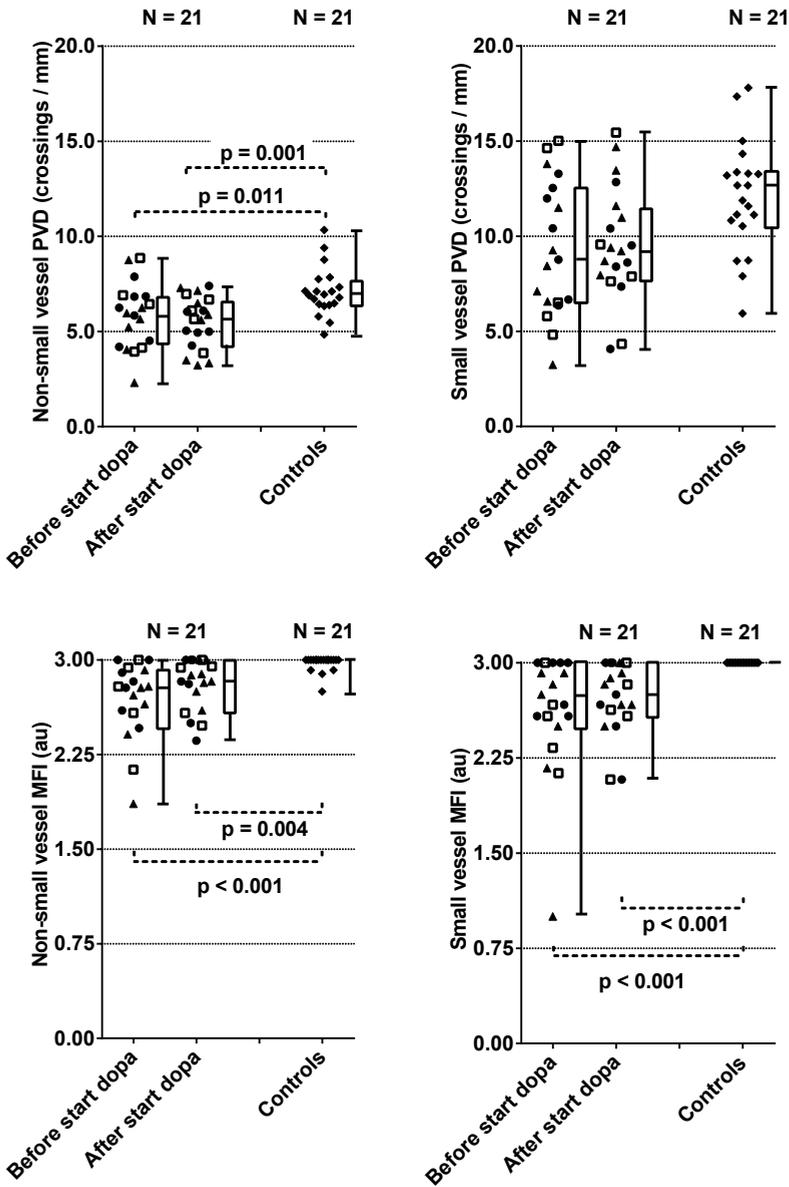


Figure 3. Scatter plots and box plots showing that the microcirculatory variables non-small vessel perfused vessel density (PVD) (upper left panel), non-small vessel microvascular flow index (MFI) (lower left panel), and small vessel MFI (lower right panel) were lower in the congenital diaphragmatic hernia patients who received dopamine (n = 21) than those in the healthy controls (n = 21). Circles represent the patients who did not require additional vasopressor support, triangles represent the patients receiving norepinephrine, squares represent the patients receiving epinephrine, and diamonds represent the healthy controls. Intergroup differences were assessed using Mann-Whitney U test.

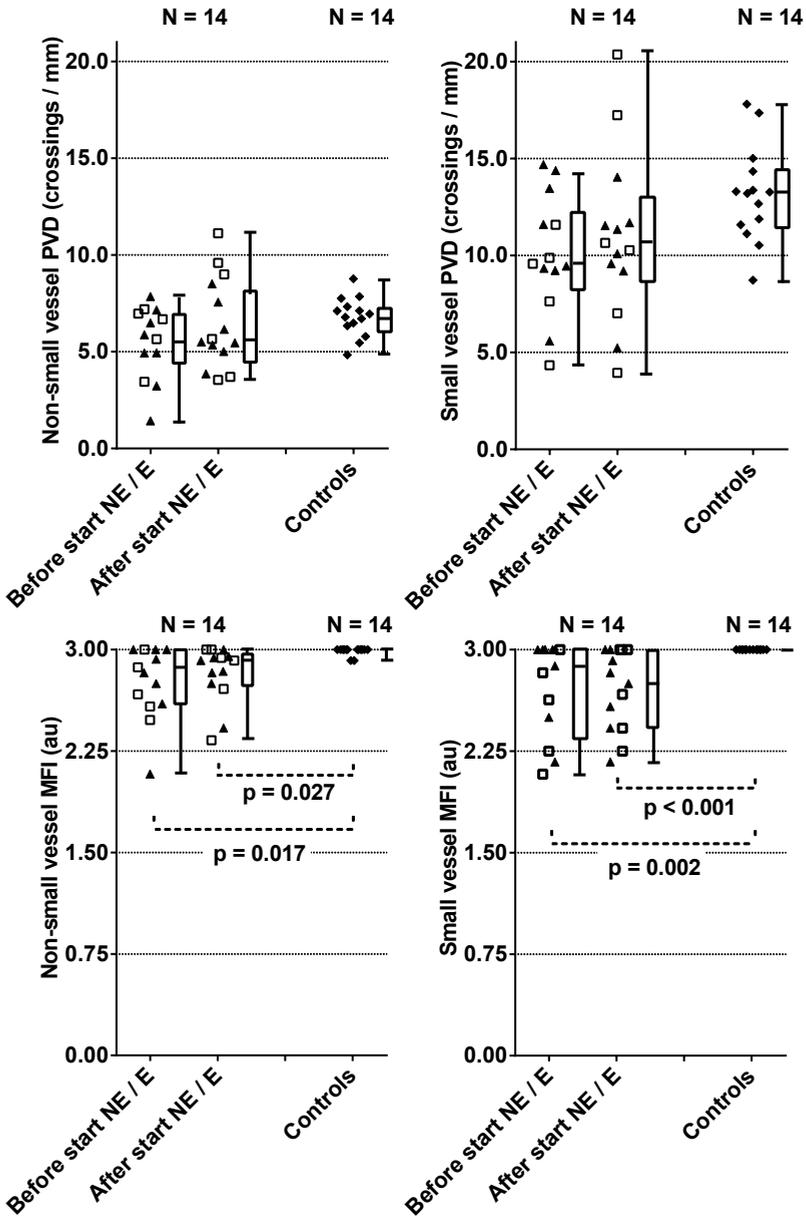


Figure 4. Scatter plots and box plots showing that the microcirculatory parameters non-small vessel MFI (upper right panel) and small vessel MFI (lower right panel) were lower in the congenital diaphragmatic hernia patients who received either norepinephrine or epinephrine (n=14) than in the healthy controls (n=14). Triangles represent the patients receiving norepinephrine, squares the patients receiving epinephrine, and diamonds the healthy controls. Inter-group differences were assessed using Mann Whitney U test. PVD: perfused vessel density.

3. The microcirculation in relation to outcome

Sub-analysis indicated that before the start of dopamine the parameters PPV NS, PPV S, MFI S, MFI NS, and HI NS were lower in the CDHvp+sub patients when compared to all other CDH patients combined (Supplemental table 1). Likewise, after the start of dopamine, the parameters PPV S, MFI S, and HI S were lower. Thus, already prior to the start of dopamine the microcirculatory measurements indicated that additional catecholaminergic support would be needed in the CDHvp+sub patients.

To further investigate this association between microcirculatory impairment and need for additional vasopressor support, the AUCs of the receiver operator characteristic curves and best cut-off values were determined for the parameters PPV S and MFI S, as these differed at both before and after dopamine start. The respective AUCs and cut-off values were 0.84 and 85%, 0.88 and 84%, 0.79 and 2.79au, and 0.74 and 2.96 au, respectively (Supplemental table 2). Before dopamine start, the sensitivity was 67% for both PPV S and MFI S and the specificity was 92% for PPV S and 69% for MFI S. After dopamine start, the sensitivity for PPV S and MFI S was 77% and the specificity for PPV S was 77% and for MFI S 69%.

The microcirculatory cut-off values were subsequently used to stratify the CDH patients according to the outcome measures oxygenation index over time and VA-ECMO receiver yes/no (Supplemental Table 3). For PPV S and MFI S measured before dopamine start, only the oxygenation index after the start of either norepinephrine or epinephrine differed. In contrast, for PPV S and MFI S after dopamine start, the oxygenation index after dopamine start and the oxygenation index before and after start of either norepinephrine or epinephrine differed. Also, the number of patients actually receiving VA-ECMO differed for the parameters PPV S and MFI S as measured after dopamine start.

DISCUSSION

This is the first clinical study in children to analyze non-invasively the actual microcirculatory effects in relation to dopamine and in relation to dopamine combined with either norepinephrine or epinephrine. Also, for the first time microcirculatory SDF-data are provided for healthy, one-day-old newborns. The main finding of this study is that catecholaminergic drug support increased mean arterial blood pressure and heart rate in CDH neonates, but not the microcirculation. The microcirculation remained abnormal in the CDH patients with catecholaminergic support when compared with healthy newborns, and more severely impaired microcirculation after the start of dopamine predicted the need for additional vasopressor support later-on and the need for VA-ECMO.

The goal of catecholaminergic drug support in CDH children is to improve cardiorespiratory function and, by doing so, to improve the microcirculation [15]. The patients in our study received a modest dose of dopamine –i.e., median 7 mcg/kg/min. Dopamine

in this dose is assumed to have predominantly inotropic effects together with renal and splanchnic vasodilatory effects through beta-adrenergic and dopaminergic stimulation [2]. There are no other clinical or experimental studies evaluating the effects of dopamine with a technique similar to ours. Nevertheless, in view of the results obtained with other techniques and/or markers, the beneficial microcirculatory effects of dopamine has been questioned [27]. More specifically, pediatric studies failed to show improvements in perfusion or oxygenation at the regional –organ– or local –cellular– level [28, 29].

Given the intrinsic alpha-adrenergic properties of norepinephrine and epinephrine, adrenergic vasopressor treatment could be deleterious for the peripheral microcirculation [2]. This has been shown for adults undergoing cardiopulmonary bypass who received phenylephrine, a selective alpha-adrenergic agonist [30]. On the other hand, the microcirculation could improve as beta-adrenergic modulation improves cardiorespiratory functioning. A clinical study by Thooft et al. in a small cohort of septic adults indeed showed that norepinephrine-induced-increments in blood pressure were accompanied by microcirculatory improvement [31]. In contrast, two other studies –describing larger cohorts– failed to demonstrate that microcirculatory parameters improved after incremental doses of norepinephrine [9, 10]. Likewise, in the non-clinical setting, norepinephrine improved blood pressure, but not the microcirculation [32]. These results resemble the results of the current study.

The microcirculation was impaired in the CDH patients with catecholaminergic support when compared to healthy neonates. These impairments predicted the need for additional vasopressor support and the need for VA-ECMO, both clinically relevant outcomes. Moreover, we observed a strong negative correlation between baseline microcirculation and the microcirculatory change from baseline after dopamine was given. This indicates that when the macrocirculatory parameters improved with dopamine, the patients with a good microcirculation at the time of dopamine start experienced a greater, negative, microcirculatory effect than the patients with a poor microcirculation at dopamine start. So, although dopamine is not suited for improving the microcirculation, the change in the microcirculation is dependent on the basal state of the microcirculation implying that, ideally, only the patients in whom low blood pressure is combined with poor microcirculation should receive treatment. Similar results have been reported for adults with septic shock [9].

In critically ill adults controversy exists regarding the routine use of vasopressor treatment [33]. Moreover, vasodilators such as nitroglycerin or levosimendan improved the microcirculation suggesting that vasodilatory rather than vasopressive therapy improves outcome [2, 34, 35]. However, while sepsis is a disease of the microcirculation, the combination of pulmonary hypoplasia and vascular abnormalities makes CDH primarily a macrocirculatory disease [13, 15, 36]. This might also explain why the micro-

circulatory impairment in CDH patients is less severe than in children with distributive or cardiogenic shock [7, 8, 37]. Likewise, other tissue perfusion parameters such as lactate were modestly increased. Moreover, the rationale for catecholaminergic support in CDH patients differs as well: improving cardiac output of predominantly the right ventricle by beta-adrenergic modulation and increasing systemic resistance by alpha-adrenergic modulation in an attempt to counteract the right-to-left shunting [13, 15]. Often, iNO is also required. Dilating the pulmonary vasculature by iNO and keeping macrocirculatory vascular resistance by inotropes in order to enhance pulmonary circulation, to increase cardiac output, and to improve systemic oxygen delivery might be the best strategy. Interestingly, iNO has been shown to improve the microcirculation in the context of PH [38]. In that study it remained unknown whether the microcirculatory improvement occurred due to the intrinsic vasodilatory properties of iNO or because it resolved persistent PH and, as such, improved microcirculatory function [38].

In our view, future research in CDH patients should focus on the relation between PH and microcirculatory impairment and should aim to elucidate the therapeutic strategy that best improves both. Also, given the specific pathophysiology of CDH, the microcirculatory effects of catecholaminergic drug support should be studied in children with distributive or cardiogenic shock in the first place. Finally, the correlation between the buccal microcirculation and other microcirculatory beds –e.g. the cerebral or splanchnic microcirculation– should be assessed in both healthy and critically ill children [39, 40].

Several limitations of this study should be addressed. Most importantly, a relatively large number of CDH patients were not treated according to protocol and were therefore excluded. Although the baseline characteristics did not differ between the excluded and the included patients, selection bias might have been introduced. A non-observational research design was deemed unethical at the start of the current study. Furthermore, although the sample size is relatively similar to that of other studies in this field, it is still small in absolute numbers. Results should thus be interpreted with caution. Also, blood pressure was moderately decreased and the oxygenation index was relatively low. The modest microcirculatory impairment and/or the modest disease severity could explain the lack of microcirculatory improvement following catecholaminergic support. Still, in spite of the small sample size and the small effect size, the microcirculation was significantly better in the healthy neonatal controls and microcirculatory impairment predicted poor outcome. Our group has shown previously that the microcirculation is also impaired prior to the start of ECMO, when disease severity is more pronounced. The use of iNO could have confounded our results. Another limitation is that there was considerable inter-individual variation. Hence, the clinical applicability of non-invasive microcirculatory visualization in CDH neonates might prove to be limited. Also, although the microcirculation was lower in the CDH patients than in the healthy controls and microcirculatory impairment was associated with poor outcome, it remains unknown to

what extend the microcirculatory impairment functions as a compensatory mechanism. Future research should focus on this. Finally, both the time interval between ICU admission and dopamine start and the time interval between dopamine start and norepinephrine or epinephrine start was short. While heart rate and blood pressure are known to react quickly, it remains to be seen whether this is also true for the microcirculation. Like arterial lactate or base excess, more time may be required for the microcirculation to adjust.

CONCLUSIONS

Catecholaminergic drug support with dopamine, norepinephrine and/or epinephrine improved macrocirculatory function, but did not improve the microcirculation in neonates with congenital diaphragmatic hernia. The microcirculation was not only impaired, it also predicted poor outcome. Future research in CDH patients should aim to elucidate the therapeutic strategy that best improves the microcirculation.

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Supplemental Table 1. The microcirculatory parameters before (T1) and after (T2) the start of dopamine that differed between the patients who required additional catecholaminergic support (CDHvp+sub) and those who did not (CDHvp+ & CDH vp-).

		CDHvp+sub	CDHvp- & CDHvp+	p-value
T1	PPV NS in %	72 (13)	84 (17)	0.036
	MFI NS in au	2.78 (0.33)	2.90 (0.22)	0.032
	HI NS in au	0.36 (0.05)	0.35 (0.36)	0.040
	PPV S in %	76 (27)	98 (8)	0.004
	MFI S in au	2.67 (0.58)	3.00 (0.29)	0.012
T2	PPV S in %	82 (18)	97 (10)	0.001
	MFI S in au	2.83 (0.42)	3.00 (0.19)	0.029
	HI S in au	0.35 (0.39)	0.00 (0.18)	0.035

The microcirculatory parameters are shown for non-small (NS; $10 \mu\text{m} \leq \text{Ø} < 100 \mu\text{m}$) and small (S; $\text{Ø} < 10 \mu\text{m}$) vessels. Inter-group differences were assessed using non-parametric tests. Data are displayed as median (IQR). Au: arbitrary units, PPV: proportion of perfused vessels, MFI: microvascular flow index, n/mm: number per millimeter, HI: heterogeneity index.

Supplemental Table 2. The microcirculatory cut-off values and the value of the microcirculatory parameters proportion of perfused vessels (PPV) and microvascular flow index (MFI) in small (S; $\text{Ø} < 10 \mu\text{m}$) vessels before (T1) and after (T2) the start of dopamine for predicting the need for additional catecholaminergic support with either norepinephrine or epinephrine in patients with congenital diaphragmatic hernia.

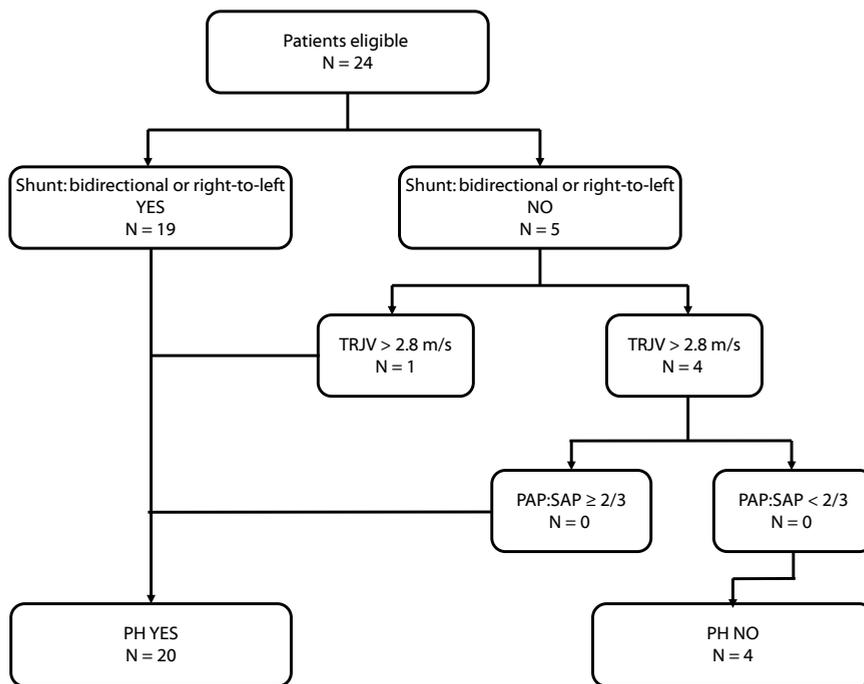
		AUC (95%-CI)	Cut-off value	Sensitivity in % (95%-CI)	Specificity in % (95%-CI)	Positive predictive value (95%-CI)	Negative predictive value (95%-CI)
T1	PPV S in %	0.84 (0.67-1.00)	85	67 (35-90)	92 (64-100)	89 (52-100)	75 (48-93)
T1	MFI S in au	0.79 (0.61-0.97)	2.79	67 (35-90)	69 (39-91)	67 (35-90)	69 (39-91)
T2	PPV S in %	0.88 (0.75-1.00)	84	77 (46-95)	77 (46-95)	77 (46-95)	77 (46-95)
T2	MFI S in au	0.74 (0.52-0.94)	2.96	77 (46-95)	69 (39-91)	71 (42-92)	75 (43-95)

AUC: area under the receiver operating characteristic, MFI: microvascular flow index, PPV: proportion of perfused vessels. Data are displayed as median (IQR).

Supplemental Table 3. The markers for disease severity –i.e., need for additional catecholaminergic support and need for extracorporeal membrane oxygenation support– stratified by abnormal and normal microcirculation –as indicated by the parameters proportion of perfused vessels (PPV) and microvascular flow index (MFI) in the small vessels– before and after start of dopamine. Microcirculatory impairment after dopamine start was associated with the higher disease severity. Abnormal microcirculatory perfusion was defined by the cut-off values that are depicted in Supplemental Table 2. Additional vasopressor support included either norepinephrine or epinephrine in addition to dopamine.

	PPV S T1		MFI S T1		PPV S T2		MFI S T2	
	Abnormal n=15	Normal n=10	Abnormal n=17	Normal n=8	Abnormal n=15	Normal n=11	Abnormal n=14	Normal n=12
Need for noradr / adr support, n (%)	11 (73)*	1 (10)*	11 (65)*	1 (13%)*	11 (73)*	2 (18)*	10 (71)*	3 (25)*
Oxygenation index at T1, median (IQR)	7 (11)	3 (19)	7 (13)	3 (16)	7 (15)	3 (12)	6 (18)	5 (11)
Oxygenation index at T2, median (IQR)	7 (9)	2 (5)	6 (8)	2 (8)	7 (9)*	3 (2)*	8 (7)*	3 (2)*
Oxygenation index at T3, median (IQR)	13 (19)	3 (21)	13 (18)	3 (23)	15 (25)*	4 (12)*	17 (24)	4 (12)
Oxygenation index at T4, median (IQR)	7 (32)*	2 (4)*	5 (24)*	2 (5)*	9 (33)*	3 (2)*	9 (34)*	3 (2)*
Need for VA-ECMO, n (%)	3 (20)	1 (10)	3 (18)	1 (13)	6 (40)*	0 (0)*	6 (43)*	0 (0)*

*Categorical variables are depicted as n (%), continuous variables as median (IQR). * p<0.05 vs. abnormal using non-parametric tests. VA-ECMO: veno-arterial extracorporeal membrane oxygenation.*



Supplemental figure 1. Flowchart showing the patients with and without pulmonary hypertension as estimated by echocardiography. PH: pulmonary hypertension, TRJV: tricuspid regurgitant jet velocity in meters per second, PAP:SAP: the ratio between pulmonary arterial pressure and systemic arterial pressure.

Chapter 7

Early microcirculatory impairment during therapeutic hypothermia is associated with poor outcome in post-cardiac arrest children: a prospective observational cohort study

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ABSTRACT

AIMS OF THE STUDY: This study aimed to evaluate if the microcirculation is impaired during and after therapeutic hypothermia (TH) in children with return of spontaneous circulation after cardiac arrest (CA) and to assess if microcirculatory impairment predicts mortality. This has been reported for post-CA adults, but results might be different for children because etiology, pathophysiology, and mortality rate differ.

METHODS: This prospective observational cohort study included consecutive, non-neonatal post-CA children receiving TH upon intensive care admission between June 2008 and June 2012. Also included were gender-matched and age-matched normothermic, control children without cardiorespiratory disease. The buccal microcirculation was non-invasively assessed with Sidestream Dark Field Imaging at the start of TH, halfway during TH, at the start of re-warming, and at normothermia. Macrocirculatory, respiratory, and biochemical parameters were also collected.

RESULTS: Twenty post-CA children were included of whom 9 died. During hypothermia, the microcirculation was impaired in the post-CA patients and did not change over time. At normothermia, the core body temperature and the microcirculation had increased and no longer differed from the controls. Microcirculatory deterioration was associated with mortality in the post-CA patients. In particular, the microcirculation was more severely impaired at TH start in the non-survivors than in the survivors –positive predictive value: 73-83, negative predictive value: 75-100, sensitivity: 63-100%, and specificity: 70-90%.

CONCLUSIONS: The microcirculation is impaired in post-CA children during TH and more severe impairment at TH start was associated with mortality. After the stop of TH, the microcirculation improves rapidly irrespective of outcome.

INTRODUCTION

Cardiac arrest (CA) in children is associated with a high mortality rate [1, 2]. In the children with return of spontaneous circulation (ROSC), significant morbidity is often present and a post-cardiac arrest syndrome (PCAS) develops [1-3]. In view of its beneficial effects on outcome in post-CA adults and asphyxiated neonates, mild therapeutic hypothermia (TH) has been clinically introduced in post-CA children [4-7]. A randomized trial in post-CA children is currently ongoing [8]. While the exact mechanism by which TH improves outcome is unknown, multiple beneficial effects have been suggested, which include balancing of vasoactive mediators and normalizing vasopermeability [9].

TH should reduce the harmful PCAS effects while adequate macrocirculation and microcirculation is ensured. The microcirculation can now be visualized non-invasively at the bedside with several techniques [10]. These have revealed that in post-CA adults the microcirculation was decreased during TH and that microcirculatory impairment existed while macrocirculatory parameters were unaltered [11, 12]. Moreover, persistent microcirculatory impairment was associated with mortality [11]. This has also been reported for children with distributive shock [13].

It is not yet known, however, whether these findings also apply to post-CA children. Extrapolating the findings in post-CA adults to children is probably inappropriate because CA etiology, post-CA pathophysiology, and post-CA mortality rate differ [3, 9, 14]. Non-invasive microcirculatory monitoring might be particularly valuable for children as the possibilities for invasive hemodynamic monitoring are limited [15]. Therefore, this study aimed to assess whether the microcirculation is impaired during and after TH in post-CA children and to evaluate whether microcirculatory impairment predicts mortality. We hypothesized that microcirculatory alterations would exist during TH and that the microcirculation would predict mortality after TH.

METHODS

Study design and Setting:

This prospective observational cohort study included patients admitted to the intensive care unit (ICU) of a level III university children's hospital between June 2008 and June 2012. The local medical ethical review board approved the study. Parental informed consent was obtained prior to the study start.

Patients:

Children eligible for inclusion were those aged between 28 days and 18 years with post-CA ROSC who received TH after admission to the study site. The exclusion criteria were: denied informed consent, start of TH in a center other than the study site, and failure to induce TH within 12h after admission. Patients in whom only one microcirculatory

measurement was done due to logistic reasons were also excluded. Age-matched and gender-matched normothermic children without cardiorespiratory disease who were hospitalized for minor, elective surgery served as controls.

Data collection:

The modified Utstein reporting templates were followed where possible [16]. Data were obtained within 12h after the start of TH (T0), at 12-24h after TH start (T1), within 12h after starting re-warming (T2), and at normothermia (T3). The primary endpoint was ICU survival. Data were obtained once in the controls.

Microcirculatory perfusion

The microcirculation was studied using Sidestream Dark Field imaging (MicroVision Medical, Amsterdam, the Netherlands) [17]. At three sites in the buccal mucosa, the microcirculation was measured according to the guidelines [18]. To avoid pressure artefacts, we adhered to the standard operating procedure as published by Trzeciak et al. [19]. Blinded, randomized video sequences were analyzed offline using dedicated software (Automated Vascular Analysis 3.0, Academic Medical Centre, Amsterdam, the Netherlands). Total vessel density (TVD), perfused vessel density (PVD), proportion of perfused vessels (PPV), microvascular flow index (MFI), and heterogeneity index (HI) were calculated for small (S; $\leq 10 \mu\text{m}$) and non-small vessels (NS; $11-100\mu\text{m}$) according to the guidelines [13, 18, 20, 21]. HI was calculated as the difference between the highest and the lowest quadrant score that is then divided by the mean score of all quadrants for one measurement. For all other scores, the mean of the video sequences per measurement was taken. The inter-observer variability between two raters was determined for all microcirculatory parameters using video sequences obtained for the current (n=60, 19%) and for another study (n=60, 19%). The Spearman's rank correlation coefficient ranged from 0.533 to 0.932 (all p-values < 0.001; mean $\rho = 0.768$), the intra-class correlation coefficient from 0.565 to 0.869 (all p-values < 0.001; mean $\rho = 0.750$).

Demographic and time-dependent parameters

Patient demographics were collected together with core body temperature, disease severity measures –the vasopressor score (VP-score), the paediatric cerebral performance category scale (PCPC) at ICU discharge, and the paediatric logistic organ dysfunction score (PELOD) at ICU day one and two–, macrocirculatory parameters, peripheral capillary refill time (pCRT), respiratory parameters, and biochemical parameters. The VP-score, PCPC score, and PELOD score, respectively serving as measures for cardiovascular, cerebral, and overall disease severity, were determined as previously described [22-24].

Hospital treatment protocol:

TH –rectal core body temperature 32.0-34.0 °C– was induced as soon as possible after admission using extracorporeal blankets (Blanketrol III, Cincinnati Sub Zero, Cincinnati, USA). Complementary intracorporeal cooling was applied if necessary. According to protocol, all children who received advanced paediatric life support, received TH. During TH, patients received continuous sedation (midazolam, 50-1000mcg kg⁻¹ hr⁻¹, morphine, 5-30mcg kg⁻¹ hr⁻¹, clonidine, 0.20-1.00mcg kg⁻¹ hr⁻¹, and/or propofol, 1-8mg kg⁻¹ hr⁻¹) and neuromuscular blockade (vecuronium, 40-100mcg kg⁻¹ hr⁻¹ and/or rocuronium, 300-700mcg kg⁻¹ hr⁻¹) if necessary. After 24h, patients were re-warmed –0.25 °C h⁻¹– to normothermia –rectal core body temperature 36.5-37.5 °C. To keep MABP within age-appropriate normal range, fluid resuscitation and cardiovascular drug support were started at the discretion of the attending intensivist. Ventilator settings were set to achieve normoxia (paO₂ 12.0-13.3 kPa) and normocapnia (pCO₂ 4.0-6.4 kPa). Patients with refractory respiratory failure despite maximal conservative treatment received extracorporeal membrane oxygenation (ECMO).

Statistical analysis:

Continuous data are presented as median (IQR); discrete data as numbers (%). The microcirculatory data were analyzed in two steps. First, differences over time were assessed using mixed effects models with time as single parameter. Linearity was assumed for all models except MFI NS. In the case of overall differences, sub-tests between time points were performed. Second, the relation between outcome and the microcirculation was assessed with non-parametric tests and through joint modeling –which incorporates both time and outcome as covariate thereby taking into account both the longitudinal and survival effects [25]. If relevant, the area under the curve (AUC) of the receiver operating characteristic (ROC) was determined. Cut-off values were identified and sensitivity, specificity, and positive and negative predictive value were calculated. With the cut-off values, the association between microcirculatory impairment and disease severity was explored. For all other data only step one was performed. Cases were compared to controls using non-parametric tests. Statistics were calculated using IBM SPSS or R statistics 2.15.2. A p-value <0.050 was considered statistically significant.

RESULTS

During the study period 55 patients with ROSC after CA were admitted to our ICU who received TH. Of those 55 eligible patients, 23 (42%) were excluded because of denied consent and 12 (22%) because of logistic reasons. Twenty children were included who received TH at the study site. These patients did not differ from the excluded patients

with regards to gender, out-of-hospital CA, survival rate, and percentage of patients with primary cardiac disease.

Table 1 shows the baseline characteristics of the included patients. In 9 (45%) patients, CA was caused by primary cardiac disease –i.e., cardiomyopathy (n=4), cardiac arrhyth-

Table 1. The baseline patient characteristics. Data are shown for the entire cohort and for the survivors and non-survivors separately.

	Total N = 20	Survivors N = 11	Non-survivors N = 9	p-value
Male gender, n (%)	15 (75)	10 (91)	5 (56)	0.127
Out-of-hospital CA, n (%)	17 (85)	10 (91)	7 (78)	0.566
Witnessed CA, n (%)	19 (95)	10 (91)	9 (100)	1.000
Bystander CPR, n (%)	12 (60)	6 (67)	6 (75)	1.000
Time first CPR – ROSC in min, median (IQR)	15 (30)	15 (25)	20 (39)	0.818
First monitored rhythm shockable ^a , n (%)	4 (20)	4 (40)	0 (0)	0.087
First admission to another hospital, n (%)	2 (10)	0 (0)	2 (22)	0.189
Age at ICU admission in y, median (IQR)	2.3 (10.6)	1.9 (11.6)	2.8 (8.9)	0.790
Weight at ICU admission in kg, median (IQR)	13.0 (31.3)	13.0 (38.3)	13.0 (24.0)	0.675
Time ICU admission – start TH in h, median (IQR)	0 (6)	4 (7)	0 (0)	0.009
Hypothermic at ICU admission, n (%)	11 (55)	4 (36)	7 (78)	0.092
First recorded core body temperature in °C, median (IQR)	33.7 (2.3)	35.0 (2.8)	33.4 (2.1)	0.057
PELOD day 1, median (IQR)	33 (10)	32 (10)	33 (14)	0.298
PELOD day 2, median (IQR)	32 (11)	31 (19)	32 (11)	0.331
Absent LPLR at start TH, n (%)	4 (20)	0 (0)	4 (50)	0.023
Fluid balance day 1 in ml kg ⁻¹ d ⁻¹ , median (IQR)	2.4 (3.5)	2.7 (3.9)	2.1 (3.0)	0.849
Fluid balance day 2 in ml kg ⁻¹ d ⁻¹ , median (IQR)	1.5 (2.2)	0.5 (2.2)	2.3 (1.9)	0.138
ECMO, n (%)	3 (15)	2 (18)	1 (11)	1.000
PCPC at ICU discharge ≤ 2, n (%)	6 (30)	6 (55)	0 (0)	NA
Length of ICU stay in days, median (IQR)	7.6 (7.1)	10.3 (25.7)	4.8 (7.9)	0.030
Diagnosis, n (%)				
- Cardiac	9 (45)	6 (55)	3 (33)	0.406
- Respiratory	11 (55)	5 (45)	6 (67)	
Cause of death, n (%)				
- Cardiac	2 (10)	-	2 (10)	NA
- Respiratory	1 (5)	-	1 (5)	
- Cerebral	6 (30)	-	6 (30)	

Categorical variables are presented as n (%), continuous variables as median (IQR). Differences assessed using non-parametric tests. CA: cardiac arrest, CPR: cardiopulmonary resuscitation, ECMO: extracorporeal membrane oxygenation, h: hours, IQR: interquartile range, LPLR: left pupillary light reflex, min: minutes, ml kg⁻¹ d⁻¹: milliliter per kilogram per day, NA: not assessed, PCPC: pediatric cerebral performance category scale, PELOD: pediatric logistic organ dysfunction score, ROSC: return of spontaneous circulation, TH: therapeutic hypothermia, y: years, °C: degrees Celsius. ^aAll shockable rhythms were due to ventricular fibrillation.

mias (n=3), congenital cardiac anomaly (n=1), and ALTE (n=1). In the other 11 patients, CA was caused by primary respiratory failure –i.e., submersion (n=5), infectious respiratory disease (n=2), neuromuscular disorder (n=1), aspiration (n=1), tracheomalacia (n=1), and hanging (n=1).

Nine (45%) post-CA children died in the ICU. The causes of death were hypoxic-ischemic brain injury (n=6), refractory cardiac failure (n=2), and refractory respiratory failure (n=1). In two out of the six patients with brain injury, the direct cause of death was uncontrollable intracranial pressure increments. In the four others, continuation of therapy was futile as they fulfilled the criteria of brain death. Two (22%) non-survivors did not reach normothermia. Three patients, one non-survivor, received ECMO. In 6 (55%) out of the 11 survivors the PCPC was ≤ 2 , indicating that at ICU discharge they had, at worst, mild neurologic deficits and that they were conscious, alert, and deemed capable of age-appropriate interactions. The median first measured body temperature of the non-survivors tended to be lower than that of the survivors. The median time between

Table 2. The macrocirculatory, respiratory, and biochemical parameters in the post-cardiac arrest children and the normothermic, healthy controls.

	T0 N = 18	T1 N = 18	T2 N = 19	T3 N = 18	c0 N = 20
Time to start TH in hours ^A	3.6 (4.2)	15.6 (4.4)	28.9 (6.4)	43.4 (8.7)	-
Core body temperature in °C	33.4 (1.6) ^b	33.8 (1.6) ^b	33.9 (1.1) ^b	37.0 (0.3) ^a	36.9 (0.3)
Vasopressor score	0 (13) ^b	8 (22) ^b	10 (20) ^b	9 (26) ^b	0 (0)
Heart rate in bpm	125 (45)	110 (56)	123 (63)	131 (51) ^a	130 (50)
MABP in mm Hg	60 (30)	64 (19)	68 (28)	66 (23)	67 (18)
pCRT in N<3 s / N≥ 3s	1 / 16	2 / 15	3 / 15	6 / 15	-
MAP in cm H ₂ O	14 (7)	15 (10)	14 (5)	14 (6)	-
Arterial saturation in %	98 (2)	99 (2)	97 (2)	97 (3)	-
pH	7.27 (0.32)	7.34 (0.12) ^a	7.34 (0.09) ^a	7.38 (0.11) ^a	-
pO ₂ in kPa	14.8 (6.4)	13.8 (8.9)	11.4 (5.0) ^a	11.5 (5.5) ^a	-
pCO ₂ in kPa	5.1 (2.1)	5.0 (1.2)	5.5 (0.9)	5.1 (1.6)	-
Base excess in mmol L ⁻¹	-10 (8)	-5 (4) ^a	-4 (4) ^a	-2 (3) ^a	-
Arterial lactate in mmol L ⁻¹	2.8 (5.8)	3.4 (4.5)	2.4 (2.4) ^a	1.5 (0.9) ^a	-
C-reactive protein in mg L ⁻¹	1 (5)	23 (51)	80 (78) ^a	73 (85) ^a	-
Hemoglobin in mmol L ⁻¹	7.1 (1.0)	7.1 (2.5)	6.6 (1.5)	6.3 (1.5) ^a	-
Hematocrit in L/L ⁻¹	0.34 (0.07)	0.35 (0.08)	0.31 (0.05) ^a	0.30 (0.05) ^a	-

All data are presented as median (IQR), except pCRT which is in n (%). ^aIndicates change over time from T0 to T3 using mixed effects models and sub-tests. ^bIndicates difference with c0 using non-parametric tests. T0: at TH start, T1: halfway during TH, T2: at re-warming start T3: at normothermia in post-cardiac arrest children, c0: normothermic, healthy controls. Bpm: beats per minute, cmH₂O: centimeter water, kPa: kilopascal, L L⁻¹: liter per liter, MABP: mean arterial blood pressure, MAP: mean airway pressure, mg L⁻¹: milligram per liter, mmHg: millimeter mercury, mmol L⁻¹: millimoles per liter, pCRT: peripheral capillary refill time, s: seconds, TH: therapeutic hypothermia, °C: degrees Celsius.

admission and start of TH was shorter for the non-survivors. More non-survivors than survivors had an absent pupillary reflex at the start of TH.

The 20 control patients without cardiorespiratory failure were all admitted to a surgical ward for minor, elective surgery –i.e., abdominal (n=7), urogenital (n=5), craniofacial (n=4), orthopaedic (n=3), and thoracic (n=1).

Macrocirculatory, respiratory, and biochemical parameters over time

Table 2 depicts the macrocirculatory, respiratory, and biochemical parameters during and after TH. Apart from core body temperature, the median values for macrocirculatory and respiratory parameters were all within the normal range. Biochemical abnormalities

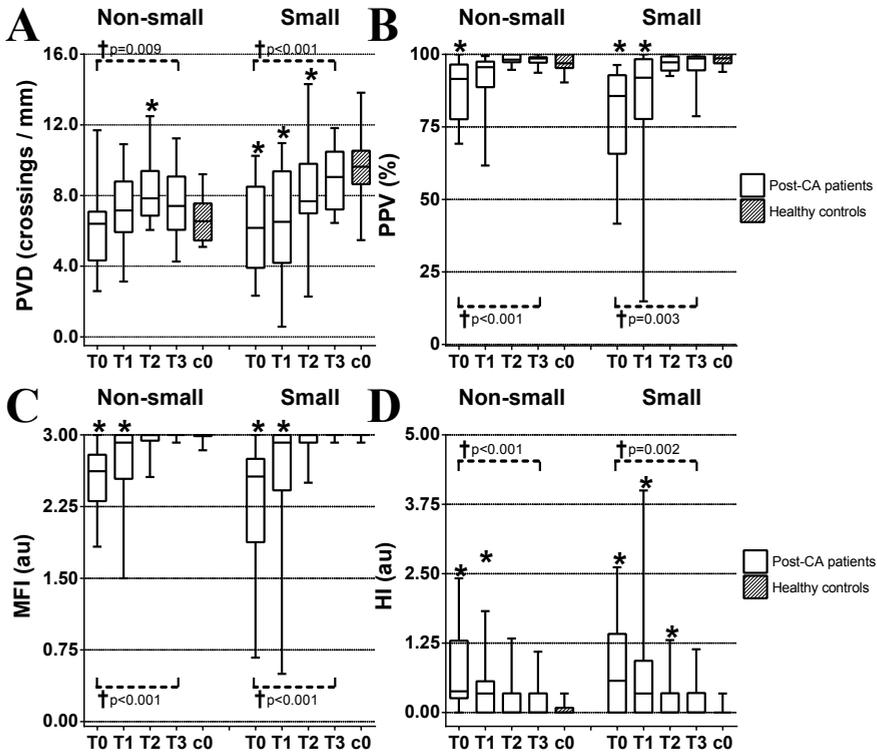


Figure 1. Boxplots showing the microcirculatory parameters perfused vessel density (A), proportion of perfused vessels (B), microvascular flow index (C), and heterogeneity index (D) for non-small (10 $\mu\text{m} \leq \text{D} < 100 \mu\text{m}$) and small ($\text{D} < 10 \mu\text{m}$) vessels in post-cardiac arrest children (n=20; blank boxplots) and normothermic, healthy controls (n=20; obliquely striped boxplots). T0: at TH start, T1: halfway during TH, T2: at re-warming start T3: at normothermia in post-cardiac arrest children, c0: normothermic, healthy controls. † indicates change over time from T0-T3 using mixed effects models, * indicates difference with c0 using non-parametric tests.

for pH, BE, and arterial lactate were manifested predominantly at the start of TH, while Hb, Ht, and CRP were abnormal predominantly at normothermia.

During TH the core body temperature was below 34.0 °C and it remained unaltered. At normothermia the median (IQR) core body temperature was significantly higher than before: 37.0 (0.3) °C. MABP, arterial saturation, MAP, pCO₂, and the VP-score did not change over time. BE and pH improved halfway TH and remained improved at normothermia. As of the start of re-warming, pO₂ and arterial lactate improved as well. CRP increased while Ht decreased. Hb was lower at normothermia and HR increased.

The microcirculation over time

The microcirculatory parameters are shown in Figure 1 and Table 3. Mixed effects models indicated that all microcirculatory parameters except TVD NS changed over time. Sub-tests for the parameters with an overall difference showed that none improved during TH. At the start of re-warming, all parameters except TVD S and HI S were improved. At normothermia, all microcirculatory parameters except PVD NS and PPV S were improved.

In comparison to the normothermic control patients, TVD S, PVD S, PPV NS, PPV S, MFI NS, MFI S, HI NS, and HI S were all lower during TH in the post-CA patients, while HR or MABP did not differ (Tables 2 and 3). At normothermia, the microcirculatory parameters in the post-CA patients did no longer differ from those in the controls. Significant correlations –range p : 0.25-0.45– existed between core body temperature and PVD S, PPV NS, PPV S, MFI NS, MFI S, and HI S.

Microcirculatory impairment and outcome

At the start of hypothermia, PVD NS, PPV NS, MFI NS, and MFI S were lower in the non-survivors than in the survivors (Figure 2, Table 3). The AUC of the ROC curves was for all four parameters 0.84 and the best cut-off points for TVD NS, PVD NS, MFI NS, and MFI S at TH start were estimated at 4.9 crossing mm⁻¹, 92%, 2.68 au, and 2.56 au, respectively (Table 4, Supplemental Figure 1). With these cut-off values, sensitivity ranged from 63 to 100% and specificity ranged from 70 to 90%. The positive and negative predictive value for mortality at ICU discharge ranged from 73 to 83% and from 75 to 100%, respectively. Joint modeling indicated that for every unit increase in PVD NS and MFI S over time –i.e. not only at the start of hypothermia, but also thereafter–, the mortality risk decreased by 5.3 and by 1.1, respectively. For the other microcirculatory parameters, there was no association with mortality over time.

At TH start, the cut-off values according to vessel size –TVD NS, PVD NS, and MFI NS vs. MFI S– then served to stratify the disease severity measures arterial lactate over time, VP-score over time, PELOD at day one and two of ICU admission, and PCPC at ICU discharge (supplemental digital content Table 1). In this way it became apparent that patients with microcirculatory deterioration in the non-small vessels –i.e., TVD NS, PVD NS, and MFI

Table 3. The microcirculatory parameters total vessel density (TVD), perfused vessel density (PVD), proportion of perfused vessels (PPV), microvascular flow index (MFI), and heterogeneity index (HI) for non-small ($10 \mu\text{m} \leq \text{D} < 100 \mu\text{m}$) and small ($\text{D} < 10 \mu\text{m}$) vessels in post-cardiac arrest children and normothermic, healthy controls.

	T0		T1		T2		T3		c0
	Whole group		Whole group		Whole group		Whole group		
	Survivors N = 10	Non-survivors N = 8	Survivors N = 10	Non-survivors N = 8	Survivors N = 10	Non-survivors N = 9	Survivors N = 11	Non-survivors N = 7	
TVD non-small in n mm^{-1}	7.0 (3.2)	7.0 (3.2)	8.0 (3.1)	8.0 (2.7) ^b	8.0 (2.7) ^b	8.0 (2.7) ^b	7.5 (3.0)	7.5 (3.0)	6.8 (1.9)
PVD non-small in n mm^{-1}	7.2 (1.8)	5.3 (3.3)	8.7 (2.7)	6.4 (3.5)	7.7 (2.8)	8.5 (3.1)	6.7 (3.6)	7.9 (1.9)	-
PPV non-small in %	6.4 (2.8)	4.3 (2.6) ^c	7.2 (2.9)	6.3 (3.8)	7.8 (2.5) ^{pb}	8.5 (2.9)	7.4 (3.0)	7.8 (2.1)	6.5 (2.1)
MFI non-small in au	92 (19) ^b	83 (19) ^c	96 (9)	92 (20)	98 (3) ^b	99 (3)	99 (2) ^a	99 (2) ^a	97 (6)
HI non-small in au	2.62 (0.49) ^b	2.38 (0.64) ^c	2.92 (0.46) ^b	2.72 (0.96)	3.00 (0.06) ^a	2.94 (0.17) ^c	3.00 (0.00) ^a	3.00 (0.00)	3.00 (0.05)
TVD small in n mm^{-1}	0.36 (0.91)	0.99 (1.24)	0.17 (0.37)	0.37 (0.86)	0.00 (0.35)	0.00 (0.35)	0.00 (0.00)	0.00 (0.35) ^a	0.00 (0.34)
PVD small in n mm^{-1}	7.4 (3.8) ^b	7.4 (3.1)	7.2 (4.7) ^b	6.7 (2.4)	8.1 (3.0)	8.3 (3.7)	9.1 (2.3)	9.3 (4.5)	9.7 (2.8)
PPV small in %	6.2 (4.6) ^b	5.6 (2.7)	6.5 (5.2) ^b	5.7 (3.7)	7.7 (2.8) ^{pb}	8.2 (3.7)	9.0 (3.3) ^a	8.9 (4.8)	9.6 (2.9)
MFI small in au	86 (27) ^b	70 (28)	92 (21) ^b	90 (29)	97 (5) ^b	95 (5)	99 (5)	98 (5)	99 (3)
HI small in au	2.72 (0.30) ^c	2.08 (1.13) ^c	2.96 (0.21)	2.79 (1.08)	3.00 (0.08)	3.00 (0.17)	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)
	0.37 (1.21)	1.24 (1.29)	0.34 (0.93) ^b	0.36 (1.60)	0.00 (0.53)	0.00 (0.35)	0.00 (0.34)	0.00 (0.38)	0.00 (0.00)

Data are presented in median (IQR) for the whole cohort (top line) as well as the post-cardiac survivors and non-survivors (bottom line). ^aIndicates change over time from T0-T3 using mixed effects models and sub-tests. ^bIndicates difference with c0 using non-parametric tests. ^cIndicates difference between survivors and non-survivors using non-parametric tests. T0: at TH start, T1: halfway during TH, T2: at re-warming start T3: at normothermia in post-cardiac arrest children, c0: normothermic, healthy controls.

NS- had higher arterial lactate levels at normothermia; had higher VP-scores halfway during TH, at the start of re-warming, and at normothermia; and had higher a PCPC score at ICU discharge. Likewise, patients with lower MFI S had microcirculatory deterioration had a higher PCPC score at ICU discharge.

At the start of TH, neither the core body temperature nor the macrocirculatory, respiratory, or biochemical parameters differed between the survivors and the non-survivors, apart from BE which was unfavorable for the non-survivors (supplemental digital content Table 2).

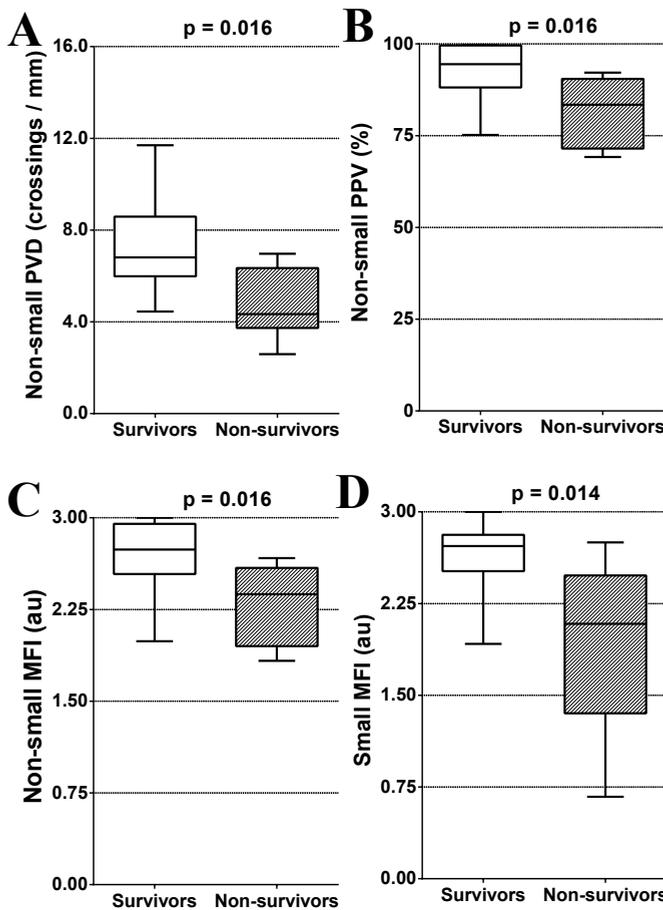


Figure 2. Boxplots showing the microcirculatory parameters non-small perfused vessel density (A), non-small proportion of perfused vessels (B), non-small microvascular flow index (C), and small microvascular flow index (D) at the start of hypothermia in survivors (n=11; blank box plots) and non-survivors (n=9; obliquely striped box plots). Differences assessed using non-parametric tests.

Table 4. The value for predicting mortality in post-cardiac arrest children with the microcirculatory parameters non-small perfused vessel density (PVD), non-small proportion of perfused vessels (PPV), non-small and small microvascular flow index (MFI).

	AUC (95%-CI)	Cut-off value	Sensitivity in % (95%-CI)	Specificity in % (95%-CI)	Positive predictive value (95%-CI)	Negative predictive value (95%-CI)
PVD non-small in $n\text{ mm}^{-1}$	0.84 (0.65-1.00)	4.9	63 (24-91)	90 (55-100)	83 (36-100)	75 (43-95)
PPV non-small in $n\text{ mm}^{-1}$	0.84 (0.65-1.00)	92	100 (63-100)	70 (35-100)	73 (34-94)	100 (59-100)
MFI non-small in au	0.84 (0.64-1.00)	2.68	100 (63-100)	70 (35-100)	73 (34-94)	100 (59-100)
MFI small in au	0.84 (0.65-1.00)	2.56	88 (47-100)	80 (44-97)	78 (40-97)	89 (52-100)

AUC: area under the receiver operating characteristic, 95%-CI: 95% confidence interval, $n\text{ mm}^{-1}$: number per millimetre, au: arbitrary units.

DISCUSSION

This study demonstrates that the buccal microcirculation was impaired during TH in post-CA children, while the cardiorespiratory parameters were relatively unaffected. After TH, the microcirculation improved to a level comparable to normothermic children without cardiorespiratory disease. Microcirculatory impairment was associated with mortality in the post-CA patients. In particular, at the start of TH, the microcirculation was more severely deteriorated in the non-survivors than in the survivors.

This study is the first to describe that the buccal microcirculation is altered during TH in post-CA children. Similar findings have been reported for post-CA adults and perinatally asphyxiated neonates [11, 12, 26]. The current study is unique in studying the microcirculation shortly after re-warming. At this point, the microcirculation had already improved substantially while neither core body temperature, nor the macrocirculatory or respiratory parameters had changed. This confirms that in post-CA children microcirculatory function cannot be estimated by macrocirculatory parameters, as was also reported for post-CA adults [11]. Interestingly, microcirculatory improvement coincided with improvements in arterial lactate and pO_2 , while pCRT remained abnormal over time.

Microcirculatory impairment before and after TH predicted mortality in post-CA adults, whereas we found the microcirculation to be lower in non-survivors at TH start [11]. Differences in etiology can explain this discrepancy: all of the adults had primary cardiac disease as opposed to 50% of the children [11]. There are also pathophysiologic differences: PCAS develops differently in children and continuous post-CA macrocirculatory failure is less prominent [3]. Accordingly, cerebral injury rather than cardiac disease is the predominant determinant for outcome in children [3, 27-29]. Our data supported this as 75% of the non-survivors were brain death and both HR and MABP were within the normal range throughout follow-up.

In critically ill adults, therapy efficacy and outcome could be predicted by early microcirculatory monitoring [30, 31]. We show that, next to mortality, microcirculatory impairment at TH start is associated with cardiovascular disease severity and neurologic

disease severity later in time. So, non-invasive microcirculatory monitoring might be clinically valuable in post-CA children. For children –and infants in particular– the possibilities for (invasive) cardiovascular monitoring are limited [15].

In the present study, microcirculatory impairment was associated with poor outcome at TH start in particular. At this point, pupillary reflexes were absent more often and BE, pH, and arterial lactate either differed or tended to differ suggesting that the non-survivors were in a worse clinical condition. Additionally, PCAS is still in its early phase at TH start and includes amongst others inflammation and continuous ischemia [3, 9, 32]. Hypothetically, both could have contributed to the microcirculatory impairment in our patients [3, 9, 32]. We, however, did not measure SvO₂, interleukins, or complement factors. Furthermore, TH is acknowledged to improve outcome in post-CA adults and perinatally asphyxiated neonates [4-7]. Yet, non-clinical studies indicate that TH in itself decreases the microcirculation [33]. During TH, microcirculatory deterioration could either be an epiphenomenon or an active mediator through which TH partly exerts its beneficial effects. The latter would contrast our observation that microcirculatory impairment relates to poor outcome. Thus, our results suggest that TH improves outcome by impacting enzymatic and metabolic processes predominantly [3, 9, 32].

Although core body temperature did not differ at the time of the microcirculatory measurements, we did observe that hypothermia was more rapidly induced in the non-survivors and that the first recorded temperature tended to be lower. This was observed before [34]. Neither the underlying diagnoses, nor the number of near-drownings differed in our study and hospital protocol applied to all patients. In contrast, our neurologic and biochemical measurements suggest that the more rapidly induced hypothermia most likely resulted from the non-survivors' poor clinical condition. Non-induced hypothermia occurs as often as hyperthermia and conveys an increased mortality risk as well [35, 36]. Endogenous factors attributing to non-induced hypothermia include altered basal metabolic rate, impaired cortisol release, hypothalamic temperature set-point alterations, vasoconstriction, and absent shivering [35].

Absent pupillary reflexes and lower pH are independently associated with mortality in post-CA children [3, 36]. Future research should focus on the combination of parameters that best predict poor outcome or monitor therapy efficacy. Our study shows that non-invasive microcirculatory monitoring could be considered as covariate in future studies, but also that the predictive accuracy of microcirculatory monitoring in post-CA children should be detailed better and that the functional role of the microcirculation during PCAS and TH should be elucidated.

Limitations

Several limitations must be acknowledged. Above all, few data regarding the period prior to ICU admission were available so it is unclear to what extent the proceedings

during the resuscitation period biased our findings. Furthermore, the included cohort was modest in size and quite heterogeneous. For instance, two patients were first admitted to another hospital. Also, CPR time and lactate levels were relatively short/low. Relatively many patients were excluded. Results should thus be interpreted cautiously, given that data were obtained only in the patients who received TH at the study site. Multicenter trials are therefore needed to substantiate our findings. Furthermore, owing to the observational design, our findings are associative rather than causal. Also, microcirculatory monitoring was limited to the very early post-CA phase whereas the follow-up for survival was longer. In addition, a reliable pre-hypothermic microcirculatory assessment was not possible.

CONCLUSIONS

In non-neonatal post-CA children, the microcirculation is impaired during TH and improves rapidly after TH discontinuation. Microcirculatory impairment early after the start of TH is associated with poor outcome. Future studies should evaluate in greater detail the accuracy by which microcirculatory monitoring predicts outcome and whether it can be used to assess therapy efficacy.

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Supplemental table 1. The disease severity measures pediatric cerebral performance category scale, pediatric logistic organ dysfunction score, the vasopressor score, and arterial lactate stratified by abnormal and normal microcirculatory perfusion in non-small and small vessels at the start of hypothermia in post-cardiac arrest children. For defining abnormal microcirculatory perfusion the cut-off values were used as presented in Table 4.

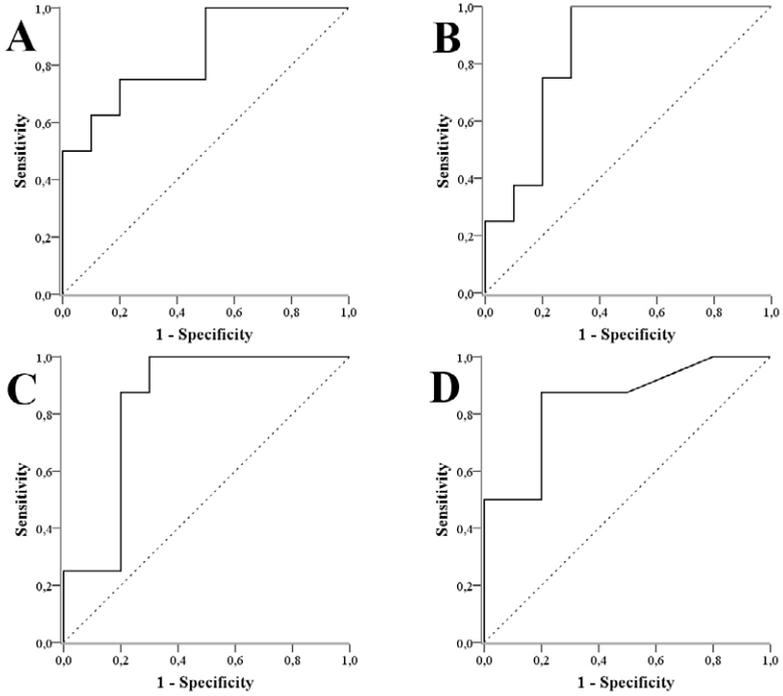
	Non-small vessels ^A		Small vessels	
	Abnormal at T0 n=7	Normal at T0 n=14	Abnormal at T0 n=11	Normal at T0 n=10
Arterial lactate T0	5.2 (12.7)	2.5 (2.7)	2.8 (6.8)	2.7 (7.3)
Arterial lactate T1	6.3 (4.4)	2.8 (3.0)	5.0 (4.0)	1.9 (3.3)
Arterial lactate T2	2.4 (4.4)	2.2 (2.2)	2.5 (2.0)	2.3 (2.6)
Arterial lactate T3	1.8 (4.0)†	1.4 (0.7)†	1.5 (0.9)	1.4 (0.6)
VP-score T0	5 (20)	0 (15)	0 (8)	5 (25)
VP-score T1	20 (37)†	0 (18)†	12 (35)	10 (26)
VP-score T2	20 (42)†	3 (18)†	5 (30)	15 (24)
VP-score T3	39 (105)†	5 (20)†	6 (46)	14 (25)
PELOD day 1	42 (15)	33 (10)	33 (10)	33 (15)
PELOD day 2	32 (11)	31 (15)	32 (11)	31 (16)
PCPC at ICU discharge	6 (0)†	3 (4)†	5 (2)†	3 (3)†

All data are displayed as median (IQR). PCPC: pediatric cerebral performance category scale, PELOD: pediatric logistic organ dysfunction score, VP-score: vasopressor score. T0: at TH start, T1: halfway during TH, T2: at re-warming start T3: at normothermia. ^A Flow in the non-small vessels was categorized as abnormal in case TVD NS, PVD NS, and MFI NS were all below the cut-off value. † indicates difference between abnormal and normal using non-parametric tests.

Supplemental table 2. The macrocirculatory, respiratory, and biochemical parameters at hypothermia start in survivors and non-survivors.

	Survivors N=11	Non-survivors N=9	p-value
Time to start TH in hours	4.3 (4.4)	2.7 (1.1)	0.424
Core body temperature in °C	33.4 (1.8)	33.1 (2.2)	0.505
Vasopressor score	3 (23)	0 (9)	0.435
Heart rate in bpm	127 (52)	121 (46)	0.657
MABP in mm Hg	60 (38)	60 (32)	0.790
pCRT in N<3 s / N≥ 3s	1 / 8	0 / 8	1.000
MAP in cm H ₂ O	14 (8)	14 (9)	0.722
Arterial saturation in %	98 (2)	97 (5)	0.349
pH	7.32 (0.27)	7.24 (0.31)	0.090
pO ₂ in kPa	15.1 (3.2)	14.4 (12.4)	0.859
pCO ₂ in kPa	5.5 (5.2)	4.9 (1.0)	0.119
Base excess in mmol/L	-6 (9)	-12 (12)	0.020
Arterial lactate in mmol/L	2.4 (2.5)	4.1 (10.9)	0.068
C-reactive protein in mg/L	3 (43)	1 (5)	0.443
Hemoglobin in mmol/L	7.4 (2.2)	7.0 (0.3)	0.075
Hematocrit in L/L	0.35 (0.10)	0.33 (0.02)	0.688

All data are presented as median (IQR), except pCRT which is in n (%). Differences assessed using non-parametric tests. Bpm: beats per minute, cmH₂O: centimetre water, kPa: kilopascal, L/L: liter per liter, MABP: mean arterial blood pressure, MAP: mean airway pressure, mg/L: milligram per liter, mmHg: millimeter mercury, mmol/L: millimoles per liter, pCRT: peripheral capillary refill time, s: seconds, TH: therapeutic hypothermia, °C: degrees Celsius.



Supplemental figure 1. The receiver operator characteristic curves for the microcirculatory parameters non-small perfused vessel density (A), non-small proportion of perfused vessels (B), non-small microvascular flow index (C), and small microvascular flow index (D) at the start of hypothermia.

PART III

ARTERIAL LACTATE MONITORING

Chapter 8

Arterial lactate as an early predictor for extracorporeal membrane oxygenation in neonates with congenital diaphragmatic hernia

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ABSTRACT

BACKGROUND: Dynamic lactate indices – which incorporate duration or trend – are early predictors for poor outcome in children with septic shock or congenital cardiac defects and outperform the predictive value of static –cross-sectional– lactate measurements. This might also be the case in children with congenital diaphragmatic hernia (CDH).

OBJECTIVE: Assessing whether static and/or dynamic lactate are an early predictor for extracorporeal membrane oxygenation requirement (ECMO; primary endpoint) and/or intensive care mortality (IC-mortality; secondary endpoint) in CDH patients.

METHODS: Static lactate levels (LACabs) were prospectively determined at 0h, at 6h, at 12h, and at 24h after IC admission. Time-weighted lactate (LACTw) and lactate change over time (LACdelta) were calculated as dynamic indices for, respectively, the duration and trend over time of lactate derangement.

RESULTS: Sixty-four inborn CDH patients were included. Twenty-two (34%) received ECMO, 14 (22%) died. 0h-LACabs and 6h-LACabs were higher in the ECMO recipients. Likewise, 6h-LACTw, 12h-LACTw, and 12h-LACdelta were all unfavorable for the ECMO recipients. LACTw and LACdelta predicted ECMO requirement better than LACabs (AUC range: 0.67-0.73, specificity range: 78-78%, negative predictive value: 72-82%). For every unit increase in 6h-LACTw, the risk for ECMO increased by 111 % (CI₉₅ 1.20-3.71, p-value 0.010). LACTw was also the best predictor for mortality.

CONCLUSIONS: Early derangements in LACabs, LACTw, and LACdelta are associated with ECMO requirement in CDH patients. The dynamic lactate indices predict adverse outcome more accurately than static lactate measurements.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is characterized by pulmonary hypoplasia and pulmonary vascular abnormalities [1]. Disease severity varies highly between patients and can include hypoxia, pulmonary hypertension (PH), and – ultimately – circulatory failure [2, 3]. Therapeutic strategies for CDH patients are optimized continuously and now comprise amongst others fetal endotracheal occlusion, inhaled nitric oxide (iNO), “gentle” ventilation with permissive hypercapnia, and – as rescue treatment – extracorporeal membrane oxygenation (ECMO) [2, 4].

As a result, it remains debatable which parameters best identify the CDH patients who benefit most from ECMO [5]. Early predictors would be highly advantageous as they allow for stratification and optimization of clinical decision-making in a timely manner [6]. For the postnatally-measured parameters that have been evaluated as prognosticator, the lack of consensus is perhaps best illustrated by the different ECMO entry criteria that are used in centers around the world [5, 7, 8]. Parameters for oxygenation are certainly important. The studies that assessed prenatal parameters showed that the observed-to-expected lung-to-head-ratio (LHR) and/or fetal lung volume (FLV) are predictive for ECMO requirement, as are gestational age, liver herniation, and 5-minute-Apgar-score (supplemental Table 1). LHR and FLV are, however, not always determined as the average prenatal CDH detection rate is merely 50-60% [9, 10].

Arterial lactate has – to the best of our knowledge – never been evaluated as a predictor for ECMO dependency. This is surprising because arterial lactate is a relatively well-established predictor for poor outcome in other patient groups [11]. Unlike pH, pO₂, or pCO₂, lactate is affected by both hypoxic and non-hypoxic causes –e.g. decreased hepatic clearance tissue [11]. These factors might be relevant for CDH patients as well. Furthermore, studies in children with septic shock or congenital cardiac defects patients showed that dynamic lactate indices – which incorporate duration or trend over time of lactate derangement– are better early predictors than static lactate measurements – which are in principle cross-sectional [6, 12-14]. Therefore, the aim of this study is to assess the value of static and dynamic arterial lactate indices as early predictors for ECMO requirement in CDH patients.

METHODS

Patients

This single center observational cohort study included all antenatally diagnosed, inborn CDH patients admitted between January 2007 and January 2012 to a level III Intensive Care (IC) of a university children’s hospital, one of two designated centers in the Netherlands for treating CDH and for delivering ECMO support. Postnatally diagnosed patients and transferred CDH patients were excluded, as were stillbirths, the patients who died

in the delivery room, the patients who were not eligible for ECMO, and the patients with less than 2 arterial lactate measurements. The local medical ethical review board approved the study and waived the need for informed consent.

Medical management

During the study period treatment complied with the internationally standardized protocol of the CDH Euro Consortium and is described elsewhere – including ECMO entry criteria [1]. The ECMO exclusion criteria were: gestational age < 34 weeks, birth weight < 2 kilograms, intracranial hemorrhage pre-cannulation, coagulopathy, and/or congenital anomalies, genetic syndromes, or other co-morbidities that are incompatible with life. All inborn CDH patients were intubated immediately after birth and thereafter transferred to the IC. Hospital staff was not blinded for the static arterial lactate levels. Interpretation of the data was left to the attending physician. The decision to start ECMO and its timing was based on our institutional protocol and international consensus [1].

Data collection

The primary endpoint was ECMO start (yes/no), the secondary endpoint was intensive care mortality (yes/no). The primary study parameters were static arterial lactate (LACabs) within 24h of IC admission and the dynamic arterial lactate indices time-weighted arterial lactate (LACTw) and lactate change over time (LACdelta). LACabs levels were determined prospectively at admission, at 6h, at 12h, and at 24h after admission using an ABL-800 flex blood gas analyzer (Radiometer Medical Aps, Copenhagen, Denmark). The data was stored digitally in the unit's Patient Data Management System (PDMS) and retrieved later in time. Non-arterial lactate measurements were discarded, as were the measurements after 24h and/or after ECMO initiation. The dynamic indices LACTw and LACdelta were calculated using LACabs [15]. LACTw –incorporating magnitude and duration of hyperlactatemia– was determined by summing the mean value of LACabs between consecutive time points multiplied by the period of time in between and then dividing by the total time. For LACdelta –incorporating magnitude and trend over time– the LACabs values were regressed against time for each individual patient, with the regression slope representing the projected change of measurements over time.

Next to lactate, we also obtained patient demographics, prenatal and perinatal parameters – i.e. gestational age, birth weight, lung-to-head ratio (LHR), presence of liver herniation, side of diaphragmatic defect, Apgar scores, and umbilical cord pH, pCO₂, and base excess (BE) –, macrocirculatory parameters – i.e. heart rate and mean arterial blood pressure –, ventilatory parameters – i.e. pre-ductal and post-ductal arterial saturation difference, ventilation type, use of inhaled nitric oxide –, blood gas analysis parameters – i.e. pH, pO₂, pCO₂, and BE. In addition, the Score for Neonatal Acute Physiology Perinatal Extension II (SNAPPE-II) was determined for the first 24h after admission.

Also, the vasopressor score – as an indicator of circulatory failure – and the oxygenation index – as an indicator of respiratory failure – were calculated at the time of each of the lactate measurements [16-18].

Statistical analysis

Continuous data are presented as median (IQR); discrete data as numbers (%). Inter-group differences were assessed with the Mann Whitney U test or the Fisher's Exact test, as appropriate. The ability of the lactate indices to predict ECMO requirement was first assessed by the area under the curve (AUC) of the receiver operating characteristic (ROC). Cut-off values – which are not available for CDH patients prior to ECMO initiation – were identified and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. To identify the patients who were likely to receive ECMO, the test had to be specific with acceptable NPV [19]. Univariate logistic regression modeling was used to calculate odds ratios with the Hosmer and Lemeshow statistic as a determinant for goodness of fit. Analyses were repeated for the secondary outcome mortality. All data were analyzed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Figure 2 was created using Graphpad PRISM v6 (Graphpad Software Inc., La Jolla, CA, USA).

RESULTS

During the 5-year study period 103 CDH patients were admitted to our IC. Of those 103 patients, 39 (38%) were excluded because in 34 cases they were outborn whereas in 5 cases there were less than 2 arterial lactate measurements (Figure 1). The excluded patients did not differ from the included patients in terms of number of right sided diaphragmatic hernias – $n_{EX}=9$ (23%) vs. $n_{IN}=9$ (14%); p -value=0.289–, number of patients receiving ECMO support – $n_{EX}=7$ (18%) vs. $n_{IN}=22$ (34%); p -value=0.113–, and number of non-survivors – $n_{EX}=5$ (13%) vs. $n_{IN}=14$ (22%); p -value=0.303.

Twenty-two (34%) out of the 64 included patients received ECMO. The baseline and the time-related patient characteristics are summarized in Tables 1 and 2, respectively. Birth weight, LHR, and 5-minute-APGAR were lower in the CDH patients in the ECMO group. Likewise, the proportion of patients with liver herniation, the SNAPPE-II score, the length of ICU stay, and the number of non-survivors were higher in the ECMO group.

Static (LACabs) and dynamic lactate indices (LACTw and LACdelta) for predicting ECMO requirement

The median (IQR) values for LACabs, LACTw, and LACdelta within the first 24h after admission in relation to outcome are presented in Figure 2. LACabs was higher in the ECMO patients at admission and at 6h after admission. LACabs at 12h and 24h after admission

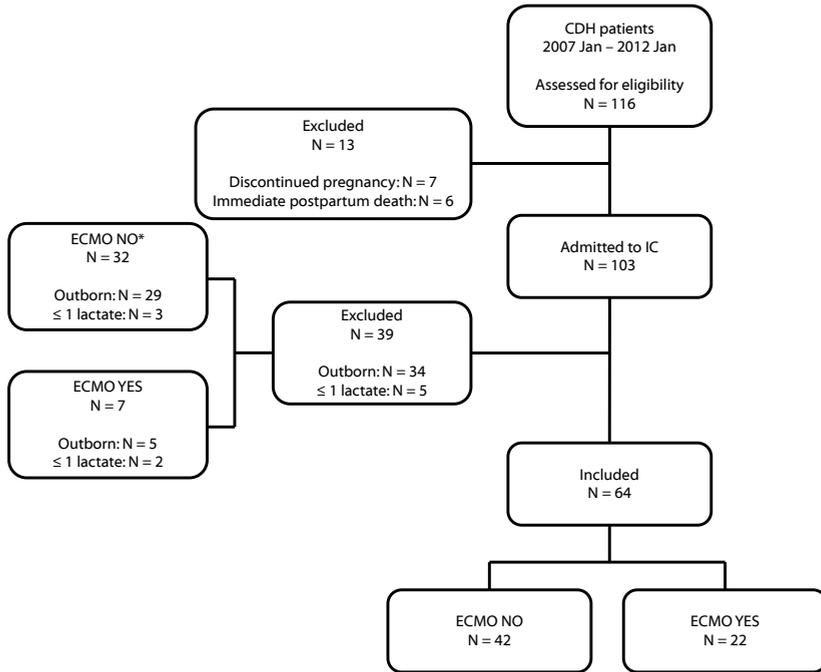


Figure 1. Flowchart for the patients with congenital diaphragmatic hernia who were assessed for inclusion in the study. *One patient was not eligible for ECMO support due to disease that was deemed irreversible, one due to prematurity.

did not differ. LACTw was higher in the ECMO patients at 6h and at 12h. LACdelta at 12h differed significantly indicating that the decline in lactate from admission to 12h after admission was greater in the ECMO patients. Univariate logistic regression showed that for every unit increase in LACabs at admission, the risk for ECMO increased by 44 % (CI₉₅ 1.05-1.98, p-value 0.024). For LACabs at 6h, LACTw at 6h, LACTw at 12h, and LACdelta at 12h, the respective results were: 97 % (CI₉₅ 1.07-3.62, p-value 0.030), 111 % (CI₉₅ 1.20-3.71, p-value 0.010), 107 % (CI₉₅ 0.99-4.32, p-value 0.052), -100% (CI₉₅ 0.00-0.47, p-value 0.022). Finally, the AUCs were determined together with the best cut-off values (Table 3 and supplemental Figure 1). In this way it became apparent that dynamic measures predicted the need for ECMO better than LACabs.

Static (LACabs) and dynamic lactate indices (LACTw and LACdelta) for predicting mortality

There were 14 non-survivors of whom one did not receive ECMO. This patient died 12 days after admission due to septic shock in combination with severe ventilator-induced, bronchopulmonary dysplasia. All other non-survivors died of cardiorespiratory failure

Table 1. Baseline patient characteristics of the patients with congenital diaphragmatic hernia who did not receive and those who did receive extracorporeal membrane oxygenation

	ECMO NO N = 42	ECMO YES N = 22	p-value
Male gender n (%)	24 (57)	8 (36)	0.188
Weight at admission in kilograms median (IQR)	3.0 (0.7)	2.9 (0.7)	0.036
GA in weeks median (IQR)	38.2 (1.3)	38.0 (2.3)	0.299
Side of diaphragmatic defect n (% of right-sided defects)	4 (10)	5 (23)	0.254
Liver herniation n (%) of liver up	8 (19)	10 (46)	0.040
LHR median (IQR)	2.0 (1.0)	1.7 (0.8)	0.009
GA in weeks at LHR calculation median (IQR)	32.1 (2.8)	32.5 (1.1)	0.412
1-minute-Apgar median (IQR)	6 (3)	6 (4)	0.090
5-minute-Apgar median (IQR)	8 (2)	7 (3)	0.010
Umbilical cord pH median (IQR)	7.29 (0.09)	7.27 (0.10)	0.289
Umbilical cord pCO ₂ median (IQR)	6.7 (1.9)	7.2 (2.3)	0.478
Umbilical cord BE median (IQR)	-2 (4)	-3 (6)	0.494
SNAPPE-II median (IQR)	26 (17)	56 (25)	<0.001
IC admission to ECMO start in days median (IQR)	-	3 (5)	NA
ECMO duration in days median (IQR)	-	9 (8)	NA
ECMO stop to IC discharge median (IQR)	-	23 (105)	NA
Length of IC stay in days median (IQR)	25 (25)	37 (101)	0.020
Non-survival at IC discharge n (%) of non-survivors	1 (2)	13 (59)	<0.001

Continuous data are presented as medians and interquartile range, discrete data as number and percentage. Differences were assessed using non-parametric tests. BE: base excess, ECMO: extracorporeal membrane oxygenation, GA: gestational age, IC: intensive care, iNO: inhaled nitric oxide, LHR: lung-to-head ratio, NA: not assessed, SNAPPE-II: Score for Neonatal Acute Physiology Perinatal Extension II.

due to therapy resistant pulmonary hypertension. In two cases ECMO weaning had to be forced due to intracranial hemorrhage.

LACabs at 12h and LACTw at 6h, at 12h, and at 24h were all higher in the non-survivors than in the survivors: median [IQR] LACabs: 2.1 [1.1] vs. 1.5 [0.7] mmol L⁻¹, median [IQR] LACTw 6h: 2.2 [1.3] vs. 2.6 [1.7] mmol L⁻¹ h⁻¹, median [IQR] LACTw 12h: 2.4 [0.8] vs. 1.9 [0.7] mmol L⁻¹ h⁻¹, median [IQR] LACTw 24h: 2.6 [0.9] vs. 1.7 [0.9] mmol L⁻¹ h⁻¹. LACTw at 24h was the best predictor: AUC (CI₉₅)= 0.75 (0.56-0.94), cut-off=2.5 mmol L⁻¹ h⁻¹, sensitivity (CI₉₅)= 67 (35-97), specificity (CI₉₅)= 84 (69-90), PPV (CI₉₅)= 46 (19-73), NPV (CI₉₅)= 93 (85-100).

DISCUSSION

This is the first study to evaluate the value of static and dynamic arterial lactate indices for predicting ECMO requirement in CDH patients. It shows that early derangements in

Table 2. The macrocirculatory, ventilatory, and blood gas analysis parameters at the time of the arterial lactate measurements over time in the patients with congenital diaphragmatic hernia who did not receive and those who did receive extracorporeal membrane oxygenation

	ADM			6H			12H			24H		
	ECMO NO	ECMO YES	ECMO NO	ECMO YES	ECMO NO	ECMO YES	ECMO NO	ECMO YES	ECMO NO	ECMO YES	ECMO NO	ECMO YES
HR in bpm median (IQR)	139 (32)*	159 (36)*	152 (50)	158 (44)	152 (46)	165 (42)	147 (36)	147 (36)	150 (39)	150 (39)	150 (39)	150 (39)
MABP in mm Hg median (IQR)	38 (10)	41 (11)	40 (13)	44 (6)	40 (7)	45 (10)	42 (9)	42 (9)	42 (10)	42 (10)	42 (10)	42 (10)
VP-score median (IQR)	0 (0)	0 (0)	13 (23)*	30 (48)*	15 (33)	30 (25)	24 (39)*	24 (39)*	55 (63)*	55 (63)*	55 (63)*	55 (63)*
iNO in ppm n (% of iNO recipients)	0 (0)*	0 (13)*	0 (0)*	20 (20)*	0 (0)*	15 (20)*	0 (0)*	0 (0)*	6 (20)*	6 (20)*	6 (20)*	6 (20)*
Ventilator type n (% of HFO recipients) ^{ab}	20 (50)	19 (86)	21 (55)	19 (86)	20 (49)	14 (88)	18 (44)	18 (44)	11 (85)	11 (85)	11 (85)	11 (85)
Δ-SAT in % median (IQR)	2 (9)*	6 (10)*	2 (4)	4 (17)	2 (3)	3 (6)	1 (2)	1 (2)	2 (5)	2 (5)	2 (5)	2 (5)
OI median (IQR)	5 (7)*	18 (12)*	3 (5)*	23 (27)*	4 (2)*	11 (23)*	4 (2)*	4 (2)*	7 (19)*	7 (19)*	7 (19)*	7 (19)*
pH median (IQR)	7.30 (0.20)*	7.10 (0.25)*	7.34 (0.13)*	7.29 (0.13)*	7.30 (0.08)	7.30 (0.09)	7.32 (0.10)	7.32 (0.10)	7.28 (0.10)	7.28 (0.10)	7.28 (0.10)	7.28 (0.10)
pO ₂ in mm Hg median (IQR)	200 (192)*	80 (76)*	138 (155)*	82 (107)*	125 (95)	122 (149)	109 (73)	109 (73)	87 (83)	87 (83)	87 (83)	87 (83)
pCO ₂ in mm Hg median (IQR)	47 (26)*	70 (43)*	41 (15)*	48 (20)*	43 (13)	42 (9)	44 (12)	44 (12)	48 (10)	48 (10)	48 (10)	48 (10)
BE in mmol/L median (IQR)	-6 (7)*	-12 (5)*	-4 (3)	-6 (3)	-4 (3)	-7 (4)	-5 (5)	-5 (5)	-4 (3)	-4 (3)	-4 (3)	-4 (3)

Data were collected at admission, at 6h, at 12h, and at 24h after admission. Continuous data are presented as medians and interquartile range, discrete data as number and percentage. * $p < 0.050$ for ECMO vs. non-ECMO at admission, at 6h, at 12h, and at 24h after admission using non-parametric tests. ΔADO_2 : alveolar-arterial oxygen gradient, BE: base excess, bpm: beats per minute, CMV: conventional mechanical ventilation, ECMO: extracorporeal membrane oxygenation, HFO: high frequency oscillation ventilation, HR: heart rate, iNO: inhaled nitric oxide, MABP: mean arterial blood pressure, mm Hg: millimeters of mercury, OI: oxygenation index, ppm: parts per million, VP-score: vasopressor score, Δ-sat: absolute difference between the pre-ductal and the post-ductal saturation. ^a28 (44%) of the patients participated in the VICE-trial and were randomized for ventilation by HFO (n=12) or CMV (n=16). ^b Not assessed for differences.

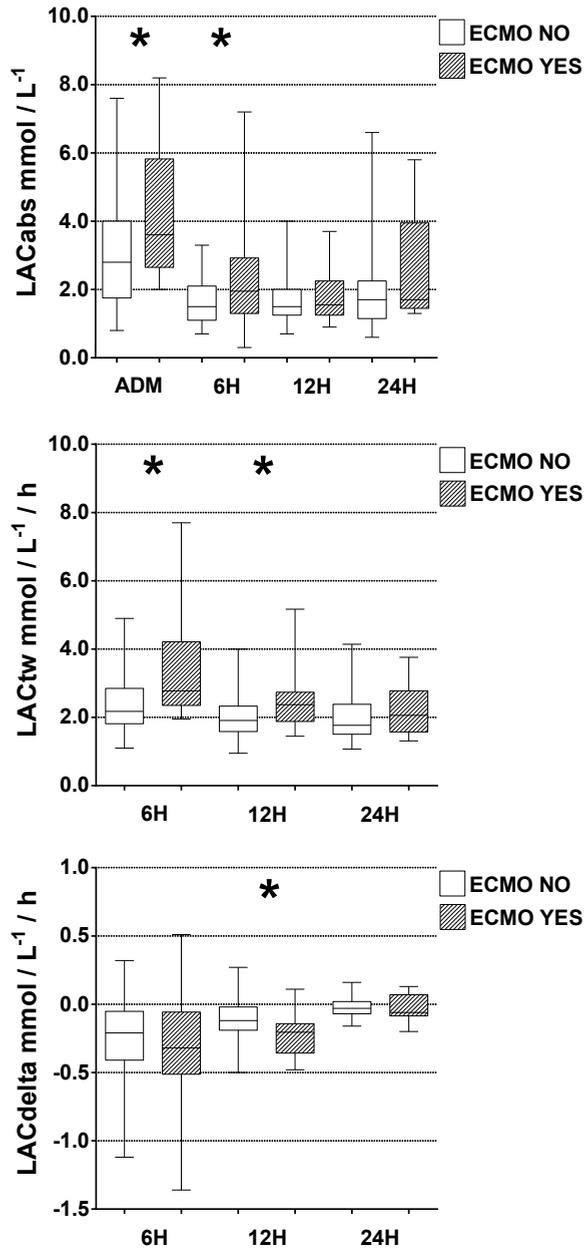


Figure 2. Box plots showing static arterial lactate (LACabs), time-weighted arterial lactate (LACTw), and lactate change over time (LACdelta) in the first 24 hours after admission in patients with congenital diaphragmatic hernia who required (obliquely striped box plots) and in those who did not require extracorporeal membrane oxygenation (blank box plots). LACabs differed at admission and at 6h after admission, LACTw differed at 6h and at 12h after admission, and LACdelta differed at 12h after admission. * p-value < 0.05 vs. ECMO NO using non-parametric tests.

LACabs, LACtw, and LACdelta are all associated with the need for ECMO and that the dynamic indices predict poor outcome more accurately than the static index.

Hyperlactatemia has been associated with poor outcome before in groups of critically ill children without CDH [11, 20]. Most studies, however, focused on mortality and used static lactate measurements. Kim et al. did focus on dynamic lactate and, in agreement with our results, showed that the lactate area –a measure for the duration of hyperlactatemia and relatively similar to LACtw– was the best predictor for mortality in pediatric patients with septic shock –mean age: 120 months, range: 1 month to 19 years children [6]. Likewise, Kalyanaraman et al. showed that the time during which lactate remained above 2 mmol/L⁻¹ was longer for pediatric non-survivors who underwent cardiopulmonary bypass for correcting congenital cardiac defects [13]. The median (range) age of participants in this study was 8 months (0-19 years). Charpie et al. (cohort median age: 6 days, range: 0-24 days) and Schumacher et al. (cohort median age: 82 days, range: 8-148 days) observed that the maximum post-surgical rate of lactate increment – which resembles the current study's LACdelta – in children with congenital cardiac defects predicts adverse outcome [12, 14]. Moreover, Rossi et al. observed retrospectively a marked decrease in mortality after implementation of lactate-guided therapy for post-cardiac surgery children (median age: 327 days) [21]. Paradoxically, LACdelta decreased more steeply from admission to 12h after admission in the non-survivors. Figure 2, however, shows that LACabs was lower in the survivors at all times, and is therefore less likely to decrease.

Moreover, the reported mean or median lactate levels in all of these studies were however markedly higher than in ours, showing that, due to amongst others pathophysiologic differences, data from non-CDH patient groups cannot be extrapolated to CDH patients. Interestingly, others have observed as well that modestly increased lactate concentration can still serve as warning signal for poor outcome: mild or relative hyperlactatemia –i.e. higher lactate concentrations within the normal reference range of 2.5 mmol /L⁻¹ – was associated with increased mortality in critically ill adults [22, 23].

CDH is characterized by pulmonary hypoplasia and pulmonary vascular abnormalities [1]. These result primarily in macrocirculatory hypoxemia and – through persistent pulmonary hypertension – in macrocirculatory failure [1]. As a consequence, tissue perfusion and tissue oxygenation are affected [24, 25]. Lactate –an end product of carbohydrate metabolism– is formed as the balance between acetyl-CoA production and lactate production shifts towards the latter during the anaerobic conditions [11]. Aerobic factors can, however, add to the lactate derangement [11]. Factors relevant in CDH patients might include: –e.g. decreased hepatic clearance tissue, inflammation-induced increments in glycolysis, heightened levels of circulating endogenous catecholamines, alkalosis, mitochondrial dysfunction, and drug infusion–e.g. epinephrine.

Table 3. The cut-off values and the value of static, absolute arterial lactate (LACabs), time-weighted arterial lactate (LACTw), and lactate change over time (LACdelta) for predicting the need for extracorporeal membrane oxygenation in patients with congenital diaphragmatic hernia within 24 hours after intensive care admission.

	AUC (CI ₉₅)	p-value	Cut-off value	Sensitivity (CI ₉₅)	Specificity (CI ₉₅)	PPV (CI ₉₅)	NPV (CI ₉₅)
LACabs adm mmol L ⁻¹	0.67 (0.54 to 0.81)	0.025	3.6	50 (28 to 72)	68 (51 to 81)	46 (26 to 67)	71 (54 to 85)
LACabs 6h mmol L ⁻¹	0.66 (0.52 to 0.81)	0.036	2.1	50 (28 to 72)	74 (59 to 87)	52 (30 to 74)	72 (55 to 85)
LACabs 12h mmol L ⁻¹	0.53 (0.36 to 0.71)	0.709	-	-	-	-	-
LACabs 24h mmol L ⁻¹	0.62 (0.45 to 0.79)	0.191	-	-	-	-	-
LACTw adm-6h mmol L ⁻¹ h ⁻¹	0.73 (0.60 to 0.86)	0.004	2.9	50 (29 to 71)	78 (64 to 91)	58 (36 to 80)	72 (57 to 86)
LACTw adm-12h mmol L ⁻¹ h ⁻¹	0.67 (0.52-0.82)	0.043	2.4	50 (35 to 65)	78 (65 to 91)	47 (23 to 71)	80 (68 to 92)
LACTw adm-24h mmol L ⁻¹ h ⁻¹	-	0.132	-	-	-	-	-
LACdelta adm-6h mmol L ⁻¹ h ⁻¹	-	0.442	-	-	-	-	-
LACdelta adm-12h mmol L ⁻¹ h ⁻¹	0.72 (0.57 to 0.87)	0.011	-0.20	56 (32 to 81)	78 (65 to 91)	50 (27 to -73)	82 (70 to 94)
LACdelta adm-24h mmol L ⁻¹ h ⁻¹	-	0.551	-	-	-	-	-

Data were collected at intensive care admission, at 6h, at 12h, and at 24h after admission. Differences were assessed using non-parametric tests. -: not determined, adm: at intensive care admission, AUC: area under the receiver operator characteristic curve, CI_{95%}: 95% confidence interval, h: hours, mmol L⁻¹: millimoles per liter, NPV: negative predictive value, PPV: positive predictive value.

Several limitations of this study should be addressed. Most importantly, a relatively large number of CDH patients were excluded. We aimed to establish an early predictor for adverse outcome and – in order to increase homogeneity – opted to exclude all postnatally-diagnosed CDH patients. As a result, the sample size for this study is modest and selection bias might have been introduced. The results should thus be interpreted with caution, although the baseline characteristics did not differ between the excluded and included patients. Moreover, the modest sample size prevented us from performing multivariate analysis that can correct for hypothetical confounders and from developing and validating a prediction model using a hold-out cohort that incorporates parameters such as LHR, gestational age, liver herniation, 5-minute-Apgar-score, and the SNAP-II score [26-29].

Yet, prospective randomized-controlled trials in critically ill adults showed that goal-directed therapy with arterial lactate as primary endpoint prevent adverse outcome [30,

31]. In the pediatric ICU such studies have not been performed. Reiss et al. have presented a European-wide consensus regarding the post-natal management of CDH patients, which improved CDH survival rates [1, 4]. This landmark report, however, also shows that goal-directed trials in the CDH population are scarce and that many therapeutic aspects are based upon expert opinion. Identifying the parameters that best select the patients at risk for clinically relevant outcomes is key in the progress towards evidence based medicine for CDH patients. Our report shows that dynamic arterial lactate indices are an early predictor for ECMO requirement. Its clinical relevance should be sought in the fact that mild lactate derangements early after birth that fail to normalize within 12h can serve as warning sign. As such this study identifies a new, early prognosticator for poor outcome in CDH patients. Also, dynamic lactate indices might now be considered as a co-variate of interest in future studies striving to develop a prediction model.

CONCLUSION

Early derangements in both the static index LACabs and the dynamic lactate indices LACtw and LACdelta are associated with ECMO requirement in CDH patients. The dynamic lactate indices predict adverse outcome more accurately than the static index.

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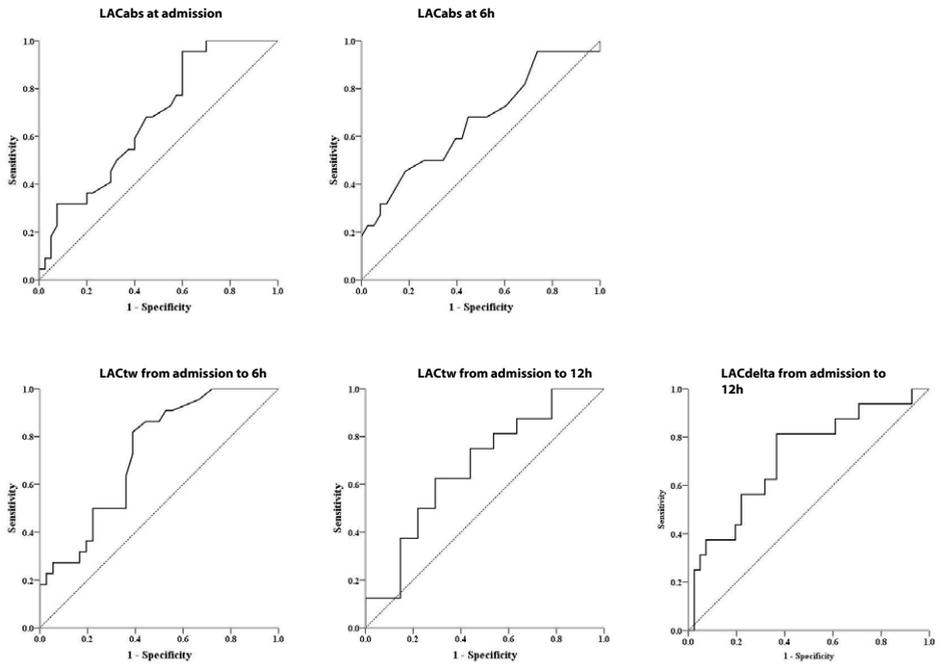
Supplemental Table 1. Overview of studies evaluating prenatal and/or perinatal parameter(s) as risk factor(s) for ECMO dependency and studies describing factors associated with ECMO dependency. The most commonly reported parameters are shown (defined as ≥ 3 reports).

Author	Size study population	LHR	FLV	Other pulmonary measures	Liver herniation	Side of diaphragmatic defect	Prenatal diagnosis	Birth weight	Gestational age	APGAR score
Tsao et al. 2010	5,022	-	-	-	-	-	-	-	Yes	-
CDHSG 1999	632	-	-	-	-	-	-	No	No	5-min APGAR: Yes
Stevens et al. 2009	628	-	-	-	-	-	-	Yes	Yes	-
Fisher et al. 2008	271	-	-	-	-	Yes	-	-	-	-
Wilson et al. 1994	173	-	-	-	-	-	No	-	-	-
Van den Hout et al. 2011	167	Yes*†	-	-	Yes	No	-	No	No	-
Lewis et al. 1997	136	-	-	-	-	-	No	-	-	-
Odibo et al. 2010	107	Yes* Not	-	-	No	No	-	-	Yes	-
Lazar et al. 2011	106	-	-	-	-	-	Yes	-	-	-
Schaible et al. 2011	106	-	Yes*	-	-	Yes	-	-	-	-
Busing et al. 2008	95	-	Yes*	-	-	No	-	-	No	-
Schaible et al. 2011	90	Yes†	Yes†	-	Yes	No	-	No	Yes	-
Kilian et al. 2009	90	Yes*	Yes*	-	No	No	-	-	-	-
Hedrick et al. 2007	89	Yes*	-	-	Yes	-	-	-	-	-
Busing et al. 2008	68	-	Yes*	-	-	No	-	-	No	-
Neff et al. 2007	65	-	Yes*	-	-	No	-	-	No	-
Metkus et al. 1996	55	No*	-	-	No	-	-	-	-	-
Albanese et al. 1998	48	-	-	-	Yes	-	-	-	-	-
Steinhorn et al. 1994	48	-	-	-	-	-	No	-	-	-
Lee et al. 2011	44	-	Yes*	-	-	-	-	-	-	-
Arkovitz et al. 2007	42	No*	-	-	No	-	-	-	-	-

Supplemental Table 1. (Continued)

Author	Size study population	LHR	FLV	Other pulmonary measures	Liver herniation	Side of diaphragmatic defect	Prenatal diagnosis	Birth weight	Gestational age	APGAR score
Kilian et al. 2009	36	-	Yes*	-	No	No	-	No	No	-
Lipshutz et al. 1997	31	Yes*	-	-	-	-	-	-	-	-
Shehata et al. 2000	30	-	-	-	-	-	-	No	No	-
Redmond et al. 1987	30	-	-	-	-	-	-	No	No	1-min APGAR : No 5-min APGAR: No
Green et al. 1995	28	-	-	-	-	-	-	No	No	1-min APGAR : Yes 5-min APGAR: No
Kitano et al. 2005	26	-	-	-	Yes	-	-	-	-	-
Tsukimori et al. 2008	25	No*	-	Lung thorax transverse area ratio: Yes	No	-	-	-	-	-
Sbragia et al. 2000	20	No*	-	-	-	-	-	-	-	-
Hayakawa et al. 2007	21	-	Yes†	-	-	-	-	-	-	-
Bamewole et al. 2007	14	-	-	Percentage of predicted lung volume: Yes	-	-	-	-	-	-
Kamata et al. 1992	14	-	-	Lung thorax transverse area ratio: Yes	-	-	-	-	-	-

- : not evaluated as risk factor, no: no difference observed between ECMO and non-ECMO patients and therefore discarded as potential risk factor, yes: difference observed between ECMO and non-ECMO patients and thus potentially suited as risk factor. * absolute LHR or TLV, † observed/expected LHR or FLV, LHR: lung-to-head ratio, FLV: fetal lung volume.



Supplemental figure 1. The receiver operator characteristic curves for LACabs at admission, LACabs at 6h, LACTw from admission to 6h, LACTw from admission to 12h, and LACdelta from admission to 12h for the patients with congenital diaphragmatic hernia who required and for those who did not require extracorporeal membrane oxygenation.

Chapter 9

Arterial lactate for predicting mortality in children requiring extracorporeal membrane oxygenation

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ABSTRACT

BACKGROUND: Dynamic arterial lactate indices predict mortality more accurately than static arterial lactate measurements in children with septic shock or congenital cardiac defects. The current study evaluates whether this also applies to children with primary respiratory disease requiring extracorporeal membrane oxygenation (ECMO).

METHODS: Static arterial lactate levels (LACabs) were prospectively collected before and during ECMO support for this single center, observational cohort study. Also, time-weighted arterial lactate (LACtw) and lactate change over time (LACdelta) were calculated as dynamic indices for, respectively, the duration and the trend over time of lactate derangement. Intensive Care mortality was the primary endpoint. Analyses were performed for neonatal and pediatric patients separately.

RESULTS: Fifty-six neonatal and 39 pediatric patients were included. Eighteen (32%) neonatal and 12 (31%) pediatric patients died. The evolution of LACabs and LACdelta differed between the pediatric survivors and the pediatric non-survivors ($p < 0.001$, $p = 0.025$). The hazard ratio was 1.23 ($CI_{95} = 1.06-1.43$, $p = 0.007$) for LACabs and 20.64 ($CI_{95} = 1.99-214.20$, $p = 0.011$) for LACdelta, indicating that higher lactate levels increase the risk for mortality. The predictive value for LACabs was 0.75 ($CI_{95} = 0.57-0.93$) and for LACdelta 0.69 ($CI_{95} = 0.51-0.87$), respectively. There were neither consistent differences for LACtw in the pediatric patients, nor for any of the static or dynamic lactate indices in the neonatal patients.

CONCLUSION: Static arterial lactate measurements and, to a lesser extent, dynamic arterial lactate indices predict mortality in pediatric, but not neonatal ECMO patients. The magnitude and trend over time rather than the duration of lactate derangement are associated with mortality.

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) can serve as rescue treatment for children with therapy-resistant, primary respiratory failure [1]. From the year 2000 to 2012, over 10,750 neonatal and 3,800 pediatric patients received ECMO worldwide [2]. Mortality rate is high and differs between neonatal (32%) and pediatric patients (44%) despite ECMO support [2].

Lactate –an end product of carbohydrate metabolism– is constantly produced during glycolysis and, thereafter, metabolized [3]. Unlike pH, pO₂, or pCO₂, the lactate concentration can increase during both anaerobic and aerobic conditions. The latter include: a) liver dysfunction resulting in reduced lactate clearance; b) enhanced glycolysis –e.g. in cytokines or due to hyperglycemia; c) increased catecholamine levels affect cellular glucose uptake; d) alkalosis causing increased cellular efflux of lactate; e) mitochondrial dysfunction; and f) drug infusion / intoxication –e.g. epinephrine, nucleosidic reverse transcriptase inhibitors, methanol–[3, 4]. Lactate concentration correlates to both severity of illness and mortality [3-5]. However, lactate concentration can also be false negatively low –e.g. sepsis-induced decrease of peripheral perfusion or necrosis-induced absence of carbohydrate metabolism– [3]. Also, age-related differences in lactate production and lactate metabolism have been described between adults and children and between newborns and older children[6-11]. Therefore, the value of lactate for predicting mortality might dependent on age, disease type, co-morbidity, and disease severity or –in other words– time [5]. It was demonstrated recently that dynamic measures of lactate derangement –i.e. incorporating duration or trend over time next to magnitude– are better predictors for survival than static lactate measurements in septic children and children with congenital cardiac defects [12-15].

Neonatal and pediatric ECMO candidates are amongst the most critically ill children conceivable. Static lactate measurements are established predictors for mortality in ECMO children with primary cardiac disease [16-20]. The few studies that focused on children with primary respiratory failure included both neonatal and pediatric patients, still included some children with primary cardiac disease, recruited patient cohorts from before the year 1997, and included patients suffering from prolonged hypoxia[21-24]. Today, however, ECMO is generally started early in order to prevent ventilator-induced lung injury whilst the ECMO population is has more co-morbidity, amongst other differences [25]. Moreover, none of the reports described dynamic lactate indices[21-24]. . Therefore, this study aimed to evaluate the predictive value of both static and dynamic arterial lactate indices obtained before and during ECMO in a general population of children with primary respiratory disease. In line with the registry maintained by the Extracorporeal Life Support Organization and the majority of scientific literature [26], and given that lactate kinetics, patient characteristics, and the crude mortality rate differ between newborns and older ECMO children, data will be presented for neonatal and pediatric patients separately.

MATERIALS AND METHODS

Study design and Setting:

This observational cohort study entails data collected prospectively in patients admitted to the intensive care (IC) of a level III university children's hospital. The local medical ethical review board approved the study and waived the need for informed consent.

Patients:

Consecutive neonatal –age at admission below 28 days– and pediatric patients –age at admission 29 days to 18 years– with primary respiratory disease receiving ECMO between 2008 and 2011 were included. This inclusion period was chosen for two reasons: a) in the end of 2007 a new protocol was implemented for CDH patients –who form the majority of the patients in the neonatal ECMO group; and b) in 2011 our department switched to another ECMO system [27]. Both factors were anticipated to lower mortality rate, which is the primary endpoint of the current study [28]. Patients with primary cardiac disease and patients with less than two lactate measurements were excluded. Only the first ECMO run was included in case patients (n=3) received multiple ECMO runs.

Data collection:

The primary endpoint was IC mortality, the primary study parameters were static arterial lactate (LACabs) and the dynamic arterial lactate indices time-weighted arterial lactate (LACtw) and lactate change over time (LACdelta). LACabs was included as it is used in most of the previous studies. The rationale for including dynamic lactate indices is threefold: 1) dynamic indices describe duration and trend over time next to magnitude and can account disease-severity-induced adjustments over time; 2) results are promising in non-ECMO children with septic shock or cardiac defects [12-15]; 3) a study in post-cardiac surgery children showed that mortality lowered after introducing lactate-guided therapy[29]. LACabs levels were determined using an ABL-800 flex blood gas analyzer (Radiometer Medical Aps, Copenhagen, Denmark) and stored unit's electronic system. From this system we retrieved all LACabs measurements before and during ECMO support. Thereafter LACtw and LACdelta were calculated [30]. LACtw –incorporating magnitude and duration of lactate derangement– was determined by summing the mean value of LACabs between consecutive time points multiplied by the time period in between and then dividing by the total time [30]. For LACdelta –incorporating magnitude and trend over time– LASabs values were regressed against time for each individual patient, with the regression slope representing the projected change of consecutive measurements over time [30].

We also obtained patient demographics and disease severity indices –i.e. pediatric risk of mortality II (PRISM II), pediatric index of mortality II (PIM II), pediatric logistic organ dysfunction (PELOD), oxygenation index (OI), and the level of vasopressor support (VP-score) [31-35]. Co-morbidity was registered using definitions described earlier [25, 36].

Renal failure was assessed only prior to cannulation because after cannulation all patients received hemofiltration. Pulmonary hypertension was assumed present in case of inhaled nitric oxide therapy combined with either echocardiographic reporting or with consistent pre-ductal to post-ductal saturation differences greater than 20%. The mode of ECMO support was scored as either venoarterial ECMO (VA-ECMO) or venovenous ECMO (VV-ECMO). If the ECMO mode was converted, the ECMO mode with the longest duration was scored.

Hospital treatment protocol:

After initial stabilization and IC admission, patients were treated according to institutional policy. Respiratory and circulatory management have been described previously, as have the ECMO inclusion and exclusion criteria, the sedative and analgesic management, the cannulation procedure, and the ECMO weaning procedure [37, 38]. In short, the ECMO criteria were: prolonged $OI > 25$ or cardiorespiratory failure for more than three hours with $pH < 7.15$ and $PaO_2 < 5.3$ kPa. VV-ECMO was preferred for patients with isolated primary respiratory failure –i.e., good myocardial function as assessed by cardiac echo and no severe circulatory failure as assessed by conventional hemodynamic parameters. VA-ECMO was preferred in patients with congenital diaphragmatic hernia (CDH) or isolated septic shock, and in patients with primary respiratory failure that was accompanied by poor myocardial function and/or circulatory failure. Both the timing of ECMO and type of ECMO modality were decided by the attending intensivist. The ECMO membrane and tubing were supplied by Medtronic (Medtronic Inc., Minneapolis, MN, USA); the ECMO roller pumps were provided by Stöckert Instrumente GmbH (Stöckert Instrumente GmbH, Munchen, Germany). The normal range for arterial lactate was: 0.5 to 2.0 mmol/L.

Statistical analysis:

Data are presented and analyzed separately for neonatal and pediatric patients (see introduction). LACabs, LACtw, and LACdelta are expressed as means with 95%-confidence interval (CI_{95}). Statistical analysis was done in three steps. Firstly, to assess evolutionary differences, a repeated measurements analysis was performed using linear mixed effects models thereby accounting for correlating measurements within each patient. For the linear mixed effects model specification, we used regression splines –i.e. natural cubic splines– for both the fixed and the random-effects parts to account for potential non-linearity. The models' assumptions were validated using residuals plots. Secondly, as a summary measure of the static and dynamic lactate indices, we calculated per patient the area under the longitudinal curve corrected for days of follow-up. Using Cox proportional hazards modeling, these were subsequently used to determine the hazard ratios (HR) with CI_{95} and the concordance index (concordindex) with CI_{95} , the latter representing the predictive value. Finally, the area (AUC) under ROC curve was calculated together with the best cut-off value and its sensitivity, specificity, positive predictive

value, and negative predictive value in case relevant. All other data are described as medians (IQR). The correlation between vasopressor score and the respective lactate indices was calculated using the spearman rank correlation coefficient. Descriptive statistics and non-parametric inferential testing were done using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The advanced statistical testing for the lactate indices was done using R2.15.2. A p-value below 0.050 was considered statistically significant.

RESULTS

We enrolled 56 neonatal and 39 pediatric patients. Seventeen patients with primary cardiac disease were excluded (Figure 1). Of those included, 18 (32%) neonatal and 12 (31%) pediatric patients died. Table 1 shows the baseline and ECMO-related patient characteristics.

VA-ECMO was used more often in the neonatal non-survivors than in the neonatal survivors while fewer non-survivors were transferred from another hospital to the study site. The time between admission and ECMO start did not differ. Vasopressor support at admission was lower in the non-survivors. The other disease indices differed neither before nor during ECMO. The non-survivors received ECMO support longer than the survivors. In one survivor, the ECMO mode was converted from VV-ECMO to VA-ECMO because the maximum VV-ECMO blood flow was insufficient to restore cardiorespiratory

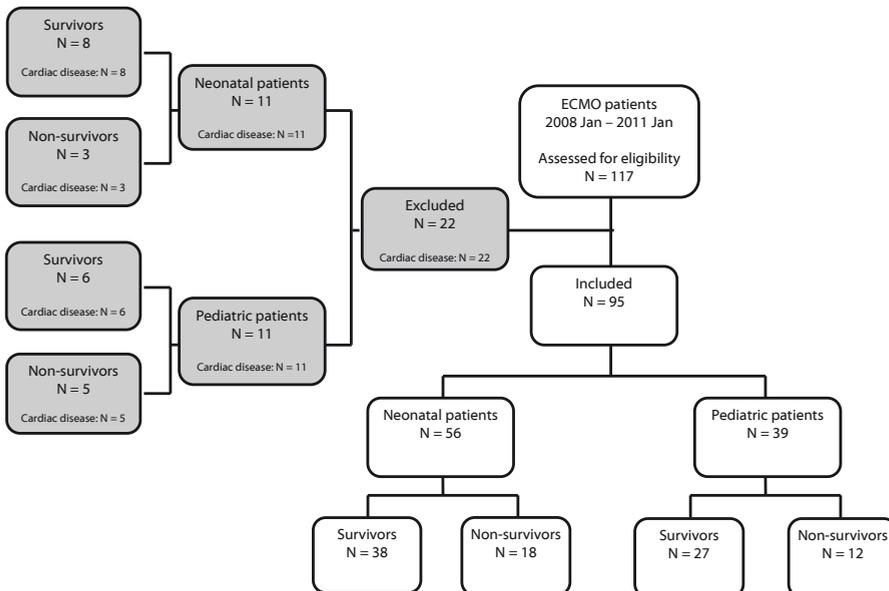


Figure 1. Flowchart for the patients with primary respiratory disease requiring extracorporeal membrane oxygenation who were assessed for inclusion in the study.

Table 1. The baseline patient characteristics for the neonatal and the pediatric extracorporeal membrane oxygenation patients who did survive and those who did not survive

	Neonatal ECMO patients			Pediatric ECMO patients		
	Survivors n = 38	Non-survivors n = 18	p-value	Survivors n = 27	Non-survivors n = 12	p-value
Male gender n (%)	21 (55)	6 (33)	NA	19 (70)	7 (58)	NA
Age at admission in days Median (IQR)	1 (1 to 1)	1 (0 to 1)	0.120	-	-	NA
Age at admission in months Median (IQR)	-	-	NA	19 (5 to 64)	94 (43 to 164)	0.028
Weight at admission in kilograms Median (IQR)	3.5 (3.0 to 4.1)	3.0 (2.2 to 3.3)	0.019	11.0 (4.2 to 17.0)	24.7 (15.0 to 51.5)	0.008
Transferred from another hospital N (%)	25 (66%)	4 (22%)	0.004	27 (100)	12 (100)	NA
Length of IC stay in days Median (IQR)	21 (8 to 79)	26 (15 to 40)	0.875	19 (11 to 24)	19 (5 to 34)	0.692
PRISM II Median (IQR)	28 (22 to 33)	25 (19 to 29)	0.147	22 (15 to 27)	21 (10 to 30)	0.776
PIM II Median (IQR)	-1.7 (-2.6 to -1.3)	-1.7 (-2.3 to -1.1)	0.757	-2.8 (-3.4 to -2.2)	-2.6 (-4.1 to -1.6)	0.776
ECMO mode N (% VA)	25 (66)	17 (94)	0.023	13 (48)	9 (75)	0.168
Conversions N (% conversions VV to VA)	1 (3)	0 (0)	1.000	4 (15)	2 (17)	1.000
IC admission to ECMO start in days Median (IQR)	1 (0 to 2)	3 (0 to 9)	0.114	1 (0 to 2)	1 (0 to 7)	0.819
Length of ECMO support in days N (%)	4 (3 to 7)	11 (6 to 15)	0.000	8 (4 to 9)	10 (3 to 21)	0.260
ECMO stop to discharge / death in days Median (IQR)	15 (4 to 58)	9 (1 to 19)	0.060	7 (2 to 18)	0 (0 to 3)	0.000
OI at admission Median (IQR)	23 (12 to 42)	18 (11 to 24)	0.368	28 (8 to 37)	18 (5 to 35)	0.548
OI after cannulation Median (IQR)	7 (4 to 14)	7 (4 to 11)	0.744	16 (9 to 29)	10 (5 to 23)	0.207
VP-score at admission Median (IQR)	6 (0 to 35)	0 (0 to 3)	0.019	0 (0 to 6)	0 (0 to 0)	0.034
VP-score after cannulation Median (IQR)	20 (4 to 47)	30 (18 to 62)	0.116	0 (0 to 17)	11 (0 to 44)	0.169
PELOD at admission Median (IQR)	21 (20 to 31)	30 (21 to 31)	0.254	22 (17 to 37)	21 (21 to 50)	0.971
PELOD after cannulation Median (IQR)	21 (13 to 22)	20 (12 to 22)	0.754	21 (17 to 31)	30 (21 to 42)	0.185

Table 1. (continued)

	Neonatal ECMO patients		p-value	Pediatric ECMO patients		p-value
	Survivors n = 38	Non-survivors n = 18		Survivors n = 27	Non-survivors n = 12	
Primary COD n (%)						
- Irreversible respiratory disease	-	14 (78)	-	5 (42)		
- Septic shock	-	2 (11)	NA	2 (17)		NA
- Irreversible neurological damage	-	1 (6)		3 (25)		
- Irreversible cardiac disease	-	1 (6)		2 (17)		
Diagnosis at the time of cannulation n (%)						
- MAS	13 (30)	1 (6)		0 (0)	0 (0)	
- CDH	10 (23)	13 (72)		0 (0)	0 (0)	
- Idiopathic PH	5 (12)	0 (0)		1 (4)	1 (8)	
- Respiratory disease infectious	1 (2)	0 (0)	NA	17 (63)	3 (25)	NA
- Respiratory disease non- infectious	4 (9)	2 (11)		6 (22)	3 (25)	
- Septic shock	5 (12)	2 (11)		3 (11)	5 (42)	
Comorbidity n (%)						
- Pulmonary hypertension	35 (92)	16 (89)		9 (33)	3 (25)	
- Neurologic disease	4 (11)	3 (17)		4 (15)	6 (50)	
- Renal failure	6 (16)	3 (17)		7 (26)	3 (27)	
- Cardiac disease	1 (3)	2 (11)		6 (22)	3 (25)	
- Cardiac arrest (yes/no)	8 (21)	3 (17)		9 (33)	7 (58)	
- Hemorrhagic / coagulation disorder	22 (58)	16 (89)	NA	5 (19)	4 (33)	NA
- Liver failure	4 (11)	2 (11)		9 (33)	9 (75)	
- Malignancy	0 (0)	0 (0)		0 (0)	0 (0)	
- Organ transplantation	0 (0)	0 (0)		1 (4)	0 (0)	
- Primary immunodeficiency	0 (0)	1 (6)		0 (0)	0 (0)	

Continuous data are presented as medians and interquartile range, discrete data as number and percentage. Differences vs. survivors at $p < 0.05$ using non-parametric tests. CDH: congenital diaphragmatic hernia, COD: cause of death, ECMO: extracorporeal membrane oxygenation, IC: intensive care, MAS: meconium aspiration syndrome, NA: differences not assessed, OI: oxygenation index, PELOD: pediatric logistic organ dysfunction, PH: pulmonary hypertension, PIM II: absolute pediatric index of mortality II, PRISM II: absolute pediatric risk of mortality II, VA: venoarterial extracorporeal membrane oxygenation, VP-score: vasopressor score, VV: venovenous extracorporeal membrane oxygenation.

parameters. Eighteen (32%) neonates died. In four patient ECMO could not be weaned due to: irreversible primary pathology (n=2; alveolar capillary dysplasia and persistent pulmonary hypertension [PPH]), hemorrhagic complication, thromboembolic complication. The 14 other non-survivors were successfully weaned off of ECMO, but died prior to IC discharge –median (IQR) time after ECMO stop: 17 (3-21) days–. Here the causes of death were PPH in CDH (n=11), septic shock (n=2), and chronic pneumonitis of infancy.

In the pediatric patients, VA-ECMO was started as often in survivors as in non-survivors. In five pediatric patients (four survivors) the ECMO mode was converted from VV-ECMO to VA-ECMO while in one pediatric patient VA-ECMO was switched to VV-ECMO. Before or during ECMO there were no differences in any of the disease severity indices, apart from a clinically irrelevant difference in vasopressor support at admission. Neither the time between admission and ECMO start, nor the duration of ECMO support differed between the survivors and the non-survivors. In nine (75%) non-survivors, ECMO could not be weaned. The causes of death included: pulmonary consolidation (n=2), PPH, septic shock, cerebral haemorrhage, ischaemic-hypoxic encephalopathy, cardiac tamponade, Waterhouse-Friderichsen syndrome, and auto-immune-induced interstitial lung disease. Three pediatric non-survivors were successfully weaned, but died before IC discharge –median (IQR) time after ECMO stop: 7 (6-11) days. The causes of death were septic shock, idiopathic PPH, and ischaemic-hypoxic encephalopathy.

Arterial lactate before & during ECMO

Neonatal patients

Fifty-six neonates were included in whom in total 3,430 arterial lactate measurements were performed (median [IQR] number per patient: 37 [17-97]). The number of lactate measurements on day 1 and day 2 of IC admission was higher in the non-survivors than in the survivors: median (IQR) number= 15 (5-18) vs. 7 (4-13). For the other days, there were no differences.

In the neonatal patients, the mean (CI95) LACabs, LACTw, and LACdelta before and during ECMO in relation to outcome are presented in Table 2. The evolution of LACabs, LACTw, and LACdelta from admission to ECMO stop is shown in Figure 2. Linear mixed effects modeling showed that, based upon the likelihood ratio, the evolution of LACabs did not differ between the survivors and the non-survivors (Table 3). Cox regression modeling showed that the hazard ratio of LACabs was not significant and that its predictive value, estimated by the concordance index, was poor (Table 3). The evolution of the dynamic lactate index LACTw in the non-survivors differed from that of the survivors. However, the hazard ratio of LACTw was not significant and its predictive value was poor. LACdelta's hazard ratio was statically different, but the predictive value was poor.

The supplemental Table 1 shows the vasopressor score in relation to LACabs, LACTw, and LACdelta for the neonatal (and the pediatric) VA-ECMO and VV-ECMO patients.

Pediatric patients

Thirty-nine pediatric were included in whom in total 3,045 arterial lactate measurements were performed (median [IQR] number per patient: 57 [29-87]). In the pediatric non-survivors, the number of lactate measurements was higher only on day 2 of IC admission when compared to the survivors: median (IQR) number=6 (9-13) vs. 4 (6-8).

For the pediatric ECMO patients, mean (CI95) LACabs, LACTw, and LACdelta before and during ECMO are presented in Table 2 and the evolution is presented in Figure 3.

Table 2. The levels of static, absolute arterial lactate (LACabs), time-weighted arterial lactate (LACTw), and lactate change over time (LACdelta) for the neonatal and the pediatric extracorporeal membrane oxygenation patients who did survive and those who did not survive

		Neonatal patients		Pediatric patients	
		Survivors n = 38	Non-survivors n = 18	Survivors n = 38	Non-survivors n = 18
Lactate levels total follow-up	LACabs in mmol L ⁻¹	1.9 (1.8 to 2.0)	1.8 (1.8 to 1.9)	2.0 (2.0 to 2.1)	2.5 (2.4 to 2.6)
	LACTw in mmol L ⁻¹	2.3 (2.2 to 2.4)	1.9 (1.9 to 2.0)	2.2 (2.1 to 2.3)	2.9 (2.8 to 3.0)
	LACdelta in mmol L ⁻¹	-0.02 (-0.06 to 0.03)	-0.04 (-0.16 to 0.07)	-0.02 (-0.06 to 0.01)	0.06 (-0.02 to 0.15)
Lactate levels pre-ECMO start	LACabs in mmol L ⁻¹	2.4 (2.2 to 2.6)	2.2 (2.1 to 2.4)	2.1 (1.7 to 2.5)	2.8 (2.4 to 3.2)
	LACTw in mmol L ⁻¹	2.4 (2.2 to 2.6)	2.2 (2.2 to 2.3)	2.1 (1.7 to 2.4)	2.5 (2.2 to 2.9)
	LACdelta in mmol L ⁻¹	0.02 (-0.08 to 0.12)	0.07 (-0.02 to 0.15)	-0.03 (-0.19 to 0.13)	0.19 (-0.08 to 0.45)
Lactate levels post-ECMO start	LACabs in mmol L ⁻¹	1.7 (1.6 to 1.8)	1.6 (1.5 to 1.7)	2.0 (1.9 to 2.1)	2.4 (2.3 to 2.6)
	LACTw in mmol L ⁻¹	2.2 (2.1 to 2.3)	1.8 (1.8 to 1.8)	2.2 (2.1 to 2.3)	3.0 (2.8 to 3.1)
	LACdelta in mmol L ⁻¹	-0.03 (-0.08 to 0.02)	-0.09 (-0.26 to 0.07)	-0.02 (-0.04 to 0.00)	0.03 (-0.05 to 0.12)

Data are presented in mean (CI_{95%}) for the total study period, for the measurements before the start of extracorporeal membrane oxygenation, and for the measurements during the course of extracorporeal membrane oxygenation support. Inter-group differences were not assessed. Mmol L⁻¹: millimoles per liter.

Table 3. The evolution (expressed by the likelihood ratio), the hazard (expressed by the hazard ratio), and the predictive value (expressed by the concordance index) of static, absolute arterial lactate level (LACabs), the dynamic measure time-weighted arterial lactate (LACTw), and the dynamic measure lactate change over time (LACdelta) in the neonatal and the pediatric patients requiring extracorporeal membrane oxygenation in relation to survival.

		Longitudinal evolution		Hazard		Predictive value	
		Likelihood ratio	p-value	Hazard ratio	CI _{95%}	p-value	Concordance index
Neonatal patients	LACabs	6.78	0.237	0.94	0.66 to 1.34	0.716	0.47
	LACTw	12.24	0.032	0.85	0.59 to 1.22	0.381	0.46
	LACdelta	2.52	0.77	0.73	0.56 to 0.97	0.028	0.39
Pediatric patients	LACabs	30.24	< 0.001	1.23	1.06 to 1.43	0.007	0.75
	LACTw	6.54	0.257	1.13	1.00 to 1.27	0.056	0.63
	LACdelta	11.11	0.025	20.64	1.99 to 214.20	0.011	0.69

The evolution of LACabs and LACdelta differed significantly between the survivors and the non-survivors (Table 3). In the pediatric non-survivors, LACabs was constantly higher before and during ECMO. Its hazard ratio was 1.23 (CI95= 1.06-1.43, $p= 0.007$) and the predictive value was good (concordance= 0.75, CI95= 0.57-0.93). The overall hazard ratio

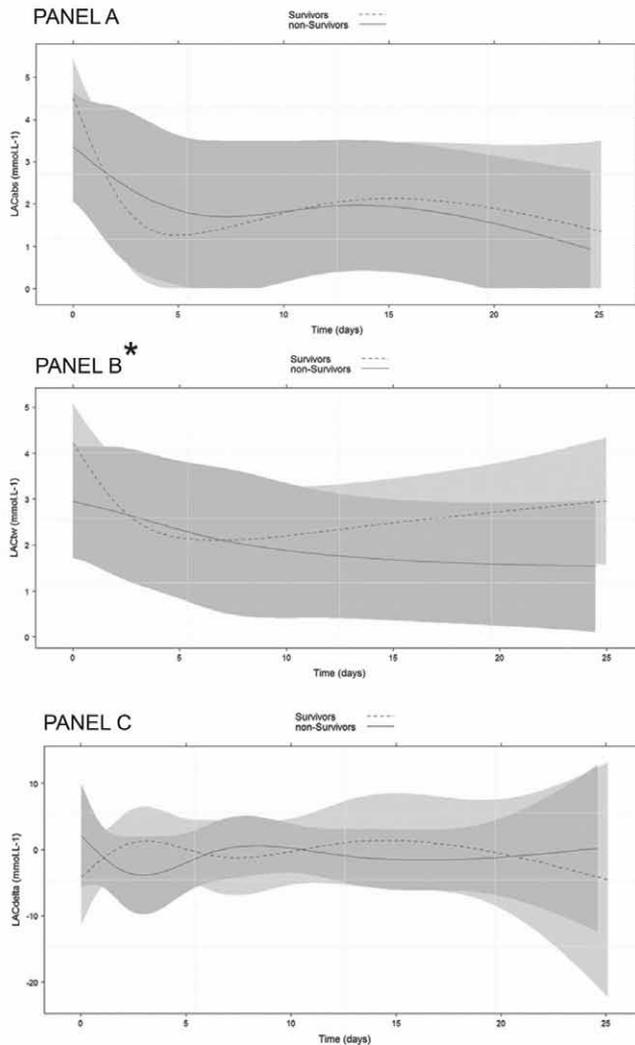


Figure 2. The evolution of static, absolute arterial lactate level (LACabs; panel A), time-weighted arterial lactate (LACTw; panel B), and lactate change over time (LACdelta; panel C) in relation to mortality in neonates with primary respiratory disease requiring extracorporeal membrane oxygenation. LACTw differed between the neonatal survivors and the neonatal non-survivors, in contrast to LACabs and LACdelta. Data are presented as mean (lines) with CI_{95} (gray areas), * $p < 0.050$ vs. survivors using mixed effects models.

of LACdelta was 20.64 (CI95= 1.99-214.20, $p= 0.011$) and the predictive value was 0.69 (CI95= 0.51-0.87). The hazard ratio of LACTw failed to reach statistical significance. Its predictive value was 0.63 (CI95= 0.47-0.79).

Figure 3 shows that the most prominent differences for LACabs and LACdelta occurred from IC admission up to day 4. These LACabs and LACdelta measurements were used subsequently to determine the AUC of the ROC together with the best cut-off value in case relevant. For LACabs, the AUC was 0.73 (p -value <0.001) and the best cut-off value

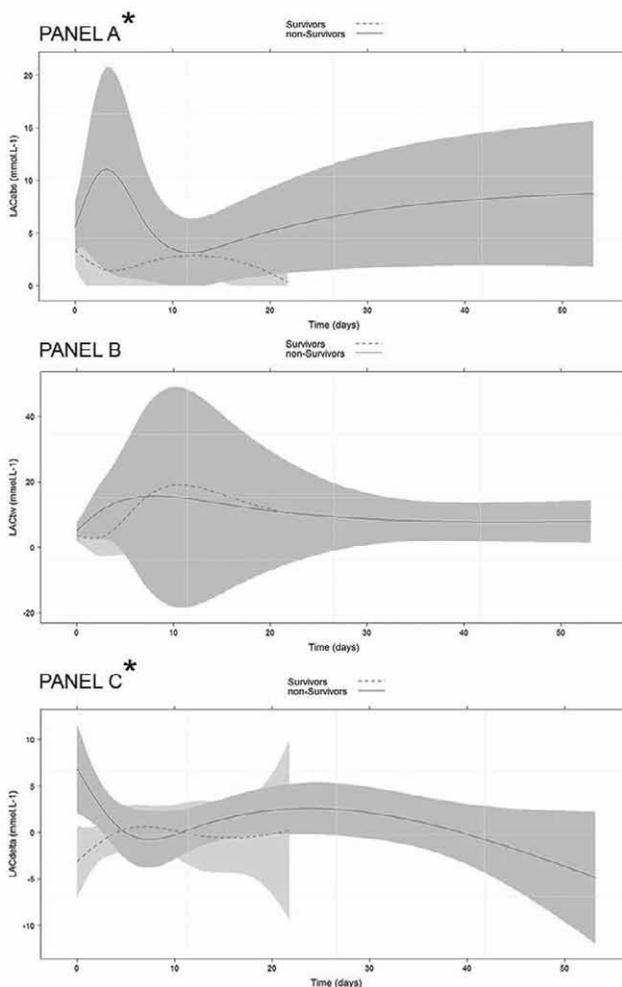


Figure 3. The evolution of static, absolute arterial lactate level (LACabs; panel A), time-weighted arterial lactate (LACTw; panel B), and lactate change over time (LACdelta; panel C) in relation to mortality in pediatric patients with primary respiratory disease requiring extracorporeal membrane oxygenation. Both LACabs and LACdelta, but not LACTw, differed between the pediatric survivors and the pediatric non-survivors. Data are presented as mean (lines) with CI_{95} (gray areas), * $p < 0.050$ vs. survivors using mixed effects models.

was 2.5 mmol/L –sensitivity: 62% (CI95:57-67), specificity: 75% (CI95:72-79), positive predictive value: 58% (CI95:53-63), and negative predictive value: 79% (CI95:75-82). For LACdelta the AUC failed to differ significantly. Hence, a cut-off value was not determined.

DISCUSSION

Our study shows that neither LACabs nor LACdelta or LACTw predicted mortality in neonatal ECMO patients. In contrast, LACabs was a good predictor in the pediatric ECMO patients. Its hazard ratio indicated that for every one unit increase, the risk for non-survival increased by 23%. Moreover, albeit less predictive than LACabs, LACdelta's hazard ratio was very high whilst LACTw did not differ significantly. So, not the duration of lactate derangement, but the magnitude and trend over time of lactate derangement are in particular associated with mortality in pediatric ECMO patients with respiratory disease.

Multiple studies have reported that static hyperlactatemia is associated with higher mortality in ECMO patients with primary cardiac disease [16-20]. For children with primary respiratory disease, there is one study that focused on a cohort of both neonatal and pediatric patients and three studies that focused on neonates exclusively [21-24]. The neonatal studies showed that, in contrast to our results, higher static lactate was associated with higher mortality [22-24]. The reported mean or median lactate levels were, however, markedly higher than ours, as was the oxygenation index and VP-score. Catecholaminergic support in itself can increase arterial lactate levels, as has been reported previously by others and as can be deduced from some of our results that are presented in the Supplemental Table 1 [3, 4]. Therefore, the discrepancy in results is most likely attributable to differences in treatment, disease severity, and, possibly, to differences in ECMO population characteristics.

The question as to why arterial lactate is associated with poor outcome in pediatric patients, but not in neonatal patients, is intriguing. In contrast to the neonatal non-survivors, relatively many pediatric non-survivors were diagnosed septic shock. Sepsis is a microcirculatory disease and lactate is more likely to increase. Moreover, when compared to the neonates, more pediatric patients were referred to our center and more pediatric non-survivors had co-morbidity –most notably liver dysfunction. . Additionally, less pediatric patients suffered pulmonary hypertension while a higher proportion of neonatal survivors was treated with VV-ECMO. Right-to-left shunting through persistent fetal pathways and mixing of deoxygenated and oxygenated venous blood in the case of VV-ECMO might increase the amount of “venous” blood in the arterial circulation. Given that venous blood is associated with higher lactate than arterial blood, both phenomena could act as confounders in the neonatal population. Thus, differences in disease type, co-morbidity, timing of treatment, and type of treatment might explain why

lactate predicts outcome only in the pediatric patients [5]. The observed differences are unlikely to be of procedural nature as the measurement error of the blood gas analyzers was small, the ECMO entry criteria remained unaltered during the study, the cannulation procedure was standardized, and the primed ECMO circuit was checked and adjusted to normal values pre-cannulation. Furthermore, the number of conversions did not differ and there were no indications that ECMO support was more often insufficient in the pediatric non-survivors.

This is the first study to focus on the predictive value of static and dynamic lactate measures in pediatric ECMO patients with primary respiratory disease. Lactate derangement has been associated with mortality in various groups of pediatric, critically ill non-ECMO patients [3, 13, 39]. Particularly interesting is the report by Rossi et al. that describes a marked decrease in mortality in post-cardiac surgery children after implementation of lactate-guided therapy [29]. Others observed that dynamic lactate indices predicted mortality better in critically ill, pediatric non-ECMO patients [12, 13]. We observed the reverse: static lactate measurements are a better predictor than dynamic indices. In our study, the arterial lactate levels were relatively low upon admission and after three or more days of ECMO support. Likewise, the oxygenation index and the VP-score were relatively low. Furthermore, all pediatric patients and approximately 50% of the neonatal patients were referred to our center. Therefore, our data are most likely attributable to the early referral of patients to our center, which is regarded good practice in our country. Moreover, estimating LACdelta and LACTw in only the first few days before and after ECMO support will result in other, probably more convincing, differences and in higher predictive value.

Interestingly, relative hyperlactatemia –i.e. higher lactate concentrations within the normal reference range– is associated with increased mortality in critically ill adults [40, 41]. While relative hyperlactatemia is not truly applicable to our study and the topic is beyond our scope, it might be interesting for future researchers to investigate whether relative hyperlactatemia is also clinically relevant in critically ill children.

The most important limitation of the current study is the modest number of included patients. This limited the possibility to correct for hypothetical confounders such as disease type at admission, co-morbidity, level of catecholaminergic support, ECMO mode, and age during statistical analysis. Results should thus be interpreted with caution. Also, we did not perform a power calculation prior to the start of the study. A reliable power calculation was deemed impossible because there are no data available on dynamic lactate indices in children receiving any form of extracorporeal support. However, statistically significant results were still obtained in spite of the small sample and effect size. Moreover, in accordance with a review by Allen we do not believe that a single biomarker should be used to amend or stop therapy and that, for correct interpretation, the cause –i.e. anaerobic or aerobic (co-) morbidity– of lactate increments should ideally be identi-

fied[3]. The clinical relevance of the current study should be sought in the fact that, for pediatric patients, mild lactate derangements that fail to normalize over time can serve as warning sign. The high hazard ratio of LACdelta shows that dynamic lactate indices – which account for disease-severity-induced adjustments over time – could be a valuable addition to clinical practice. Therefore, future prospective studies should substantiate the value of absolute and dynamic arterial lactate levels, preferably in homogeneous ECMO patient groups, and with respect to type, timing, and level of therapy delivered. Ideally, arterial lactate should then be evaluated together with other biomarkers such as microcirculatory perfusion [39]. In septic shock children, microcirculatory perfusion has been associated with mortality [42]. For neonatal ECMO patients with primary respiratory disease, future research should elucidate whether arterial lactate monitoring in neonates may be used for other clinically relevant purposes.

CONCLUSIONS

Static arterial lactate measurements and, to a lesser extent, dynamic arterial lactate indices predict mortality in pediatric ECMO patients with primary respiratory disease. The magnitude and trend over time of arterial lactate levels, but not the duration of lactate derangement predict mortality. In contrast, the value of arterial lactate for predicting outcome in neonatal ECMO patients is limited. A prospective multicenter study should substantiate the findings presented here in relation to intervention and a panel of biomarkers.

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Supplemental table 1. The vasopressor score in relation to LACabs, LACtw, and LACdelta for the neonatal and pediatric VA-ECMO and VV-ECMO patients.

		Neonatal patients		Pediatric patients	
		VA-ECMO N = 42	VV-ECMO N = 14	VA-ECMO N = 22	VV-ECMO N = 17
VP-score at admission	Median (IQR)	0 (11)	15 (27)	0 (0)	0 (5)
LACabs at admission	Median (IQR)	3.2 (3.9)	2.6 (5.0)	2.5 (3.3)	1.2 (1.1)
	Correlation coefficient	-0.08	0.11	0.16	0.15
LACtw at admission	Median (IQR)	2.9 (3.1)	3.6 (4.5)	2.1 (3.3)	1.4 (1.4)
	Correlation coefficient	0.17	0.20	0.13	0.00
LACdelta at admission	Median (IQR)	-0.24 (1.23)	0.00 (1.73)	-0.06 (1.25)	0.00 (0.36)
	Correlation coefficient	0.65*	0.06	-0.35	0.29
VP-score after cannulation	Median (IQR)	28 (54)	20 (32)	10 (46)	0 (8)
LACabs after cannulation	Median (IQR)	5.5 (3.7)	4.3 (2.5)	5.1 (4.7)	3.2 (3.1)
	Correlation coefficient	-0.17	0.06	0.49*	0.24
LACtw after cannulation	Median (IQR)	2.6 (2.7)	3.7 (2.9)	2.7 (4.4)	1.9 (1.2)
	Correlation coefficient	-0.26	0.38	0.80*	-0.07
LACdelta after cannulation	Median (IQR)	-0.39 (0.84)	-0.38 (0.78)	-0.62 (0.86)	-0.44 (1.54)
	Correlation coefficient	0.00	-0.54*	-0.15	-0.31

For all parameters, data are presented at admission as well as after cannulation –in line with Table 1– and both the median (IQR) scores as well as the Spearman rank correlation coefficients are calculated. $p < 0.050$ for correlation coefficient*

PART IV

DISCUSSION & SUMMARY

Chapter 10

General discussion

The microcirculation

Critical illness in neonatal or pediatric patients most often involves cardiovascular or respiratory dysfunction. As argued in the introductory comments, cardiovascular dysfunction can occur at three different levels: the systemic circulation, the regional circulation, and/or the microcirculation. The latter is elusive to monitor in children.

The thesis' subtitle –which reads: “Go with the flow?”– can be interpreted in two ways. The first interpretation is literally: should we measure microcirculatory flow? The answer is, of course, confirmative because the microcirculation is essential for maintaining the DO_2 - VO_2 balance, for conserving homeostasis, and, therefore, for sustaining health [1, 2]. This brings us to the figurative interpretation: should one adhere to what the majority is doing or saying? Well, from a researcher's perspective the answer is no. Although one should always keep in mind what is already known, the progress that is inherent to science would halt when researchers fail to pursue novel ideas or fail to develop new concepts or techniques. When it comes to research focusing on hemodynamic monitoring, the concept of classifying hemodynamic markers as “upstream”, microcirculatory, or “downstream” markers (see Introduction, Figure 2) is not innovative in itself, but the search for and the validation of new markers for each of these three categories is innovative indeed, particularly in the neonatal and pediatric age group. When attaining a historical viewpoint to the field of cardiovascular research, it becomes clear that the proportion of published reports primarily focusing on the microcirculation steadily increased from nearly 0% in 1950 to approximately 10% in 2010. Some may say that this percentage is still disappointingly low and argue that, in line with the figurative interpretation of this thesis's subtitle, attempts should be intensified to develop and/or validate techniques for monitoring the microcirculation in children to the extent that these might replace classical circulatory parameters in the (near) future.

This thesis focuses on two, relatively novel, parameters to monitor the microcirculation in children with either primary respiratory failure or primary cardiac failure. The first parameter is non-invasive video-microscopy for visualizing the actual microcirculation. The video-microscopy device Orthogonal Polarization Spectral imaging (OPS) was used for one study, whereas for the other studies the second generation device Sidestream Dark Field imaging (SDF) was used. The second parameter that is central in this thesis is arterial lactate and, in particular, the so-called dynamic lactate indices which incorporate duration or trend over time of lactate derangement next to magnitude of lactate derangement. Lactate is regarded as a “downstream” microcirculatory marker. The benefit that lactate has over routinely measured macrocirculatory parameters such as blood pressure, is that it also conveys information on tissue perfusion.

The aims of this thesis were threefold:

1. to study whether the microcirculation is altered in children who are critically ill
2. to evaluate if these microcirculatory alterations normalize over time with therapeutic intervention
3. to assess whether microcirculatory alterations are related to outcome.

Microcirculatory imaging

Non-invasive microcirculatory imaging in adults

The interest in bedside microcirculatory imaging with the use of OPS and/or SDF in children was propelled by promising findings in critically ill adults. In the beginning of the 21st century the first reports appeared in high impact journals. These discussed the observation of microcirculatory abnormalities in adults with brain tumors or distributive shock that, in the latter case, could be reversed by nitroglycerin –a paradoxical intervention for a disorder characterized by low vascular resistance [3-5]. Hereafter, more than 180 clinical studies followed, all describing the microcirculation observed by OPS and/or SDF in adults *in vivo*. The medical topics that were covered include the four forms of shock, oncology, venous insufficiency, and wound healing. The effects of various interventions were investigated: e.g. vasodilatory and vasoconstrictive drug treatment, fluids and blood products, cardiovascular extracorporeal assist devices, and hypothermia. From these studies it became apparent that a) OPS and SDF are feasible and promising, b) microcirculatory deterioration is present during disease and related to poor outcome, c) improvement of macrocirculatory parameters was not always accompanied by microcirculatory improvement, and d) microcirculatory resuscitation should be considered as a clinical endpoint.

Non-invasive microcirculatory imaging in children

Dr. Genzel-Boroviczeny and colleagues were the first to report on the use of OPS in children [6]. Next to the studies presented in this thesis, there are 19 reports available to date that describe studies using OPS or SDF in children [7-26]. These are summarized in Table 1. Most studies either compared the microcirculation in two groups of patients or described the microcirculatory effects of a therapeutic intervention –e.g. vasoactive drugs. Microcirculatory studies were performed across all age groups –i.e. preterm neonates, term neonates, and pediatric patients. Also, OPS and SDF reference data –obtained in healthy children– became available for both neonatal and pediatric patients [17, 22, 24, 25], and chapters 2, 4, 6, and 7. Unless specified otherwise, for term neonates and older children microcirculatory imaging data were obtained from the buccal mucosa and for preterm neonates from the skin of the inner-upper arm near the axilla.

Table 1. Overview of the studies using the non-invasive, video microscopy techniques Orthogonal Polarization Spectral imaging or Sidestream Dark Field imaging to study the microcirculation in children. Studies are categorized according to age group and date of manuscript appearance.

First author	N	Age group	Type of disease	Intervention	Main outcome and main conclusions
Genzel-Boroviczeny, 2004 [7] *	13	Preterm	Anemia	BTx	BTx improves the MC for at least 24h in anemic, preterm infants
Kroth, 2008[8] *	25	Preterm	-	-	The MC is higher in 1-week-old preterms than in 4-week-old preterms
Weidlich, 2009[9]*	25	Preterm	Proven infection	-	Compared to infants without infection, the MC decreased in infants with proven infections from d5 to d1 before starting antibiotics
Hiedl, 2010[10]#	25	Preterm	Significant PDA	Indomethacin or ibuprofen	The MC of PDA+ patients was lower than the MC of PDA- patients. The MC differences disappear after closing PDA+. The MC is better in the left than in the right arm, irrespective of treatment and PDA type
Schwepcke, 2013[11]#	21	Preterm	Hypotension	-	The MC is increased early after birth in hypotensive preterms when compared to normotensive preterms.
D'Souza, 2011[12]*	44	Preterm Term	LBW	-	The MC is increased in LBW infants when compared to normal weight infants
Top, 2009[13]*	14	Term	Primary RF Critical illness without RF	VA	Before VA, the MC is lower in VA patients than in non-ventilated controls, but it does not differ from ventilated controls. After VA, the MC is increased in VA patients whereas it is not in ventilated control patients
Ergenekon, 2011[14]#	15	Term	Severe polycythemia	PET	The MC is improved after PET
Antonios, 2012[15]*	22	Term	Maternal hypertension	-	While the MC is increased in preterm neonates born from hypertensive mothers, it is decreased in term neonates born from hypertensive mothers when compared to neonates born from normotensive mothers.
Alba-Alejandre, 2013[16]*	16	Term	Infection without shock	-	The MC is impaired in neonates with infection that does not cause shock
Ergenekon, 2013[17]#	7	Term	Perinatal asphyxia	TH	The MC is decreased during TH
Raghuraman, 2013[18]*	26	Term	-	-	The MC is higher in twin infants than in singleton infants
Tytgat, 2013[19]#	12	Term	Pyloric stenosis requiring laparoscopic pyloromyotomy	Pneumo-peritoneum	Pneumoperitoneum impairs the MC
Top, 2011[20]*	18	Term Ped	Septic shock	-	The MC does not differ at ICU d1 and increases thereafter in the survivors, but not the non-survivors. MC impairment predicts mortality more accurately than the PRISM-II

Table 1. (continued)

First author	N	Age group	Type of disease	Intervention	Main outcome and main conclusions
Top, 2011[21]*	8	Term Ped	Primary respiratory failure	iNO	The MC increases after iNO whereas macrocirculatory and ventilatory parameters are unaltered
Top, 2011[22]*	45	Term Ped	-	-	The MC is higher in neonates aged 0d to 7d when compared to all older children
Den Uil, 2009[23]#	3	Ped	Congenital heart disease	-	Alveoli can be visualized when a MC imaging device is placed against the visceral pleural surface
Milstein, 2012[24]#	11	Ped	Alveolar cleft gingiva	-	The MC is impaired in patients with an alveolar cleft
Paize, 2012[25]#	20	Ped	MCD	-	The MC is impaired in MCD patients and resolves as MCD regresses MC impairment at admission predicted independently the length of ventilation
Caixeta, 2013[26]#	2	Ped	Dengue shock	-	The MC is severely impaired during dengue shock
This thesis					
Top, 2012*	21	Term	Primary respiratory failure	VA	The MC is maintained, but not improved immediately after starting VA
Buijs, 2014#	28	Term	CDH	Dopa ± E or NE	Whereas HF and/or MABP rise after dopa ± E or NE, the MC fails to improve Abnormal MC is associated with need for E or NE and with need for VA
Van den Berg, 2014#	28	Term	-	-	Buccal MC measurements in term newborns are highly reproducible in contrast to cutaneous MC measurements There is no correlation between buccal and cutaneous MC
Buijs, 2014#	48	Term Ped	Primary respiratory failure	VA VV	The MC is impaired prior to both VA and VV and it requires 24h of VA or VV support to improve MC The MC evolution does not differ between VA and VV There is no relation between MC impairment and mortality
Buijs, 2014#	20	Ped	Post-cardiac arrest	TH	The MC is impaired during TH and increases thereafter to a level comparable to normothermic, healthy controls At TH start, MC impairment is associated with poor outcome

* indicates studies using orthogonal polarization spectral imaging, # indicates studies using Sidestream Dark Field imaging (SDF). BTx: blood transfusion, CDH: congenital diaphragmatic hernia, d: days, dopa: dopamine, E: epinephrine, ECMO: extracorporeal membrane oxygenation, h: hours, HF: heart frequency, ICU: intensive care unit, iNO: inhaled nitric oxide, LBW: low birth weight, MABP: mean arterial blood pressure, MC: microcirculatory, MCD: meningococcal disease, NE: norepinephrine, Ped: pediatric patients, PET: partial exchange transfusion, PDA: patent ductus arteriosus, PNA: post-natal age, Preterm: preterm neonatal patients, PRISM-II: absolute pediatric risk of mortality II, RF: respiratory failure, Term: a term neonatal patients, TH: therapeutic hypothermia, VA: venoarterial extracorporeal membrane oxygenation, VV: venovenous extracorporeal membrane oxygenation, w: weeks, yrs: years.

Some interesting similarities were observed with regard to the microcirculation in preterm and term children. One example is an age-related decrease in microcirculatory density, observed both in preterm infants and term infants [8, 22]. Another example is that the microcirculation is impaired prior to infection in preterm infants [9]. Microcirculatory impairments were also observed with OPS or SDF in term neonates with infection, in neonatal and pediatric patients with septic shock, in pediatric patients with meningococcal disease, and in pediatric patients with Dengue-induced septic shock [16, 20, 25, 26]. Yet another similarity is that the microcirculation improved in anemic preterm infants receiving blood transfusion and in neonates with polycythemia that required partial exchange transfusion [7, 14].

From the study on hypothermia in pediatric post-cardiac arrest (post-CA) patients that is presented in this thesis emerges a remarkable similarity when compared to a study on hypothermia in neonates with perinatal asphyxia [17] and chapter 7. The microcirculation as assessed by SDF was impaired during hypothermia and it improved after hypothermia was withdrawn in both the neonatal and the pediatric patients. It is, however, not clear whether the impairment originated from hypothermia-induced “physiological” vasoconstriction or from the underlying pathology for which hypothermia was initiated. Thus, the functional role of the microcirculation during hypothermia in the pediatric post-cardiac arrest context is paradoxical and should be better characterized.

The studies presented in chapters 3 and 4 focus on the microcirculation of children with primary respiratory failure who require extracorporeal membrane oxygenation (ECMO). With OPS we observed that the microcirculation of neonates is impaired prior to venoarterial ECMO (chapter 3). This is in line with an earlier report and it was also confirmed in our second study that used SDF and focused on both neonatal and pediatric patients [13] and chapter 4. Moreover, while venoarterial ECMO provides full macrocirculatory support once started, we showed that the microcirculatory impairment does not improve immediately after ECMO initiation (chapter 3). Approximately 24 hours of venoarterial ECMO support is required for improving the microcirculation (chapter 4). It could be that OPS and SDF are not sensitive enough to detect microcirculatory alterations within the first 24 hours of ECMO support. Yet, remarkably consistent results were obtained in the subsequent studies despite the fact that children of different age groups and with different pathology were included and that the microcirculation was studied with either OPS or SDF. Furthermore, the study presented in chapter 4 is novel in the sense that it shows that microcirculatory impairment is also present in patients who require venovenous ECMO, that the pattern of microcirculatory evolution during ECMO is similar between venovenous and venoarterial ECMO patients, and that with venovenous ECMO the microcirculation can be improved as well. These observations deserve attention because venovenous ECMO has intrinsic advantages over venoarterial ECMO and it is increasingly used.

Chapter 6 focuses exclusively on patients with congenital diaphragmatic hernia. With SDF it was found that microcirculatory density was lower in hypotensive term CDH neonates when compared to normotensive term controls. We hypothesized that this originated primarily from pulmonary hypertension, which results in decreased cardiac output, increased systemic resistance, and redistribution of blood away from the buccal microcirculation towards the microcirculation of the more vital organs. Using OPS microcirculatory impairment has been observed as well in adults with pulmonary hypertension [27]. Moreover, although information on the pulmonary circulation was only available indirectly through cardiac ultrasound data in our study, we observed that catecholaminergic treatment increased the macrocirculatory parameters heart rate and mean arterial blood pressure, but failed to improve the microcirculation. Thus, optimization of the macrocirculation does not always imply a reciprocal improvement of the microcirculation as assessed by SDF in both CDH neonates (chapter 6) and children with primary respiratory failure requiring ECMO (chapters 3 and 4).

A strong negative correlation was observed in the CDH patients between the microcirculation at baseline and the microcirculatory change from baseline after catecholaminergic therapy was started (chapter 6). In the post-CA patients, microcirculatory impairment at the start of hypothermia was associated with cardiovascular disease severity, with neurologic disease severity, and with mortality later in time (chapter 7). Accordingly, Top et al. and Paize et al. have reported that buccal and sublingual microcirculatory deterioration is related to mortality in neonatal or pediatric patients with septic shock and to prolonged duration of mechanical ventilation in pediatric patients with meningococcal disease, respectively [20, 25]. Together with the observed discrepancy between macrocirculatory and microcirculatory parameters, we feel these observations with OPS or SDF are important: although originating from different *in vivo* models, the combined results presented in this thesis suggest that microcirculatory monitoring with OPS or, preferably, SDF could, in itself, be clinically relevant as it identifies patients at risk for poor outcome. Moreover, microcirculatory monitoring could be an endpoint for therapy suggesting that both macrocirculatory failure and microcirculatory failure should be treated. In other words, measuring the microcirculation with OPS or SDF could serve as a stratification and/or a surrogate treatment-monitoring marker in the (near) future [28].

Non-invasive microcirculatory imaging in the obstetric setting

Neonatal patients, and to a lesser degree pediatric patients, are a unique patient group in the sense that prenatal factors can have a significant effect on postnatal health. Congenital anomalies are the most apparent example of this, but there are other examples as well, such as extreme low birth weight infants born at a post-menstrual age of 24 weeks. With respect to the field of hemodynamics, it is, for instance, acknowledged that

maternal hypertensive disorders during pregnancy are associated with increased risk of hypertension and stroke in the offspring [15]. The microcirculation is considered key in the development of hypertension and persistent microcirculatory impairment early in life has been implicated in the pathogenesis of cardiovascular disease and metabolic disorders later in life [29-31]. Yet, not until recently, maternal or non-maternal intra-uterine effects on the “post-natal” microcirculation have been studied sparsely using OPS or SDF. Starting in the year 2011, Antonios and colleagues have published some intriguing studies by using non-invasive video microscopy. They observed with OPS that, other than in infants with normal-birth weight, capillary density is increased in low birth weight infants [12]. Of note, 75% of the low birth-weight group was born preterm. Prematurity in itself might affect the microcirculation as assessed by video microscopy [6]. However, the increased capillary density could, hypothetically, also be the result of an intra-uterine compensatory microcirculatory mechanism to the placental insufficiency that led to the low-birth weight. The observation with OPS that singleton infants have more capillary rarefaction than twin infants seems in line with this hypothesis [18]. More than twin infants, singleton infants enjoy access to abundantly available nutrients which presumably triggers a process of capillary rarefaction. Even more interesting is the study that used OPS and found that the microcirculation is lower post-natally in term neonates born from hypertensive mothers than in those born from normotensive mothers [15]. This provides evidence that maternal cardiovascular disease affects cardiovascular function –including the microcirculation– in children even after they are born.

It also raises the question whether maternal hemodynamic treatment therapy affects not only the maternal microcirculation, but also the microcirculation of the offspring? In this light, the departments of pediatric surgery and gynecology & obstetrics in Erasmus MC-Sophia have joined their research efforts. As a first step, the maternal microcirculation was characterized using SDF in pre-eclamptic women with or without HELLP-syndrome [32]. The microcirculation was lowered only in the women with HELLP syndrome [32]. Also, with SDF we showed that the anti-hypertensive drug nicardipine effectively improved the maternal systemic parameters without affecting the maternal microcirculation, the uteroplacental macrocirculation, and the fetal macrocirculation [33]. In our view, one of the next steps should be to characterize the microcirculatory effects of maternal treatment in both mother and child as a model for trans-placental effects of maternal drug therapy and its potential (temporary) consequences for the newborn.

Technical aspects, methodology, and limitations of non-invasive microcirculatory imaging

The technologic aspects behind OPS and/or SDF have been described by Groner et al. and Goedhart et al., respectively [34, 35]. OPS and SDF use light at a wavelength at

which it is absorbed by both oxygenated and deoxygenated hemoglobin whilst it is not or to a lesser extent absorbed by other structures, it becomes possible to visualize the microcirculation. The resultant is an image in which erythrocytes are visualized as dark globules against a white-grayish background. In OPS, light is first polarized –i.e. light waves are parallelally-aligned using a filter– and thereafter emitted while with the use of a lens the light can be focused onto a region of interest of approximately 1mm in diameter [34]. Thereafter, the light that is transmitted back either remains polarized –low number of scattering events, for instance due to reflection by superficial non-microcirculatory structure – or becomes depolarized –i.e. high number of scattering event, for instance light penetrating relatively deep within the region of interest. Only the depolarized light is captured and used to form an image. OPS was found to outperform intravital fluorescence video microscopy –the gold standard at that time. SDF is the technologically-superior successor of OPS and provides the better image quality [35]. Specifically, SDF emits light in a stroboscopic –or pulsed– fashion preventing smearing of moving erythrocytes and mitigating confounders such as device movement or patient movement. Other advantages include separation of the reflected light from the emitted light through a different light guide which prevents signal interference and a shallower focus depth leading to less light absorption in the region of interest. Together these features results in brighter images with greater contrast and sharpness where the capillaries are more clearly visible and flow velocity can be estimated better. Also, light-emitting-diodes (LEDs) are used in SDF. These consume less power and therefore improve the portability and the clinical availability of the SDF device.

The light emitted by OPS and/or SDF does not penetrate to tissues deeper than 1 millimeter [34, 35]. OPS and/or SDF have been used in humans at various sites including sublingual mucosa, buccal mucosa, gingiva, transcutaneous –e.g. nailfold, axilla, ear conch–, conjunctiva, vaginal mucosa, and rectal mucosa, [4, 6, 13, 16, 36-40]. In the operating theater, many more sites become available and cerebral, pancreatic, hepatic, intestinal, and renal microcirculatory data have been reported [3, 41-44]. Most microcirculatory OPS and SDF data in adults have been obtained using the sublingual site. In children –and this is particularly true for ventilated neonates– size constraints prevent sublingual microcirculatory measurements and, consequently, buccal OPS or SDF measurements are the most feasible. In preterms –whose size prevents both sublingual and buccal measurements and whose skin architecture differs notably from that of the adults– microcirculatory OPS and SDF data have been obtained most often from cutaneous measurements in the inner-upper arm near the axilla. The microcirculatory architecture varies between the various sites. Likewise, it could be questioned to which extent the buccal microcirculation is representative for other microcirculatory beds –e.g. the splanchnic or cerebral microcirculation. With SDF we found (chapter 2) no correlation between the buccal and cutaneous inner-upper arm microcirculation in healthy

neonates, which may have been due to lower feasibility of the cutaneous measurements in the term neonates. Wan and colleagues showed, by using OPS or SDF in two separate, non-clinical studies that cardiogenic and hemorrhagic shock invoke severe alterations in the buccal microcirculation whilst the cerebral microcirculation is maintained [5, 6]. In contrast, Verdant et al. and Boerma et al. reported that, at least during sepsis, in adults the sublingual microcirculation observed with OPS is correlated significantly to the splanchnic microcirculation [45, 46]. Microcirculatory SDF data in pregnant women suggest that the correlation between sublingual and buccal microcirculation is equal to or greater than 0.80 (*non-published data*).

The cross-sectional data that OPS and SDF produce are generally recorded on DV-tape and thereafter digitized and stored on a computer's hard drive. Either way, video clips are produced. A round table conference was held in order to achieve consensus on optimal image acquisition and data analysis [47]. For optimal image acquisition, there are five key points:

1. During a measurement, the microcirculation should be visualized at a minimum of three different sites, given the intrinsic variability of microvascular perfusion.
2. Eliminate all that is obscuring the image –e.g. secretions, lanugo.
3. Provide adequate focus and contrast.
4. Minimize device and/or patient movement in order to produce an image in which the same vessels are observable for a period of at least 20 seconds.
5. Given that microcirculatory vessels are collapsible, avoid excessive pressure that might result in pressure artefacts by slowly retracting the OPS or SDF device until contact is lost and then advancing the probe again up to the point at which contact is restored and visualization is regained.

Similar to cardiac ultrasound, obtaining high-quality images requires an extensive training period [48]. Interestingly, image-quality scores have been developed [48, 49]. Routinely incorporating such a score in reports, would help assessing the reliability of the paper's results and conclusions. Others have developed an image-acquisition stabilizer, which indeed improved image quality without affecting microcirculation [50].

The analysis of OPS and/or SDF data is based on the principle that vessel density and blood flow velocity are both prerequisites for adequate microcirculatory function [47]. For vessel density, the score that is most widely used is based on the principle that the density of microcirculatory vessels is proportional to the number of vessels crossing three equidistant horizontal and three equidistant vertical lines superimposed on the image [4]. For blood flow velocity, a semi-quantitative score is most often reported [5, 43]. This score is based on determining the predominant type of flow –i.e. absent, intermittent, sluggish, or continuous– in four quadrants generated by two right-angled lines superimposed on the image. Several important additions and/or adjustments

were suggested as progressive insight was gained over the years. One such suggestion that became widely accepted is that it is important to distinguish between functional density –i.e. vessels with continuously moving erythrocytes– and non-functional density – vessels with stagnant erythrocytes– [47]. Likewise, for the blood flow velocity, it is important to not only observe the predominant type of flow, but also the other types of flow that are present [47]. Hence, for the density score it is recommended to quantify the total number of vessels –total vessel density– together with number of vessels that are “perfused” –perfused or functional vessel density–. This thus allows determination of the proportion of perfused vessels for vessel density, and to calculate the microcirculatory heterogeneity index for the blood flow velocity. Another important addition to the quantification of microcirculatory data is that microcirculatory deterioration might cluster in the capillaries whilst in the greater vessels relatively few alterations are observed [35]. Hence, many investigators now report on small vessels and non-small vessels using a diameter cut-off value –e.g. for term children the cut-off is generally 10 micrometers [20, 51].

Multiple dedicated software packages have been developed for analyzing microcirculatory OPS or SDF data –e.g. CapImage software (Dr. Zeitl software Engineering, Heidelberg, Germany), CapiScope (KK Technology, Honiton, UK), Automated Vessel Analysis (Microvision Medical BV, Amsterdam, the Netherlands) [52]. With these software programs, manual correction is still needed and data analysis remains semi-automated. Two examples may serve to illustrate this point. One, in practice the quality of the OPS or SDF video clips is often suboptimal (e.g. slight movement artefacts); two, OPS and SDF have a relatively low temporal resolution –i.e. a low imaging rate– compared to the actual erythrocyte velocity (e.g. causing smearing) [53]. As a result, although OPS and SDF are applicable at the bedside and point-of-care assessments have been reported [54], the full microcirculatory data analysis is presently: a) best done off-line; b) time-consuming; and c) subject to inter-observer and intra-observer variability. For the studies describing the buccal SDF measurements in the critically ill children (chapters 3, 5, and 6) and in the healthy neonates (chapter 2), the mean inter-observer variability –i.e. averaged for all microcirculatory parameters– was determined using the intra-class correlation coefficient (ICC). For the studies in the critically ill children and for the study in the healthy neonates, the mean ICC was 0.750 and 0.930, respectively. The intra-observer variability (*non-published data*) approximated the inter-observer variability. Both intra-observer and inter-observer variability can be regarded as reasonable.

Also, due to the lack of a gold standard in analysis software and in spite of the consensus paper, over the years many reports have appeared all describing either some of the outcome parameters or describing outcome parameters that methodologically differ from each other. Aside from the technological differences between OPS and SDF, this hinders the generalizability of studies and precludes meta-analysis. This is particularly

undesirable for research in critically ill children, as sample size and sparse data are most often a point of concern.

Arterial lactate

Lactate is one of the normal end products of carbohydrate metabolism; the other main product is acetyl-CoA [55]. In normal aerobic conditions, lactate is constantly being produced during glycolysis from pyruvate by lactate dehydrogenase [56]. Hereafter, most of the lactate is converted back to either pyruvate by oxygenation to serve once again as energy substrate or to serve as precursor for gluconeogenesis [56]. Together, these three processes –i.e. glycolysis (lactate production), oxidation (lactate exchange), and gluconeogenesis (lactate use)– have been termed the lactate shuttle [56]. As a result, the reference range for arterial lactate concentration is in children 0.5 to 2.0 mmol/L⁻¹. As stated in the introduction, in critically ill children the lactate concentration can increase due to both aerobic and anaerobic causes [56, 57]. As such, we hold lactate to be a “downstream” microcirculatory parameter.

Hyperlactatemia has been related to greater disease severity and to increased risk for mortality in critically ill adults [57, 58]. Moreover, mild hyperlactatemia and even relative hyperlactatemia –i.e. higher lactate levels within the reference range– have been related to mortality as well [59, 60]. In adults, landmark randomized controlled trials showed that goal-directed therapy with lactate as a primary endpoint resulted in improved outcome in patients with septic shock, in post-cardiac surgery patients, and in a general critically ill patient population [61–64].

For children, such randomized controlled trials have not been performed. Rossi et al. have reported that, when compared to a historical control group, the introduction of goal-directed therapy –which incorporated lactate as one of the endpoints, but is otherwise unspecified– resulted in a lower mortality rate in post-cardiac surgery children [65]. Furthermore, approximately thirty prospective or retrospective studies have focused on lactate as the primary parameter of interest and its relation to outcome [66–95]. The majority of these studies concluded that increased lactate concentration –i.e. hyperlactatemia– is associated with poor outcome. However, results obtained in pediatric post-cardiac surgery patients might not be relevant for, for instance, neonatal patients with primary respiratory failure because it has been suggested that the prognostic value of lactate depends on, amongst others, disease type, disease severity, comorbidity, and age. With respect to disease type and disease severity, it is important to realize that, in order for arterial blood lactate concentration to rise, energy metabolism is required. Lactate concentration might remain false negatively low in children with severe tissue necrosis –e.g. those with meningococcal sepsis or intestinal volvulus due to malrotation. Alternatively, children with hepatic disease might be particularly sus-

ceptible for increments in lactate [56]. Also, when microvascular perfusion is absent, the intra-cellular-formed lactate cannot be released to circulation. A paradoxical increase in lactate concentration after starting cardiopulmonary bypass has been described for children with congenital cardiac defects [83]. Also, age-related differences in lactate concentration due to both intrinsic and extrinsic factors have been described [96-104]. Younger children, for instance, produce less lactate at equal levels of exercise –intrinsic factor– while lactate can be false positively increased in the peri-partum period in children that are otherwise healthy –extrinsic factor– [96, 104].

Given that the arterial lactate concentration can vary over time in critically ill children, dynamic lactate indices were developed that –in contrast to the static, cross-sectional measurement of lactate– incorporate duration and trend over time next to magnitude of lactate derangement (Table 2). Two dynamic lactate indices have been developed, although slight mathematical differences exist between the various studies. These are: “time-weighted lactate”, which incorporates the magnitude and the duration of lactate derangement, and “delta lactate”, which incorporates the magnitude and change over time of lactate derangements.

Charpie et al. and Schumacher et al. reported that delta lactate predicts adverse outcome in neonatal and pediatric patients with congenital cardiac defects [92, 94]. Kalyanaraman et al. showed that, in comparison to the survivors, the time during which lactate remained above 2 mmol/L^{-1} –i.e. time-weighted lactate– was longer for pediatric non-survivors who underwent cardiopulmonary bypass for correcting congenital cardiac defects [93]. Likewise, Kim et al. observed that the lactate area –which is comparable to time-weighted lactate– was the best predictor for mortality in pediatric patients with septic shock [95]. All studies demonstrated that the dynamic lactate indices were better predictors than the measurement of static lactate –e.g. lactate concentration at admission, which does not allow assessment of therapy efficacy, or peak lactate concentration, which can only be determined retrospectively.

The promising results of the studies mentioned in the previous paragraph inspired us to study the predictive value of dynamic lactate indices next to the predictive value of static, cross-sectionally measured lactate in neonates and pediatric patients with primary respiratory failure –for whom the prognostic value of lactate has been studied sparsely. In chapter 8 we show that “time-weighted lactate” –a dynamic lactate index for the duration of lactate derangement– is a better predictor for the need for ECMO in CDH patients than static lactate. In contrast, in Chapter 9 it is discussed that in pediatric ECMO patients the measurement of static arterial lactate was the better predictor for mortality. In neonatal ECMO patients, neither static nor dynamic lactate predicted mortality. This study illustrates that results cannot be extrapolated easily when different patient groups, treatment forms, or outcome measures are considered.

Table 2. Overview of the studies focusing on dynamic arterial lactate indices in children. Studies are categorized according to date of manuscript appearance.

First author	N	Age group	Type of disease	Timing	Primary endpoint	Main outcome and main conclusions
Charpie, 2000 [92]	47	Neo	CHD	Post-surgical	IC Mortality ECMO	A change in "LACdelta" $\geq 0.75 \text{ mmol L}^{-1} \text{ h}^{-1}$ predicts mortality, in contrast to "LACabs" at admission
Rossi, 2005 [65]	2,366	Neo Ped	CHD	Post-surgical	IC Mortality	GDT –including lactate amongst other endpoints– lowered mortality when compared to historical controls
Kalyanaraman, 2008 [93]	129	Neo Ped	CHD	Post-surgical	IC Mortality	"LACTw" predicts mortality best, in comparison to initial, post-surgical "LACabs" ^{***} and peak post-surgical "LACabs"
Schumacher, 2013 [94]	231	Neo Ped	CHD	Post-surgical	IC Mortality	"LACdelta" $> 0.6 \text{ mmol L}^{-1} \text{ h}^{-1}$ predicts mortality better than "LACabs"
Kim, 2013 [95]	65	Ped	Septic shock	24h after IC admission	IC Mortality	"LACTw" predicts mortality best, in comparison to 24h "LACdelta" and "LACabs" at admission
This thesis						
Buijs, 2014	64	Neo	CDH	24h after IC admission	ECMO	Early derangements LACabs, LACTw, and LACdelta are associated in CDH patients requiring ECMO. LACTw predicts outcome best
Buijs, 2014	22	Neo Ped	Primary RF requiring ECMO	Before and during ECMO	IC Mortality	LACabs predicts mortality best in pediatric patients LACdelta, while LACTw is not associated with outcome in pediatric patients. Neither LACabs, nor LACTw or LACdelta predicts mortality in neonatal patients

CDH: congenital diaphragmatic hernia, CHD: congenital heart defect, ECMO: extracorporeal membrane oxygenation, GDT: goal-directed therapy, h: hours, IC: intensive care, LACabs: static absolute arterial lactate, LACdelta: lactate change over time, LACTw: time-weighted arterial lactate, $\text{mmol L}^{-1} \text{ h}^{-1}$: millimoles per liter per hour, Neo: neonatal patients, Ped: pediatric patients, RF: respiratory failure.

Linking microcirculatory imaging to arterial lactate

It was reported recently that both the microcirculation –as measured by OPS or SDF– and arterial lactate are early independent predictors for poor outcome in adults with septic shock [105]. As discussed in the previous section, arterial lactate might be false negatively low due to severely altered microvascular perfusion and/or decreased energy metabolism [106]. OPS and/or SDF have the potential to reveal microcirculatory alterations already at an early stage, before arterial lactate has had the chance to increase. OPS and/or SDF, however, visualize erythrocytes irrespective of their oxygenation status [34, 35]. Hence, these imaging techniques can be used to monitor tissue perfusion, but not tissue oxygenation. Given their characteristics, arterial lactate and OPS and/or SDF in particular might act synergistically as a diagdisnostic or treatment-monitoring biomarker [107].

De Backer et al. observed that, after infusing dobutamine or after starting drotrecogin alfa in septic shock adults, the increment in capillary perfusion –as measured by OPS– was proportional to the decrease in lactate concentration [108, 109]. A similar pattern was noted by Thooft et al. after norepinephrine infusion in septic shock adults [110]. Others showed that, by stratifying patients with the use OPS or SDF, the patients with the poorest microcirculation also had the highest lactate levels [111]. Likewise, microcirculatory imaging has been reported to correlate inversely with lactate in adults receiving cardiac surgery, in adults admitted with cardiogenic shock, and in adults with malaria [40, 112-118]. Jung et al. developed an interesting multimodal model for adults with cardiogenic shock receiving intra-aortic balloon pump. The model incorporated cardiac index, mean arterial blood pressure, and the microcirculation measured by SDF and this model correlated strongly with lactate levels [119]. Moreover, lactate increments could be predicted at 3 hours and at 24 hours after measuring the model's parameters. Yeh et al. also used SDF and reported that in adults receiving general or thoracic surgery the microcirculation measured pre-surgically and 1 hour post-surgically predicts lactate increments at 24 hours after surgery [120]. These observations might explain why Lauten et al. found microcirculatory deterioration at admission –by using SDF in adults with acutely decompensated heart failure– whilst lactate remained within the normal spectrum. Similarly, Morelli et al. observed with SDF that the microcirculation improved 6 hours after starting terlipressin while lactate remained modestly elevated [121]. Follow-up measurements confirming that microcirculatory improvement precedes lactate lowering –and that lactate is a slower reactant–, were, unfortunately, not performed. In contrast, others used SDF and found that in adults with hyperdynamic septic shock, the microcirculation did not differ between the patients (n=10) with a lactate clearance exceeding 10% and those (n=5) without a lactate clearance exceeding 10% [122]. Also, it should be noted that several reports –not primarily focusing on the relation between on the one hand OPS and/or SDF and on the other hand lactate– described that either

the microcirculation or arterial lactate, but not both, differed between groups or after intervention. Thus, the relation between these two markers is not straightforward.

The association between SDF measurements on the one hand and lactate measurements on the other hand has been sparsely studied in children. Hiedl et al. observed with SDF that the microcirculation was lower in preterm neonates with a hemodynamically significant patent ductus arteriosus, whilst none of the other parameters –including arterial lactate– differed [10]. Top et al. observed that two days after the admission of children with septic shock, lactate and the microcirculation –at that time measured by OPS– both improved significantly in the survivors whilst there were no significant improvements in non-survivors. In this thesis, we report a concomitant improvement in lactate and the microcirculation observed by SDF in neonatal and pediatric patients with primary respiratory failure 2 days after starting ECMO and in pediatric post-cardiac arrest patients after stopping therapeutic hypothermia (chapters 6 and 7). Moreover, after defining cut-off values for abnormal SDF microcirculation in the post-CA children, we observed that an abnormal microcirculation at the start of hypothermia predicts heightened lactate levels –as well as increased need for vasopressors and poor neurologic outcome– once normothermia had been reached. In CDH patients requiring vasopressor support, we observed that ECMO requirement could be predicted with SDF whilst lactate did not differ (chapter 6). Yet, in another study describing a larger CDH cohort, we also showed that arterial lactate measured within 24 hours of IC admission is related to ECMO requirement in CDH patients (chapter 8).

Conclusions

The use of the video microscopy techniques OPS and SDF in particular is feasible in critically ill children and shows that the buccal microcirculation is impaired in children with primary respiratory or primary circulatory failure. Moreover, these impairments are associated with disease severity –e.g. oxygenation index, vasopressor score, and pediatric cerebral performance category scale– and poor outcome –e.g. need for ECMO and mortality. Furthermore, our microcirculatory imaging results indicate that microcirculatory alterations can persist whilst macrocirculatory parameters are corrected. Also, the buccal microcirculation as assessed by SDF in term neonates correlates poorly with the cutaneous inner-upper arm microcirculation.

With respect to the dynamic arterial lactate indices that incorporate duration or trend over time of lactate derangement, we conclude that alterations of moderate magnitude are present in children with primary respiratory failure, that these alterations normalize over time, and that these alterations can be related to poor outcome –e.g. need for ECMO. When compared to the cross-sectional measurement of lactate, the dynamic lactate indices can be superior in predicting poor outcome, but this superiority is likely

to depend on the patient group, the treatment form, and/or the outcome measure that is considered.

The possibilities for microcirculatory monitoring are limited in children. Thus, the data provided in this thesis could be clinically relevant as evidence is provided that suggests that microcirculatory monitoring with SDF and, to a lesser extent, with dynamic lactate indices might serve as stratification markers and/or a surrogate treatment-monitoring markers in critically ill children. First, however, technological improvements should be gained together with increased insight in pathophysiological phenomena and adequately-power validation studies before these markers can be used in daily clinical practice to monitor changes in therapy such as vasoactive drug dosing.

Future perspectives and recommendations

Randomized controlled trials (RCTs) provide the highest level of scientific evidence. In adults, RCTs using goal-directed therapy with microcirculatory endpoints such as arterial lactate support the concept that not only the macrocirculation should be monitored and treated, but the microcirculation as well or –in time– even predominantly [61-64]. For improving outcome, such trials should be performed for critically ill children as well. Rather than just one parameter, these studies should, ideally, incorporate a panel of markers consisting of both macrocirculatory and microcirculatory markers as well as markers for oxygenation [123]. This, however, first requires additional studies in order to identify the markers that are most valuable for multimodal monitoring. Also, for each endpoint cut-off values should be defined that account for potential age-related changes over time. An additional advantage of multimodal monitoring is that pathophysiological insight can be gained in local microcirculatory differences. For instance, while assessing the buccal microcirculation with video microscopy, the skin, kidney, and cardiac circulation can be assessed using respectively the peripheral perfusion index, laser speckle imaging, and contrast-enhanced ultrasound (see introduction).

The non-invasive video microscopy technique SDF is highly valuable as it aids in providing novel pathophysiologic insight in critically ill children. Not only is SDF capable of visualizing the actual microcirculation, it is also unique in the sense that this modality can discriminate between capillaries on the one hand and arterioles or venules on the other hand [124, 125]. Furthermore, the non-invasiveness of SDF (and OPS) is regarded particularly important for children, for whom invasive monitoring or contrast-based techniques is often impossible or undesired. As discussed, SDF has significant limitations as it cannot be used as point-of-care assessment tool yet. Many scores, all calculated slightly different, have been described. We strongly recommend to standardize the endpoints for the microcirculatory video microscopes this is imperative for increasing generalizability of studies and for allowing future meta-analysis. Knowing

that neonatal and pediatric studies are prone to modest sample sizes, this would propel scientific progress. Moreover, the lack of bedside numerical microcirculatory data significantly hinders the clinical applicability SDF (and OPS). Recently, a new imaging device has been introduced: Cytocam, Braedius Medical BV, Huizen, the Netherlands. Most importantly, this third generation handheld video microscope has a higher spatial and temporal resolution in combination with computer-controlled imaging sensors providing digital output [126]. Also, improved optics in the Cytocam can identify 10-20% more vessels than OPS and SDF. Together with the development of more advanced computer software [53, 127], it is expected that fully automated and quantitative image analysis will become available providing the needed microcirculatory functional parameters at the bedside. Once this new microcirculatory imaging parameter has been properly validated, video-microscopy-guided trials in the neonatal and pediatric intensive care setting would become methodologically and ethically justifiable.

Finally, it should be mentioned that a Neonatal and Pediatric Microcirculatory Research Working Group was founded at the ESPNIC congress held June 2013. Joining this working group is recommended for all researchers in the field of microcirculatory research in children. This initiative aims to coordinate research efforts, to facilitate collaboration on specific projects, and to exchange ideas and information. With respect to research involving video microscopy in critically ill children, the following fields in medicine are to date largely unexplored, yet highly interesting: cardiology, surgery, obstetrics, and epidemiology –e.g. prevalence studies such as the microSOAP study [128]–. Likewise, interventions that have been studied in the adult intensive care setting, but not in the neonatal or pediatric intensive care include: fluids and blood products, cardiopulmonary bypass, and selective vasoactive drug treatment –e.g. NO-donors, ACE-inhibitors. These are all awaiting to be evaluated using a systematic approach that should incorporate the microcirculation, thereby taking the treatment of critically children a step further on the basis of increased insight in the pathophysiological derangements during respiratory and circulatory failure.

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Chapter 11

Summary / Samenvatting

Summary

The aims of this thesis are to study whether the microcirculation – as assessed by Orthogonal Polarization Spectral imaging (OPS), by Sidestream Dark Field imaging (SDF), and/or by arterial lactate – is altered in children who are critically ill; to evaluate if any alterations normalize over time with therapeutic intervention; and to assess whether microcirculatory alterations are related to outcome.

PART I. Introduction

In **Chapter 1** it is argued that the microcirculation is essential for maintaining an adequate balance between oxygen consumption and oxygen delivery, but that the possibilities for bedside monitoring of the actual microcirculation have long been limited in critically ill children. Consequently, clinicians have focused on either “upstream” markers –i.e. the macrocirculation–, or “downstream” markers –i.e. microcirculatory derivatives. Together, these are valuable as they represent the patient’s hemodynamic status, but they do not directly represent the critical intermediary: the actual microcirculation. The presently available techniques that could fill the “gap” in circulatory monitoring are discussed and the non-invasive imaging techniques OPS and SDF –a second generation monitoring tool that is central in this thesis– are introduced. Also, recent developments regarding one of the promising “downstream” microcirculatory markers –i.e. arterial lactate– are reviewed.

PART II. Microcirculatory imaging

It is shown in **Chapter 2** that the feasibility and reproducibility of buccal, microcirculatory measurements with SDF is acceptable in term neonates, in contrast to cutaneous inner-upper arm measurements. This observation is important for the remaining studies in this part, because: 1) to date, the analysis of microcirculatory imaging data requires manual intervention and is, therefore, subject to inter-observer variability; 2) size constraints prevent sublingual measurements –routinely performed in adults– in neonates, for whom apparently the buccal measurements, but not the cutaneous measurements –routine in preterm neonates– form an acceptable alternative; and 3) the feasibility of SDF measurements has been questioned in incapacitated, un-sedated, healthy neonates.

Healthy, term neonates might serve as controls for patients in one of the three patients groups –each receiving a distinct form of treatment that could potentially impact the microcirculation. Children with primary respiratory failure who required extracorporeal membrane oxygenation (ECMO) are discussed in **Chapter 3** and **Chapter 4**. ECMO temporarily provides full cardiopulmonary support that allows time for evaluation, diagnosis, and treatment of the condition causing the respiratory failure. **Chapter 3** shows that

microcirculatory function –assessed by OPS– does not improve immediately after starting venoarterial ECMO in neonates. This is surprising because macrocirculatory blood flow and oxygenation are restored instantaneously with the start of venoarterial ECMO. In **Chapter 4** SDF was used, which produces images of higher quality and is therefore technologically superior to OPS. With SDF it became apparent that the microcirculation is impaired prior to ECMO and that after the start of ECMO it requires approximately 24 hours to restore. Thereafter the microcirculation improves and this improvement is sustained up to after decannulation irrespective of the patient’s outcome. Interestingly, the pattern of microcirculatory evolution was rather similar between those treated with venovenous ECMO and those treated with venoarterial ECMO. The patients who require venovenous ECMO usually suffer less macrocirculatory failure prior to cannulation, whilst during venovenous ECMO physiologic blood flow –including pulmonary circulation– is maintained.

Chapter 5 reviews the current knowledge of the distribution and the function of catecholaminergic receptors in the heart and the peripheral vasculature in children of various ages –i.e. from fetus to adolescence. Although catecholamines are used on a daily basis by neonatologists and pediatric intensivists around the world, little is known about the characterization of catecholaminergic receptor distribution in the vasculature of children. Also, and this is based upon *in vitro* studies using cardiac tissue, alterations in receptor distribution might not be reciprocal to alterations in receptor function. To partly elucidate *in vivo* catecholaminergic receptor function in children, we used SDF in **Chapter 6**. SDF was used to evaluate the microcirculatory effects of catecholaminergic treatment in neonates with congenital diaphragmatic hernia (CDH; second patient group of interest). This study shows that whilst dopamine, norepinephrine, and epinephrine increase heart rate and/or mean arterial blood pressure, the microcirculation fails to improve. The microcirculation of the CDH patients remained lower when compared to healthy neonates. Moreover, microcirculatory impairment was related to poor outcome –i.e. the need for ECMO.

Pediatric post-cardiac arrest (post-CA) patients receiving therapeutic hypothermia comprised the third patient group of interest in the microcirculatory imaging studies. The study described in **Chapter 7** indicates that –when compared to the survivors– the microcirculation is more severely impaired during hypothermia in the post-CA patients who ultimately died in the pediatric intensive care unit. Also, abnormal microcirculation as assessed by SDF at hypothermia start was associated with increased hemodynamic and neurologic dysfunction. However, therapeutic hypothermia, which is known to improve outcome in post-CA adults, decreases the microcirculation as well. Paradoxically, it is not until after therapeutic hypothermia is stopped that the microcirculation increases to a level that is comparable to that of healthy, normothermic children.

PART III. Arterial lactate monitoring

Recent studies in septic and post-cardiac surgery children showed that dynamic arterial lactate indices are better predictors for outcome than the measurement of static –cross-sectional– arterial lactate levels that are conventionally determined. These findings inspired the two studies that are described in **Chapter 8** and **Chapter 9**. The results presented in **Chapter 8** indicate that “time-weighted lactate” –a dynamic index incorporating the magnitude and the duration of hyperlactatemia– is a better predictor for the need for ECMO in CDH patients than both static lactate and “delta lactate” –a dynamic index incorporating magnitude and change over time. In contrast, **Chapter 9** shows that in pediatric ECMO patients the measurement of static arterial lactate was the better predictor for mortality. This study illustrates that results cannot be extrapolated easily across different patient groups, treatment forms, or outcome measures.

PART IV. General discussion

Chapter 10 contains the general discussion. The contents of this thesis are reviewed in connection with the literature. It is concluded that a) the non-invasive video microscopy techniques OPS and SDF are feasible in critically ill children, b) microcirculatory deterioration is present in children with primary respiratory or primary circulatory failure, c) that the improvement of macrocirculatory parameters through therapy interventions was not always accompanied by microcirculatory improvement, and d) that microcirculatory impairment is related to poor outcome. A heightened arterial lactate concentration is also related to poor outcome. The dynamic lactate indices can be superior to static, cross-sectional lactate measurements in predicting poor outcome, but this superiority is not uniform for all critically ill neonatal or pediatric patient groups.

Perspectives are to set up trials to substantiate the predictive value of microcirculatory monitoring and to characterize, preferably, a panel of microcirculatory markers. This multimodal panel should include microcirculatory imaging and arterial lactate amongst other parameters. Ultimately, multimodal microcirculatory-guided trials are the real challenge in the future.

Samenvatting

Het doel van dit proefschrift is om te bestuderen of de microcirculatie –geëvalueerd met Orthogonal Polarization Spectral imaging (OPS), met Sidestream Dark Field imaging (SDF), en/of met arterieel lactaat– is veranderd; om te bepalen of deze eventuele veranderingen normaliseren over de tijd na therapeutische interventie; en om te evalueren of veranderingen in de microcirculatie gerelateerd zijn aan slechte uitkomst.

DEEL I. Introductie

In **Hoofdstuk 1** wordt beargumenteerd dat de microcirculatie essentieel is voor een goede balans tussen zuurstofconsumptie en zuurstofaanvoer, maar dat het lange tijd lange tijd nauwelijks mogelijk was om de daadwerkelijke microcirculatie te bestuderen aan het bed van de patiënt. Hierdoor focusten artsen zich ofwel op “stroomopwaartse” parameters –d.w.z. de macrocirculatie– ofwel op “stroomafwaartse” parameters –d.w.z. microcirculatoire afgeleiden. Tezamen zijn deze parameters waardevol omdat zij een indruk geven van de hemodynamische status van de patiënt, maar niettemin zijn zij geen directe representatie van de daadwerkelijke microcirculatie. Nieuwe technieken die het hiaat in circuloire bewaking zouden kunnen opvullen worden besproken en de niet-invasieve imaging technieken OPS en SDF –een 2^e-generatie imaging techniek die centraal staat in dit proefschrift– worden geïntroduceerd. Daarnaast worden recente ontwikkelingen beschreven op het gebied van één van de veelbelovende “stroomafwaartse” microcirculatoire parameters: arterieel lactaat.

DEEL II. Microcirculatoire imaging

Hoofdstuk 2 toont dat de bruikbaarheid en de reproduceerbaarheid van de buccale, microcirculatoire metingen met SDF in à terme neonaten acceptabel zijn, in tegenstelling tot de cutane bovenarm metingen. Dit is een belangrijke observatie voor de hiernavolgende studies in dit gedeelte omdat: 1) de analyse van microcirculatoire imaging data tot op heden deels mensenwerk is en daarom onderhevig is aan inter-observer variabiliteit; 2) sublinguale metingen –die gewoonlijk uitgevoerd worden in volwassenen– vanwege de grootte van de SDF-probe niet mogelijk zijn in neonaten, voor wie kennelijk de buccale metingen maar niet de cutane metingen –die gewoonlijk uitgevoerd worden in preterme neonaten– een acceptabel alternatief vormen; 3) er twijfels bestaan omtrent de bruikbaarheid van SDF-metingen bij niet-wilsbekwame en niet-gesedeerde, gezonde neonaten.

Gezonde, à terme neonaten zouden kunnen dienen als controle patiënten in één van de drie patiënt groepen –die elk een andere vorm van therapie ontvangen die de microcirculatie zou kunnen beïnvloeden. Kinderen met primair respiratoir falen

die extracorporele membraanoxygenatie (ECMO) behoeven vormen het onderwerp in **Hoofdstuk 3** en **Hoofdstuk 4**. ECMO levert tijdelijk volledige cardiopulmonale ondersteuning waarmee tijd wordt gewonnen voor evaluatie, diagnostisering, en behandeling van de conditie die het respiratoir falen veroorzaakt. **Hoofdstuk 3** toont dat de microcirculatie –bestudeerd met OPS– niet direct verbetert na het starten van venoarteriele ECMO in neonaten. Dit is opvallend omdat de macrocirculatoire doorbloeding en oxygenatie wel onmiddellijk verbeteren. In **Hoofdstuk 4** werd de microcirculatie bestudeerd met SDF, dat beelden van hogere kwaliteit produceert en daarmee technologisch superieur is aan OPS. Met SDF werd het duidelijk dat de microcirculatie verstoord is voorafgaand aan ECMO en pas herstelt na ongeveer 24 uur na de start van ECMO. Hierna blijft de microcirculatie verbeterd tot na het moment van decannulatie en deze verbetering is niet gerelateerd aan de uitkomst voor de patiënt. In dit opzicht was er ook geen verschil tussen de patiënten die werden behandeld met venoveneuze ECMO en de patiënten die behandeld werden met venoarteriele ECMO. Dit is opvallend omdat de eerstgenoemden in de regel minder macrocirculatoir falen hebben voor canulatie, terwijl de fysiologische doorbloeding –inclusief pulmonale circulatie– in stand blijft tijdens venoveneuze ECMO.

Hoofdstuk 5 vat de huidige kennis samen over de distributie en het functioneren van catecholaminerge receptoren in het hart en de perifere vasculatuur van kinderen van verschillende leeftijden –d.w.z. van foetus tot adolescent. Alhoewel catecholaminen dagelijks worden voorgeschreven door neonatologen en kinderintensivisten over de gehele wereld, is er weinig bekend omtrent de karakterisatie van de catecholaminerge receptor distributie in de vasculatuur van kinderen. Wel is bekend via *in vitro* studies die gebruik maken van hartweefsel, dat veranderingen in receptordistributie mogelijk niet leiden tot reciproke veranderingen in het functioneren van diezelfde receptor. Het *in vivo* functioneren van catecholaminerge receptoren in kinderen hebben wij indirect bestudeerd middels SDF in **Hoofdstuk 6**. SDF werd gebruikt om de effecten van catecholaminerge behandeling in neonaten met congenitale hernia diafragmatica (CDH; de tweede patiëntgroep centraal in dit proefschrift) te evalueren. Deze studie toont dat dopamine, norepinephrine, en epinephrine weliswaar de hartslagfrequentie en/of de gemiddelde arteriële bloeddruk verhogen, maar niet de microcirculatie. De microcirculatie van de CDH-patiënten bleef lager in vergelijking met gezonde neonaten. Belangrijker, de microcirculatoire verslechtering was gerelateerd aan een slechte uitkomst –d.w.z. de noodzaak tot het starten van ECMO.

Pediatrische patiënten die na een hartstilstand therapeutische hypothermie ontvingen vormen de derde patiëntgroep die centraal staat in de microcirculatoire imaging studies. De studie beschreven in **Hoofdstuk 7** geeft aan dat –in vergelijking met de patiënten die uiteindelijk overleven– de microcirculatie meer verslechterd is tijdens therapeutische hypothermie in de patiënten die na een hartstilstand uiteindelijk komen

te overlijden op de IC Kinderen. Een abnormale microcirculatie –zoals beoordeeld met SDF– is bij de start van hypothermie tevens geassocieerd met slechter hemodynamisch en neurologisch functioneren. Ondanks dat therapeutische hypothermie gekend is de uitkomst in volwassenen met een hartstilstand te verbeteren, verstoort therapeutische hypothermie op zichzelf de microcirculatie ook. De microcirculatie verbetert, paradoxaal genoeg, tot een niveau vergelijkbaar met dat van gezonde, normotherme kinderen pas nadat therapeutische hypothermie gestopt wordt.

DEEL III. Arteriële lactaat bewaking

Recente studies in septische en post-cardiale chirurgie kinderen tonen dat dynamische arteriële lactaat indices betere voorspellers voor morbiditeit en mortaliteit zijn dan de gebruikelijke statische –cross-sectionele– arteriële lactaat metingen. Deze bevindingen vormden de inspiratie voor de twee studies beschreven in **Hoofdstuk 8** en **Hoofdstuk 9**. De resultaten die gepresenteerd worden in **Hoofdstuk 8** geven aan dat “tijd-gewogen lactaat” –een dynamische index die de grootte en de duur van hyperlactatemie incorporeert– een betere voorspeller is voor de noodzaak van ECMO bij CDH-patiënten dan zowel statisch lactaat als “delta lactaat” –een dynamische index die de grootte en het verschil over de tijd incorporeert. **Hoofdstuk 9** daarentegen toont dat bij ECMO-patiënten het meten van statisch arterieel lactaat de betere voorspeller is voor mortaliteit. Deze studie illustreert dat de resultaten niet gemakkelijk geëxtrapoleerd kunnen worden naar andere patiëntgroepen, vormen van behandeling, of patiënt-uitkomstmaten.

DEEL IV. Discussie

In **Hoofdstuk 10** worden de bevindingen van dit proefschrift besproken in het licht van de literatuur. Er wordt geconcludeerd dat a) de non-invasieve videomicroscopie technieken OPS en SDF gebruikt kunnen worden in kinderen, b) microcirculatoire verslechtering aanwezig is in kinderen met primair respiratoir of primair circulatoir falen, c) dat het verbeteren van macrocirculatoire parameters middels therapeutische interventie niet gepaard hoeft te gaan met microcirculatoire verbetering, en dat d) microcirculatoire verstoring gerelateerd is aan hogere morbiditeit en/of mortaliteit. Een verhoogde arteriële lactaat concentratie is eveneens gerelateerd aan een slechte patiënt uitkomst. Dynamische lactaat indices kunnen superieur zijn aan statische, cross-sectionele lactaat metingen in het voorspellen van de patiënt uitkomst, maar deze superioriteit is niet uniform voor alle kritisch zieke neonatale of pediatrie patiënt groepen.

Het toekomst perspectief is dat trials opgezet moeten worden teneinde de voorspellende waarde van microcirculatoire bewaking te substantiëren en een doeltreffende set van microcirculatoire markers te karakteriseren. Deze multimodale set zou moeten

bestaan uit onder meer microcirculatoire imaging en arterieel lactaat. Trials met multimodale microcirculatoire therapeutische eindpunten vormen uiteindelijk de echte uitdaging voor in de toekomst.

APPENDICES

Curriculum vitae

Erik Antonius Bernardus Buijs was born on March 24, 1984 in Utrecht. He grew up in Woerden, where he completed secondary school (Gymnasium) in 2002. Before starting his medical training, he attended one year of Psychology at the department of Social Sciences, Leiden University. From 2003 onwards, he started his medical training at the Erasmus MC, University Medical Center in Rotterdam. Erik combined his study with several extra-curricular activities and administrative functions, including co-founder and treasurer of the Stichting Museumnacht Leiden 2008-2009. In January 2009, he passed his medisch doctoraal examen (master's degree in medicine).

In March 2009 he commenced his PhD project by studying non-invasive microcirculatory monitoring in children at the Intensive Care, Erasmus MC-Sophia, under supervision of Prof.dr. D. Tibboel and Prof.dr. C. Ince. During this research, he also participated in projects at the department of Obstetrics and Gynecology and the department of Pediatric Surgery. In 2014, he started his internship at the Erasmus MC, University Medical Center in Rotterdam.

List of publications

1. **Buijs EA**, Danser AH, Meijer NI, Tibboel D. Cardiovascular catecholamine receptors in children: their significance in cardiac disease. *J Cardiovasc Pharmacol* 2011;58(1): 9-19.
2. Top AP, **Buijs EA**, Schouwenberg PH, van Dijk M, Tibboel D, Ince C. The microcirculation is unchanged in neonates with severe respiratory failure after the initiation of ECMO treatment. *Crit Care Res Pract* 2012;372956.
3. **Buijs EA**, Zwiers AJ, Ista E, Tibboel D, de Wildt SN. Biomarkers and clinical tools in critically ill children: are we heading toward tailored drug therapy? *Biomark Med* 2012;6(3):239-257.
4. **Buijs EA**, Verboom EM, Top AP, Andrinopoulou ER, Buysse CM, Ince C, Tibboel D. Early microcirculatory impairment during therapeutic hypothermia is associated with poor outcome in post-cardiac arrest children: a prospective observational cohort study. *Resuscitation* 2014;85(3):397-404.
5. Cornette J, Herzog E, **Buijs EA**, Duvekot JJ, Rizopoulos D, Hop WC, Tibboel D, Steegers EA. Microcirculation in women with severe pre-eclampsia and HELLP syndrome: a case-control study. *BJOG* 2014;121(3):363-370.
6. **Buijs EA**, Reiss IK, Kraemer U, Andrinopoulou ER, Zwiers AJ, Ince C, Tibboel D. Increasing mean arterial blood pressure and heart rate with catecholaminergic drugs does not improve the microcirculation in children with congenital diaphragmatic hernia: a prospective cohort study. *Pediatr Crit Care Med* 2014, **in press**.
7. **Buijs EA**, Houmes RJ, Rizopoulos D, Wildschut ED, Reiss IK, Ince C, Tibboel D. Arterial lactate for predicting mortality in children requiring extracorporeal membrane oxygenation. *Minerva Anestesiologica*, accepted for publication.
8. **Buijs EA**, Ince C, Zwiers AJ, Andrinopoulou ER, Mooij MG, Verboom EM, Houmes RJ, Wildschut ED, Reiss IK, Tibboel D. The microcirculation in children with primary respiratory disease requiring venoarterial or venovenous extracorporeal membrane oxygenation: a prospective cohort study. Submitted.
9. Cornette J, **Buijs EA**, Duvekot JJ, Herzog E, Roos-Hesselink J, Rizopoulos D, Meima ME, Steegers EA. Hemodynamic effects of intravenous nicardipine in severe pre-eclamptic women with a hypertensive crisis. Submitted.
10. van den Berg VJ, van Elteren HA, **Buijs EA**, Ince C, Tibboel D, Reiss IK, de Jonge RC. Reproducibility of microvascular vessel density assessment in Sidestream Dark Field (SDF) derived images of healthy term newborns. Submitted.
11. **Buijs EA**, Reiss IK, Kraemer U, Wildschut ED, Tibboel D. Arterial lactate as an early predictor for extracorporeal membrane oxygenation in neonates with congenital diaphragmatic hernia. Manuscript with co-authors.

PhD portfolio

Summary of PhD training and teaching

Name PhD student: E.A.B. Buijs
 Erasmus MC department: Intensive Care, Dept. of Pediatric Surgery
 Research School: COEUR
 PhD period: 2009-2014
 Promoters: Prof.dr. D. Tibboel, Prof.dr. C. Ince
 Co-promoters: -

1. PHD TRAINING	WORKLOAD	
	YEAR	ECTS
GENERAL COURSES		
- BROK (Basiscursus Regelgeving Klinisch Onderzoek) Erasmus University Rotterdam	2009	1.0
- Systematisch Literatuuronderzoek in Pubmed	2009	0.3
- Systematisch Literatuuronderzoek in andere databases	2009	0.3
- Classical Methods for Data-analysis (CC02)	2009	5.7
- Biomedical Writing Course	2010	4.0
- Introduction Course on Statistics & Survival Analysis for MD's	2010	0.4
- Cursus Integriteit in Wetenschappelijk Onderzoek	2012	1.5
SPECIFIC COURSES		
- Video Microscopy Training in AMC and MCL	2009	0.6
- PhD Training Course Vascular Biology	2010	3.0
- Principles of Clinical Pharmacology (NIH webconference course)	2010-2011	2.0
- Business Intelligence Center Data Retrieval Training	2011	0.3
- COEUR post-graduate course Cardiovascular Medicine	2011	1.5
- Repeated Measurements (CE08)	2012	1.4
- Courses for the Quantitative Researchers (EP17)	2012	1.4
- COEUR post-graduate course Arrhythmia Research Methodology	2012	1.5
SEMINARS AND WORKSHOPS		
- Minisymposium Let it Flow	2009	0.3
- COEUR PhD Day in Leiden	2011	0.3
- Presenting Skills for junior researchers 2nd series	2012	1.0

NATIONAL CONFERENCES

- Nederlandse Intensivisten dagen 2013 (1x poster), Ede	2009	0.9
- Erasmus Critical Care Days (1x invited oral)	2013	0.6
- WES Rotterdam Symposium (1x invited oral)	2014	0.6

INTERNATIONAL CONFERENCES

- Functional Hemodynamics and Fluid Therapy (1x poster) Istanbul, Turkey	2011	0.6
- 24 th annual meeting of ESPNIC (3x oral) Rotterdam, the Netherlands	2013	0.9

OTHER

- Pharmacology Research Meeting (weekly) (multiple oral presentations)	2009-2013	2
- Pharmacology Days, Erasmus MC-Sophia, Intensive Care and Dept. of Pediatric Surgery (annually) (1x oral)	2010-2013	0.3
- Secretary Pharmacology Research Meeting	2010-2011	0.3
- Secretary Principles of Clinical Pharmacology (NIH webconference course)	2010-2011	0.3
- F1000 evaluations (n=16)	2011-2013	2

COMMITTEES

- F1000 Associate Faculty Member	2011-2013	-
- ESPNIC Neonatal and Pediatric Microcirculatory Research Working Group	2013	-

2. TEACHING

SUPERVISING MEDICAL STUDENT MASTER'S THESIS

- E. van der Kooij	2010	0.8
- E. Herzog	2010	0.8
- DME de Snoo	2011	0.8
- EM Verboom	2012	0.8

TRAINING RESEARCHERS

- Methods for microcirculatory data-analysis (multiple researchers)	2009-2013	0.3
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MEDICAL PERSONAL

- Research presentations, e.g. on progress or results (multiple orals)	2009-2013	0.3
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ECTS: European Credit Transfer and Accumulation System
1 ECTS represents 28 hours

Dankwoord

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