NEONATAL CARDIAC SURGERY: PREVENTING COLLATERAL DAMAGE

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Preventie van bijkomende schade
(met een samenvatting in het Nederlands)

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ABBREVIATIONS

aEEG amplitude-integrated electro-encephalography
ACP antegrade cerebral perfusion
ASD atrial septal defect
AUC area under the curve
BT Blalock-Taussig
CHD congenital heart disease
CNV continuous normal voltage
CoA coarctation of the aortic arch
CPB cardiopulmonary bypass
CPR cardiopulmonary resuscitation
DHCA deep hypothermic circulatory arrest
DWI diffusion-weighted imaging (on MRI)
HLHS hypoplastic left heart syndrome
IAA interrupted aortic arch
IL interleukin
ICU intensive care unit
LCOS low cardiac output syndrome
LOS length of stay
MDI mental development index
MRI magnetic resonance imaging
NIRS near-infrared spectroscopy
NSE neuron-specific enolase
PDI psychomotor development index
PICU pediatric intensive care unit
RCT randomized controlled trial
SIRS systemic inflammatory response syndrome
SSI surgical site infection
SWC sleep-wake cycling
TGA transposition of the great arteries
TMS total maturational score
UBC University of British Columbia, Vancouver, Canada
UCSF University of California, San Francisco, USA
VSD ventricular septal defect
WMI white matter injury

1

GENERAL INTRODUCTION

Congenital heart disease (CHD) is the most common congenital disorder in newborns, with a prevalence of 6-13 per 1000 live births[1]. In the Netherlands, this approximates 1250 cases per year[2]. One-third of all cases of CHD need an intervention in the first year of life[3]. Since the first pediatric cardiac surgery in 1938, major advancements in antenatal detection, surgical possibilities and improvements in intensive care, have resulted in a current survival to adulthood of over 85%[4]. With atrial and/ or ventricular septal defects on one side of the CHD spectrum, the other end is comprised of the more complex anomalies. Examples are transposition of the great arteries (TGA) and hypoplastic left heart syndrome (HLHS).

Especially the development of cardiopulmonary bypass (CPB) has contributed to the surgical possibilities to repair even the most complex types of CHD, as it enables the surgeon to perform surgery on a non-beating heart (see **Figure 1**). However, specific defects such as obstructions in the aortic arch, call for further refinements of CPB techniques. As normal CPB necessitates the placement of the arterial cannula in the aortic arch, this is not feasible during surgery on the arch itself. Therefore, deep hypothermic circulatory arrest was developed in the 1960's, in which cooling to 18-20 °C was performed using CPB. This way, basal metabolism was kept at a minimum, which provided a time window period of approximately one hour during which the CPB cannulae could be removed from the surgical field, the aortic arch repaired, and subsequently the patient was rewarmed using CPB. This technique made the palliation of the most severe type of aortic arch obstruction, namely HLHS, a feasible option, and the procedure was first performed using DHCA by Norwood in 1983[5].

Although these procedures are often life-saving, and optimal cardiac repair remains the primary goal, the surgery itself invariably has an impact on other organ systems, of which the brain is the most important example. Neonates are at the greatest risk of cerebral injury, due to the immaturity of the brain, the often complex cardiac anomalies, and subsequently the extensive surgery they need.

During childhood, 30-50% of those who once underwent complex cardiac surgery as a neonate, encounter problems in different domains, varying from motor problems

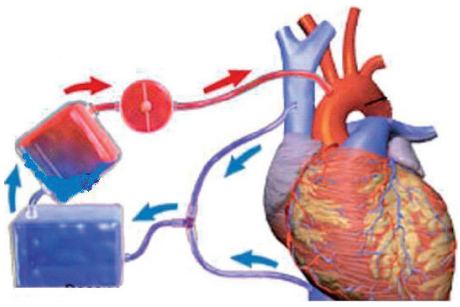

Figure 1. In full-body CPB, oxygen-rich perfusate is pumped into ascending aorta, providing systemic perfusion. The desaturated perfusate is subsequently drained from the superior and inferior venae cavae and transported back to the CPB machine.

to behavioral problems or problems with executive functioning[6-8]. If nowadays already 4 in 1000 adults live with a once repaired CHD, with the ever increasing surgical possibilities, this number is likely to go up. This makes it of paramount importance to prevent collateral damage from occurring in early life.

PART I: CEREBRAL INJURY IN NEONATAL AORTIC ARCH OBSTRUCTIONS

Cerebral injury occurs both due to modifiable factors (such as the type of surgery or choices made during on the intensive care unit), and non-modifiable factors. The latter includes the socio-economic background of the family, and the genetic background. Especially in this subgroup of CHD, this is important as the cardiac deformities may present as part of a genetic syndrome, such as 22q11 deletion, which in itself carries a varying risk of neurodevelopmental delay[9].

In this thesis, we discuss the modifiable factors which may influence the burden of cerebral injury. Due to the complexity of potential risk factors, these are separated into pre, intra- and postoperative time periods. Most of the knowledge on when, which factors play a role, is based on studies using pre- and postoperative MRI, but also studies have used (early) neurodevelopment as outcome.

We focus on neonates specifically with aortic arch obstructions, as this is the group with the highest rates of cerebral injury and delayed neurodevelopment. Diagnoses include HLHS and its milder variants, and other defects presenting with a hypoplastic aortic arch, such as interruption of the aortic arch and coarctation.

Pre-operative injury

It has become clear that already during fetal life, there is an increased susceptibility for injury. Thanks to prenatal screening programs which identify cases of congenital heart disease, fetal MRI performed in research settings have been able to show an abnormal intra-uterine brain development. Specifically, in cases of HLHS and transposition of the great arteries, reduced cerebral blood flow, restricted brain volume and evidence of hypoxia (seen as lactate on MR spectroscopy) have been observed (see **Figure 2**)[10, 11]. This is likely the basis for the immature aspect of the brain in these cases of CHD[12].

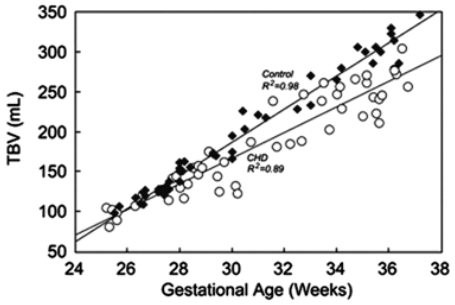

Figure 2. The increase in total brain volume (TBV) in fetusses with TGA and HLHS (open circles) is restricted compared to those of fetuses without CHD (black diamonds). These measurements were performed with fetal MRI (from Limperopoulos et al, 2010 [10].

Morphological features of the brain such as depth of sulci and operculisation show more resemblance to the premature than the term brain.

Also, just as in premature neonates, white matter injury (WMI) is the most common type of injury. It has been reported in 5-27% of neonates before surgery[13-16] (see **Figure 3** for an example). In premature neonates, WMI is thought to manifest itself due to increased vulnerability of the immature oligodendrocytes to hypoxia, hypoperfusion and inflammation[17]. From what we know, this is no different in neonates with CHD[18]. Indeed, the more immature the brain appears, the more likely it is to see WMI[19, 20]. Suboptimal cerebral perfusion is also likely to play a role; as patients with a diminished perfusion seen by MRI have more WMI. This translates into the fact that cardiopulmonary resuscitation and less severe markers of hemodynamic instability such as a high lactate value, are associated with an increased risk for WMI[15, 19]. The widespread implementation of prenatal screening for CHD is hoped to have a protective effect in this respect, ultimately resulting in a more stable situation after birth and hence a lower risk of WMI.

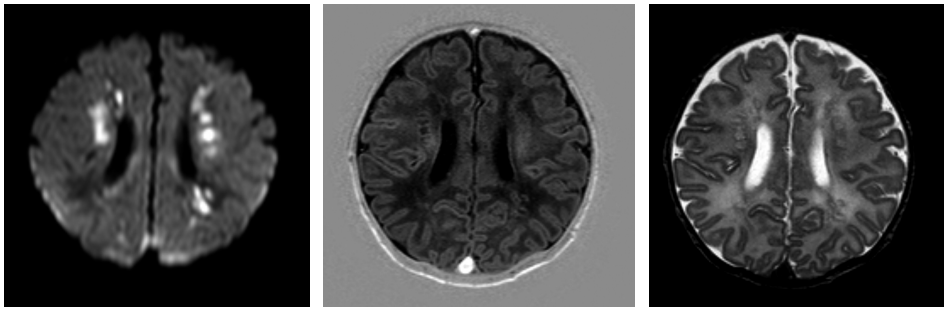

Figure 3. An example of severe WMI in a patient with an aortic arch obstruction. Left:diffusion weighted imaging (DWI) shows areas of restricted diffusion in white, which suggest recent injury; middle: T1; right: T2 imaging.

The second most common type of injury is focal infarction, usually referred to as stroke, which is seen in 5-23% of patients with transposition of the great arteries (TGA) or HLHS-type deformities (see **Figure 4**). Clinical risk factors for stroke have been studied most extensively in patients with transposition of the great arteries, where the need for balloon atrioseptostomy (BAS) has been shown to show a strong association[21, 22]. However, the debate on whether this is due to the embolic risk associated with TGA, or the duration and depth of the hypoxia preceding the BAS, highlights the gap in knowledge of the exact mechanisms. In aortic arch obstructions, BAS is rarely necessary. Risk factors have not been established in this group. One difficulty in unraveling the etiology of stroke versus that of WMI is the fact that different centres currently use different definitions to separate the two types of cerebral injury[14].

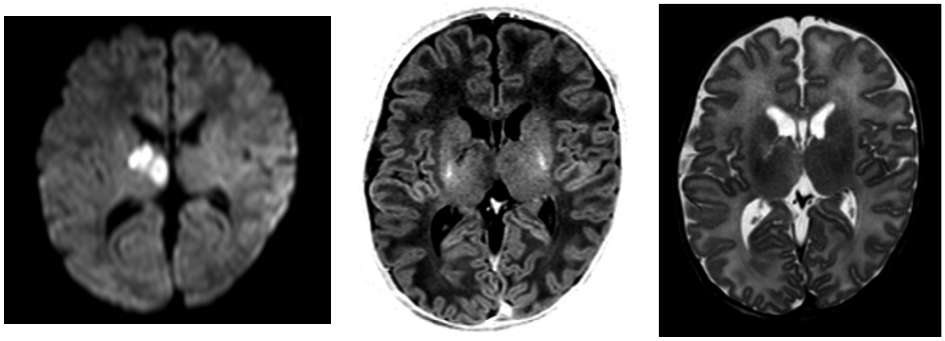

Figure 4. Stroke in the right thalamus and part of the internal capsule, much more apparent on DWI (left image, lesions in white) than on T1 (middle) or T2 (right).

Intra-operative injury

Cardiac surgery has traditionally been viewed as the most perilous for the brain. There are many potential dangers encompassed in these often complex procedures. Firstly, the use of anesthesia is an area currently under large investigation, as there have been reports of neurotoxicity, especially to the developing brain[23]. The cardiac repair itself can cause tissue or vascular particles to dislodge and embolize into the cerebral circulation. Also, the manipulation of the heart and vessels and complications during CPB can result in significant variations of blood pressure and thereby fluctuations in cerebral perfusion. However, most attention goes out to the risks associated with the use of CPB. A number of aspects which may contribute to the risk of cerebral injury include the following:

1. Even though there are filters in the arterial line of the CPB, air emboli or micro-particles (from tissue or thrombi) can still enter the circulation and obstruct cerebral perfusion[24-29].
2. The surgery itself, as well as the contact of blood with the foreign material of the CPB tubing initiates systemic inflammation which may increase the risk for cerebral injury, especially WMI[17, 30-33].
3. There is always some hemodilution while on CPB, which may result in less oxygenative capacity and therefore may lead to hypoxic injury[34]. Although it has been established that a hematocrit of 20% results in an adverse neurodevelopment compared to 30%, the exact threshold is unknown[35].
4. Cooling during CPB, especially to deep hypothermia, has been shown to increase cerebral vascular resistance and thereby diminish cerebral perfusion. This is thought to remain an issue throughout rewarming to normothermia[36, 37].

Specifically during arch repair, as full-body CPB is not a feasible option, DHCA may be used during the procedure. Although the hypothermia lowers the metabolic rate of the brain as a protection from the ischemic insult, the use of DHCA has still been shown to result in cerebral apoptosis in animal models[38-40]. After pediatric cardiac surgery, a higher risk of neurodevelopmental sequelae is seen after procedures including DHCA[40-42]. However, often the use of DHCA also imply marks the category

of patients undergoing the most complex procedures. In the landmark trial of the Boston Circulatory Arrest Study, DHCA was compared to a low-flow (but full-body) CPB strategy. Although initially the DHCA group fared worse in terms of especially motor outcome, later follow-up of these children (who are now adolescents), failed to reveal a meaningful differences[8, 43]. However, the first conclusions of this trial did indicate that especially DHCA duration longer than 41 minutes, increased the risk of neurological damage. This supported the existing notion that DHCA should be kept as short as possible, or at least under 60 minutes.

As an alternative to DHCA, antegrade cerebral perfusion (ACP) was developed more recently, in the 1980's. This involves the advancement of the arterial CPB cannula into the brachiocephalic artery, by which the brain is perfused at a flow of approximately 25% of full-flow CPB flow (see **Figure 5**). Providing there is a patent Circle of Willis, all regions of the brain should be perfused in this manner[44]. However, this selective perfusion of the brain has the risk of air emboli or micro-particles directly entering the cerebral circulation[28]. Also, the increased exposure to the CPB circuit induces more inflammation, which may have an adverse effect on cerebral injury[45].

Although animal models show less cerebral apoptosis after ACP than DHCA, in humans, no differences have been found[40]. A randomized controlled trial (RCT) comparing DHCA to ACP in neonates undergoing the Norwood procedure for HLHS, assessed neurodevelopment at 1 year of age and found similar results in both groups, although especially motor outcome was delayed in both groups[46]. A study in adults undergoing carotid endarterectomy also failed to find a difference between the two perfusion methods [47]. However, both trials used neurocognitive testing as outcome variable, which may not reveal subtle differences in cerebral injury, especially when small groups are used. In addition, testing is always performed months to years after the procedure, giving postoperative complications and subsequent surgeries a chance to obscure results. Hence, the superiority of either DHCA or ACP was unresolved.

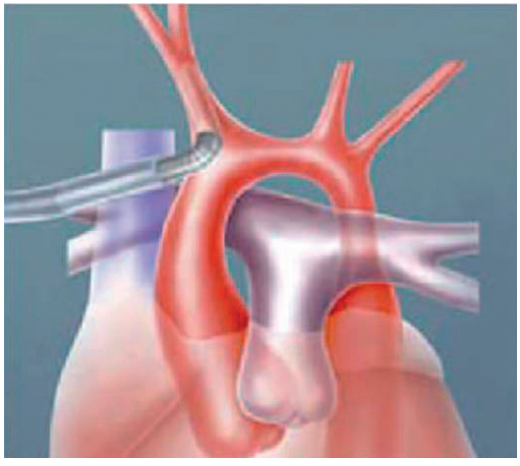

Figure 5. Selective antegrade cerebral perfusion (ACP) during aortic arch surgery, performed by advancement of the arterial CPB cannula into the brachiocephalic artery.

Postoperative injury

When surgery has been successful, postoperative recovery on the intensive care unit starts and the newly repaired heart must prove itself worthy. Most postoperative complications occur during the first 24 hours after surgery; i.e. bleeding, tamponnade or arrhythmias[48]. This often causes a (temporarily) lower cardiac output and thereby compromised cerebral perfusion. Indeed, a lower blood pressure postoperatively has been observed to result in more WMI[49]. After the first day, cardiac function and circulation are generally more stable, and the greatest danger will have passed. However, especially after the Norwood procedure for HLHS, recovery on the intensive care unit can be as long as weeks. This is mostly due to the univentricular physiology, where a new balance between pulmonary and systemic blood flow must be found, which and can prove a difficult task.

After all cardiac surgery, but especially when shunts or other artificial material are implanted, there is an increased risk for thrombosis, which can have cerebral consequences. This is also due to the hypercoagulative state by activation by the CPB, which is possible exaggerated in neonates due to the limited capacity to counteract these mechanisms[25, 50].

Also, especially with increasing postoperative recovery and more indwelling material, the risk of postoperative infections increases. This has been shown as a risk factor for WMI postoperatively, likely via systemic inflammation[51].

Furthermore, there may be more factors than those named here. The fact that the duration of recovery is associated with later neurodevelopment warrants the search for further risk factors on the intensive care unit. Due to the many variables which likely play a role, and the complex hemodynamic interactions, teasing out the exact mechanisms is a challenge. Currently literature is lacking a study which has simultaneously measured all potentially important physiological parameters which may have an effect (i.e. blood pressure, carbon dioxide pressure, oxygen saturation, temperature) and has assessed the differential effects on cerebral injury.

Cerebral monitoring at the bed side

It is clear that there are many periods before, during and after surgery which are potentially dangerous for the brain. Hence, there is increasing attention for brain monitoring at the bed-side during these complex procedures.

Amplitude-integrated encephalography (aEEG) is valuable monitoring device which can continuously record cerebral activity for days on end (see **Figure 6**, top monitor in the picture). It is an adaptation of the ('normal') raw EEG, but condensed in time and with 2 channels instead of 16 in the standard EEG. Apart from displaying the basal activity of the brain, it can alert the physician of subclinical seizures, which is especially important in heavily sedated neonates. Developed and validated in the full-term infant who suffered from perinatal asphyxia, it was proven to reliably assess function real-time and also provide a reliable prediction of neurological outcome [52]. During the cardiac procedures described here, it may be also be used to monitor the electrographic response of the brain to cooling the deep hypothermia, which results

in a virtually absent electrical activity. The activity level can subsequently be seen to normalize during rewarming back to normothermia (see **Figure 7**).

Near-infrared spectroscopy (NIRS), another method of cerebral monitoring, provides insight into cerebral oxygenation and is used routinely during surgery in most centres nowadays (see **Figure 6**, bottom monitor). The NIRS electrode is often placed fronto-

Figure 6. Peri-operative cerebral monitoring at the bed-side. The top monitor displays the aEEG, the bottom monitor shows cerebral and somatic NIRS values.

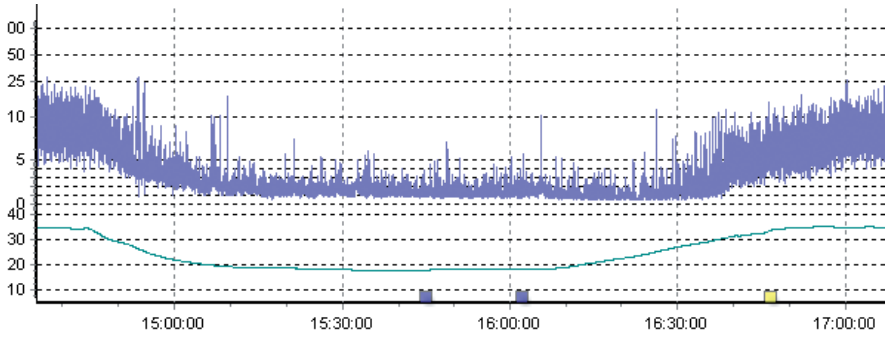

Figure 7. Registration of nasopharyngeal temperature (below) and aEEG (top) during cardiac surgery with deep hypothermia. When temperature decreases to approximately 18°C, aEEG activity is at a minimum, showing a burst suppression pattern. During rewarming, normal aEEG activity re-appears.

parietally, where it provides an estimation of the oxygenated haemoglobin in mostly the venous capillary bed of the cerebral parenchyma, 2-3 cm below the electrode (Figure 8). NIRS can provide a warning sign in the event of sudden compromise in cerebral blood flow, which is useful during these complex cardiac surgeries[53]. Also, as the change in NIRS correlates with central venous oxygen saturation, NIRS can also be used to monitor cardiac output during surgery and while on the intensive care unit[54, 55]. This makes NIRS an ideal monitor to indicate whether cerebral perfusion is sufficient and is hoped to aid in the prevention of hypoperfusion injury, such as WMI[15, 56].

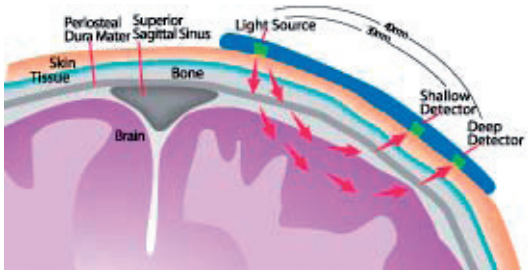

Figure 8. The principle of NIRS. Two wavelengths of near-infrared light are emitted from the electrode, and the difference between the two received signals represents the cerebral tissue oxygenation.

Finally, serum-based biomarkers have the potential to provide the clinician with an indication of the extent of cerebral injury. Markers such as 100b and neuron-specific enolase (NSE) are released into the circulation in case of neuronal or astroglial injury. Studies validating these markers, of which s100b and NSE are best-known, are appearing for neonates after perinatal asphyxia, where both a relationship with injury on MRI and later neurodevelopment has been seen. In adults with stroke, the same relationship has been reported. However, the many other sources of these proteins other than the brain may limit their value in cardiac surgery.

Imaging modalities

Ultrasound

In the first reports of cerebral injury in cases of CHD, cranial ultrasound was used to objectify the lesions. Incidences of abnormalities before surgery ranged from 3% to 59%, and most lesions were seen in patients with HLHS or other aortic arch obstructions[57-60]. Types of abnormalities included increased ventricular volumes, and infarctions in the central regions of the brain. However, it is known that ultrasound quality is heavily influenced by the experience of the involved technician or physician. Also, keeping the different degrees of WMI in mind, it is known from the premature population that only the more severe degrees of WMI are readily recognized on ultrasound, as opposed to milder injury[61]. Focal infarctions may also require repeated ultrasounds in the first

postnatal weeks, to become apparent and smaller cortical infarct are also likely to be missed. Indeed, a recent report shows that although much cheaper and less fraught with logistical issues, a single ultrasound pre-operatively unfortunately lacks sensitivity and specificity to identify cerebral injury, compared to MRI[59].

Magnetic resonance imaging

Due to the large amount of detail of cerebral structures, MRI has provided most information on the exact cerebral injury in cases of CHD. It is currently the gold standard in cerebral imaging and is used to validate other modalities. The traditional sequences, T1 and T2, reveal the size and location of the abnormalities in great detail. A worthwhile addition is diffusion weighted imaging (DWI), which allows us to visualize cytotoxic edema within hours after the insult. An increased signal intensity is present for about a week after the insult, following which pseudonormalisation will occur[62]. So, DWI is of importance to direct thoughts on timing of the injury.

In total, literature states that between 19-40% of infants show some kind of injury pre-operatively[13, 15, 20]. After surgery, most postoperative MRI scans of the brain are performed approximately one week after surgery, when patients are stable enough to be transported to the MRI suite. Thirty-four to 72% of complex cardiac surgeries (HLHS-related or TGA) will have new lesions, most again being WMI (23-47%) or strokes (4-38%)[13, 14, 20, 49, 63]. Incidences between studies vary widely, which is likely to be caused in part by different radiologic classifications[64]. As noted previously, although there is a general consensus that WMI is generally thought to be due to hypoperfusion, and stroke is of an embolic nature, there is currently no consensus in literature on how to radiologically differentiate between the two. This importantly limits the identification of risk factors for both types of injury.

PART II: INFLAMMATION AND SOMATIC EFFECTS OF PERFUSION TECHNIQUES

There are also side-effects of cardiac surgery with the use of CPB for other organ systems than the brain. These vary from systemic inflammatory response, with edema and pulmonary consequences, to hematological effects, acute kidney failure, and finally multiple organ failure. All these complications can delay postoperative recovery and consequently prolong ICU and hospital stay. Of note, these 'somatic' complications can also affect the brain, via hypoperfusion, hypoxia, or inflammatory or thrombotic effects. Indeed, the length of postoperative intensive care stay is closely related to later neurodevelopment[65].

Postoperative infections

Surgery with the use of CPB inevitably causes systemic inflammation. Both the contact of blood with foreign material of the CPB apparatus and tissue damage by the surgery itself contribute to the systemic inflammatory response syndrome (SIRS)[66]. This is characterized by a cytokine storm produced by both innate and adaptive cells of the immune system, the endothelium and damaged or ischemic tissues. Pro- and anti-inflammatory cytokines are both abundant after surgery (see **Figure 9**). Clinically, the

SIRS is most apparent by an elevated body temperature and the accumulation of fluid in the interstitium due to capillary leak, not sparing the pulmonary bed and carrying the risk of acute respiratory distress syndrome. Fortunately, this overwhelming inflammation is usually self-limiting. On the other hand, it is considered to result in a 'hypo-inflammatory' phase, characterized by de-activated immune cells, which predisposes to infections[67].

Of note, there are indications that these hyper- and hypo-inflammatory phases are not sequential, but simultaneous. Whichever the case, the hypo-inflammation seems most clinically relevant in the current surgical era, resulting in postoperative

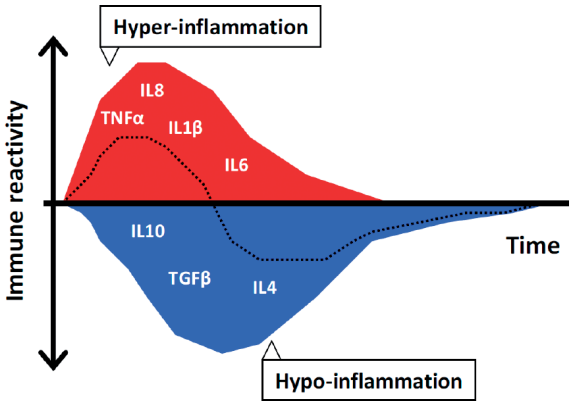

Figure 9. Cardiac surgery with CPB initiates a systemic inflammatory response, characterized by an abundance of both pro- and anti-inflammatory cytokines. From: Thesis Alvin Schadenberg, 2013.

infections in as many as 8-30% of children[68-71]. This suggests that there are risk factors for infections which need to be dealt with, or at least a high-risk population which needs identification in order to lower the incidence of these infections, as these can importantly complicate postoperative recovery[72].

Abdominal injury

The use of CPB, and especially DHCA, is thought to result in substantial abdominal organ injury, assumed to be both due to hypoperfusion during or after CPB, as well as the systemic inflammation[73, 74]. Fortunately, severe damage such as end-stage renal failure or necrotizing enterocolitis is rare. However, regarding renal injury, virtually all neonates undergoing cardiac surgery have increased creatinine values in the days following surgery and diuretics are frequently prophylactically administered [75]. Gastro-intestinal problems are often seen as difficulties in initiating oral feeding after surgery[76].

As DHCA inevitably results in full-body circulatory arrest, despite the lower temperature, more renal dysfunction has been observed after adult cardiac procedures with DHCA, compared to ACP[77]. It is suggested that ACP may provide partial perfusion of the abdominal region by flow through thoracic collaterals. Indeed, during surgery with ACP, some backflow of blood can be observed in the surgical field, coming from the descending aorta. Also, studies have shown a higher abdominal NIRS value when

ACP flow is increased [78] (**Figure 10**). However, it is unknown whether ACP indeed results in less injury to the abdominal and perhaps also thoracic organs, and whether this impacts postoperative recovery.

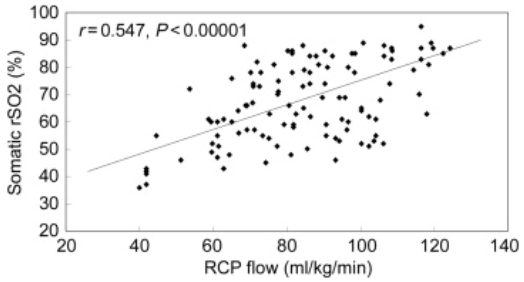

Figure 10. As ACP-flow (named 'RCP' here) is increased, the somatic NIRS, measured in the renal region, also increases. From: Miyaji et al, 2010 [77].

Seeing as the types of CHD which necessitate either the use of DHCA or ACP during surgery are also often the most complex defects (i.e., HLHS), with the longest postoperative recovery, all efforts to minimize complications after surgery are worthwhile. As the heart must recover and adapt to the new hemodynamic situation, the risk of disturbing this precarious situation should be kept as low as possible.

OUTLINE OF THE THESIS

The first part of this thesis focuses on the various effects of complex congenital heart diseases, specifically aortic arch obstructions, on the brain. In chronological order (Chapters 2-5) the pre-, intra- and postoperative aspects are discussed. In Chapter 2 we compare the incidence of pre-operative brain injury between a European and two North-American centres, highlighting the role of prenatal diagnosis. In Chapter 3, in a randomized controlled trial, we compare the effect of DHCA versus ACP during aortic arch surgery, with regard to new postoperative cerebral injury. Chapter 4 highlights the importance of a complete Circle of Willis for optimal cerebral perfusion during ACP, and after the Norwood procedure. Chapter 5 discusses the role of bed-side cerebral monitoring (aEEG, NIRS and biomarkers) in risk assessment for adverse neurological outcome during and after surgery.

The second part of this thesis focuses on the effects of cardiac surgery with CPB on systemic inflammation and somatic organs. Chapter 6 reveals the immediate effect of cerebral ischemia during cardiac surgery on inflammatory markers in the cerebral circulation. In Chapter 7 we describe the incidence of postoperative infections after pediatric cardiac surgery. To identify those patients at the highest risk for infection, a simple bed-side prediction rule was developed. In Chapter 8, the somatic effects of DHCA versus ACP are investigated. Is there partial perfusion of abdominal organs during ACP, and can this protect from organ injury? Subsequently, in Chapter 9, the clinical postoperative recovery after DHCA and ACP on the ICU are compared.

In Chapter 10, the above named articles are discussed in light of the current literature. Chapter 11 provides a summary of the most important findings in this thesis.

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PART I:
CEREBRAL INJURY IN NEONATAL AORTIC ARCH OBSTRUCTIONS

2

MINIMIZING THE RISK OF PRE-OPERATIVE BRAIN INJURY IN NEONATES WITH AORTIC ARCH OBSTRUCTION

Submitted

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ABSTRACT

Objective

Neonates with aortic arch obstruction are at high risk of brain injury. Prenatal diagnosis of arch obstruction improves preoperative hemodynamic stability. To determine if prenatal diagnosis also lowers the risk of pre-operative brain injury, we assessed differences in the incidence of preoperative brain injury across three centres.

Study design

From two prospective cohorts of newborns with complex congenital heart disease studied with pre-operative cerebral magnetic resonance imaging(MRI) (University Medical Center Utrecht, the Netherlands; and a combined cohort from University of California, San Francisco and University of British Colombia, Vancouver), patients with aortic arch obstruction were selected and their imaging and clinical course reviewed.

Results

Birth characteristics were comparable between Utrecht (n=33) and UCSF/UBC (n=54) cohorts. All patients had a hypoplastic aortic arch with either a coarctation/interruption or hypoplastic left heart syndrome. In subjects with a prenatal diagnosis, a significant difference in the incidence of white matter injury (WMI) between centres was demonstrated (Utrecht 11/22 [50%] vs. UCSF/UBC 4/30 [13%]; $p<0.01$). In the UCSF/UBC cohort, prenatal diagnosis was significantly protective for WMI (13% vs. 50% [prenatal vs. postnatal diagnosis]; $p<0.01$), but not in Utrecht (50% vs. 46%; $p>0.99$). Differences in clinical practice between prenatally diagnosed subjects at Utrecht versus UCSF/UBC included older age at surgery (median 9 vs. 6 days [$p<0.01$]), less time spent on the ICU (25% vs. 100% [$p<0.01$]), more diuretics (59% vs. 17% [$p<0.01$]), less total parenteral nutrition (9% vs. 60% [$p<0.01$]) and more infections (23% vs. 0% [$p=0.01$]). In patients diagnosed postnatally, incidence of WMI was similar between centres (46% vs. 50% [$p>0.99$]). Stroke incidence was similar between centres regardless of prenatal diagnosis (prenatal diagnosis: 4.5% vs. 6.7% [$p=0.75$]; postnatal diagnosis: 9.1% vs. 13% [$p>0.99$]).

Conclusion

Prenatal diagnosis can be protective for WMI, but protection may be dependent upon specific clinical management practices that differ across centres.

BACKGROUND

Neonates with complex congenital heart disease (CHD) are at high risk for cerebral injury. Newborns with aortic arch obstruction, particularly those with single ventricle physiology, have some of the highest rates of injury[1]. At school age, approximately one-third of these children go on to manifest problems varying from motor problems to difficulties in executive functions[2, 3]. Imaging studies have revealed that pre-operatively, 28-43% have evidence of injury as seen on cranial MRI, with this percentage increasing to 34-72% after surgery[4-7]. Most lesions seen on MRI are white matter injury (WMI) and a small proportion are strokes.

Brain injuries occur as the cumulative result of altered circulation during fetal, pre-, intra- and postoperative periods, and involve many different risk factors, including clinical management practices such as balloon atrial septostomy and timing of surgery[8, 9]. A surprising amount of clinical practice pattern variability exists across major pediatric congenital heart surgery programs. In the recent Single Ventricle Reconstruction trial testing the effects of different Norwood shunt types among North-American centers, there was significant variation in rates of common clinical practices including prenatal diagnosis (55-85%), preoperative intubation (29-91%) and enteral feeding (1-100%)[10].

Prenatal diagnosis in particular affects clinical practice, allowing for planned delivery and perinatal management in a tertiary care centre. Changes in clinical care afforded by prenatal diagnosis have been postulated to influence both surgical and neurodevelopmental outcomes[11, 12]. Particularly for newborns with aortic arch obstruction, prenatal diagnosis allows for early initiation of prostaglandin E2 to maintain ductal patency and results in improved preoperative clinical status[13]. Surgical mortality does not seem to have been affected. For newborns with transposition of the great arteries (TGA), prenatal diagnosis did not improve early neurodevelopmental outcomes[14]. At school age however, although IQ, language and memory was normal in both groups, children with postnatally diagnosed TGA had higher prevalence of neurocognitive deficits and worse executive function[15]. The effect of prenatal diagnosis on risk of brain injury has not been reported.

In this study, we focused on rates of pre-operative brain injury in neonates with aortic arch obstruction, as they relate to presence of prenatal diagnosis and clinical practice differences across centres.

METHODS

Data from 2 prospective cohorts at three centers performing MRI scans before and after neonatal cardiac surgery were used: the University Medical Center Utrecht (UMC Utrecht, the Netherlands), and a longstanding collaboration of the University of California, San Francisco (UCSF) and the University of British Columbia (UBC, Vancouver, Canada)[1, 7, 16]. UCSF and UBC were treated as one centre owing to the smaller sample size of patients with arch obstruction at UBC (n=10 and after initial comparisons showed similarity in peri-operative management (see Supplementary

Table 1). Informed consent was obtained from all participating parents and from the institutional medical ethical boards.

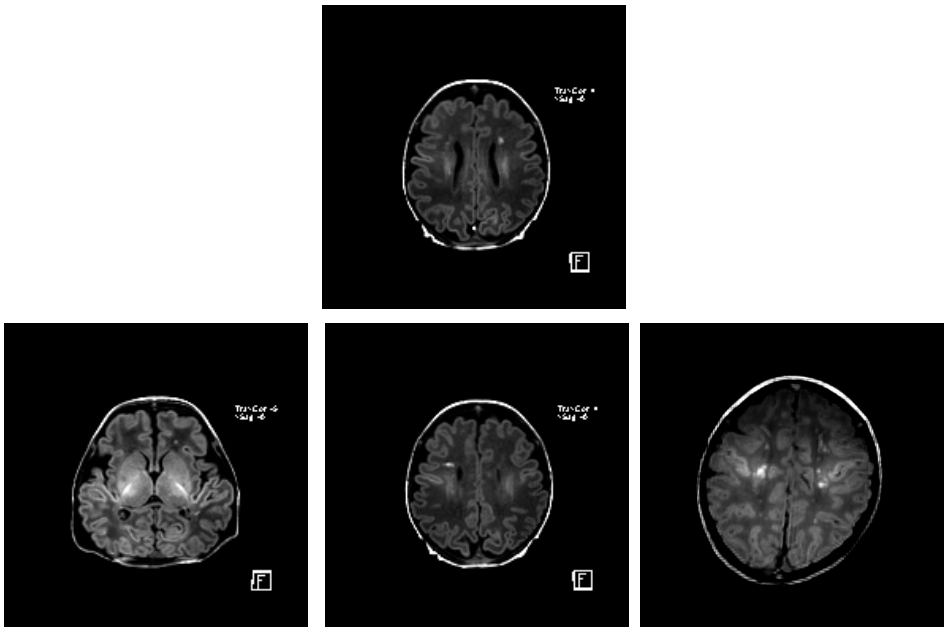
Patient data and MRI analysis

For this study, the pre-operative scans of all enrolled neonates with aortic arch obstruction were used. The three centres had comparable MRI protocols resulting in a similar sensitivity to identify abnormalities.

At Utrecht, MRI was performed on a 1.5-Tesla scanner (Philips Medical Systems, Best, the Netherlands). MR imaging included 2-mm-thick sagittal T1-, transverse T2-, and inversion recovery-weighted sequences. An echo-planar imaging technique was used for diffusion weighted imaging (DWI) (repetition time msec/echo time msec = 3800-5200/89), with a 180 x 180-mm field of view, 4-mm-thick sections, 0-mm section gap, and *b* factors of 0 and 1000 sec/mm^2 (1.5-T). At UCSF, a 1.5-tesla Signa Echo-Speed system (GE Medical Systems, Waukesha, Wis) was used. Imaging included and included (TR/TE/FieldOfView/SliceThickness/Gap): (1) T1-weighted sagittal spin echo images (600/8/20 cm/3 mm/1 mm), (2) dual-echo T2-weighted spin echo (3000/60/120/8 3 13.5 cm/4 mm/2mm), (3) coronal volumetric 3-dimensional gradient echo images with radiofrequency spoiling images (36/3.5/22 cm/1 mm/0), and (4) average diffusivity maps echo-planar acquisition (8000/150/36 3 27 cm/5 mm/0). At UBC, MRI studies were carried out on a Siemens 1.5-tesla Avanto system using VB 13A software and included: (1) 3-dimensional coronal volumetric T1-weighted images (36/9.2/200 mm/1 mm/0) and (2) axial fast spin echo T2-weighted images (4610/107/160 mm/4 mm/0.2 mm). Average diffusivity maps were generated from diffusion tensor imaging acquired with a multirepetition, single-shot echo planar sequence with 12 gradient directions (4900/104/ 160 mm/3 mm/0), b ¼ 0, 600 and 700 s/mm2, and an in-plane resolution of 1.3 mm.

Scans were assessed for evidence of stroke and WMI by a single reviewer (K.P.), using conventional T1 and T2 and DWI. White matter injury was scored as previously defined[17] and as depicted in Figure 1: mild WMI was defined as no more than 3 lesions each no larger than 2mm; moderate by 3 or more lesions or areas larger than 2mm; severe WMI if approximately more than 5% of the hemisphere was involved.

Clinical data were collected by retrospective chart review. All daily physician's progress notes and transfer notes were used as well as all available laboratory data. The presence of low cardiac output syndrome (LCOS) was defined either by cardiopulmonary resuscitation, or 3 or more of the following variables: clinical signs of LCOS such as tachycardia, cool extremities, poor pulses or oliguria; laboratory data showing an increased base deficit >3 mEq/L or a lactate >3 mmol/l; or an intervention such as administration of inotropes, high-dose prostaglandin or HCO3-[18]. LCOS was only scored for patients with sufficient clinical records to establish the diagnosis. Infections were assessed as previously defined[16]. All clinical and imaging data were analysed separately by category of prenatal diagnosis.



Mild WMI
(≤3 lesions, each ≤2mm)

Moderate WMI
(>3 areas of abnormality
or areas >2 mm)

Severe WMI
(>5% of hemisphere involved)

Figure 1. Classification of mild, moderate and severe WMI.

Statistics

For comparison of binary variables between groups, Fisher's exact test was used. For comparison of continuous variables, the Mann-Whitney U test was used. Given the sample size, univariate analyses were performed. SPSS version 15 was used for all analyses.

RESULTS

Eighty-eight cases with aortic arch obstruction were available for the study; 33 from Utrecht and 54 from UCSF/UBC (of which 44 from UCSF and 10 from UBC). Of the prenatally diagnosed population (n=52), 25 (48%) had HLHS, the rest had other aortic arch obstructions resulting in uni- or biventricular repair. Of the postnatally diagnosed group (n=35), 12 (34%) ha d HLHS (p=0.27 between pre- and postnatal group for diagnosis of HLHS). Specific cardiac diagnoses are listed in **Supplementary Table 2**.

Pre-operative cerebral injury and prenatal diagnosis

Table 1 shows the incidence of WMI and stroke in both centres by prenatal diagnosis. In the UCSF/ UBC cohort, 4 of 30 (13%) prenatally diagnosed patients had WMI, compared with 12 of 24 (50%) of the postnatally diagnosed, a risk reduction of 37% (p<0.01). In

Table 1. Pre-operative injury

	Utrecht	UCSF/UBC	p
Prenatal diagnosis	n=22	n=30	
Any WMI	11 (50)	4 (13)	<0.01
Mild	7 (32)	3 (10)	0.05
Moderate	4 (18)	1 (3.3)	0.07
Stroke	1 (4.5)	2 (6.7)	0.75
Postnatal diagnosis	n=11	n=24	
Any WMI	5 (46)	12/24 (50)	>0.99
Mild	0 (0)	6/24 (25)	0.15
Moderate - severe	5 (46)	6/24 (25)	0.26
Stroke	1 (9.1)	3/24 (13)	>0.99

Values stated as number of patients (% of centre).
WMI= white matter injury.

Utrecht, 11 of 22 (50%) prenatally diagnosed patients had pre-operative WMI, which was not significantly different from subjects with a postnatal diagnosis, 5 of 11(46%) (p>0.99). There was a significant difference in incidence of injury between Utrecht and UCSF/ UBC in patients diagnosed prenatally (50 vs. 13% [p<0.01]). In both centres, mild WMI was most common (Utrecht: 7 of 11 patients; UCSF/UB: 3 of 4 patients), and there were no cases of severe WMI. In Utrecht, 6 of the 11 cases with WMI had decreased Apparent Diffusion Coefficient (ADC) values on DWI, suggesting recent injury. At UCSF/ UBC, none of the four patients with WMI had areas of restricted diffusion.

Status of prenatal diagnosis did not affect the occurrence of stroke in either centre (Utrecht: 1 of 22 [4.5%] prenatally diagnosed vs. 1 of 10 postnatal [p>0.99]; UCSF/ UBC: 2 of 30 (6.7%) prenatally diagnosed vs. 3 of 21 [13%] postnatal [p=0.64]). Stroke incidence in prenatally diagnosed patients was not different between centres (Utrecht: 1/22 [4.5%] vs. UCSF/UBC: 2/30 [6.7%]; p=0.75).

Clinical practice pattern variation

In order to explore the risk factors accounting for the differences in rates of brain injury across centres, we examined clinical management practices by status of prenatal diagnosis. Differences in perinatal data and in postnatal management are outlined in Table 2. There were no differences between groups regarding birth characteristics, although there was a trend towards more diagnoses of HLHS at UCSF/UBC (Utrecht: 32% versus UCSF/UBC: 60%; p=0.06). Apgar scores were significantly higher in Utrecht, both at 1 and 5 minutes (median 1-minute Apgar in Utrecht 9 versus 8 at UCSF/UBC; p=0.02; and 5-minute 9 versus 9; p=0.02).

Utrecht had a different policy of ICU-admittance compared to UCSF/UBC. In Utrecht, patients diagnosed prenatally with their cardiac condition were admitted to the

pediatric ICU immediately after birth for stabilisation, and once stable (usually the next day), transferred to the pediatric cardiology ward (with less intensive monitoring). Re-admittance to the ICU before surgery only occurred in case of hemodynamic instability. In contrast, at UCSF/UBC, all patients were cared for in the pediatric cardiac ICU or intensive care nursery until they underwent surgery. These management differences are reflected in the percentage of pre-operative time spent on the ICU. In Utrecht, this was a median of 25%, versus 100% at UCSF/UBC ($p<0.01$). Also, the number of non-ICU days was different; in Utrecht this was a median of 3 days versus none at UCSF/UBC ($p<0.01$).

The timing of surgery was later in Utrecht than UCSF (median of 9 versus 6 days; $p<0.01$). As MRI scans are usually planned shortly before surgery in Utrecht, the day of the MRI scan was therefore also later than at UCSF (median 7 versus 3 days; $p<0.01$). Rates of cardiopulmonary resuscitation and LCOS were not different between the centres (with data available for 18/22 (81%) Utrecht patients and 25/30 (83%) UCSF/UBC patients). Despite an increased admission to ICU at UCSF/UBC, the number of patients requiring mechanical ventilation and inotropic medications were similar. However, more patients in Utrecht received diuretics (59% of Utrecht patients versus 17% of UCSF/UBC; $p<0.01$). At UCSF/UBC, more patients received total parenteral nutrition (parenteral nutrition 9.1% in Utrecht versus 64% at UCSF/UBC; $p<0.01$). Enteral feeding was more common in Utrecht (100% vs. 10%, $p<0.01$). Finally, infections prior to surgery were more common in Utrecht 23% versus none at UCSF; $p=0.01$). Infections included 3 culture-confirmed blood stream infections (2 with *S. Aureus* and one with a coagulase-negative *Staphylococcus)* and 2 pneumonias (documented on chest X-rays), all treated with antibiotics for at least 5 days.

Of note, when excluding the UBC patients from the analyses, hence comparing Utrecht only to UCSF, all above noted differences remained significant.

Patients with postnatal diagnosis: Cerebral injury

In this subgroup, there was no difference between centres in incidence of WMI (46 vs. 50% [$p>0.99$]), see **Table 1**. Regarding severity, all of the Utrecht cases had moderate WMI and all had decreased ADC values. At UCSF/ UBC, the severity was evenly distributed over mild and moderate to severe (1 case of severe WMI), and 5 of 12 cases (42%) had decreased ADC values. Stroke incidence was similar between centres (Utrecht:1/11 [9.1%] vs. UCSF/UBC: 3/24 [13%]; $p>0.99$).

Patients with postnatal diagnosis: Pre-operative management

As listed in **Table 2**, in neonates diagnosed with CHD postnatally (n=35), again, birth characteristics were similar between the two centres, but there was a trend towards more HLHS-patients at UCSF/UBC (9.1% in Utrecht versus 46% at UCSF/UBC, $p=0.06$). Caesarean section also showed a trend towards being more common at UCSF/UBC (none in Utrecht versus 29% at UCSF/UBC; $p=0.07$) and Apgar score at 5 minutes was higher in Utrecht (median 10 in Utrecht versus 9 at UCSF/UBC; $p=0.03$).

Patients often presented at a level 2 centre, and were then transferred to a level 3 centre (Utrecht or UCSF/UBC). The day of the initial presentation (at any hospital,

Table 2. Clinical Characteristics and management practices per centre

	Prenatal diagnosis		
	Utrecht (n=22)	UCSF/ UBC (n=30)	*p* (Utrecht vs. UCSF/UBC)
Birth characteristics			
Gestational age	39.0 (38.3-40.0)	38.7 (38.0-39.3)	0.28
Birth weight	3320 (3030-3660)	3245 (2971-3688)	0.99
Male sex	19 (86)	21 (70)	0.20
Genetic syndrome	2 (9.5)	2 (6.7)	0.71
Diagnosis: HLHS*	7 (32)	18 (60)	0.06
Perinatal course			
Caesarean section	3/22 (13.6)	9 (30)	0.20
Apgar 5 min	9 (9-10)	9 (8-9)	0.02
Neonatal course			
Age at presentation	0 (0-0)	0(0-0)	>0.99
Age at MRI scan	7 (6-9)	3 (2-5)	<0.01
Age at surgery	9 (7-13)	6 (4-8)	<0.01
% of time spent on ICU	25 (16-70)	100 (100-100)	<0.01
Number of non-ICU days	3 (2-6)	0 (0-0)	<0.01
Hemodynamics and management			
LCOS ‡	4 (22)	4 (17)	0.71
CPR	0 (0)	0 (0)	>0.99
Mechanical ventilation	6 (27)	13 (43)	0.26
Ventilator strategy**	0 (0)	4 (13)	0.13
Sedatives ‡	7 (31.8)	16 (57)	0.09
Prostaglandin	19 (86)	29 (97)	0.17
Inotropes	4 (18)	2 (6.9)	0.38
Diuretics ‡	13 (59)	5 (17)	<0.01
Total parenteral nutrition ‡	2 (9.1)	16 (64)	<0.01
Enteral feeding ‡	22 (100)	3 (10)	<0.01
Lowest haemoglobin	14.6 (13.0-17.6)	13.3 (12.5-14.6)	0.13
Inflammation			
Infection	5 (23)	0 (0)	0.01
Antibiotics	7 (32)	4 (13)	0.11

Values stated as number of patients (% of centre) or median (interquartile range).
*Specific diagnoses are listed in Supplementary Table 2.
**Special ventilator strategies included ventilation with hypoxic gas or permissive hypercapnia.

	Postnatal diagnosis		
	Utrecht (n=11)	UCSF/ UBC (n=24)	*p* (Utrecht vs. UCSF/UBC)
	39.1 (38.6-40.1)	39.3 (38.5-40.3)	0.89
	3400 (3240-3516)	3278 (3043-3528)	0.39
	8 (73)	14 (58)	0.48
	1 (9.1)	3 (13)	>0.99
	1 (9.1)	11 (46)	0.06
	0 (0)	7 (29)	0.07
	10 (9-10)	9 (8-9)	0.03
	3 (1-9)	0 (0-4)	0.06
	11 (7-13)	6 (5-10)	0.03
	12 (10-15)	9 (7-12)	0.07
	29 (18-46)	60 (31-86)	0.03
	8 (3-9)	2 (1-4)	0.04
	9 (90)	14 (67)	0.22
	3 (27)	2 (8.3)	0.14
	8 (73)	20 (83)	0.65
	0 (0)	0 (0)	>0.99
	8 (73)	21 (88)	0.35
	10 (91)	23 (96)	0.54
	9 (82)	14 (58)	0.26
	6 (54)	9 (39)	0.48
	1 (9.1)	16 (80.0)	<0.01
	11 (100)	16 (73)	0.08
	13.0 (11.6-15.3)	12.1 (11.3-13.3)	0.06
	1 (9.1)	3 (13)	>0.99
	4 (36)	22 (92)	<0.01

‡ Data was available for a limited number of patients: LCOS, n=74; sedatives, n=84; diuretics n=86; total parenteral nutrition, n=79 and enteral feeding, n=85. Percentages shown are only of patients with sufficient data.
HLHS=hypoplastic left heart syndrome. LCOS=low cardiac output syndrome. CPR= cardiopulmonary resuscitation.

Table 3. Risk factors for WMI

	Prenatal diagnosis		
	No WMI (n=37)	WMI (n=15)	P (No WMI vs. WMI)
Perinatal course			
Gestational age at birth	39 (38-39)	39 (38-40)	0.55
Birth weight	3290 (3010-3695)	3237 (2739-3655)	0.44
Caesarean section	10 (27)	2 (13)	0.47
Apgar 5 min	9 (9-9)	9 (8-9)	0.55
Cardiac diagnosis: HLHS	19 (51)	6 (40)	0.55
Neonatal course			
Age at presentation	0 (0-0)	0 (0-0)	>0.99
Age at MRI scan	4 (2-6)	6 (4-8)	<0.01
Age at surgery	7 (5-8)	9 (6-12)	0.07
% of time spent on ICU	100 (64-100)	67 (25-100)	0.16
Non-ICU days	0 (0-3)	2 (0-6)	0.09
Hemodynamics and management			
LCOS ‡	5 (15)	3 (33)	0.22
CPR	0 (0)	0 (0)	>0.99
Balloon atrioseptostomy	1 (2.7)	0 (0)	>0.99
Mechanical ventilation	11 (30)	8 (53)	0.13
Ventilator strategy**	3 (8.1)	1 (6.7)	>0.99
Sedatives ‡	15 (43)	8 (53)	0.55
Prostaglandin	35 (95)	13 (87)	0.57
Inotropes	3 (8.3)	3 (20)	0.34
Diuretics ‡	10 (28)	8 (53)	0.11
Total parenteral nutrition ‡	13 (39)	5 (36)	>0.99
Enteral feeding ‡	14 (39)	11 (73)	0.03
Lowest hemoglobin	13.9 (13.0-16.2)	13.0 (12.3-17.1)	0.46
Inflammation			
Infection	1 (2.7)	4 (27)	0.02
Antibiotics	5 (14)	6 (40)	0.06

Values stated as number of patients (% of centre) or median (interquartile range).
CPR= cardiopulmonary resuscitation.
**Special ventilator strategies included ventilation with hypoxic gas or permissive hypercapnia.
‡ Data was available for a limited number of patients: LCOS, n=74; sedatives, n=84; diuretics n=86; total parenteral nutrition, n=79 and enteral feeding, n=85. Percentages shown are only of patients with sufficient data.
LCOS=low cardiac output syndrome. CPR= cardiopulmonary resuscitation.

	Postnatal diagnosis		
	No WMI (n=18)	WMI (n=17)	P (No WMI vs. WMI)
	39 (39-40)	40 (39-41)	0.14
	3234 (2941-3375)	3412 (3140-3569)	0.10
	4 (22)	3 (18)	>0.99
	9 (8-9)	9 (9-9)	0.80
	4 (22)	8 (47)	0.16
	1 (0-8)	2(0-5)	0.81
	8 (5-12)	7 (5-12)	0.53
	11 (7-14)	11 (8-15)	0.84
	31 (19-85)	60 (32-76)	0.13
	6 (1-10)	3 (1-7)	0.31
	8 (53)	15 (94)	0.02
	0 (0)	5 (31)	0.02
	0 (0)	1 (5.9)	0.49
	11 (61)	17 (100)	0.008
	0	0	
	12 (67)	17 (100)	0.02
	16 (89)	17 (100)	0.49
	9 (50)	14 (82)	0.08
	6 (35)	9 (53)	0.49
	7 (47)	10 (63)	0.48
	13 (77)	14 (88)	0.66
	12.2 (11.4-14.3)	12.2 (11.3-13.3)	0.63
	3 (17)	1 (5.9)	0.60
	13 (72)	13 (77)	>0.99

usually the level 2 hospital) showed a trend towards being later in the Utrecht group than UCSF/UBC (Utrecht: median 3 days versus 0 [day of birth] at UCSF/UBC; p=0.06).

Similar to the group diagnosed prenatally, MRI scans were performed at a later age in Utrecht, with a trend for day of surgery to be later (age at MRI scan in Utrecht: 11 days versus 6 days at UCSF/UBC; p=0.03; day of surgery at Utrecht: 12 days versus 9 days at UCSF/UBC; p=0.07). Also, the time spent on the ICU was less in the Utrecht group compared to UCSF/UBC (29% of time in Utrecht versus 60% at UCSF/UBC; p=0.03; and 8 non-ICU days in Utrecht versus 2 at UCSF/UBC; p=0.04).

Potential risk factors for WMI

Combining the patients of both centres, we assessed which pre-operative factors were associated with presence of WMI. Results are listed in Table 3. In the overall cohort, protective effect of prenatal diagnosis was just short of significance (p=0.07).

Specifically regarding prenatally diagnosed patients, increased age at MRI and surgery showed a significant association with more WMI (patients without WMI had an MRI at a median age of 4 days and underwent surgery at 7 days, versus 6 days and 9 days, respectively, in the group with WMI [p<0.01 and p=0.07, respectively). Enteral feeding was associated with occurrence of WMI (39% in no WMI group versus 73% in WMI group with WMI [p=0.03]), and finally an infection was also associated with increased risk (2.7% in no WMI group versus 27% in WMI group [p=0.02]).

For postnatally diagnosed patients; significant risk factors for WMI were cardiopulmonary resuscitation, LCOS and need for mechanical ventilation and sedatives (p-values are respectively p=0.02; p=0.02; p<0.01; p=0.01 and p=0.02).

DISCUSSION

In the current study of neonates with an aortic arch obstruction, prenatal diagnosis was protective for the development of preoperative WMI in the combined UCSF/UBC cohort, although this effect not observed at Utrecht. Development of WMI in patients without prenatal diagnosis was strongly related to variables indicating hemodynamic instability (e.g. cardiac arrest, LCOS and need for mechanical ventilation). Presumably, prenatal diagnosis reduces the risk of WMI by improving preoperative hemodynamic and clinical condition through early initiation of prostaglandin E2. At Utrecht however, this protective effect was not observed. Differences in clinical practice were identified which may mitigate protection, including longer time to surgery, less time admitted to a high level of care, increased use of diuretics, enteral feeding and increased infections. These data suggest that although prenatal diagnosis has the potential to protect against pre-operative WMI, pre-operative clinical management must be optimized to effectively minimize the risk of injury.

The fact that the two cohorts had very similar birth characteristics, but still a very different incidence of WMI, makes this a highly suitable group to study the potential of different postnatal care strategies to reduce the burden of brain injury. In the course leading up to the surgical procedure, the most apparent difference between the two

centres was the difference in timing of surgery (and consequently, of the pre-operative MRI). The question arises as to whether the white matter lesions were more established in the Utrecht patients as they were scanned later. This is not likely, as sensitive MRI techniques such as DWI was used in both centres, which will show injury, such as WM lesions, as early as hours after the insult. Furthermore, in the Utrecht population, the abundance of lesions which were DWI-positive on MRI, suggests that at least part of the injury has a postnatal onset, as lesions remain DWI-positive for up to 7-8 days and the median day of scanning was 7 and 11 days in the pre- and postnatally diagnosed Utrecht patients, respectively.

Taking the above into account, we hypothesize that the increasing abundance of WMI while awaiting surgery may be partly due to the ongoing suboptimal hemodynamic state. Indeed, in newborns with TGA, lower oxygen saturation and longer time to surgery are also associated with more WMI[4, 8]. In patients with single ventricle physiology such as HLHS, there is a delicate balance between pulmonary and systemic perfusion, and in the first days of life, as pulmonary vascular resistance falls and pulmonary blood flow increases, this may be in expense of the systemic and cerebral circulation. With increasing pulmonary blood flow, there is a risk of cerebral hypoperfusion. In the Utrecht patients, patients underwent surgery at a median of 9 days, as opposed to 6 days at UCSF/UBC. The longer time until surgery may place these neonates at higher risk of systemic and cerebral hypoperfusion, especially when they are not continuously admitted on the ICU and closely monitored. Of note, in addition to close hemodynamic monitoring, further efforts can be made to prevent systemic hypoperfusion, for instance by using hypoxic gas during mechanical ventilation, or a permissive hypercapnic ventilator strategy require admission to an intensive care unit. Both of these therapies were used in the UCSF/UBC groups, albeit rarely (n=4) [19].

Other management differences between the 2 centres must also be taken into account, as these may also have an important effect on cerebral injury. The first is the higher incidence of pre-operative infections before surgery in Utrecht. The relationship with WMI has been observed before both in cardiac surgery and premature neonates [16, 20]. Furthermore, use of diuretics was much more common in Utrecht. While commonly given for tachypnea and pulmonary edema, diuretics do have the risk of reducing preload and further compromising systemic perfusion. Consequently, this may result in a higher incidence of WMI. Finally, total parenteral nutrition, instead of enteral nutrition, was more common at UCSF/UBC. As it is becoming increasingly evident that nutrition is essential for brain protection in neonates, total parenteral nutrition may deliver more trophic factors to the brain which help to minimize risk of WMI. Alternatively, enteral feeding may lead to more abdominal perfusion, in expense of cerebral blood flow in ductal-dependant circulations.

In patients diagnosed after birth, the incidence of WMI was approximately the same (50%) across both centres. The risk factors for WMI in this group were all markers of the state in which these patients presented with their cardiac lesion: LCOS and cardiac arrest with subsequent need for mechanical ventilation and sedation. These abnormal circulatory states presumably result in a primary brain injury at the time of

presentation. Further centre-specific management differences did not seem to affect the overall risk of injury. However, there was a trend towards milder forms of WMI at UCSF/UBC in this group, perhaps reflective of the same pre-operative management differences between the centres as in the prenatally diagnosed group (i.e. time spent on the ICU and total parenteral nutrition). Another explanation may be the later age at which the Utrecht patients were recognized to have a cardiac condition (3 days versus the day of birth at UCSF/UBC), which may reflect the more common practice of home births in the Netherlands.

Although there was a large discrepancy in WMI burden between centres, this did not apply to stroke incidence. This was very similar in both centres, suggesting less influence of pre-operative management to this phenomenon. This may be due to the fact that thrombo-embolic processes are the most important cause of stroke. Balloon atrial septostomy (BAS) has been identified as a risk factor for stroke in newborns with TGA. In neonates with aortic arch obstruction, BAS is much less common[9].

The findings in this study have important limitations. Although the included number of cases is quite substantial for this very specific cardiac group, the separate assessment of pre- and postnatally diagnosed patients, precluded multivariate testing of risk factors for WMI. Furthermore, although we made every effort to collect extensive data to explore possible important clinical factors, we cannot exclude that other important information may have been overlooked. In particular, given the differences in care location (e.g. ICU versus ward), important physiological data (such as blood pressure), and laboratory data (lactate, blood gasses) were not collected uniformly at each centre, making meaningful analysis of these data impossible. In future, a prospective study in which this valuable hemodynamic data is continuously recorded during the entire pre-operative course, will better inform us of possible risk factors for cerebral injury, especially WMI. Only then can we effectively intervene, aiming for the lowest possible burden of injury before these neonates undergo their necessary cardiac surgery.

In conclusion, the variability in the burden of WMI in neonates with aortic arch obstruction appears related to centre-specific pre-operative management, particularly in those neonates diagnosed prenatally. These data suggest that optimizing the care of neonates with aortic arch obstruction before they undergo surgery, may provide an opportunity to fully realize the potential of prenatal cardiac diagnosis to improve the brain health of these vulnerable neonates.

SUPPLEMENTARY TABLES

Supplementary Table 1. Characteristics of UCSF versus UBC

	UCSF (n=44)	UBC (n=10)	p
Pre-operative injury			
Any WMI	14 (31)	2 (20)	0.71
Mild	7 (16)	2 (20)	0.66
Moderate or severe	7 (16)	0	0.33
Stroke	2 (4.4)	2 (10)	0.15
Birth characteristics			
Gestational age	39.0 (38.0-39.9)	39.0 (38.0-40.0)	0.92
Birth weight	3227 (2982-3516)	3590 (2758-4355)	0.35
Male sex	28 (62)	8 (80)	0.47
Genetic syndrome	5 (11)	0 (0)	0.57
Prenatal diagnosis	25 (56)	6 (60)	>0.99
Diagnosis: HLHS	25 (57)	4 (40)	0.49
Perinatal course			
Caesarean section	11 (25)	5 (50)	0.14
Apgar 5 min	9 (8-9)	9 (9-9)	0.11
Neonatal course			
Age at presentation	0 (0-0)	0 (0-0)	0.44
Age at MRI scan	5 (3-6)	2 (1-8)	0.10
Age at surgery	7 (6-11)	6 (2-15)	0.14
% of time spent on ICU	100 (69-100)	100 (34-100)	0.71
Number of non-ICU days	0 (0-2)	0 (0-6)	0.92
Hemodynamics and management			
LCOS	16/36 (44)	2/9 (22)	0.28
CPR	2 (4.4)	0	>0.99
Mechanical ventilation	30 (68)	3 (30)	0.04
Ventilator strategy [4]	4 (8.9)	0	>0.99
Sedatives	34/43 (77)	4/9 (44)	0.10
Prostaglandin	45 (100)	8 (80)	0.03
Inotropes	15/43 (34)	1 (10)	0.25
Diuretics	12/43 (27)	2/9 (22)	>0.99
Total parenteral nutrition	30/37 (81)	2/9 (22)	<0.01
Enteral feeding	13/41 (32)	6 (60)	0.15
Lowest hemoglobin	12.7 (11.5-13.4)	13.7 (12.6-15.4)	0.05
Inflammation			
Infection	2 (4.4)	1 (10)	0.47
Antibiotics	24 (53)	3 (30)	0.30

Values stated as number of patients (% of centre) or median (interquartile range).
WMI=white matter injury. HLHS=hypoplastic left heart syndrome. IAA=interrupted aortic arch.
LCOS=low cardiac output syndrome. CPR= cardiopulmonary resuscitation.

Supplementary Table 2. Cardiac diagnoses and performed procedures

Diagnosis	Surgery	Utrecht prenatal dx (n=22)	postnatal dx (n=11)	UCSF/UBC prenatal dx (n=30)	postnatal dx (n=24)
HLHS					
HLHS	Norwood procedure	7	1	18	11
Other diagnosis					
HRHS (TGA with TA atresia or stenosis)	Norwood procedure	2	0	3	1
DILV, TGA, CoA	Norwood procedure	1	0	0	0
DILV with CoA or hypoplastic aortic arch	Norwood procedure	1	0	1	0
Complete AVSD, LVOTO, hypoplastic MV, left atrial isomerism	Norwood procedure	0	0	1	0
HLHC [21]	Biventricular repair	3	4	0	0
CoA (with or without VSD and/or ASD)	Aortic arch reconstruction, ASD and/or VSD closure	1	3	2	5
IAA (with or without VSD and/or ASD)	Aortic arch reconstruction, ASD and/or VSD closure	0	1	1	2
Taussig-Bing anomaly with CoA or IAA	Arterial switch, aortic arch reconstruction, VSD closure	4	0	3	1
TGA with IAA or CoA, VSD and/or ASD	Arterial switch, aortic arch reconstruction, VSD and/or ASD closure	2	0	1	0
DORV, CoA, VSD	VSD tunnel repair, aortic arch reconstruction	0	1	0	1
Complete AVSD, LVOTO, hypoplastic aortic arch, situs inversus	AVSD repair, LVOTO resection, aortic arch reconstruction	1	0	0	0
IAA type B with hypoplastic aortic arch, severe LVOTO, VSD	Ross-Konno, aortic arch reconstruction, VSD closure	0	1	0	0
DORV, AVSD, CoA, MV atresia	Hybrid procedure: PA banding, aortic arch and PDA stenting	0	0	0	1
IAA, large A-P window	Patch plasty aortic arch, repair A-P window	0	0	0	1
Truncus arteriosus, IAA, VSD	Aortic arch reconstruction, RV-PA conduit, VSD closure	0	0	0	1
Total		15	10	12	13

Values stated as number of patients (% of centre).
dx: diagnosis; HLHS: hypoplastic left heart syndrome; HLHC: hypoplastic left heart complex[21]; HRHS: hypoplastic right heart syndrome; IAA: interrupted aortic arch; CoA: coarctation of the aortic arch; ASD: atrial septal defect; VSD: ventricular septal defect; AVSD: atrioventricular septal defect; LVOTO: left ventricular outflow tract obstruction; DORV: double outlet right ventricle; MV: mitral valve; PA: pulmonary artery; PDA: patent ductus arteriosus; A-P: aortopulmonary; RV-PA: right ventricle to pulmonary artery; TGA: transposition of the great arteries; DILV: double inlet left ventricle.

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3

NEUROLOGICAL INJURY AFTER NEONATAL CARDIAC SURGERY: A RANDOMIZED CONTROLLED TRIAL OF TWO PERFUSION TECHNIQUES

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ABSTRACT

Background

Complex neonatal cardiac surgery is associated with cerebral injury. Especially aortic arch repair, requiring either deep hypothermic circulatory arrest(DHCA) or antegrade cerebral perfusion(ACP), entail a high risk of peri-operative injury. It is unknown, whether ACP results in less cerebral injury than DHCA.

Methods and results

Thirty-seven neonates with an aortic arch obstruction presenting for uni- or biventricular repair, were randomized to either DHCA or ACP. Pre-operatively and one week after surgery, magnetic resonance imaging(MRI) was performed in 36 patients(one patient died during hospital stay). The presence of new postoperative cerebral injury was scored, and results were entered into a sequential analysis, which allows for immediate data analysis. After the 36th patient, it was clear that there was no difference between DHCA and ACP in terms of new cerebral injury. Pre-operatively, 50% of patients had evidence of cerebral injury. Postoperatively, 14/18(78%) of DHCA patients had new injury, versus 13/18(72%) of ACP(p=0.66). White matter injury(WMI) was the most common type of injury in both groups, but central infarctions occurred exclusively after ACP(0 vs. 6/18[33%];p=0.02). Early motor and cognitive outcome at 24 months was assessed and was similar between groups(p=0.28 and p=0.25,respectively). Additional analysis revealed lower postoperative arterial pCO2 as risk factor for new WMI(p=0.04).

Conclusions

In this group of neonates undergoing complex cardiac surgery, we were unable to demonstrate a difference in the incidence of peri-operative cerebral injury after ACP compared to DHCA. Both techniques resulted in a high incidence of new WMI, with central infarctions occurring exclusively following ACP.

Clinical Trial Registration Information http://clinicaltrials.gov; NCT01032876.

BACKGROUND

Survival after neonatal cardiac surgery has improved over the past decades to over 90%, due to major advances in surgical techniques and peri-operative management. However, it has also become evident that neurodevelopment is impaired in approximately one-third of children who underwent surgery at a neonatal age[1]. As seen on magnetic resonance imaging (MRI), 23-40% of neonates presenting with a complex cardiac defect already have evidence of cerebral injury pre-operatively[2-6]. After surgery, 36-73% of patients have evidence of new cerebral lesions on MRI[2-8]. This suggests that much of the injury develops peri-operatively.

Neonates diagnosed with aortic arch obstruction (i.e., hypoplastic left heart syndrome (HLHS) or other complex cardiac defects) are consistently at the highest risk of cerebral injury[5, 7, 9]. This may be due to the fact that full-flow cardiopulmonary bypass (CPB) is not feasible during the reconstruction of the aortic arch, obligating the use of either deep hypothermic circulatory arrest (DHCA) or antegrade cerebral perfusion (ACP). Despite initial reports on the adverse cerebral effects after DHCA and the intuitive benefit of ACP, previous studies have not been able to show superiority of ACP in studies of neurodevelopmental outcome at one year of age[10, 11]. However, these results may be confounded by the reoperations which frequently take place in the interim.

Therefore, in the current randomized controlled trial, we used pre- and postoperative MRI, as the most sensitive measure, to assess peri-operative cerebral injury. Specifically, in neonates undergoing aortic arch reconstruction, the incidence of new postoperative injury on MRI was compared between DHCA and ACP.

METHODS

All neonates presenting for aortic arch reconstruction between January 2009 and May 2012 were assessed for enrollment in the current study. Exclusion criteria were: 1) age more than four months; 2) high suspicion of a genetic syndrome; 3) need for sedation and intubation exclusively for the pre-operative MRI or 4) an expected time needed for aortic arch reconstruction for more than 60 minutes. Written informed consent was obtained from both parents of all included patients. The current study protocol was approved by the local medical-ethical committee and is registered at clinicaltrials.gov, number NCT01032876.

Once a patient was enrolled, the day before surgery, a sealed and numbered envelope was opened to reveal the allocated perfusion technique and the surgical team was informed. The randomization was performed with sequentially numbered, opaque sealed envelopes according to a standardized protocol[12]. Block randomization was used to prevent large discrepancies in group size as the trial continued.

Aortic arch reconstruction and intracardiac repairs were performed as described in detail in the Supplemental data. CPB was performed using a modified alpha-stat strategy, with a target pCO2 between 45 and 55 mm Hg. Target hematocrit was 24-28%, which increased to 32-40% at the end of CPB for univentricular repair, and 28% for biventricular repair. Before initiation of DHCA or ACP, all patients were cooled

to a nasopharyngeal temperature of approximately 18°C, ACP was instituted either by direct advancement of the arterial cannula from the ascending aorta into the brachiocephalic artery or via a Blalock-Taussig shunt sewed to the brachiocephalic artery. ACP was set at 25% of full-flow, with right radial artery pressures not exceeding 40 mm Hg. Further CPB details are described in the Supplemental data. All patients received 1 mg/kg dexamethasone i.v. before initiation of CPB. Standard anesthetic protocols during surgery included sevoflurane for induction of anesthesia, midazolam and sufentanil for continuous sedation and pancuronium for muscle paralysis. Postoperative sedation was routinely ensured by midazolam, morphine, and clonidine.

Bilateral near-infrared spectroscopy (NIRS) and amplitude-integrated electroencephalography (aEEG) were used to monitor cerebral oxygen saturation and cerebral function, respectively (as described in the Supplemental data). Four and 24 hours after surgery, samples were drawn for determination of s100b and neuron-specific enolase levels (see Supplemental data).

Pre- and postoperatively, MRI was performed on a 1.5-Tesla scanner (Philips Medical Systems, Best, the Netherlands). Details of the classifications of injury types are described in the Supplemental data. As primary outcome of the trial, assessment of presence of new postoperative cerebral injury on the MRI was performed by two observers blinded for perfusion technique. Both have extensive experience in neonatal cerebral imaging (L.d.V. and F.G.).

Initially, a classical sample size based on a chi-square analysis between two groups was calculated, resulting in an anticipated number of 50 patients needing enrollment, when ACP was hypothesized to reduce new cerebral injury from 75% to 35% compared to DHCA (with a one-sided alpha of 5% and power of 80%)[2]. However, in 2010, the local medical ethical committee granted a request to start the use of sequential analysis, so as not to unnecessarily continue an already conclusive trial. From then on, results from MRI analysis (new lesions; yes or no) were forwarded to the independent biostatistician (I.v.d.T.) as soon as these were obtained or after approximately every five new patients, who entered the data into the sequential analysis. The study team could then be informed immediately if a boundary had been crossed, either showing that there was a clear beneficial effect of ACP compared to DHCA, or that there was no difference between DHCA and ACP. Sequential analysis was performed in a triangular test using PEST software (MPS Research Unit, University of Reading, England). The analysis was corrected for uni- or biventricular repair and an intention-to-treat principle was applied. **Figure 1** depicts the sequential analysis of this trial. On the horizontal axis (V), the cumulative amount of information is represented. The vertical axis (Z) shows the cumulative effect size, where a higher value corresponds to superiority of ACP compared to DHCA (i.e.; ACP results in lower incidence of new postoperative lesions than DHCA). Each cross represents cumulative data of groups of patients. When the test statistic based on the cumulative data crosses the upper (red) boundary, the null hypothesis of indifference is rejected. In this case, ACP would be beneficial to DHCA. When the lower (purple) boundary is crossed, the null hypothesis is accepted,

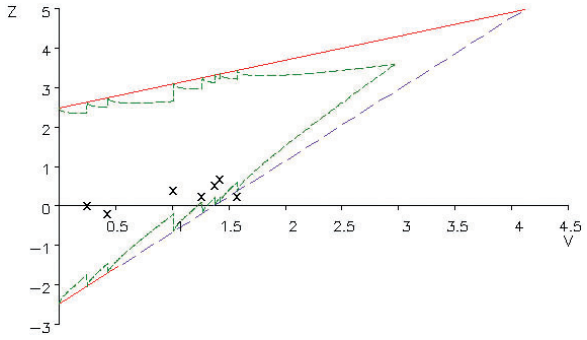


Figure 1. Results of sequential analysis. This graph depicts the sequential analysis of this trial. On the horizontal axis (V), the cumulative amount of information is represented. The vertical axis (Z) shows the cumulative effect size, where a higher value corresponds to superiority of ACP compared to DHCA (i.e.; ACP results in lower incidence of new postoperative lesions than DHCA). Each cross represents cumulative data of groups of patients. If the upper (red) boundary would be crossed, this would mean ACP is indeed beneficial compared to DHCA. If the lower (purple) boundary was crossed, this would indicate that there is no difference between DHCA and ACP. In the current trial, the last cross indicates that the lower boundary is crossed and therefore the result is that ACP does not result in less new postoperative injury than DHCA. Note that the inner green serrated boundaries act as a continuity correction, the so-called Christmas tree correction. These boundaries are the real stopping boundaries. DHCA:deep hypothermic circulatory arrest; ACP:antegrade cerebral perfusion.

hence indicating that there is no difference between DHCA and ACP. Note that the inner green serrated boundaries act as a continuity correction, the so-called Christmas tree correction. These boundaries are the real stopping boundaries.

Early neurodevelopment was assessed by a pediatric physiotherapist at approximately 24 months of age, using the Bayley Scales of Infant and Toddler Development, version III (BSITD; San Antonio, USA).

All group characteristics are listed as median (ranges) or number of patients (% of group). All secondary analyses assessing differences between groups were performed using the Fisher exact test for dichotomous variables, or the Mann-Whitney U test for continuous variables. SPSS version 19.0 was used (SPSS Inc., Chicago, IL, USA).

RESULTS

Thirty-seven patients were included in the trial and were randomized to either DHCA or ACP. Details of the inclusion process are shown in **Figure 2.** Total survival to date is 35/37 (95%), with a median follow up of 2.4 years (range 0.8-4.0). One patient, originally diagnosed with a complete atrioventricular septum defect, situs inversus and a hypoplastic aortic arch, died six weeks after corrective surgery due to an ongoing mitral valve insufficiency without further surgical options. The other patient had a sudden cardiac arrest after the Norwood procedure one week after discharge home. Both patients were from the DHCA group. The first patient was the only case in whom

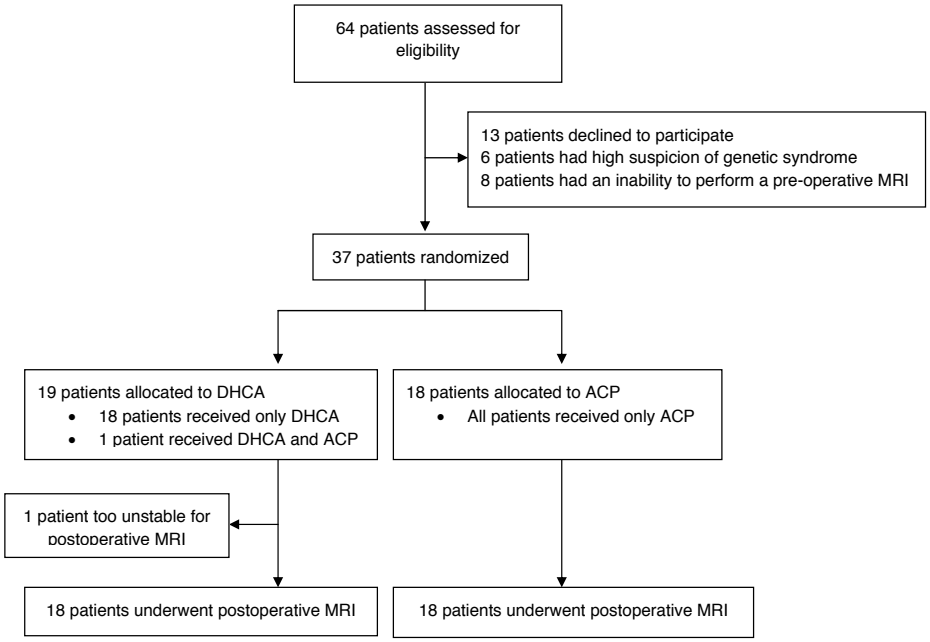


Figure 2. Patient inclusion. MRI: magnetic resonance imaging; DHCA:deep hypothermic circulatory arrest; ACP:antegrade cerebral perfusion.

a postoperative MRI scan could not be performed, resulting in 36 patients available for the primary outcome variable. However, in line with the intention-to treat principle, the patient was included in all other analyses.

Baseline clinical characteristics are shown in Table 1. This shows that DHCA and ACP groups were comparable; although the proportion of patients with a univentricular repair was higher in the DHCA group. As this discrepancy was anticipated, a correction for univentricular was included in the sequential analysis (see Methods).

All patients except one underwent the allocated perfusion technique without crossovers to the other technique. This patient had been randomized to undergo the Norwood procedure using DHCA. During surgery, aortic arch reconstruction was indeed completed during 35 minutes of DHCA, but soon after there appeared to be severe pulmonary valve insufficiency for which the area needed immediate revision. ACP was then instituted to avoid risk of a very long DHCA time.

General surgical outcome for the DHCA and ACP groups is listed in Table 2. This shows that the postoperative course was very comparable between groups.

Primary outcome: MRI findings

For the sequential analysis, the presence of new injury on the postoperative MRI scan was the primary outcome. Results of the analysis are depicted in Figure 1. This shows that when the 36th MRI result was entered, the lower boundary was crossed,

Table 1. Baseline characteristics and surgical data

	DHCA (n=19)	ACP (n=18)
Birth characteristics		
Male sex	16(84)	14(78)
Prenatal diagnosis	13(68)	11(61)
Caesarean section	3(16)	1(5.6)
Gestational age at birth(weeks)	39.1(36.3-40.6)	39.0(35.3-41.0)
Birth weight(g)	3400(2690-3970)	3340(2040-4055)
1-minute Apgar score	9(7-10)	9(3-10)
5-minute Apgar score	9(8-10)	10(8-10)
Genetic syndrome	1(5.3)	3(17)
Non-Caucasian race	1(5.3)	2(11)
Cardiac diagnosis		
Biventricular repairs		
Hypoplastic left heart complex[13]	2(11)	3(17)
IAA/CoA and hypoplastic aortic arch,with:		
No intracardiac lesions	1(5.3)	1(5.6)
ASD and/or VSD	2(11)	6(33)
Multiple VSD's (for which PAB)	0(0)	1(5.6)
DORV,TGA, VSD(Taussig-Bing)	2(11)	2(11)
TGA	2(11)	0(0)
DORV	1(5.3)	0(0)
Severe aortic valve stenosis	0(0)	1(5.6)
Severe LVOTO, VSD(Ross-Konno)	1(5.3)	0(0)
Univentricular repairs		
Hypoplastic left heart syndrome	6(32)	2(11)
Tricuspid atresia	2(11)	0(0)
Double inlet left ventricle	0(0)	2(11)
Pre-operative course		
Balloon atrioseptostomy	1(5.3)	0(0)
Need for inotropic medication	9(47)	4(22)
Cardiopulmonary resuscitation	2(11)	1(5.6)
Pre-operative infection	3(16)	3(17)
Length of ICU stay(days)	3(1-12)	2(0-8)
Duration of mechanical ventilation(days)	1(0-12)	0(0-6)
Surgical data		
Age at surgery(days)	9(5-29)	12(5-34)
CPB duration(min)	166(113-376)	164(111-249)
Duration of DHCA or ACP (min)	34(20-45)	39(25-68)
ACP flow (ml/kg/min)		46(33-59)
Myocardial ischemia duration (min)	70(35-167)	62(34-147)
Cooling duration (min)	30(20-70)	30(24-42)
Rewarming duration (min)	50(33-70)	45(26-75)
Lowest nasopharyngeal temperature (°C)	17.9(15.8-19.4)	17.6(14.7-19.1)

Values stated in median (ranges) or number of patients (% of group). Durations during CPB are in minutes.
DHCA:deep hypothermic circulatory arrest; ACP:antegrade cerebral perfusion; IAA:interrupted aortic arch; CoA:coarctation of aortic arch; ASD:atrial septum defect; VSD:ventricular septum defect; PAB:pulmonary artery banding; DORV:double-outlet right ventricle; TGA:transposition of the great arteries; LVOTO:left ventricular outflow tract obstruction; ICU:intensive care unit; CPB:cardiopulmonary bypass.

Table 2. Surgical outcome data

	DHCA (n=19)	ACP (n=18)	p
Postoperative course			
Duration of mechanical ventilation	7(1-51)	6(2-20)	0.25
Duration of open sternum	2(0-5)	3(0-6)	0.91
Maximum inotrope score[14]	21(6-67)	24(13-67)	0.50
Need for dialysis	1(5.3)	1(5.6)	>0.99
Length of stay on ICU	8(2-52)	7(3-21)	0.71
Length of hospital stay	20(7-70)	14(8-79)	0.50
Mortality during hospital stay	1(5.3)	0(0)	>0.99
Later surgical outcome			
Number of unplanned reinterventions*	0(0-2)	0(0-3)	0.94
Total mortality to date	2(11)	0(0)	0.49

Values stated in median (ranges) or number of patients (% of group). All durations are in days.
*Included both interventional heart catheterizations and cardiac surgeries.
DHCA:deep hypothermic circulatory arrest; ACP:antegrade cerebral perfusion.

meaning that there was no difference between DHCA and ACP in terms of new postoperative cerebral injury.

Detailed analysis of pre- and postoperative MRI scans is listed in Table 3. Pre-operative MRI was performed at a median age of eight days (range 4-34). In 51% of all patients, there was evidence of any kind of pre-operative cerebral injury. White matter injury (WMI) was most common, affecting 49% of all patients. Three patients (8.1%) had an infarction (a cortical infarction in one patient and a lentriculostriate infarction in two).

Postoperatively, MRI was performed at a median of seven days after surgery in the DHCA group, versus six days after ACP (p=0.41). Fourteen patients (78%) of the DHCA group had evidence of any kind of new injury, versus 13 (72%) of the ACP group (p=0.66). The relative risk for new postoperative MRI injury in the ACP group was 0.86 (95% confidence interval 0.16 – 4.36). When separating the cohort in univentricular and biventricular groups, in the univentricular group, 7 of 8 DHCA patients (88%) had new MRI lesions, versus 3 of 4 ACP patients (75%). In the biventricular group, 7 of 10 DHCA patients (70%) had new MRI lesions, versus 10 of 14 ACP patients (71%).

Most injury was WMI, of which the severity was not different between the two perfusion groups (see Supplemental data for examples of new WMI). WMI showed a bilateral distribution, and was only present in the frontal and medial territories. One patient in the DHCA group (the same patient in which ACP was also used during surgery) had a large left-sided middle cerebral artery infarction. Six patients, all from the ACP group, and all biventricular repair patients, had areas of infarction involving the thalamus, basal ganglia and/ or internal capsule (p=0.02 between DHCA and ACP). These were

restricted to the right side in five patients, and bilateral in one patient. Examples of these centrally located infarctions are shown in **Figure 3**. All new lesions appeared ischemic, with increased signal intensity (SI) on IR and reduced SI on T2SE), except for one case with large bilateral intraventricular hemorrhages and associated hemorrhagic lesions in the periventricular white matter (this was the only patient with new abnormalities on susceptibility-weighted imaging). Nine of DHCA patients (50%), and eight of ACP patients (44%) had lesions apparent on diffusion-weighted imaging, not visible on susceptibility-weighted imaging and therefore suggestive of ischemic injury.

Secondary cerebral outcome markers

Results of other measures of cerebral injury are listed in **Table 4**. These include those recorded by amplitude-integrated electro-encephalography (aEEG) and near-infrared spectroscopy (NIRS), which was continued for a median of 71 hours after surgery (range 48-186).

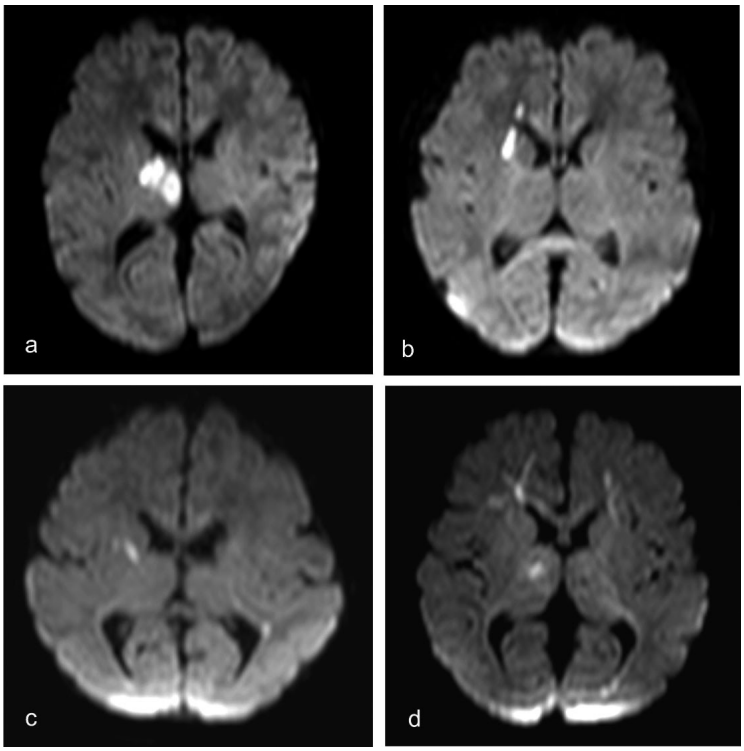


Figure 3. Central stroke after ACP. Four examples of diffusion weighted images patients from the ACP group with a new postoperative infarction located centrally on the right. a: Infarction of the right thalamus and part of the internal capsule, two days after surgery. b: Infarction of the head of the right caudate nucleus, five days after surgery. c: This patient had an ischemic area involving the right internal capsule, four days postoperatively. d. Apart from the severe WMI located especially frontally, this image also shows a small right thalamic infarction, 11 days postoperatively.

Table 3. MRI results

Pre-operative MRI	DHCA (n=19)	ACP (n=18)	
Any injury	12(63)	7(39)	
Severe injury*	3(16)	0(0)	
WMI	12(63)	6(33)	
Mild	2(11)	3(17)	
Moderate	6(32)	3(17)	
Severe	4(21)	0(0)	
Focal infarction	3(16)	0(0)	
Thalamus/ basal ganglia	2(11)	0(0)	
Cortical/ hemispheric	1(5.3)	0(0)	
Watershed injury	0(0)		
Intraparenchymal hemorrhage	1(5.3)	0(0)	
Cerebellar hemorrhage	0 (0)	0(0)	
Intraventricular hemorrhage	3(16)	2(11)	
Other abnormalities			
Subdural hemorrhage	9(47	4(22)	
Subarachnoidal hemorrhage	1(5.3)	2(11)	
Partial CSVT	3(16)	0(0)	
Total maturation score	13(11-14)	13(11-15)	
Incomplete circle of Willis	8(42)	1(5.6)	
Postoperative MRI ‡	**DHCA (n=18)**	**ACP (n=18)**	**p**
Any new injury	14(78)	13(72)	0.66†
Severe injury*	12(67)	11(61)	>0.99
WMI	13(72)	9(50)	0.31
Mild	4(22)	2(11)	0.66
Moderate	4(22)	3(17)	>0.99
Severe	5(28)	4(22)	>0.99
Focal infarction	1(5.6)	6(33)	0.09
Thalamus/ basal ganglia	0(0)	6(33)	0.02
Cortical/ hemispheric	1(5.6)	0(0)	>0.99
Watershed injury	0(0)	2(11)	0.49
Intraparenchymal hemorrhage	1(5.6)	0(0)	>0.99
Cerebellar hemorrhage	1(5.6)	0(0)	>0.99
Ventricular enlargement	0(0)	2(11)	0.48
Intraventricular hemorrhage	1(5.6)	0(0)	>0.99
Other new abnormalities			
Subdural hemorrhage	0(0)	1(5.6)	>0.99
Subarachnoidal hemorrhage	1(5.6)	0(0)	>0.99
Partial or complete CSVT	3(17)	2(11)	>0.99

Values stated in median (ranges) or number of patients (% of group).
*Severe injury was defined as severe WMI and/ or an infarction involving the basal ganglia, thalamus or internal capsule, or a large hemispheric infarction. †Result of sequential analysis. All other p-values are results of Mann-Whitney U testing. ‡All stated postoperative lesions are newly acquired lesions. Patients could have more than one type of lesion at a time. DHCA:deep hypothermic circulatory arrest; ACP:antegrade cerebral perfusion.
WMI= white matter injury. MRI= magnetic resonance imaging. CSVT= Cerebral sinovenous thrombosis.

Table 4. Secondary cerebral outcome data

Pre-operative	DHCA (n=19)	ACP (n=18)	
Clinical seizures	2(11)	0(0)	
aEEG			
Seizures on aEEG	2(11)	0(0)	
Continuous normal voltage	15(79)	16(89)	
Sleep-wake cycling	9(47)	9(50)	
Mean cerebral saturation (%)	63(51-81)	64(50-89)	
Injury biomarkers			
s100b (pg/ml)	23(4-79)	38(0-76)	
Neuron-specific enolase (ng/ml)	172(0-301)	152(0-400)	
Intra-operative	**DHCA (n=18)**	**ACP (n=18)**	**p**
Cerebral saturation			
Mean cerebral saturation during DHCA or ACP (%) (%)	52(46-65)	76(33-95)	<0.01
Lowest cerebral saturation (%)	43(15-56)	46(21-63)	0.04
Duration cerebral saturation <45% (min)	46(0-178)	8(0-164)	<0.01
aEEG			
Areas suspect for seizure activity	3(16)	2(11)	>0.99
Early postoperative	**DHCA (n=18)**	**ACP (n=18)**	**p**
Clinical seizures	2(11)	1(5.6)	>0.99
aEEG			
Seizure activity	3(17)	4(22)	0.69
Time to continuous normal voltage (hours)	5.8(0.0-30)	1.7(0.0-5.5)	<0.01
Time to sleep-wake cycling (hours)	16(7.0-125)	14(4.1-58)	0.17
Cerebral saturation (0-48 hours)			
Mean cerebral saturation (%)	66(46-83)	71(30-90)	0.39
Lowest cerebral saturation (%)	44(16-71)	49(24-74)	0.13
Injury biomarkers	**DHCA (n=16)**	**ACP (n=14)**	
s100b at 4 hours (pg/ml)	60(0-172)	62(0-124)	0.60
s100b at 24 hours (pg/ml)	33(16-87)	41(10-84)	0.48
Neuron-specific enolase at 4 hours (ng/ml)	137(0-488)	110(0-648)	0.95
Neuron-specific enolase at 24 hours (ng/ml)	99(0-704)	28(0-290)	0.10
Neurodevelopment	**DHCA (n=12)**	**ACP (n=11)**	**p**
Age at testing (months)	24(24-26)	24(23-26)	0.57
Motor function			
Cerebral palsy	2(17)	1(5.6)	>0.99
Motor composite score*	105(79-121)	103(85-118)	0.98
Score < 85	2(17)	0(0)	0.48
Fine motor scaled score †	13(7-18)	12(6-18)	0.83
Gross motor scaled score †	9(2-18)	8(6-10)	0.83
Walking age	18(13-26)	18(12-24)	0.93
Cognitive function			
Cognitive composite score*	105(70-140)	100(90-145)	0.61
Score < 85	1(8.3)	0(0)	>0.99

Values stated in medians (ranges) or number of patients (% of group).
[1] Motor and cognitive composite scores have an average of 100 (SD 15) in the normal population.
†Fine and gross motor scaled scores have an average of 10 (SD 3) in the normal population.
DHCA:deep hypothermic circulatory arrest; ACP:antegrade cerebral perfusion; aEEG:amplitude-integrated electro-encephalography.

During surgery, cerebral saturation was lower in the DHCA group than in the ACP group (p<0.01). Short periods suspect for seizure activity were detected by aEEG in three DHCA patients and two ACP patients (p>0.99). After surgery, three patients of the whole cohort (8.3%) had clinical seizures during postoperative hospital stay, without a difference between groups. aEEG detected additional subclinical seizures; making the total number of patients with clinical and subclinical seizures 7/36 (19%), with no difference between groups. The time to a normal background pattern on aEEG was longer after DHCA than ACP (median 5.8 hours compared to 1.7 hours; p<0.01). Mean cerebral saturation as measured by NIRS was not different after DHCA or ACP.

Markers of brain injury, s100b and neuron-specific enolase were also similar at four and 24 hours after surgery.

Early neurodevelopmental outcome

So far, 23 of the 35 survivors had reached the age of 24 months and could be tested for neurodevelopment. No differences in outcome were observed between groups. Three patients developed cerebral palsy; one with a unilateral spastic cerebral palsy (USCP) due to a large left sided middle cerebral artery infarction; another also with a USCP due to severe bilateral WMI with involvement of the internal capsule and the last patient had a bilateral spastic cerebral palsy due to severe bilateral WMI and a focal infarction in the right thalamus. Further markers of motor function (motor composite scores, fine and gross motor scaled scores and walking age) were not different between DHCA and ACP (p=0.98; p=0.83; p=0.83 and p=0.49, respectively). Regarding cognitive outcome, there were also no differences in composite score between the two groups (p=0.61). When the above analyses were repeated excluding patients with a genetic syndrome (n=3), results remained the same (motor composite score, p=0.92; cognitive composite score, p=0.55).

Clinical risk factors for new white matter injury (WMI)

Due to the observed incidence of new WMI postoperatively, an additional analysis was performed to assess which peri-operative clinical factors could be associated with new WMI. Results are presented in **Table 5**. This showed that of the demographic variables, only male sex showed a trend towards a relationship with new WMI (p=0.08). Surgical, anesthetic or perfusion-related issues, such as undergoing univentricular repair were not significantly different. Postoperatively, general measures of cardiac output such as blood pressure or inotrope score were very similar between groups, as well as cerebral saturation, and infection incidence. The only variable which showed a relationship with new WMI was postoperative arterial pCO2, which was lower in the group with new WMI (p=0.04). Of note, the day of performing postoperative scan was not different in the group with or without evidence of new WMI (median 7 [range 2-12] versus 6 days [2-27]; p=0.28).

Table 5. Risk factors for new white matter injury

	No new WMI (n=14)	New WMI (n=22)	p
Pre-operative			
Gestational age(weeks)	39.5(37.6-41.0)	39.0(35.3-41.0)	0.29
Birth weight(grams)	3420(2395-4055)	3340(2040-3970)	0.53
Male sex	9(64)	20(91)	0.08
Genetic syndrome	0(0)	3(14)	0.28
Total maturation score	13(11-14)	13(11-15)	0.34
Pre-operative WMI	7(50)	10(46)	>0.99
Intra-operative			
Univentricular repair	4(29)	8(36)	0.73
Age at surgery(days)	9(8-15)	9(5-34)	0.62
DHCA duration(min)	34(20-35)	35(21-45)	0.44
ACP duration(min)	36(25-48)	39(25-68)	0.44
CPB duration(min)	162(120-259)	160(96-376)	0.86
Cooling duration(min)	30(20-40)	30(20-61)	0.36
Rewarming duration(min)	41(26-75)	50(32-70)	0.21
Lowest nasopharyngeal temperature(°C)	17.8(15.8-19.1)	17.4(14.7-19.4)	0.36
Lowest cerebral saturation(%)	44(15-63)	44(21-58)	0.99
Duration cerebral saturation <45%(min)	22(0-178)	15(0-149)	0.65
AUC cerebral saturation <45%(%*min)	34(0-1570)	43(0-1765)	0.58
Mean O2 saturation(off CPB, %)	93(59-99)	95(57-100)	0.54
Mean arterial BP(off CPB, mm Hg)	51(38-58)	50(39-64)	0.83
Arterial pCO2(off CPB, mm Hg)	40(31-53)	41(26-48)	0.62
Mean end-tidal CO2(off CPB, kPa)	3.7(2.5-4.6)	3.8(2.4-4.8)	0.69
Postoperative			
Maximum inotrope score *[14]	21.5(11-67)	22(6.0-67)	0.91
Postoperative MV duration(days) *	6(2-12)	5(1-14)	0.40
Postoperative infection*	0(0)	1(4.5)	>0.99
Mean arterial BP(mm Hg)	56(46-65)	55(44-66)	0.43
Lowest arterial BP(mm Hg)	45(33-50)	43(35-50)	0.40
Mean systolic arterial BP(mm Hg)	73(51-92)	72(45-90)	0.99
Lowest systolic arterial BP(mm Hg)	58(45-73)	57(44-73)	0.68
Mean diastolic arterial BP(mm Hg)	46(36-55)	45(35-56)	>0.99
Lowest diastolic arterial BP(mm Hg)	35(27-43)	34(24-43)	0.96
Mean arterial oxygen saturation(%)	96(67-99)	96(60-99)	0.47
Lowest arterial oxygen saturation(%)	92(50-99)	92(45-98)	0.64
Mean arterial lactate(mmol/l)	1.9(0.7-6.0)	2.1(1.0-4.3)	0.91
Highest arterial lactate(mmol/l)	3.3(0.0-10)	3.8(1.4-13)	0.47
Mean arterial pCO2(mm Hg)	40(35-43)	38(34-43)	0.04
Lowest arterial pCO2(mm Hg)	34(28-40)	31(27-39)	0.12
Mean end-tidal CO2(kPa)	4.8(4.0-5.5)	4.3(2.1-5.5)	0.08
Lowest end-tidal CO2(kPa)	3.6(3.1-4.7)	3.5(2.0-4.9)	0.38
Mean cerebral saturation(%)	64(39-77)	63(35-87)	0.47
Lowest cerebral saturation(%)	49(24-71)	44(16-74)	0.19
Duration cerebral saturation <45%(min)	0(0-1367)	83(0-1272)	0.40
AUC cerebral saturation <45%(%*min)	0(0-19327)	156(0-17782)	0.22
Anesthetics**			
Clonidine(mg/kg)	44(0-240)	0(0-192)	0.38
Sufentanil(mg/kg)	6.8(3.9-11.3)	6.5(4.0-12.5)	0.91
Midazolam(mg/kg)	30.3(6.7-6..0)	20.8(0.7-48.1)	0.34
Morfine(mg/kg)	1.5(0.52-2.7)	1.6(0.68-3.8)	0.75
Pancuronium(mg/kg)	0.24(0.00-0.56)	0.30(0.00-0.65)	0.28
Fentanyl(ug/kg)	1.4(0.0-24.4)	1.7(0.0-19.2)	0.99
Sevoflurane(inhaled, end-tidal %*min)	4(0-90)	39(0-375)	0.12
Isoflurane(in CPB, %*min)	53(0-130)	45(0-150)	0.96

Values stated in median (ranges) or number of patients (% of group).
* Before postoperative MRI. **Includes all anesthesia between pre- and postoperative MRI.
All postoperative physiologic parameters were calculated over the first 48 hours postoperatively. All 'lowest' values were those which sustained for 10 minutes or more.
WMI:white matter injury; Min:minutes; BP:blood pressure.

DISCUSSION

In the current randomized controlled trial, the use of DHCA and ACP during neonatal cardiac surgery was compared with respect to cerebral injury. A number of previously performed studies formed the basis for this study. The landmark trial in which the effect of DHCA was first compared to an alternative perfusion technique, was the Boston Circulatory Arrest Study[15]. Patients with TGA were randomized to DHCA or low-flow CPB (which also included short periods of DHCA). While the DHCA group initially fared worse in terms of neurodevelopment, the children who are now adolescents, subsequently failed to show any differences in a battery of elaborate neurodevelopmental tests[1, 15]. However, the original findings did lead to a proposed 'limit' for DHCA duration (41 minutes), above which DHCA was considered to have an adverse effect on cerebral function[16]. This was confirmed in a very recent study by Beca et al, who showed a higher rate of postoperative WMI when DHCA was used during aortic arch repair[6]. There have been two studies in which DHCA was directly compared to ACP in a randomized manner. In the first study, by Goldberg et al, neonates undergoing the Norwood procedure were assigned to either DHCA or ACP. Neurodevelopment was tested at the age of one year, following the second stage of the Norwood procedure[11]. Infants generally showed a substantially delayed development, without differences between DHCA and ACP [17]. The second trial, performed in adults undergoing pulmonary endartertectomy, also failed to find any difference in cognitive tests after surgery. However, both studies used neurocognitive outcome as outcome parameter, which may not be as sensitive a measure of injury as imaging techniques can be, and may be influenced by adverse events or additional surgeries performed in the interim.

Therefore, we used pre- and postoperatively acquired MRI to assess the direct effects of DHCA versus ACP, and did not find a significant difference in incidence of new cerebral injury between the two perfusion techniques. Three-quarters of all patients developed evidence of new injury, of which WMI was most common. However, after ACP, a higher incidence of central infarctions was observed compared to DHCA. There was no difference with regard to surgical or other cerebral outcome measures postoperative seizure incidence, regional cerebral saturation, brain injury biomarkers and neurodevelopment at two years.

Already pre-operatively, 50% of all patients had evidence of cerebral injury, which is high compared to the published rates in literature (23-40%)[2-6]. The reason for this is unknown, but the slightly later age at surgery at our centre (median of ten days), may play a role as these range between four and seven days in the above named studies. The ongoing pre-operative hemodynamic insufficiency may result in a cumulative risk for more cerebral injury, as has been seen before surgery in patients with transposition of the great arteries[18].

However, most attention should go out to the high incidence of new postoperative injury. After both DHCA and ACP, WMI was the most common type of injury, which added to the already present WMI often observed before surgery. However, in approximately one-third of the ACP population, MRI also revealed central infarctions, mostly on the right side. This phenomenon has been noted previously in the study of McQuillen et al, in which

patients with TGA or HLHS were included and postoperatively the infarcts were solely seen in the HLHS patients, who had undergone surgery with ACP[4]. We hypothesize that the infarcts are directly attributable to the use of ACP, as this involves selective perfusion of the right brachiocephalic artery. This may introduce micro-particles or air emboli directly into the right carotid artery, which likely travel via the middle cerebral artery to the lenticulostriate arteries perfusing the basal ganglia, thalami and internal capsule[19]. During full-body CPB, these particles would otherwise end up in the abdominal circulation[20].

The high incidence of new WMI in our study deserves further attention (67% of the whole cohort), as this affected three-quarters of all patients. Previous studies of mostly univentricular patients in whom pre- and postoperative MRI were performed, reported new WMI in 16-47% of patients[2-5]. The reasons for the high incidence in the current study are not entirely clear. Regarding technical issues, MRI scanners and protocols and the timing of scanning are comparable to those of other studies. Therefore, differences in peri-operative management may underlie the increased incidence of postoperative WMI. Surgical and perfusion protocols do not show a discrepancy with those published in similar studies; i.e. various aspects such as cooling depth, cooling duration, ACP perfusion rate, surgery times, and surgical outcome are comparable to those used at other centres[3, 5-8]. The anesthetic medication of our centre is similar to those used in other studies[3, 5-8]. There is an ongoing debate on whether pH-stat strategy is superior to alpha-stat strategy, and similarly it is unclear whether a low (20%) or high (30%) hematocrit is preferable[21]. Finally, we do not know the effect of the pre-operative dexamethasone which is routinely used at our institution, even though similar doses have been associated with a delayed later development in premature infants with chronic lung disease[22].

It is known from studies in premature neonates that cerebral hypoperfusion, hypoxia and inflammation are all considered to contribute to WMI[23]. Especially since neonates with complex cardiac disease seem to have delayed cerebral development, making them susceptible to these premature types of cerebral injury, it is likely that the same causative factors play a role[4]. Similarly, previous MRI studies performed after neonatal cardiac surgery have found that lower postoperative blood pressure, regional cerebral saturation and a preceding infection, are associated with the occurrence of new lesions [5, 8, 24]. Animal models of CPB underscore this and have found lower oxygenation and higher inflammation to lead to more WMI. Hence, as proposed by Andropoulos et al, a targeted protocol aiming for acceptable postoperative regional cerebral saturation values, may be necessary to ensure adequate cerebral perfusion[5]. Interventions include increasing blood pressure and actively increasing pCO2 values (i.e., to 45-55 mm Hg), as these changes have been shown to have a direct effect on cerebral saturation in the early postoperative period and a higher pCO2 post-surgery has been associated with improved cerebral function postoperatively[25]. Indeed, when we performed an additional analysis of our cohort to assess the impact of known risk factors for WMI, lower arterial pCO2 levels were modestly associated with an increased risk of new WMI. Although the difference between the two groups may seem trivial, it may indicate the 'tipping point' at which WMI manifests itself. Most likely, there is a complex interplay of carbon dioxide

pressure with other factors which influence cerebral perfusion and oxygenation. On the other hand, the susceptibility of oligodendrocytes, and their specific maturational subsets, will define the eventual injury. For example, males may be more predisposed to WMI, as has been seen in premature neonates[26]. This may explain why male sex shows a trend towards being more common in the group with new WMI in our study.

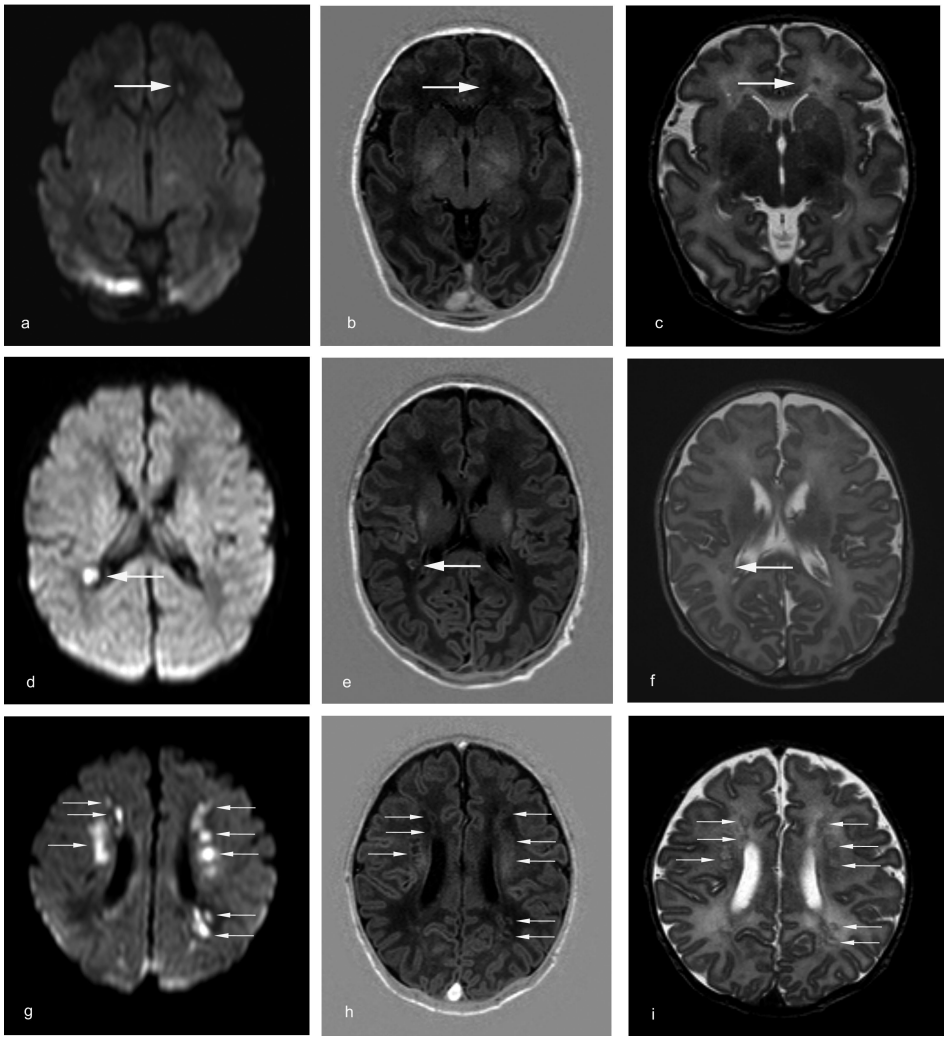
In general, early neurodevelopmental outcome was well within the normal range in the current cohort. However, three patients did develop cerebral palsy. With regard to the other cerebral outcome variables, aEEG took longer to recover to a normal background pattern after DHCA compared to ACP. This was also observed in the Boston Circulatory Arrest Study, of which the underlying mechanism remains to be elucidated[15]. Though , in the absence of significantly more cerebral lesions in the DHCA group, we hypothesize that this delayed recovery is a reflective of the cerebral recovery after profound ischemia. Serological markers of brain injury, s100b and neuron-specific enolase, were similar between groups and in the range of those of other neonates after cardiac surgery[27]. Furthermore, surgical outcome was also comparable between the two groups.

Sequential analysis was used to assess whether ACP could decrease the incidence of new postoperative lesions compared to DHCA. This powerful statistical tool is suitable for studies in patient groups with rare conditions, which limit the number of available patients within a certain time period[28, 29]. Especially when the outcome parameter is available shortly after inclusion of the patient (as is the case in MRI studies), the sequential analysis of accumulating data can prevent further inclusion of individuals while results are already conclusive. In this RCT, the data of the 36th patient showed that the lower boundary had been crossed and that there was no clear difference between DHCA and ACP with regard to incidence of new MRI lesions. This resulted in an earlier end of the trial than if classical statistical techniques had been used (i.e., 50 patients). Especially in this specific patient group, where there is a high burden of disease in a relatively rare population, use of these statistical techniques can help to investigate more research hypotheses in the same time frame, and subsequently help to improve outcome[30].

The choice for sequential analysis did however result in a relatively small group of patients, limiting definite conclusions of secondary analyses as these may be underpowered. For the detailed assessment of the exact size and location of the different MRI lesions, it may have been more informative to study more patients. Also, the results of the potential risk factors for new WMI are not conclusive and the role of CO2 must be confirmed. Finally, neurodevelopmental testing of the full cohort, also later in childhood, must be awaited to reveal the full consequences of the injury seen on MRI, as the relationship between new lesions and later outcome is as yet an unresolved issue.

In summary, the results of this randomized controlled trial between DHCA and ACP in neonates with aortic arch obstructions demonstrate that there is no significant difference between the two perfusion techniques with regard to new cerebral lesions as evident on MRI. ACP is no more neuroprotective than DHCA, and may even lead to more focal infarcts in central regions of the brain. Furthermore, WMI remains the most common type of injury acquired peri-operatively, and strict postoperative monitoring of the pCO2 may help to reduce this burden.

SUPPLEMENTAL DATA



Supplemental figure 1. Severities of white matter injury.Examples of different severities of new postoperative white matter injury (WMI). a-c: Mild WMI in the left frontal lobe, shown on DWI, IR and T2 images, respectively. d-f: Moderate WMI (one right periventricular lesion larger than 2 mm). g-h: Severe WMI with a bilateral distribution. This patient also had a cerebral sinovenous thrombosis.

Supplemental methods

Surgery and CPB

In all patients, surgery was performed through a median sternotomy and standard cannulation techniques with double venous cannulation were applied. In case of interrupted aortic arch, double cannulation of the distal ascending aorta and the pulmonary trunk was performed. Depending on the individual anatomic situation, aortic arch reconstruction was performed with direct end-to-end or end-to-side anastomosis, reverse subclavian flap and patch plasty or patch plasty alone. All procedures were performed by a single surgical team.

CPB was conducted using a Stöckert S5 mast-mounted roller pump (Stöckert, Munich, Germany), a hollow-fiber membrane oxygenator (Capiox RX05; Terumo Corp., Tokyo, Japan), pediatric arterial filter (Capiox AF02; Terumo Corp.), hemoconcentrator BC20 (Maquet CP, Germany) and tubing set from Sorin Group (Italy). A total prime volume of 350 ml was used. Isoflurane (up to 2%) was used to improve cooling while on CPB.

Cerebral monitoring

Cerebral near infrared-specstrocopy (NIRS) and amplitude-integrated electro-encephalography (aEEG) were measured peri-operatively (NIRS: Invos 5100C, Somanetics Inc., Troy, MI, USA; aEEG: BRM2 monitor, BrainZ, Natus, San Carlos, CA, USA). Both were routinely started the night before surgery, or at least one hour before surgery. Pediatric NIRS sensors were placed bilaterally on the forehead. aEEG was performed using 5 intracutaneous electrodes. Data was simultaneously recorded on the BrainZ monitor, together with physiological data via a Philips Intellivue MP70 monitor. Analysis of the collected data was performed in SignalBase SignalBase version 7 (University Medical Center Utrecht, the Netherlands).

Recordings of aEEG were assessed for seizure activity by two neonatologists with longstanding experience in interpretation of aEEG patterns (LdV and MT). The start of (imminent) sleep-wake cycling was also assessed, and background patterns were defined as described in the Atlas of Amplitude-integrated EEGs in the Newborn (second edition; de Vries, Westas and Rosen; Informa Healthcare, London, England).

Biomarkers

Samples of four and 24 hours after surgery were collected in 30 patients for determination of biomarker concentrations. These were analysed at HaemoScan in Groningen, The Netherlands. s100b and neuron-specific enolase levels were measured using using ELISA assay by means of antibodies and standards obtained from Abnova (Taipei city, Taiwan) and Hytest (Turku, Finland), respectively.

Magnetic Resonance Imaging

MR imaging was performed using a 1.5-T magnet (Gyroscan ACS-NT; Philips Medical Systems, Best, The Netherlands). MR imaging included sagittal T1-, transverse T2-, and inversion recovery-weighted sequences. An echo-planar imaging technique was

used for DW imaging (repetition time msec/echo time msec = 3800-5200/89), with a 180 x 180-mm field of view, 4-mm-thick sections, 0-mm section gap, and *b* factors of 0 and 1000 sec/mm^2 (1.5-T). Susceptibility Weighted Imaging (SWI), MR angiography (MRA) were also included in the protocol.

Classification of MRI lesions

Newly acquired injury was defined as new white matter injury (WMI), focal infarct, watershed injury, intraparenchymal hemorrhage, intraventricular hemorrhage (IVH) or other evident ventricular enlargement (possibly due to loss of cerebral tissue). Hence, subdural or subarachnoidal hemorrhages (SDH and SAH respectively) and sinovenous thromboses (SVT), in the absence of structural cerebral injury, were not included in the classification of newly acquired injury.

All lesions in the WM were classified als WMI, except for those in continuum with the basal ganglia or other central structures; these were classified as areas of infarction. Regarding classification of WMI, 'mild' was defined by the presence of not more than 3 lesions, all smaller than 2 mm ('punctate lesions'); 'moderate' as 4-5 punctate lesions or 1 or 2 lesions of 2-4 mm; and 'severe' was defined as 6 or more punctate lesions or 2 or more lesions of 4 mm or larger.

Ventricular enlargement was defined as the enlargement of 1 or both lateral ventricles; from normal (<8mm) to at least moderately enlarged (8-10mm); or moderately enlarged to too large (>10mm).

Severe cerebral injury was defined as severe WMI or an infarction involving the basal ganglia, thalamus or internal capsule.

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4

NORWOOD PROCEDURE USING MODIFIED BLALOCK-TAUSSIG SHUNT: BEWARE OF THE CIRCLE OF WILLIS

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Neonates undergoing complex cardiac surgery are at high risk of developing cerebral damage[1]. In the past decades, surgical and cardiopulmonary bypass strategies have been modified to improve neurodevelopmental outcome. One example is the introduction of antegrade cerebral perfusion (ACP) during aortic arch repair, instead of deep hypothermic circulatory arrest. Although it is not yet known whether this indeed is a superior strategy, ACP is now widely used in congenital heart surgery[2]. In this case report we show that when ACP is performed, cerebral near infrared spectroscopy (NIRS) can provide important information about the Circle of Willis, which may influence the surgical strategy.

CLINICAL SUMMARY

A term infant presented with double inlet left ventricle, transposition of the great arteries, and an obstruction of the aortic arch. He was scheduled to undergo the Norwood procedure. According to our standard protocol, cerebral MRI was performed the day before surgery and NIRS electrodes were placed on both sides of the forehead, showing similar values on right and left hemisphere (mean regional oxygen saturation right 76%, left 72%).

During surgery, a modified Blalock-Taussig (BT) shunt was placed on the brachiocephalic artery, just distal of the bifurcation, to perform ACP (43 ml/kg/min). During this period (50 minutes) right NIRS was continuously 20% higher than left (mean right: 92%, left: 72%). Following arch repair, full CPB was resumed and right and left NIRS values approximated each other. However, when the patient was weaned off bypass, asymmetric NIRS values emerged again, but now with lower NIRS values on the right hemisphere. NIRS was monitored until 48 hours postoperatively, right NIRS continuing to show a 15-20% lower value than left (mean right: 41%, left: 55%). Continuous electro-encephalography showed no abnormalities or asymmetries during the entire perioperative period.

Cerebral MRI performed 1 week after Norwood palliation did not show any ischemic or hemorrhagic lesions. MR angiography revealed that there was no flow through the right carotid artery (pre-operatively 71 ml/min), opposed to a flow of 50 ml/min and 27 ml/min through the left carotid and basilary artery, respectively. The Circle of Willis was incomplete as there was no left posterior communicating artery (**figures 1** and **2**).

Three weeks post-surgery, the asymmetry was re-assessed by NIRS and ultrasound of the carotid arteries. Both showed no differences between right and left side. Neurological follow-up at three and nine months of age did not reveal any abnormalities.

DISCUSSION

The modified BT-shunt is frequently used for Norwood stage I procedure. In literature, it has been suggested that this may result in a reduced flow through the right carotid artery, but this has not been confirmed using modern vascular imaging techniques[3]. In the present case we have demonstrated a reduced perfusion of the right cerebral hemisphere using multiple modalities.

The reduced flow through the right carotid artery after stage I Norwood, is likely due to the combination of a 'steal' by the BT-shunt, and a fetal-type (incomplete) variant of the

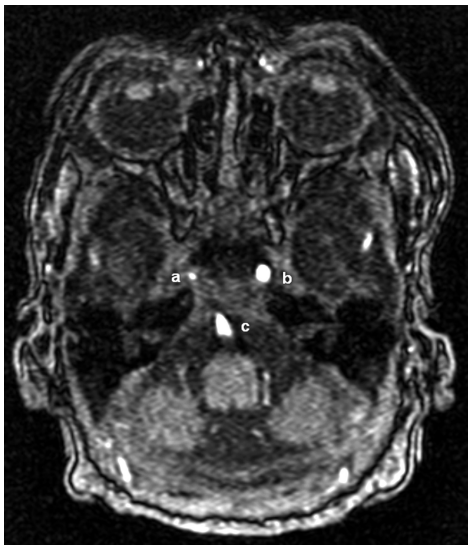

Figure 1. MR angiography of the cerebral arteries. a: Right carotid artery, b: left carotid artery, c: basilary artery.

Figure 2. MR angiography of Circle of Willis. Arrow indicates absent left posterior communicating artery.

Circle of Willis. This occurs in approximately 25% of the healthy adult population, and is even more common in premature neonates[4]. It need not be a problem, if the other (carotid and basilary) arteries are able to compensate. In the present case, the left carotid artery pre-operatively already additionally perfused the posterior part of the left hemisphere. After placement of the BT-shunt, perfusion of a major part of the right hemisphere also depended on the left carotid artery. This resulted in a lower perfusion of the right frontal region and subsequently in lower NIRS values on the right. This very large strain on the left carotid artery put this child at high risk for cerebral ischemia. Placement of a right ventricle to pulmonary artery shunt may have prevented this situation.

The substantially lower NIRS values on the left hemisphere during ACP were a warning sign that in our patient, there was an incomplete Circle of Willis. Asymmetries during ACP in neonatal arch reconstructions have been observed before, however without vascular imaging studies[5].

In conclusion, a more than 20% lower left than right cerebral NIRS during ACP is suggestive of an incomplete Circle of Willis. In addition, the placement of a modified BT-shunt can cause an arrest of flow through the right carotid artery. The combination of these two is highly undesirable, although this did not have clinical consequences for this patient. Therefore we propose that when a large asymmetry in NIRS values is noticed during ACP, a right ventricle to pulmonary artery shunt deserves consideration, to avoid risk of cerebral damage of the right hemisphere.

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5

ROLE OF BED-SIDE CEREBRAL MONITORING TO PREDICT CEREBRAL INJURY IN NEONATES UNDERGOING COMPLEX CARDIAC SURGERY

Article in preparation

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ABSTRACT

Background

Neonates undergoing aortic arch reconstruction are at risk to acquire cerebral injury. Imaging techniques, in particular magnetic resonance imaging (MRI) are the gold standard for assessment of the burden of injury. However, this is an expensive and time-consuming modality. Therefore, we assessed whether bed-side cerebral monitoring techniques could also predict the development of new postoperative injury as seen on MRI. Furthermore, the relationship with early neurodevelopment was assessed.

Methods

In thirty-six neonates admitted for aortic arch repair, pre- and postoperative MRIs were performed. The presence of new cerebral injury was scored by two observers. Throughout surgery until 48-72 hours thereafter, amplitude-integrated encephalography (aEEG) and near-infrared spectroscopy (NIRS) were used to monitor cerebral function and oxygenation, respectively. Serum biomarkers s100b and neuron-specific enolase (NSE) were assessed before and after surgery. Neurodevelopment was assessed at a minimum age of 15 months.

Results

Twenty of 36 patients (55%) had substantial new injury on the postoperative MRI. Postoperative electrographic seizures on aEEG occurred in 11 patients (31%, of which 9 were subclinical) and were significantly associated with both new cerebral injury on MRI, as well as motor and cognitive outcome at a median age of 24 months ($p<0.01$, $p=0.04$ and $p=0.03$, respectively). Neither electrographic seizures occurring *during* surgery, nor time to a normal background pattern or sleep-wake cycling after surgery were related to new injury or neurodevelopment. The same applied for intra- and postoperative NIRS. However, cerebral biomarkers s100b and NSE after surgery, were associated with neurodevelopment ($p=0.02$ and $p<0.01$, respectively).

Conclusion

Continuous aEEG monitoring is useful to predict postoperative development of cerebral injury and neurodevelopmental outcome in neonates undergoing aortic arch reconstruction. In this cohort, NIRS was not found to be predictive of injury. Measurement of serum biomarkers may prove effective in the identification of high-risk patients.

BACKGROUND

It is becoming increasingly clear that complex cardiac surgery performed in neonates is associated with a high risk of cerebral injury. Especially neonates with hypoplastic left heart syndrome (HLHS) and other types of aortic arch obstruction have a high burden of injury[1, 2]. A school age, approximately one in three children has a delay in one or more developmental domains.

Cerebral MRI studies have revealed that already before surgery, abnormalities can be seen in 23-40% of neonates with a complex cardiac defect[3-7]. Newly acquired lesions are observed after surgery in 36-73%[3, 4, 6-10]. These are mostly seen in the white matter, thought to be due to the relative immaturity of the brain due to an delayed cerebral development in utero[11]. Additional postnatal triggers include hypoperfusion, hypoxia and inflammation. The second type of injury is stroke, which is likely to have an embolic etiology[12].

MRI is currently the gold standard in assessing peri-operative injury in these neonates. However, it is a very time-consuming and expensive modality, not only due to the scanning apparatus, but also due to the logistical issues fraught with transporting a relatively unstable neonate to the MRI suite. Therefore, bed-side cerebral monitoring techniques can play an important role in the risk assessment. Examples are amplitude-integrated electro-encephalography (aEEG), near-infrared spectroscopy (NIRS) and serum-based markers of cerebral injury.

aEEG is a time-compressed type of EEG, which records cerebral activity for days at the bed-side. aEEG can detect subclinical electrographic seizures, which is especially important in heavily sedated patients such as those who undergo cardiac surgery who rarely have clinical symptoms. Also, the time to recover to a normal background pattern after perinatal asphyxia or cardiac surgery, and the presence of sleep-wake cycling can be assessed. All the above have been shown to correlate with cerebral injury and later outcome in neonates after perinatal asphyxia, and several studies suggest that these also are predictive of mortality and neurodevelopmental outcome in neonates undergoing cardiac surgery[13-16].

Cerebral NIRS provides a measure of regional tissue oxygen saturation. NIRS can therefore be useful as a general trend for cardiac output and oxygenation[17]. Regarding cerebral injury, lower NIRS values have been associated with increased peri-operative cerebral injury, both in neonates and adults undergoing cardiac surgery[3, 18-20]. However, this association has not been consistently found in all cohorts, which prevents definite conclusions on the clinical effectiveness specifically for cerebral injury[6].

Finally, serum-based biomarkers are hoped to be of use in the identification of neonates at high risk of cerebral injury. s100b and neuron-specific enolase (NSE) are products of neuronal or astroglial injury, which enter the circulation after cerebral injury. Especially in cases of perinatal asphyxia, these markers were noted to have a strong association with the extent of injury as seen either on MRI, or in later neurodevelopment[21, 22]. Also in adults with stroke, biomarkers have provided an insight into the later consequences of the injury[23].

The aim of the current study was to assess the above three types of cerebral monitoring modalities for their relationship with cerebral injury. In a cohort of neonates undergoing cardiac surgery for aortic arch obstruction, MRI was used as

the gold standard to assess brain injury, being the most sensitive modality available. Early neurodevelopment was also taken into account to assess whether the observed relationships proved to be clinically relevant.

METHODS

The current study is based on data collected in a randomized controlled trial comparing the cerebral effects of deep hypothermic circulatory arrest (DHCA) versus antegrade cerebral perfusion (ACP), of which the results will be published elsewhere. The trial was performed between January 2009 and May 2012, and all neonates presenting for aortic arch reconstruction were assessed for enrolment. Exclusion criteria were: 1) age more than four months; 2) high suspicion of a genetic syndrome; 3) need for sedation and intubation exclusively for the pre-operative MRI or 4) an expected time needed for aortic arch reconstruction for more than 60 minutes. Written informed consent was obtained from both parents of all included patients. The current study protocol was approved by the local medical-ethical committee and is registered at clinicaltrials.gov, number NCT01032876.

Cardiac surgery and cardiopulmonary bypass

Aortic arch reconstruction and intracardiac repairs were performed as described previously[24]. After the start of cardiopulmonary bypass (CPB), patients were cooled to a nasopharyngeal temperature of approximately 18°C, and either DHCA or ACP was used during the aortic arch reconstruction. ACP was performed either by direct advancement of the arterial cannula from the ascending aorta into the brachiocephalic artery or via a Blalock-Taussig shunt sewed to the brachiocephalic artery. ACP was set at 25% of full-flow, with right radial artery pressures not exceeding 40 mm Hg. After rewarming to normothermia (at least 35°C) on full-flow CPB and later weaning from CPB, patients were transported to the intensive care unit for postoperative recovery. Standard anesthetic protocols during surgery included sevoflurane for induction of anesthesia, midazolam and sufentanil for continuous sedation and pancuronium for muscle blockade. Postoperative sedation was routinely ensured by midazolam, morphine, and clonidine.

Cerebral data

For the above named study, pre- and postoperative MRI was performed on a 1·5-Tesla scanner (Philips Medical Systems, Best, the Netherlands). The postoperative scan was planned 3 to 10 days after surgery, depending on the hemodynamic stability of the patient. In this study, MRI injury was regarded as the presence of any of the following abnormalities: moderate or severe white matter injury ('moderate': 4-5 punctate lesions or 1 or 2 lesions of 2-4 mm; 'severe': 6 or more punctate lesions or 2 or more lesions of 4 mm or larger); a focal infarction, watershed injury, intraparenchymal hemorrhage or intraventricular hemorrhage with enlarged ventricles. Hence, other mild abnormalities were not regarded as 'injury', i.e. mild WMI (3 or less punctate lesions), or small subdural or subarachnoidal hemorrhages, or small intraventricular hemorrhages. Images included sagittal T1-, transverse T2-, and inversion recovery-weighted sequences. An

echo-planar imaging technique was used for DW imaging (repetition time msec/echo time msec = 3800-5200/89), with a 180 x 180-mm field of view, 4-mm-thick sections, 0-mm section gap, and *b* factors of 0 and 1000 sec/mm^2 (1.5-T).

Cerebral monitoring was performed peri-operatively using amplitude-integrated encephalography (aEEG; BRM2 monitor, BrainZ, Natus, San Carlos, CA, USA) and near-infrared spectroscopy (NIRS. Invos 5100C, Somanetics Inc., Troy, MI, USA). Both were routinely started the night before surgery, but at least one hour before surgery, and continued throughout the procedure until 48-72 hours postoperatively. aEEG was performed using 5 needle electrodes on positions P (parietal) 3 and 4 and F (frontal 3 and 4). Pediatric NIRS sensors were placed bilaterally over the fronto-parietal region. 'Lowest NIRS' was defined as the minimum value which was sustained for at least 10 minutes[6].

Interpretation of the aEEG recordings for electrographic seizures, background pattern and sleep-wake cycling was performed by two neonatologists with longstanding experience in interpretation of aEEG, who were blinded to the patient and imaging data (LdV and MT).

In a subgroup of patients, biomarkers of cerebral injury, s100b and neuron-specific enolase (NSE) were measured in serum. Samples were drawn pre-operatively and at 4 and 24 hours and 1 week after surgery. Laboratory analysis was performed at HaemoScan in Groningen, The Netherlands. s100b and neuron-specific enolase levels were measured using using ELISA assay by means of antibodies and standards obtained from Abnova (Taipei city, Taiwan) and Hytest (Turku, Finland), respectively.

Finally, early neurodevelopment was assessed by a team of neonatologists and child physiotherapists between 15 and 26 months of age, using either the Griffiths Mental Development Scales (GMDS, mean developmental quotient 100, SD ±12)) at approximately 18 months and the Bayley Scales of Infant and Toddler Development, version III (BSITD, mean composite score [CS] 100, SD ±15) at approximately 24 months[25, 26].

Statistics

All group characteristics are listed as median (ranges) or number of patients (% of group). Differences between groups were tested using the Fisher exact test for dichotomous variables, or the Mann-Whitney U test for continuous variables. The relationship between two continuous variables was tested by linear regression, with log transformation if necessary. SPSS version 19·0 was used (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 37 patients were originally included in the randomized controlled trial on which this study was based. One patient however, diagnosed with a complete atrioventricular septum defect, situs inversus and a hypoplastic aortic arch died six weeks after corrective surgery due to an ongoing mitral valve insufficiency without further surgical options. This patient was the only case in whom a postoperative MRI scan could not be performed, resulting in 36 patients available for the current study. Another patient died one week after discharge due to a sudden cardiac arrest after the Norwood procedure one week after discharge. Hence, total survival to date of the current cohort is 35/36 (97%).

Patient characteristics are outlined in Table 1. Twenty-four patients had a hypoplastic aortic arch with associated anomalies for which a biventricular repair could be performed, and 12 patients underwent univentricular repair for primarily hypoplastic left heart syndrome. Patient underwent surgery at e median age of 10 days (range 5-34), all with cardiopulmonary bypass including deep hypothermia. Either deep hypothermic circulatory arrest (DHCA) or antegrade cerebral perfusion (ACP) was used during aortic arch reconstruction, except for one patient in which both DHCA and ACP were used. After surgery, patients remained in the intensive care unit for a median of 8 days (range 1-52). Cumulative dosages of anesthetics used during and after surgery are listed in Table 1.

MRI results and early neurodevelopment

Pre- operative MRI was performed at a median age of eight days (range 4-34). Thirteen patients (35%) had injury pre-operatively, excluding cases of mild WMI, intraventricular hemorrhage and small subdural or subarachnoidal hemorrhage. Injury consisted of white matter injury in all, with additionally a thalamic and/or basal ganglia infarction in two patients, a cortical infarction in one patient and multiple small periventricular hemorrhages in another patient.

Postoperative MRI was performed at a median of 6 days after surgery (range 2-27). Twenty patients (55%) had new injury on their postoperative MRI scan. Sixteen patients had new WMI, with or without other abnormalities, of which 7 had areas of infarction (6 in the basal ganglia or thalamic regions, and one large middle cerebral artery infarction), 2 had watershed injury, one severe intraventricular and periventricular hemorrhages and one had multiple small cerebellar hemorrhages.

To date, 25 patients have reached an age suitable for neurodevelopmental testing, at median of 24 months (range 15-26). Of these, three patients (12%) developed cerebral palsy; one with a unilateral spastic cerebral palsy (USCP) due to a large left sided middle cerebral artery infarct; another also with a USCP due to severe bilateral WMI with involvement of the internal capsule and the last patient had a bilateral spastic cerebral palsy due to severe bilateral WMI and a focal infarction in the right thalamus. Stated otherwise, of the whole cohort, one patient (4%) had a mild motor delay (PDI <85), and three (12%) had a severe motor delay (PDI<70). Regarding cognitive outcome, one patient (4%) had a mild cognitive delay (MDI<85) and one (4%) a severe cognitive delay (MDI<70).

There was a trend towards a relationship between the pre-operative MRI and early motor outcome (no injury, median score 107 [85-121]; with injury, 96 [79-112]); p=0.10). This was also the case for newly acquired postoperative MRI injury (110 [85-121], versus 102 [79-118], p=0.07). Pre- and postoperative MRI injury was not associated with cognitive scores (pre-operative 105 [90-145]; vs. 100 [70-110]; p=0.27; and postoperative 105 [90-140], versus 100 [70-145], p=0.52).

Amplitude-integrated EEG

Pre-operative

Pre-operatively, two patients had clinical seizures accompanying their electrographic seizures; one with a left cortical infarction and the other with bilateral moderate WMI.

Table 1. Patient characteristics

Male sex	30 (81)
Prenatal diagnosis	24 (65)
Gestational age at birth (weeks)	39.0 (35.3-41.0)
Birth weight (g)	3340 (2040-4055)
Pre-operative mechanical ventilation duration(days)	0 (0-12)
Cardiac diagnosis:	
Biventricular	
Hypoplastic left heart complex[31]	5 (14)
IAA/CoA and hypoplastic aortic arch, with:	
No intracardiac lesions	2 (5.6)
ASD and/or VSD	8 (22)
DORV with or without TGA, VSD	5 (14)
TGA	2 (5.6)
Severe LVOTO	2 (5.6)
Univentricular	
Hypoplastic left heart syndrome	8 (22)
Tricuspid atresia	2 (5.6)
Double inlet left ventricle	2 (5.6)
Age at surgery (days)	10 (5-34)
CPB duration (min)	161 (96-376)
Duration of DHCA and/or ACP (min)	36 (20-68)
Lowest nasopharyngeal temperature (°C)	17.6 (14.7-19.4)
Duration of mechanical ventilation	6 (1-51)
Duration of postoperative ICU stay	8 (2-52)
Duration of open sternum	3 (0-6)
Cumulative dose of pre-operative sedation:	
Midazolam (mg/kg)	0.1 (0.0-11)
Morphine (mg/kg)	0.2 (0.0-1.6)
Cumulative dose of intra-operative sedation:	
Sevoflurane (volume%*min)	15 (0.0-375)
Sufentanil (mg/kg)	6.8 (3.9-13)
Midazolam (mg/kg)	1.2 (0.5-3.9)
Cumulative dose of postoperative sedation:	
Midazolam mg/kg	8.3 (0.2-19)
Morphine (mg/kg)	1.6 (0.50-20)
Fentanyl (ug/kg)	1.7 (0.0-24)

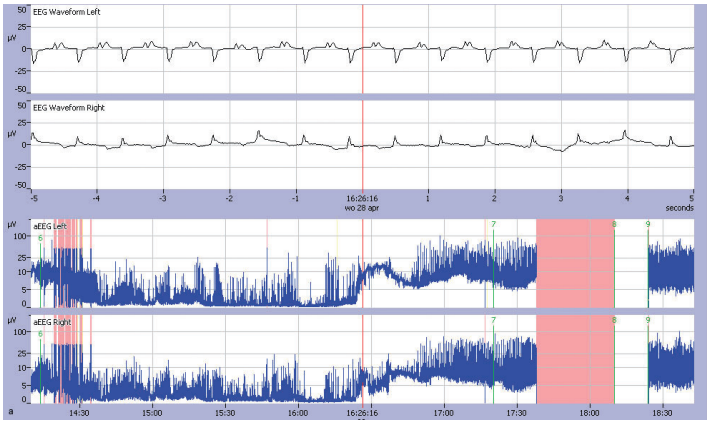
Values stated as median (range) or number of patients (% of injury group).
IAA: interrupted aortic arch; CoA: coarctation of the aortic arch; ASD: atrial septal defect; VSD: ventricular septal defect; DORV: double outlet right ventricle; TGA: transposition of the great arteries; LVOTO: left ventricular outflow tract obstruction; CPB: cardiopulmonary bypass; DHCA: deep hypothermic circulatory arrest; ACP: antegrade cerebral perfusion.

In the hours before surgery, 31 patients (86%) had a continuous normal voltage (CNV) background pattern and five (14%) had discontinuous normal voltage (DNV). Sleep-wake cycling (SWC) was observed in 18 patients (50%).

Intra-operative

During surgery, aEEG recordings could not be assessed in three patients during (part of) surgery, presumably due to interference of surgical apparatus. In nearly all patients, artefacts due to electrocardiographic (ECG) interference could be seen (see **Figure 1a** for an example). After cooling to deep hypothermia, 20 patients (61%) had a flat

A



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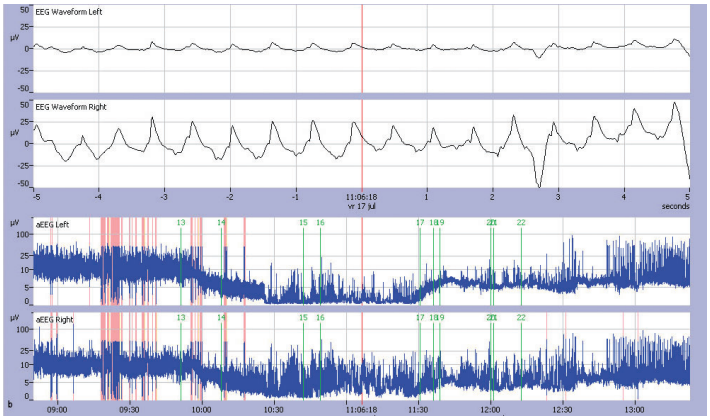

Figure 1. Examples of intra-operative aEEG abnormalities. a. Artifact due to interference of the ECG, resulting in a 'drift of the baseline' at 16:26. In the raw EEG (top), the rhythmicity and waveform of the discharges are very suggestive of an ECG artefact. b. Bilaterally suspected seizure activity, during surgery, at 11:06. This occurred 15 minutes after the start of DHCA. The aEEG remained suspicious for epilepsy throughout the period of DHCA (35 minutes). Thereafter, the aEEG was normal and no further epileptic activity was seen. Pre-operatively, the MRI scan had been normal and postoperatively mild WMI was seen in the left frontal region.

trace and 13 (39%) had a burst suppression pattern. Bilateral electrographic seizures, without any clinical correlates, were seen in five patients (14%), see **Figure 1b** for an example. Two cases occurred during induction of anesthesia [with isoflurane in one patient, and sevoflurane in the other]; another patient after 15 minutes of DHCA; during rewarming in another and after weaning from cardiopulmonary bypass (CPB) in the last patient.

Postoperative

Postoperatively, aEEG data was collected for a median of 71 hours (range 42-186). It took a median of 2.2 hours (range 0-30) for the background pattern to recover to CNV and 31 [86%] recovered within 12 hours. All patients eventually attained a CNV pattern before the end of the recording. SWC was evident after a median of 16 hours (4.1-120) (so, 27 [75%] of patients had SWC within 24 hours and 30 [83%] within 48 hours). All patients except for one had a SWC pattern before the end of the recording.

Electrographic seizures occurred in 11 patients (31%) at a median of 36 hours (range 1.5-120) after surgery, of which two patients had clinical epileptic symptoms.

Table 2. Description of aEEG abnormalities and new postoperative MRI findings

Electrical discharge type	Left/ right	Clinical correlate	MRI
Status epilepticus			
Patient 1	left	yes	large left medial cerebral artery infarction
Patient 2	bilateral	yes	severe bilateral intra- ventricular hemorrhage and severe WMI
Patient 3*	right	no	severe right WMI and a focal thalamic and internal capsule infarction
Repetitive electrographic seizures			
Patient 4	bilateral	no	moderate bilateral WMI
Patient 5	bilateral	no	moderate right WMI
Patient 6	right	no	right watershed injury
Patient 7	right	no	infarction right internal capsule and severe bilateral WMI
Multiple single electrographic seizures			
Patient 8		no	infarction right caudate nucleus and severe WMI
Patient 9	right	no	infarction right thalamus and severe WMI
Patient 10	right	no	right watershed injury and left moderate WMI
One single, electrical discharge			
Patient 11	right	no	no injury

* This patient is depicted in **Figure 2**.
aEEG: amplitude-integrated electro encephalography; MRI: magnetic resonance imaging; WMI: white matter injury

Details of the specific findings are listed in **Table 2**. An example of a patient with a status epilepticus on aEEG (without any clinical signs) and subsequent severe MRI injury is depicted in **Figure 2**.

Relationship with postoperative MRI

The relationship between aEEG findings and new injury on the postoperative MRI scan are listed in **Table 3**. This shows that intra-operative electrographic seizures were not associated with MRI injury, but postoperative electrographic seizures did show a significant association ($p>0.99$ and $p<0.01$, respectively). Hence, as depicted in **Figure 3a**, 10/11 (91%) of all cases with electrographic seizures had new injury on their MRI, whereas this occurred in 9/24 (37%) of cases with a normal aEEG ($p<0.01$). The time to first CNV and SWC were not related to MRI injury ($p=0.88$ and $p=0.46$).

Relationship with neurodevelopment

Postoperative electrographic seizures showed a relationship with motor and cognitive outcome ($p=0.04$ and $p=0.03$), resulting in a median reduction of 14 and 18 points on the motor and cognitive composite scores, respectively (see **Figure 3b**). The other aEEG variables were all without a significant association with neurodevelopment, as listed in **Table 4**.

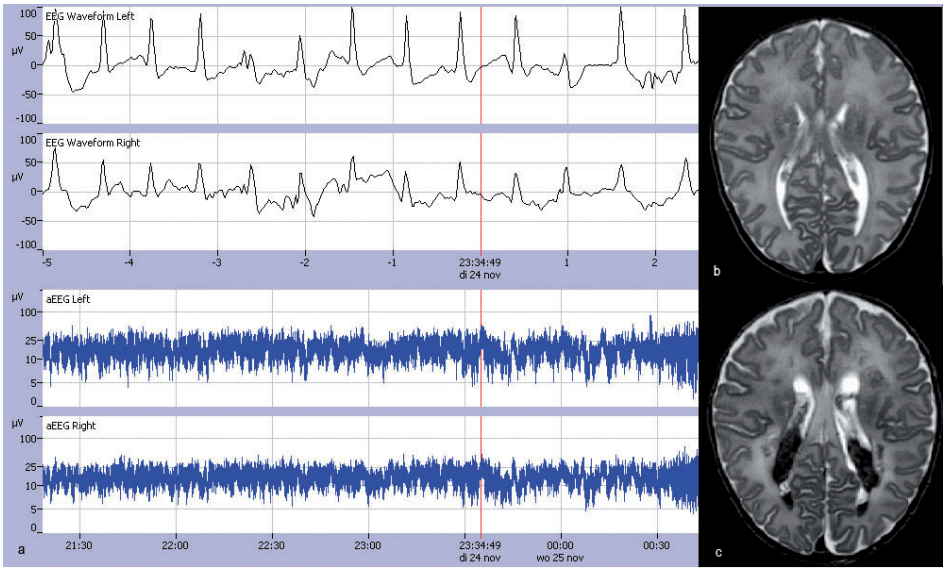

Figure 2. Example of postoperative status epilepticus. a: Approximately 24 hours after surgery, this patient showed a status epilepticus bilaterally on the aEEG, which was treated with phenobarbital. b and c: The pre-operative MRI (b) was unremarkable, whereas postoperatively severe intraventricular hemorrhage and WMI was seen bilaterally (c).

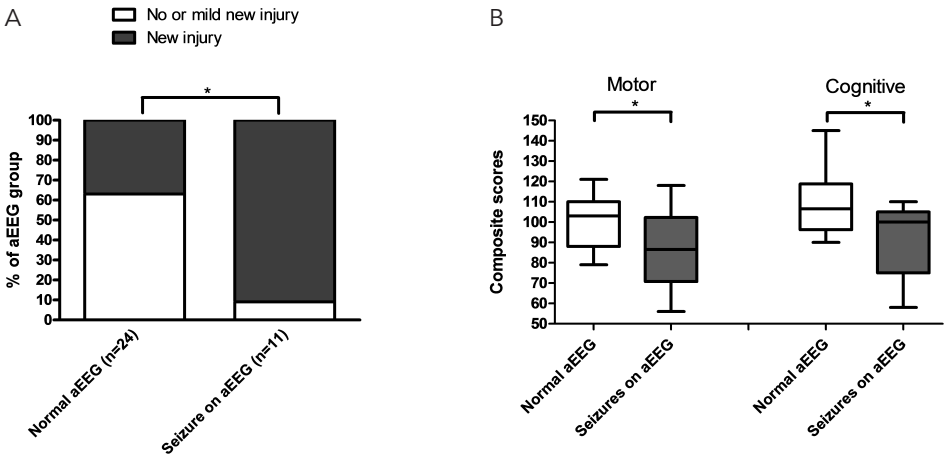

Figure 3. Electrographic seizures on aEEG are associated with MRI injury and neurodevelopment. a: Of all cases with electrographic seizures on postoperative aEEG, 10/11 (91%) had new injury on MRI, whereas this occurred in 9/24 (37%) of cases with a normal aEEG ($p<0.01$). b: Electrographic seizures on postoperative aEEG are associated with lower motor and cognitive composite scores ($p=0.04$ and 0.03, respectively).

Factors influencing postoperative time to CNV and SWC

Due to the lack of a relationship between time to CNV or SWC and MRI or neurodevelopment, an additional analysis was performed to investigate which factors influenced duration to CNV and SWC. Regarding time to CNV, this was only associated with use of DHCA (longer time to CNV with DHCA, $p<0.01$), as reported on previously. Hence, the following variables did not play a role: univentricular repair ($p=0.39$), mean NIRS ($p=0.28$), mean arterial blood pressure ($p=0.34$), doses of intra- or postoperative sedation (isoflurane $p=0.08$, sufentanil $p=0.37$, midazolam $p=0.30$; morphine $p=0.79$. Regarding time to SWC, none of the above named variables were associated with time to SWC (mean NIRS $p=0.41$, mean arterial blood pressure, $p=0.48$, midazolam $p=0.37$, morphine $p=0.85$, fentanyl $p=0.74$).

Of note, there was also no relationship between sedation dose and the occurrence of postoperative electrographic seizures (midazolam dose between group with and without seizures; $p=0.74$; morphine $p=0.34$ and fentanyl $p=0.46$).

Near-infrared spectroscopy

Before surgery, a median cerebral near-infrared spectroscopy (NIRS) value of 60% (range 46-81) was measured. During surgery, NIRS values increased to 79% (44-95), and during DHCA and ACP, respectively, these were 52% (46-65) and 76% (33-95), respectively. In the first 24 hours after surgery, median NIRS was 63% (35-87); from 24-48 hours after surgery this was 72% (53-94).

Table 3. aEEG, NIRS and biomarkers: Relationship with new postoperative MRI injury

	No or mild new injury on MRI* (n=16)	New injury on MRI (n=20)	p
aEEG			
Pre-operative electrographic seizures			
Intra-operative electrographic seizures	2 (13)	3 (15)	>0.99
Postoperative electrographic seizures	1 (6.3)	10 (50)	<0.01
Time to CNV (hours)	3.1 (0.0-30)	2.2 (0.7-23)	0.88
Time to SWC (hours)	14 (6.0-107)	17 (4.1-125)	0.46
NIRS			
Pre-operative mean NIRS (%)	59 (46-78)	62 (55-81)	0.11
Intra-operative lowest NIRS (%)	44 (21-56)	44 (15-63)	0.57
Intra-operative duration NIRS<45% (min)	42 (0-178)	13 (0-149)	0.27
Intra-operative AUC< 45% (min*%)	73 (0-1764)	42 (0-1570)	0.46
0-48h postoperative mean NIRS (%)	64 (46-83)	69 (30-90)	0.79
0-48h postoperative lowest NIRS (%)	42 (16-71)	47 (20-74)	0.86
0-48h postoperative NIRS<45% (min)	91 (0-1367)	61 (0-1252)	0.58
0-48h postoperative AUC< 45% (min*%)	341 (0-19327)	68 (0-17782)	0.81
Serum biomarkers			
s100b			
Pre-operative	23 (0-76)	28 (5-79)	0.23
4h postoperative	59 (39-124)	71 (0-172)	0.61
24h postoperative	41 (21-73)	36 (10-87)	0.71
1w postoperative	37 (4-143)	32 (7-66)	>0.99
NSE			
Pre-operative	121 (0-400)	183 (0-277)	0.98
4h postoperative	110 (0-329)	139 (0-648)	0.70
24h postoperative	56 (0-488)	69 (0-704)	0.79
1w postoperative	48 (0-360)	98 (0-496)	0.38

Values stated as median (range) or number of patients (% of injury group).
*Mild new injury was defined as mild white matter injury, ventricular enlargement without apparent injury, or intraventricular hemorrhage without ventricular enlargement or intraparenchymal involvement.
aEEG: amplitude-integrated electro encephalography; NIRS: near-infrared spectroscopy; MRI: magnetic resonance imaging; CNV: continuous normal voltage; SWC: sleep-wake cycling; AUC: area under the curve; NSE: neuron-specific enolase; min=minutes

Table 4. aEEG, NIRS and biomarkers: Relationship with neurodevelopmental outcome

	Motor composite score p	B (95% CI)	Cognitive composite score p	B (95% CI)
aEEG				
Pre-operative electrographic seizures	0.41		0.95	
Intra-operative electrographic seizures	0.35		0.48	
Postoperative electrographic seizures	0.04	-14 (-28 - -0.8)	0.03	-18 (-33 - -2.4)
Time to CNV (hours)	0.59		0.84	
Time to SWC (hours)	0.92		0.86	
NIRS				
Pre-operative mean NIRS (%)	0.99		0.45	
Intra-operative lowest NIRS (%)	0.76		0.62	
Intra-operative duration NIRS<45% (min)	0.55		0.51	
Intra-operative AUC< 45% (min*%)	0.53		0.59	
0-48h postoperative mean NIRS (%)	0.18		0.63	
0-48h postoperative lowest NIRS (%)	0.11		0.91	
0-48h postoperative NIRS<45% (min)	0.73		0.62	
0-48h postoperative AUC< 45% (min*%)	0.85		0.70	
Serum biomarkers				
s100b				
Pre-operative	0.35		0.22	
4h postoperative	0.53		0.02	-0.3 (-0.5 - -0.1)
24h postoperative	0.16		0.78	
1wpostoperative	0.78		0.13	
NSE				
Pre-operative	0.07		0.97	
4h postoperative	<0.01	-3.6 (-6.0- -1.1)	0.16	
24h postoperative	0.07		0.98	
1wpostoperative	0.64		0.67	

p-values represent results of linear regression.
B=regression coefficient; CI: confidence interval; aEEG: amplitude-integrated electro encephalography; NIRS: near-infrared spectroscopy; MRI: magnetic resonance imaging; CNV: continuous normal voltage; SWC: sleep-wake cycling; AUC: area under the curve; NSE: neuron-specific enolase; min=minutes.

Association with MRI

As listed in Table 3, neither pre-, intra- nor postoperative NIRS values were associated with the occurrence of new injury on MRI. Mean NIRS values were tested, as well as the lowest value, the duration under 45%, and the area under the curve under 45%.

Also, separate analyses of the different peri-operative phases did not yield any significant results (for example, mean NIRS in the first hour after CPB; p=0.22). Furthermore, inclusion of new WMI only, instead of all kinds of cerebral injury, resulted in similar results (intra-operative lowest NIRS, p=0.99; duration <45%, p=0.65; AUC, p=0.58; postoperative lowest NIRS, p=0.19; duration <45%, p=0.40; AUC <45%, p=0.22).

Association with neurodevelopment

NIRS values were not associated to motor or cognitive outcomes, as listed in Table 4. Separate assessment of the various intra-operative phases also did not yield an association with neurodevelopmental outcome (i.e. first hour after CPB; motor p=0.97; cognitive p=0.96).

Serum biomarkers

The cerebral biomarkers s100b and neuron-specific enolase (NSE) were assessed pre- and postoperatively in 24 to 28 patients, depending on the time point.

Association with MRI and neurodevelopment

As listed in Table 3, s100b and NSE values were not different in the group with or without new postoperative MRI injury.

Table 4 shows the relationship between the biomarkers and early neurodevelopment. This shows that an increase in s100b, measured at 4 hours after surgery, was associated with a reduced cognitive score (p=0.02). At the same time point, an increase in NSE was associated with a worse motor score (p<0.01).

DISCUSSION

In the current study, bed-side cerebral monitoring techniques, aEEG and NIRS were assessed for their relationship with cerebral injury as seen on MRI. We were able to show that postoperative electrographic seizures were the only variable associated with both lesions on MRI and early neurodevelopmental outcome. Other aspects of aEEG; namely the time to recovery of the background pattern after surgery, and the onset of sleep-wake cycling, did not show this relationship. NIRS was also not associated with cerebral injury. Finally, the serum biomarkers s100b and NSE did show a significant relationship with early neurodevelopment, but not with post-operative MRI injury.

We observed a lower incidence of intra-operative electrographic seizures (14%) than previously reported by Gunn et al, who found seizures during 23% of Norwood procedures[16]. Specifically, the authors noted a preference for left-sided seizures, mostly during use of ACP, during which the right carotid artery is selectively perfused. We did not observe such a trend; moreover, we did not find any abnormal aEEG

activity during ACP. However, the reading of the aEEG recording during CPB with deep hypothermia proved to be a challenging task, as it is very difficult to make a distinction between artifacts due to CPB or ECG effects, and seizure discharges.

aEEG revealed postoperative electrographic seizures in 31% of patients. This is amongst the highest reported. The earliest reports of continuous encephalographic monitoring was by the Boston Circulatory Arrest study, in which seizure activity was found in 20% of neonates undergoing surgery for transposition of the great arteries[27]. Subsequent studies of neonatal cardiac surgery found seizures in 3-18%[16, 28, 29]. Reasons for the varying incidence have been proposed to be due to differences in anesthetic protocols, but the types of sedatives used in our study are very similar to those of others, although (cumulative) dosages are difficult to compare. A more likely reason for the large proportion of cases with electrographic seizures may be to the fact that the surgeries in this cohort are among the most complex, which likely influenced the high overall rate of new MRI lesions (present in 55% of the patients).

Indeed, we found a strong association between the occurrence of these electrographic seizures and new injury on cerebral MRI, and also a worse motor and cognitive outcome. This has been observed before by Clancy et al[30]. The recording of seizure activity using continuous (a)EEG seems important to identify patients at high risk of extensive cerebral injury, especially as these seizures tend to be subclinical due to sedation. In 10 of 11 patients in our cohort with electrographic seizures, new injury on MRI was observed, varying from watershed injury to severe WMI. On the other hand, of the patients without any suspicious periods on their aEEG recording, 37% still had MRI injury, which suggests that the modality has a high specificity, but lower sensitivity for new injury.

We did not observe any relationship between the time to a normal (CNV) background pattern after surgery, or sleep-wake cycling, and parameters of cerebral injury. This in contrast to the findings of Gunn et al, where CNV and SWC recovery were associated with mortality and neurodevelopmental outcome[15]. In their study, however, it remains unclear whether these aEEG variables are merely a marker of cardiac output and, subsequently, both mortality and cerebral outcome, or whether these aEEG markers are specific for cerebral injury. Another reason for not finding an association between time to CNV or SWC and injury in our study may lie in the low incidence of mortality, and generally good neurodevelopmental outcome in this cohort, which reduces the chance of finding an association. Finally, the time to recovery was much shorter in our cohort than in the described study (2 hours versus 13 hours), which reveals the large differences between centres. Of note, an additional analysis of our results did not show any effect of anesthetics, for instance, on the time to CNV or SWC, so the reason for this difference between cohorts remain elusive.

NIRS was not found to be associated with cerebral injury in our study, and neither was there any indication that this would be the case in a larger group. There have been contradictory reports on the relationship between NIRS and cerebral injury. In two large studies of neonatal cardiac surgery, lower NIRS values were found to be associated with worse neurodevelopmental outcome at one or two years of age. However, in the first study, this was only true for the period immediately after CPB

disconnection, which may highlight the most complex cases, in which hemodynamic instability is maximal after surgery[20]. A more recent study by Hoffman et al showed a relationship between duration of NIRS below 45% in the first 48 hours post-surgery, and specifically a higher chance of delayed visual-motor integration (which occurred in a small group of three patients[18]). In a larger group of 67 neonates after cardiac surgery, Andropoulos et al failed to find any relationship between NIRS and new postoperative injury on MRI; similar to our results[6]. NIRS may prove more effective in assessing trends for cardiac output, than specifically providing an insight into evolving cerebral injury[17].

The results of the serum biomarkers s100b and NSE suggest that these biomarkers may be useful in the assessment of cerebral injury at the bed-side. Both markers, when measured early after surgery, showed a significant relationship with neurodevelopmental outcome. Although median values did seem to be higher in the group with new injury on MRI, this was clearly not significant, possibly due to the small number of patients. There have been no previous reports of the association between these markers and cerebral imaging or neurodevelopment in the field of neonatal cardiac surgery. There are multiple studies however on neonates with perinatal asphyxia, in which both s100b and NSE have shown a clear relationship with MRI abnormalities and early neurodevelopment[21, 22]. However, the many extracerebral sources of 100b and NSE (renal, muscular and cardiac in s100b, and hemolysis in NSE) can importantly affect this pattern, as has been seen in adult surgery[23].

The most important limitation of this study is the small number of patients, which does not allow more elaborate statistical methods to assess predictive capacities of the various modalities. This also limits definite conclusions on associations between cerebral monitoring and eventual cerebral injury. However, contrary to the many studies including a wide range of age groups and procedure types, the current study did commit to one specific patient group, and tested a broad array of cerebral parameters. Although multi-centre studies are often needed to attain an adequate sample size, these have the important difficulty of different surgical and anesthetic protocols which may mask important relationships, making small but in-depth studies like the current one, important for further research.

In conclusion, in neonates undergoing aortic arch reconstruction, we have assessed the relationship between aEEG, NIRS and serum biomarkers and subsequent cerebral injury as seen both on MRI and early neurodevelopmental outcome. The aEEG proved most effective in identifying patients at risk for injury, as seen by an increased number of electrographic seizures in the group with injury on MRI. This underscores the need for peri-operative cerebral monitoring. Although NIRS has proven to be an effective bed-side marker of cardiac output and thereby may be important for cerebral protection, it was not directly related to cerebral injury in our cohort. Finally, s100b and NSE may prove effective early biomarkes in the identification of high-risk patients, but this needs confirmation in a larger group of patients.

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PART II:
INFLAMMATION AND SOMATIC EFFECTS OF PERFUSION TECHNIQUES

6

CEREBRAL ISCHEMIA INITIATES AN IMMEDIATE INNATE IMMUNE RESPONSE IN NEONATES DURING CARDIAC SURGERY

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ABSTRACT

Background

A robust inflammatory response occurs in the hours and days following cerebral ischemia. However, little is known about the immediate innate immune response in the first minutes after an ischemic insult in humans. We utilized the use of circulatory arrest during cardiac surgery to assess this.

Methods

Twelve neonates diagnosed with an aortic arch obstruction underwent cardiac surgery with cardiopulmonary bypass and approximately 30 minutes of deep hypothermic circulatory arrest (DHCA, representing cerebral ischemia). Blood samples were drawn from the vena cava superior immediately after DHCA and at various other timepoints from pre-operatively to 24 hours after surgery. The innate immune response was assessed by neutrophil and monocyte count and phenotype using FACS, and concentrations of cytokines IL-1β, IL-6, IL-8, IL-10, TNFα, sVCAM-1 and MCP-1 were assessed using multiplex immunoassay. Results were compared to a simultaneously drawn sample from the arterial cannula. Twelve other neonates were randomly allocated to undergo the same procedure but with continuous antegrade cerebral perfusion (ACP).

Results

Immediately after cerebral ischemia (DHCA), neutrophil and monocyte counts were higher in venous blood than arterial ($p=0.03$ and $p=0.02$ respectively). The phenotypes of these cells showed an activated state(both $p<0.01$). Most striking was the increase in the 'non-classical' monocyte subpopulations ($CD16^{intermediate}$; arterial 6.6% vs. venous 14%; CD16+ 13% vs. 22%, both $p<0.01$). Also, higher IL-6 and lower sVCAM-1 concentrations were found in venous blood (both $p=0.03$). In contrast, in the ACP group, all inflammatory parameters remained stable.

Conclusions

In neonates, approximately 30 minutes of cerebral ischemia during deep hypothermia elicits an immediate innate immune response, especially of the monocyte compartment. This phenomenon may hold important clues for the understanding of the inflammatory response to stroke and its potentially detrimental consequences.

BACKGROUND

It is well established that cerebral ischemia induces an immune response on many different levels. Both in the parenchyma and the systemic circulation, within hours after the insult, cytokines are produced in vast amounts and leukocytes are activated and migrate into the injured brain[1-5]. The various immune cells and their subpopulations have very different effects on the lesion and thus the outcome for the patient[6].

The exact mechanisms in the pathway from the cessation of blood flow to the establishment of stroke and its outcome have predominantly been studied in animal models. These have shown the infiltration of neutrophils and monocytes into the cerebral tissue starting from 4-6 hours after the ischemic insult, with these cells showing phenotypical changes in the periphery for weeks following the event[2, 3]. In adults with a cerebrovascular accident or a transient ischemic attack, it is the systemic compartment which has been studied most. Pro-inflammatory cytokine concentrations have been associated with poor neurological outcome after stroke, as have innate immune cell numbers and phenotypes[7-9]. For example, Smith et al have found high TNFα and activated neutrophils to be correlated to worse symptoms after stroke[9]. Others have shown that monocytes are highly associated to prognosis, with the different monocyte subpopulations having variable effects, likely due to their specific characteristics and functions[4]. For example, the abundance of 'classical' (CD16-) monocytes in the peripheral circulation following stroke were shown to be associated with a worse outcome[8]. Data on the immune response to cerebral ischemia in neonates is scarce. There have been studies on the effects of perinatal asphyxia, which show a burst in cytokines and activation of neutrophils and monocytes within 24 hours, similar to the response in adults[10-15].

Although many studies have characterized the immune response to stroke in the hours and days after the insult, the immediate effect of cerebral ischemia has never been assessed. The unique opportunity to assess this arises in the field of cardiac surgery, where procedures performed with the use of cardiopulmonary bypass (CPB) may include a period of global circulatory arrest at a deep hypothermic temperature ('deep hypothermic circulatory arrest', DHCA). This allows for the study of the immediate response to approximately 30 minutes of induced cerebral ischemia. We assessed the inflammation in the cerebral circulation by characterization of neutrophils and monocytes and measurement of cytokines IL-1β, IL-6, IL-8, IL-10, TNFα, sVCAM-1 and MCP-1, as these markers are known to respond within hours after cerebral ischemia[1, 3, 7, 8, 10-14, 16-20]. The results were compared to those from patients undergoing the same surgical procedure at the same temperature, but with continuous cerebral perfusion, and thus without any apparent ischemia ('antegrade cerebral perfusion', ACP). We hypothesized that DHCA would have an immediate 'pro-inflammatory' effect on innate immune cells and cytokines, whereas ACP would not affect these markers of systemic inflammation.

METHODS

Patients and surgical procedures

Neonates presenting with a hypoplastic aortic arch between 2009 and 2011 were assigned to undergo aortic arch reconstruction. This procedure entails the use of either DHCA or ACP during CPB. The current study was performed as part of a prospective randomized controlled trial comparing DHCA and ACP in terms of neurological outcome (clinicaltrials.gov number NCT01032876). In the subgroup reported here, data on cerebral inflammatory markers were collected. Surgery and CPB was performed as previously described [21]. The institutional medical ethics committee approved the study and all parents gave informed consent for enrolment.

Sample collection

Blood samples were drawn at various time points (see **Figure 1**); 1) 'Pre-operative': before the start CPB and after the administration of dexamethasone 1 mg/kg to the patient; 2) 'Start DHCA/ACP': after cooling, at deep hypothermia on full-flow CPB, just before the start of either DHCA or ACP; 3) 'End': immediately [0-3 minutes] following DHCA or ACP; 4) '30 min reperfusion': 30 minutes after recommencement of full body CPB (during rewarming); 5) '4h postoperative': 4 hours after the end of DHCA or ACP, with the patient off CPB and on the intensive care unit; 6) '24h postoperative': 24 hours after surgery.

At the 'Start DHCA/ACP' time point (at deep hypothermia, on full-flow CPB), leukocyte counts are lower than before surgery, due to the expansion of the circulating

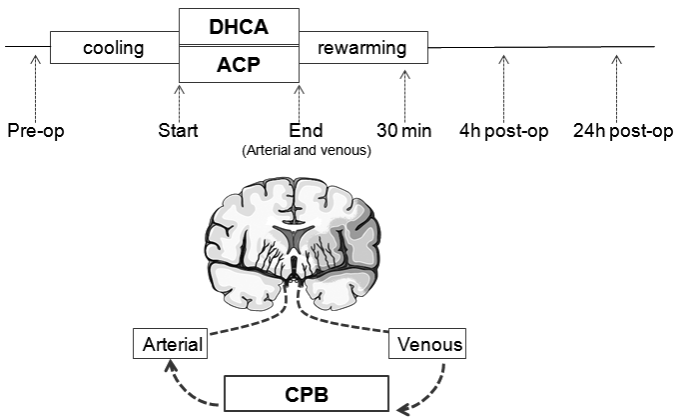

Figure 1. Sample collection during CPB. Upper picture shows all time points at which blood samples were collected. The first was before CPB ('Pre-op'). Once on CPB, patients were cooled to deep hypothermia and DHCA or ACP was initiated ('Start'). At the end of DHCA or ACP ('End'), full-flow CPB was recommenced and the patient was rewarmed to normothermia, during which the '30 min' sample was drawn. 4 and 24 hours after surgery, the final samples were collected ('4h post-op' and '24h post-op'). The lower picture depicts the arterial and venous samples drawn at the 'End' timepoint. Samples were simultaneously drawn from the arterial and venous cannulae, transporting blood to and from the brain, respectively.

volume after connection to the CPB circuit. For better comparison with pre-operative leukocyte counts, the count during CPB at 'Start' was corrected for this dilution, based on the volume of the CPB circuit. For example, a child of 3 kg is expected to have 270 ml of circulating volume pre-operatively, and when a CPB circuit volume of 810ml is added, the total circulating volume makes for a total volume of 1080 ml, hence a 4-times dilution. The leukocyte count at 'Start' can then be corrected for dilution by multiplied by 4 to attain the cell count corrected for dilution. The same applies to the 'End' and '30 minutes' timepoints. We depicted these corrected counts in grey in **Figure 3a** and b.

At the 'End' time point, blood samples were drawn from two different locations of the CPB circuit, named the 'arterial' and 'venous' samples (see **Figure 1**). The arterial sample represents blood going to the brain, whereas the venous sample represents blood exiting the brain. The arterial sample was drawn from the arterial line, which connects the CPB machine to the aorta of the patient. The venous sample was drawn from the venous cannula, which is situated in the vena cava superior of the patient, where the venae azygos and anonyma were temporarily occluded in order to obtain blood specifically coming from the brain, and the first 10 ml of blood was discarded as it approximates the dead space of the cannula.

Laboratory analyses

Blood samples were kept on ice during all handling and staining procedures. Plasma samples were analyzed by multiplex immunoassay to measure levels of IL-1β, IL-6, IL-8, IL-10, TNFα, sVCAM-1 and MCP-1 with the Bio-Plex suspension array system (Bio-Rad Laboratories), as previously described[22]. Neutrophil and monocyte counts were performed by manual microscopy, hematocrit and thrombocyte counts were analyzed by an automated cell counter.

As shown in **figure 2**, monocytes were categorized into three monocyte subpopulations (CD16-, CD16^{intermediate} [CD16int], and CD16) as proposed by Ziegler-Heitbrock et al [23, 24].

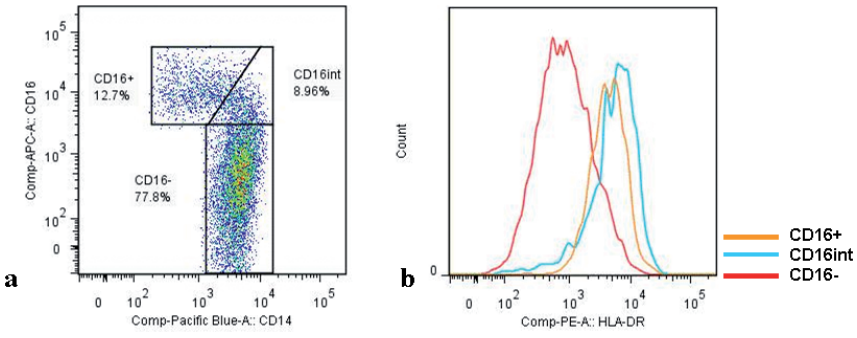

Figure 2. Monocyte subpopulations. a: Gating of monocytes into CD16+, CD16int (intermediates) and CD16+ subpopulations. b: CD16- monocytes show a low expression of HLA-DR, whereas CD16int and CD16+ show a high expression (median MFIs of 663 [IQR 337-867], 2174 [1189 – 4884] and 3903 [1372 – 8393], respectively).

For neutrophil phenotyping, fluorescently labeled monoclonal antibodies (mAb) directed against CD11b (RPE, clone 2LPM19c; Dako, Glostrup, Denmark) and CD62L (FITC, clone Dreg56; BD, Franklin Lakes, New Jersey, USA) were used for flow cytometry. For monocyte phenotyping, CD14 (Pacific Blue, 301815 clone M5E2; Biolegend, San Diego, California, USA), HLA-DR (PE, 347401; BD), CD16 (APC, MHCD1605, clone 3G8, CalTag, Buckingham, UK) and CD62L (FITC, 555543, clone Dreg 56; BD), The FACSCantoflow cytometer (BD) was used to record cells and FacsDiva Software (BD), FacsExpress (De Novo Software, Thornhill, Ontario, Canada) and FlowJo (Ashland, Oregon, USA) used for analysis. Neutrophils were identified according to their specific forward- and sideward-scatter signals. Expressions of CD11b and CD62L were calculated as activational markers (with CD11b increasing, and CD62L decreasing during activation).

Statistical analyses

Values in graphs and text are stated as numbers (% of population) or medians (interquartile range [IQR]). Differences between arterial and venous samples were tested using Wilcoxon Signed Ranks test. Differences between DHCA and ACP for timepoints from '30 minutes reperfusion' onwards were tested by Mann-Whitney U test. IBM SPSS Statistics version 19 (SPSS.) was used for statistical calculations and Prism version 5.03 was used to depict graphs (GraphPad Software, San Diego, California, USA).

RESULTS

Baseline characteristics

Data were collected from 24 neonates (n=12 for DHCA, n=12 for ACP). All neonates underwent aortic arch reconstruction as well as intracardiac procedures for specific diagnoses listed in Table 1. Surgical and CPB data were similar at the start of DHCA or

Table 1. Patient characteristics

	DHCA (n=12)	ACP (n=12)
Male sex	8/12 (67)	9/12(75)
Age at surgery (days)	9 (8 – 11)	12 (8 – 15)
Diagnosis:		
Hypoplastic left/ right heart syndrome or complex	8 (67)	5 (42)
Aortic arch interruption/ coarctation	3 (25)	5 (42)
Double outlet right ventricle or double inlet left ventricle	1 (8)	2 (17)
Duration of cooling (minutes)	33 (20 – 43)	30 (28 – 36)
Nasal temperature at deep hypothermia (°C)	17.7 (17.1 – 18.1)	17.5 (16.6 – 17.9)
Duration of DHCA or ACP (minutes)	33 (23 – 38)	44 (33 – 53)
Dilution factor circulating volume during CPB	3.8 (2.3 – 4.5)	4.2 (2.6 – 4.6)

Values represent number (% of group) or medians (interquartile ranges).

ACP. Also, inflammatory profiles were comparable pre-operatively and at the start of either DHCA or ACP, as shown in **Table 2**.

Table 2. Baseline inflammatory characteristics

Neutrophils and monocytes	DHCA	ACP
Pre-operative		
Monocyte count	1.6 (1.3 – 2.1)	1.9 (1.1 – 2.1)
%CD16- monocytes	83 (71-91)	74 (52-83)
%CD16int monocytes	7.0 (5.0-14)	12 (6.7-22)
%CD16+ monocytes	7.0 (4.5-15)	10 (5.5-22)
Neutrophil count	8.2 (5.7 – 10.6)	6.0 (5.4 – 8.8)
Neutrophil CD62L expression	166 (103 – 257)	294 (221 – 329)
Neutrophil CD11b expression	426 (264 – 717)	523 (265 – 685)
Start DHCA or ACP		
Monocyte count	0.17 (0.09 – 0.32)	0.20 (0.13 – 0.27)
%CD16- monocytes	81 (70-86)	78 (66-85)
%CD16int monocytes	6.5 (3.5-9.0)	6.6 (3.7-8.6)
%CD16+ monocytes	14 (7.4-19)	14 (7.3-23)
Neutrophil count	1.4 (0.8 – 2.2)	1.0 (0.5 – 1.4)
Neutrophil CD62L expression	189 (104 – 262)	205 (146 – 288)
Neutrophil CD11b expression	519 (322 – 743)	673 (468 – 792)
Cytokines and chemokines		
Start DHCA or ACP		
IL-1β	0.18 (0.00-1.1)	0.18 (0.00-0.62)
IL-6	18 (1.0 – 21)	8.1 (2.3 – 16)
IL-8	7.2 (5.6 – 35)	5.3 (3.0 – 10)
IL-10	42 (9.7 – 61)	12 (6.3 – 32)
Values corrected for dilution		
IL-1β	0.82 (0.72-18)	0.81 (0.78-12)
IL-6	59 (15-103)	36 (8.1-65)
IL-8	28 (18-67)	26 (11-65)
IL-10	105 (42-337)	74 (23-135)

Values represent medians (interquartile ranges). Neutrophil and monocyte counts in 10^9/L, neutrophil expressions in median fluorescence intensity (MFI), cytokine and chemokine concentrations in pg/ml.

Deep hypothermic CPB decreases neutrophil and monocyte counts

Cell numbers were available for all 24 neonates (n=12 for both groups). **Figure 3** shows the peri-operative change in neutrophil and monocyte counts for the DHCA and ACP groups separately. In grey, the counts during deep hypothermic, full-flow CPB are depicted, corrected for the dilution of the CPB circuit. At 4 hours after surgery, neutrophil and monocyte numbers are back to their pre-operative values and rise further at 24 hours.

Cerebral ischemia increases neutrophils and monocyte counts

To assess the direct effect of cerebral ischemia on leukocyte concentrations, we compared the absolute cell counts of the cerebral venous sample to the arterial sample, both obtained immediately after DHCA. Results are depicted in **Figure 4a**, which shows significantly increased neutrophil numbers in venous blood compared to arterial (median arterial 1.6 x 10^9/L [IQR 1.0 – 2.2] vs. venous 1.9 x 10^9/L [1.4 – 2.1]; p=0.03). Monocyte counts also showed an increase from arterial to venous (median arterial 0.18 x 10^9/L [0.09 – 0.28] vs. venous 0.30 x 10^9/L [0.21 - 0.38], p=0.02). In neonates with continuous cerebral perfusion (ACP), leukocyte numbers did not change (neutrophils p=0.72 and monocytes p=0.40; **Figure 4b**).

To rule out an effect of hyperviscosity on the increased leukocyte number after cerebral ischemia, hematocrit and thrombocyte numbers were compared in arterial and venous blood. As shown in **Figure 4c** and d, these were stable after both DHCA and ACP (DHCA: hematocrit p=0.07 and thrombocytes p=0.15; ACP: hematocrit p=0.44 and thrombocytes p=0.96).

Cerebral ischemia leads to an immediate activation of neutrophils and monocytes

Next, we questioned whether neutrophils exiting the cerebral circulation after ischemia were not only more abundant, but also more activated.

To this end, we assessed neutrophil phenotype using CD62L (which is shed during activation) and CD11b (increases during activation). As shown in **Figure 5a**, after cerebral ischemia (DHCA), although CD62L did not change (p=0.48), CD11b did show a small, but significant increase from arterial to venous (arterial median fluorescence intensity [MFI] 341 [286 – 543], venous 382 [313 – 638]; p<0.01). In continuous cerebral perfusion (ACP), no changes in expression of CD62L and CD11b were observed (CD62L p=0.06, CD11bb p=0.26; see **Figure 5b**).

Furthermore, we assessed changes in the composition of the monocyte compartment after cerebral ischemia. As depicted in **Figure 5c** and **d**, after cerebral ischemia (DHCA), the percentage of CD16-negative monocytes decreased significantly in the venous sample compared to arterial (median arterial 78% [IQR 67 - 88], venous 57% [45 - 71], p<0.01; see **Figure 5c**).In contrast, the $CD16^{intermediate}$ and CD16+ populations increased from arterial to venous, reflecting increased monocyte activation ($CD16^{intermediate}$, median arterial 6.6% [4.5 – 8.7], venous 14 [11 – 17]; CD16+ median

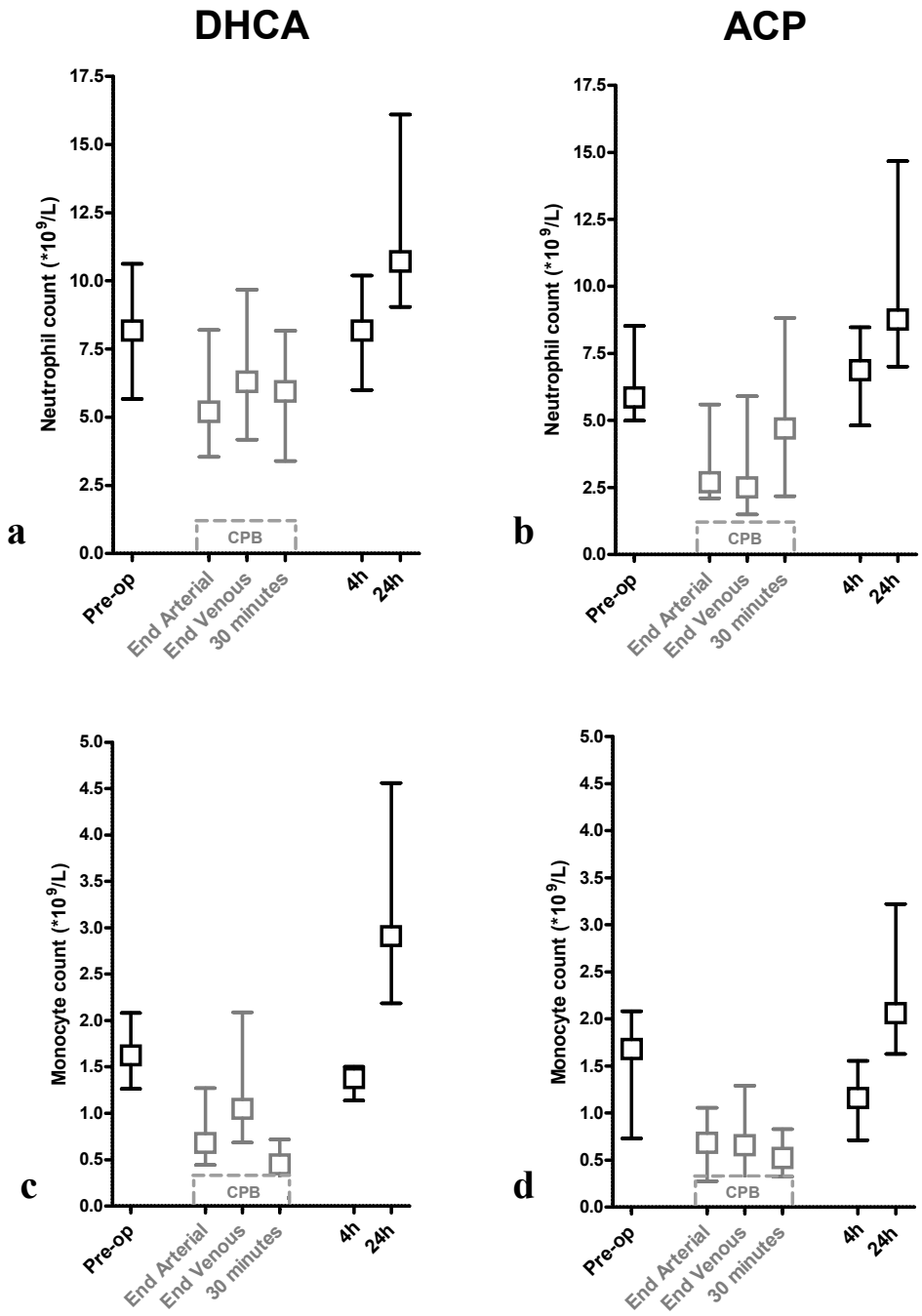

Figure 3. Deep hypothermic CPB decreases neutrophil and monocyte counts. Peri-operative time course of neutrophil and monocyte and counts, in DHCA and ACP groups separately (DHCA; figure a and c; ACP; b and d). During CPB, counts were corrected for the dilution of the CPB circuit (shown in grey).

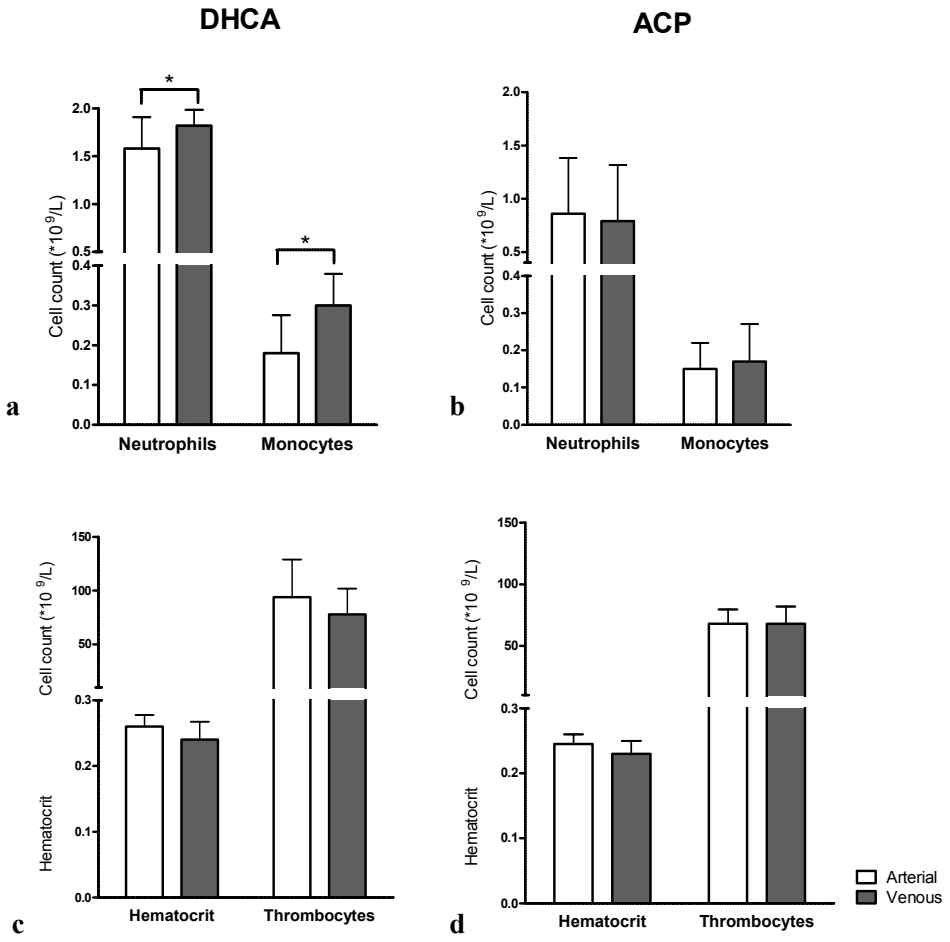

Figure 4. Cerebral ischemia increases neutrophils and monocyte counts. a: Immediately after cerebral ischemia (DHCA), neutrophil and monocyte counts are increased in the venous sample compared to arterial (p=0.03 and p=0.02, respectively). b: In continuous cerebral perfusion (ACP) there is no difference in neutrophil and monocyte counts. c: To rule out the effect of hyperviscosity in the venous sample after cerebral ischemia, hematocrit and thrombocyte numbers were assessed revealing no difference after DHCA. d: Also after ACP there is no difference in hematocrit and thrombocyte number.

arterial 13% [IQR 8.6 - 19], venous 22% [17 - 36]; both p<0.01). After ACP, monocyte subpopulations remained unchanged (CD16-negative p=0.57, $CD16^{intermediate}$ p=0.48, CD16+: p=0.51; see **Figure 5d**).

Cerebral ischemia increases IL-6, and decreases sVCAM-1 concentrations

To assess whether the pro-inflammatory profile of the cells was also reflected in the soluble compartment, cytokines and chemokines were measured in the first 18 neonates included in this study (n=10 DHCA, n=8 ACP). **Figure 6** shows arterial

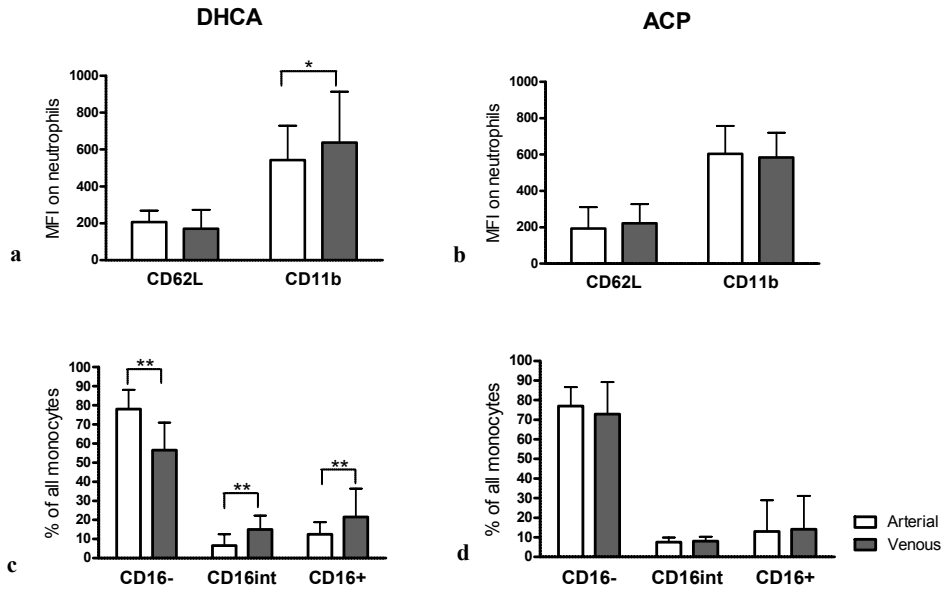

Figure 5. Cerebral ischemia leads to an immediate activation of neutrophils and monocytes. a: After cerebral ischemia (DHCA), CD62L expression is the same in arterial and venous blood, but CD11b expression is higher in the venous sample compared to arterial ($p<0.01$). b: After continuous cerebral perfusion (ACP), neutrophil CD11b and CD62L expressions remain stable. c: After cerebral ischemia (DHCA), in the venous sample, the proportion of monocytes which is CD16- is decreased compared to arterial, due to an increase in CD16intermediate and CD16+ monocyte subpopulations (all $p<0.01$). d: After continuous cerebral perfusion (ACP), the monocyte subpopulations remain unchanged.

and venous concentrations after DHCA and ACP. After cerebral ischemia (DHCA), IL-6 levels significantly increased from arterial to venous (median arterial 16 pg/ml [0.6 – 22]; venous 19 pg/ml [1.1 – 39]; p=0.03).

Furthermore, soluble VCAM-1 significantly decreased in this group (median arterial 1.0 x 10^6 pg/ml [0.83– 1.3 x $10^{6]}$; vs. venous 0.8 x 10^6 pg/ml [0.8 – 9.4 x 10^6], p=0.03). These cytokines did not show any change in concentration in the ACP group (IL-6 p=0.49; sVCAM-1 p=0.26). Regarding the other cytokines IL-1β, IL-8, IL-10 and MCP-1, these did not change significantly from arterial to venous in either the DHCA or ACP group (DHCA, IL-1β p=0.08, IL-8 p=0.17, IL-10 p=0.31, MCP p=0.37; ACP, IL-1β p=0.48, IL-8 p=0.16, IL-10 p=0.16, MCP p=0.48). TNFα was below the detection threshold in all patients.

Differences between DHCA and ACP subside at later timepoints

As listed in Table 3, after 30 minutes of reperfusion, there were no differences to be found in neutrophil and monocyte count or phenotype between DHCA and ACP. The same applies to 4 hours postoperatively, although at 24 hours there was a slight difference in monocyte subpopulations, with less CD16+ monocytes in the DHCA group (p=0.01).

Table 3. Inflammatory results for later timepoints

Neutrophils and monocytes	DHCA	ACP	p
30 min reperfusion			
Monocyte count	0.13 (0.06 – 0.23)	0.12 (0.07 – 0.14)	0.76
%CD16- monocytes	68 (60-85)	74 (50-88)	0.84
%CD16int monocytes	7.4 (5.7-14)	6.1 (2.4-10)	0.36
%CD16+ monocytes	19 (9.2-33)	16 (6.0-43)	0.76
Neutrophil count	1.5 (1.2 – 1.9)	1.1 (0.7 – 1.7)	0.24
Neutrophil CD62L expression	193 (67 – 227)	187 (134 – 307)	0.44
Neutrophil CD11b expression	610 (469 – 816)	672 (508 – 926)	0.85
4h postoperative			
Monocyte count	3.1 (1.7 – 4.8)	2.0 (1.5 – 2.4)	0.15
%CD16- monocytes	86 (85-95)	87 (78-94)	0.74
%CD16int monocytes	6.8 (4.9-8.7)	7.5 (3.2-11)	0.72
%CD16+ monocytes	6.0 (1.7-8.5)	4.5 (2.4-9.9)	0.88
Neutrophil count	8.2 (6.0-10.2)	6.9 (4.8-8.5)	0.43
Neutrophil CD62L expression	281 (253 – 300)	292 (257 – 356)	0.43
Neutrophil CD11b expression	478 (276 – 621)	462 (237 – 594)	0.65
24h postoperative			
Monocyte count	1.4 (1.0 – 2.2)	0.9 (0.7 – 1.3)	0.29
%CD16- monocytes	88 (78-94)	81 (73-91)	0.23
%CD16int monocytes	10 (6.0-21)	10 (6.0-26)	0.61
%CD16+ monocytes	2.9 (1.7-3.0)	6.0 (3.0-7.0)	0.01
Neutrophil count	11 (9.0 – 16)	8.8 (7.0 – 15)	0.32
Neutrophil CD62L expression	260 (196 – 322)	300 (227 – 336)	0.35
Neutrophil CD11b expression	359 (227 – 491)	288 (176 – 368)	0.22
Cytokines and chemokines			
4h postoperative			
IL-1β	0.25 (0.25-0.25)	0.25 (0.25-4.2)	0.61
IL-6	91 (48 – 114)	49 (41 – 65)	0.14
IL-8	77 (59 – 109)	53 (29 – 64)	0.04
IL-10	2760 (2474 – 7077)	3112 (768 – 5758)	0.42
24h postoperative			
IL-1β	0.25 (0.00-3.9)	2.2 (0.51-3.5)	0.48
IL-6	19 (11 – 34)	23 (7.6 – 33)	0.76
IL-8	44 (27 – 67)	15 (11 – 37)	0.05
IL-10	24 (16 – 33)	15 (7.4 – 31)	0.20

Values represent medians (interquartile ranges). Neutrophil and monocyte counts in 10^9/L, neutrophil expressions in median fluorescence intensity (MFI), cytokine and chemokine concentrations in pg/ml.

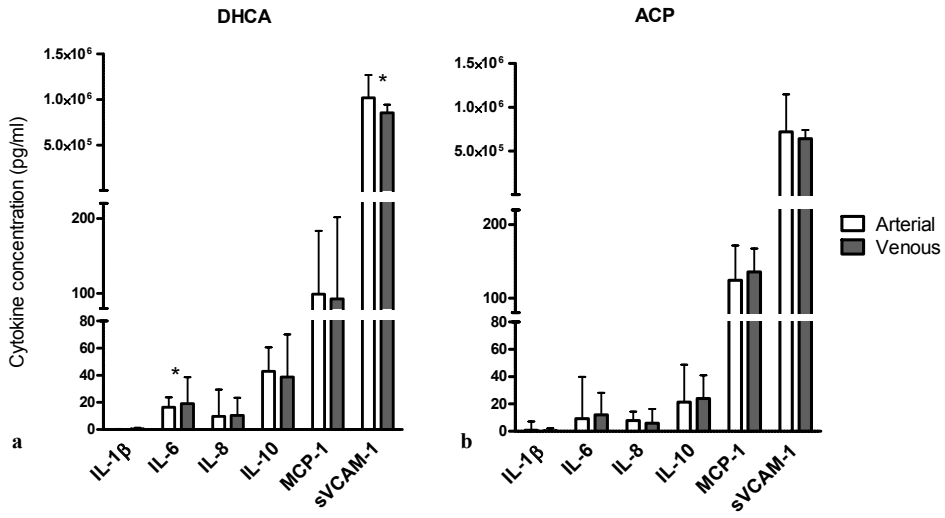

Figure 6. Cerebral ischemia increases IL-6, and decreases sVCAM-1 concentrations. a: After cerebral ischemia (DHCA), serum IL-6 is mildly increased, and sVCAM-1 decreased, in the venous sample compared to arterial (both p=0,03). b: After continuous cerebral perfusion (ACP), all cytokine levels remain stable.

Regarding cytokines and chemokines, absolute concentrations were much higher after surgery than during surgery, but when the intra-operative values are corrected for dilution, IL-6 values remain approximately stable. When comparing the 2 groups, only at 4 hours postoperatively there was a higher IL-8 concentration in the DHCA group (p=0.04), other cytokines were not significantly different.

DISCUSSION

In the present study, we observed that in neonates undergoing cardiac surgery, cerebral ischemia during deep hypothermia elicited an immediate inflammatory response in the cerebral circulation. Not only were there significant changes in the soluble milieu; neutrophils and monocytes acquired an activated phenotype and were even increased in number upon leaving the cerebral circulation. This was not the case in the situation where the brain was continuously perfused. This phenomenon has not been reported on previously and may hold important clues for further research on the inflammatory response to stroke.

Previous studies involving cerebral ischemia and inflammation, both in neonates and adults, have found changes in peripheral immune cells within hours after the ischemic insult [1, 3, 8, 10-14, 16]. In the same time frame, leukocytes are reported to start their migration into the ischemic lesion[2-4]. However, to our knowledge, the immediate effect of ischemia on the cerebral circulation has not been assessed previously. There have been reports of other models of ischemia in other organ systems, for example in limb

ischemia and reperfusion during orthopedic surgery. These have found neutrophils and monocytes to acquire a more activated phenotype after approximately 30 minutes of ischemia [25, 26]. In contrast, during coronary bypass operations in adults, less activated cells were observed distal to the ischemic coronary region than proximally [27]. Here, we report the opposite (more activated cells and increased cell numbers leaving the ischemic brain), which may suggest that this is an organ-specific effect.

The most striking finding in this study is the abundance of 'non-classical' ($CD16^{intermediate}$ and CD16+) monocytes in venous blood directly after cerebral ischemia. The concentration is much higher than the concentration of cells in the arterial sample, which was drawn simultaneously. The question arises as to where these cells have come from. To systematically address this question, a number of options are possible: 1) these cells have undergone proliferation, 2) the venous sample has a higher viscosity, which increases the concentration of immune cells, and/or 3) cells have migrated from the parenchyma into the intravascular compartment. The first option (proliferation) seems highly unlikely in the short period of half an hour that the increase was observed. The second option, of a temporarily more viscous venous sample, could be an option for example by leakage of plasma into the cerebral parenchyma, or an uptake of fluid by other cells. However, both hematocrit and thrombocyte numbers show stable values and thus argue against this. Therefore, the most valid explanation would be that the cells have migrated into the intravascular compartment, from elsewhere. The cells may have come from the 'perivascular space', which is located outside of the endothelial cell layer and inside the parenchymal basal membrane. Histopathologic studies of the brain have revealed that the perivascular space contains macrophages, neutrophils and possibly also lymphocytes [6, 28, 29]. We hypothesize that the neutrophils and monocytes migrate from their perivascular location, into the vascular space and thereby are increased in the venous sample. The increased permeability of the blood-brain barrier due to the cessation of blood flow may facilitate the migration of cells into the circulation [30-32]. A similar phenomenon has been observed in models of exercise, where within 30 minutes after infusion of adrenaline, amongst others, the 'non-classical' monocytes ($CD16^{intermediate}$ and CD16+) are dramatically increased in the systemic circulation [33]. In this case, the release of these monocytes from the perivascular space is thought to be a direct effect of adrenaline, whereas in the current study, it seems plausible that the ischemia has initiated this process. The phenomenon has also been described in transendothelial models of monocyte trafficking, where the specifically the non-classical monocyte populations 'reverse-migrate' from the tissue into the vascular lumen[34].

The decreased concentrations of neutrophils and monocytes during deep hypothermic CPB may play a role in the above described perivascular phenomenon. As is depicted in **Figure 3**, neutrophil and monocyte concentrations were much lower than may be expected due to the expansion of the circulating volume by connection to the CPB circuit. Hence, cells may be migrating out of the circulation in response to another, as yet unknown, trigger. At this point, these cells may occupy the perivascular space, only to be released again promptly after cerebral ischemia has occurred.

Regarding the soluble effects of cerebral ischemia, although subtle, significant differences were found after DHCA in IL-6 and sVCAM-1. The higher IL-6 concentration in venous blood after DHCA is presumably a direct effect of ischemia on the endothelium and the intra- and perivascular leukocytes. In line with the activated phenotypes of neutrophils and monocytes, these may rapidly release vast amounts of IL-6. The lower sVCAM-1 concentration may represent the uptake of sVCAM by these cells, as their activated subsets are known to highly express the receptor CD49d [8].

We cannot predict if the observed changes in inflammation are of any clinical relevance. Generally, a more pro-inflammatory milieu in the first hours of reperfusion predisposes to a more detrimental cerebral outcome [5, 35]. However, the difference in inflammation between DHCA and ACP was limited to the time point immediately after the insult, suggesting that the effect of the ischemia was temporary. Of note, this may only be the case in this specific context, as the effect of other inflammatory triggers during surgery (such as the ongoing surgical damage, the use of CPB and cyanosis) are likely overwhelming [36, 37].

We acknowledge that this work has important limitations. First, this is a model of cerebral ischemia during deep hypothermia, a temperature not directly applicable to the clinical situation in which adults or children have a stroke. Similarly, although still a matter of debate, neonates may have a different permeability of the blood-brain barrier than adults[38, 39]. Secondly, as dexamethasone was administered to all patients before start of CPB, we cannot exclude that this may have mitigated the inflammatory response to the ischemia[40]. Finally, due to the many triggers for systemic inflammation which accompany the surgery of these patients, later effects of the ischemia are impossible to tease out. In an animal model, a more 'clean' model of cerebral ischemia can be performed, and the evolution of the inflammation can be studied at all desired timepoints. However, the translation to the human situation is fraught with difficulties, making studies like the current study essential to gain insight into these complex mechanisms.

CONCLUSIONS

In neonates undergoing cardiac surgery at deep hypothermia, we observed an enhanced innate immune response immediately after cerebral ischemia. Most notably, a high number of activated neutrophils and monocytes were found exiting the cerebral circulation. Although the findings are not extreme, this is the first report of such a phenomenon in the very early period after cerebral ischemia and it may hold important clues for the understanding of the immune response to stroke. Further investigation is needed to determine the underlying mechanisms and the further consequences of this rapid immune response, as a better understanding can hold clues for new biomarkers and potentially novel therapeutic options.

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BED-SIDE PREDICTION RULE FOR INFECTIONS AFTER PEDIATRIC CARDIAC SURGERY

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ABSTRACT

Purpose

Infections after pediatric cardiac surgery are a common complication, occurring in up to 30% of cases. The purpose of this study was to develop a bed-side prediction rule to estimate the risk of a postoperative infection.

Methods

All consecutive pediatric cardiac surgery procedures between April 2006 and May 2009 were retrospectively analysed. The primary outcome variable was any postoperative infection, as defined by the Center of Disease Control (2008). All variables known to the clinician at the bed-side at 48 hours post cardiac surgery were included in the primary analysis, and multivariable logistic regression was used to construct a prediction rule.

Results

A total of 412 procedures were included, of which 102 (25%) were followed by an infection. Most infections were surgical site infections (26% of all infections) and blood-stream infections (25%). Three variables proved to be most predictive of an infection: age <6 months, postoperative PICU stay >48 hours and open sternum >48 hours. Translation into prediction rule points yielded 1, 4 and 1 point for each variable, respectively. Patients with a score of 0 had 6.6% risk of an infection, whereas those with a maximal score of 6 had a risk of 57%. The area under the receiver operating characteristic curve was 0.78 (95% confidence interval 0.72 – 0.83).

Conclusions

A simple bed-side prediction rule designed for use at 48 hours post cardiac surgery can discriminate between children at high and low risk for a subsequent infection.

INTRODUCTION

Infections occurring after pediatric cardiac surgery are a frequent complication, with the reported incidence varying widely from 16% to 31%[1-4]. These infections are an important burden in the recovery after surgery, as they cause significant morbidity and lengthen pediatric intensive care unit (PICU) and total hospital stays[5].

The incidence of postoperative infections in children after cardiac surgery is higher than may be expected in the general PICU population (6-15%) [5-7]. This is presumed to be due to the hypo-inflammatory phase of the systemic inflammatory response to cardiac surgery[8]. The most common types of infections are those occurring at the surgical site and blood stream infections [1-4]. Peri-operative risk factors have been identified previously, yielding younger age at surgery, higher surgical complexity, longer surgical and cardiopulmonary bypass (CPB) duration, a longer PICU stay and delayed sternal closure as important risk factors [1-4, 9-15]. Although these factors have helped to unravel the etiology of the infections, they do not necessarily reliably identify those patients which are most at risk. This creates the need for a prediction model, which aims to assist the clinician in the decision-making process. In the case of postoperative infections, the question is; which preventive measures need to be taken, and in which patients?

In this study we used retrospective data from a recent pediatric cardiac surgery cohort from our centre, in order to identify which variables are predictive of postoperative infections. The aim was to develop a prediction rule to estimate the risk of an infection during hospital stay. The rule was designed for use at 48 hours postoperatively, as the current protocol at our centre is to stop prophylactic antibiotics at this time.

METHODS

Patient population

In this retrospective study we included all consecutive cardiac surgery procedures performed with CPB in patients under the age of 18 years, between April 2006 and May 2009 at the Wilhelmina Children's Hospital, University Medical Center Utrecht, The Netherlands. The Wilhelmina Children's Hospital is a university teaching hospital with a level III PICU, and is one of the four pediatric heart centres in the Netherlands. Patients were excluded if they died during or within 48 hours after surgery, if charts were incomplete or if long-term broad-spectrum antibiotics were already started pre-operatively due to blood stream infections. All data were collected by retrospective chart review using standardised case report forms. The local Institutional Medical Ethics Committee approved the study and waived the need for consent.

All patients received peri-operative antibiotic prophylaxis, consisting of cefazolin 100 mg/kg a day, starting at the induction of anesthesia and continued until 48 hours postoperatively or until sternal closure. A single dose of dexamethasone (1 mg/kg) was administered to all patients at the induction of anesthesia. Pre -operative disinfection of the skin of the thorax was performed with Chlorhexidine solution 0.5% in 70% alcohol. After correction of the heart defect and directly before skin closure, disinfection

of the skin and wound margins was repeated with Chlorhexidine solution 0.5% in 70% alcohol. During the study period no specific *Staphylococcus Aureus* eradication protocols were performed. All procedures were performed by the same surgical and anesthesiology team, the latter being responsible for care in the operating room as well as at the PICU.

Clinical variables

All known risk factors for postoperative infection from literature were considered for use in the prediction model. However, only those readily available at the bed-side were included in the analyses, and these were subsequently dichotomized (into a yes/no variable) using accepted thresholds from literature. The eligible variables were age <6 months [2, 4, 9-11, 13, 14, 16]; pre-operative admission at the PICU [3, 4, 9, 10, 13, 16]; surgical complexity [2-4, 10, 15]; previous cardiac surgery with the use of CPB [3, 4, 10, 14]; duration of surgery (timed from first incision until closure) >3 hours [9, 14]; CPB duration >2 hours [2, 3, 9, 14, 17, 18]; lowest nasopharyngeal temperature <25°C [16, 19, 20]; use of inotropes [2, 11, 13], endotracheal tube [5, 11, 13, 14], open sternum and rethoracotomy [2, 3, 5, 12, 14, 16] and PICU stay >48 hours postoperatively [2, 3, 5, 6, 11, 13], red blood cell transfusion (total of intra- and postoperative transfusion) >50 ml/kg [3, 11, 14, 16, 17] and peak glucose >10 mml/L in the first 24 hours postoperatively [21, 22]. Surgical complexity was the only variable to be categorized into three groups, which was a simplified version of the RACHS-1 and Aristotle score [23, 24]. The 'low complexity' group (reference group) consisted of atrial and/or ventricular septal defect closures with or without ductus arteriosus ligation; the 'high complexity' group comprised all neonatal procedures resulting in a functionally univentricular heart. All other procedures were classified as 'medium complexity'.

Outcome definition

The primary outcome of this study consisted of all infections presenting between 48 hours after surgery until discharge from hospital. The occurrence of a postoperative infection was defined by the criteria of the Centre of Disease Control (CDC), revised by Horan et al in 2008[25]. If the origin or presence of an infection was not clear during chart review, the case was presented to an expert panel of two pediatric intensivists (NJGJ and AJvV) blinded for patient name and potentially predictive variables. When pneumonia was considered, chest X-rays were assessed by a pediatric radiologist (MG), similarly blinded. If there was consensus that a patient had an apparent infection, but insufficient data were available to classify the infection according to CDC criteria, it was classified as 'infection not otherwise specified'. Blood stream infections include both confirmed cases ('laboratory confirmed'), and unconfirmed cases, where clinical symptoms suffice ('clinical sepsis'), in accordance with the CDC criteria. Also, blood stream infections were only classified as such when they were not related to an infection at another site. Surgical site infections (SSI) were categorised into incisional SSI or organ/ space SSI. Incisional SSI consist of superficial and deep incisional SSI, where the *superficia*l incisional SSI involve only skin and subcutaneous tissue, and the *deep* incisional SSI involve deep soft tissues (i.e. fascial and muscle layers). Due to the difficulty of retrospective assignment

of tissue layers, these were combined into one category (namely, incisional SSI). Organ/space SSI are defined as infections occurring in organs or spaces which are opened during the procedure, excluding skin, fascia and muscle (e.g. mediastinitis, pleuritis). Microbiologic and virology data were collected from charts and the hospital database. Proven infections were defined as infections confirmed either by culture or polymerase-chain reaction, and these infections were used as secondary outcome.

Statistical analysis

All variables were assessed for their association with infections using 2-sided Pearson Chi-Square or Fisher's Exact tests, as appropriate. For missing data, imputation techniques were used as complete case analysis would probably introduce bias[26]. A p-value <0.15 in the univariable analysis allowed the variable to be used in the multivariable logistic regression model, providing it was not co-linear with other variables (Pearson correlation >0.7). Manual stepwise backward logistic regression was then performed. The choice for the final model was based on a balance between a high AUC and the clinical utility. The discriminative potential of the final model was assessed using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. A useless predictive model has an ROC-AUC of 0.5 and a perfectly predicting model results in an ROC-AUC of 1.0. The calibrative potential was assessed using the Hosmer-Lemeshow test. The final model was internally validated using bootstrap techniques, which provide a 'shrinkage factor' for the variable estimates. Alternative models that included more variables were compared with the final, fully reduced model. Furthermore, the robustness of the model was tested by using the secondary outcome (proven infections) and by repeating the analysis for complete cases only.

The final model was translated into a prediction rule, yielding 'rule points' for each variable. Rule points were calculated by dividing the multivariable regression coefficient by the lowest coefficient and rounding to the nearest integer. All patients were categorized according to their scores and the corresponding positive and negative predictive values were calculated. The calibration of the model was assessed by calculating the predicted risks of all cases belonging to one risk group.

Bootstrap techniques were performed with R for Windows version 2.10.1(R Foundation for Statistical Computing, Vienna, Austria). SPSS version 15.0 (SPSS Inc., Chicago, Illinois) was used for all other statistical analyses.

RESULTS

A total number of 426 procedures were performed in the inclusion period; with 412 procedures performed in 364 patients remaining for analysis (with incomplete files in 3 procedures, 4 patients who already had long-term antibiotics started pre-operatively and 7 patients who died within 48 hours postoperatively). Patient characteristics are outlined in Table 1.

Postoperative infections occurred after 102 procedures, yielding an incidence of 25% (95% confidence interval [CI] 21 – 29). The total number of infections was 127,

Table 1. Patient characteristics

	All procedures (n=412)	Procedures without infection (n=310)	Procedures with infection (n=102)
Male	226 (55)	167 (54)	59 (58)
Age (months)	6.8 (1.7 – 44)	11.3 (3.0 – 68)	3.2 (0.5 – 9.2)
Weight (kg)	6.7 (4.1 – 15)	8.5 (4.6 – 17.6)	4.6 (3.5 – 7.1)
Postoperative PICU stay (days)	2 (1 – 6)	2 (1 – 4)	7 (3 – 14)
Postoperative hospital stay (days)	7 (5 – 14)	6 (5 – 9)	20 (13 – 30)
Surgical complexity [a]	8.0 (6.5 – 10.0)	8.0 (6.0 – 9.0)	9.0 (7.0 – 11.5)

Values stated as n (% of [infection] group) or median (inter-quartile range). [a] Surgical complexity was calculated using the Aristotle score[24].

with 81 procedures being followed by a single infection, 17 procedures by two, and 4 procedures by three infections. The median start of infection was 7 days after surgery (inter-quartile range [IQR] 4 - 12).

Of all infections, 72 (57%) were proven by culture or PCR, with seven cases yielding two different micro-organisms (Table 2). The two most common types of infection were surgical site infections, responsible for 33 (26%) of all infections, and blood stream infections, responsible for 32 (25%) of all infections. Surgical site infections were mostly incisional SSI (n=24, consisting of both superficial and deep incisional SSI), and nine organ/ space SSI (mediastinitis in 5 cases, and pleuritis in 4 cases) (Table 2). Most SSI were caused by *Staphylococcus aureus* (71% of all proven infections), whereas in blood stream infections, coagulase-negative *Staphylococcus* was most abundant (44%). In urinary tract infections, *Escherichia coli* was isolated most often (33%).

Differences between the group with, versus without infections regarding various peri-operative risk factors for infections are shown in Table 3. After screening for colinearity, where surgery and CPB duration were co-linear, as well as inotrope and endotracheal tube duration and PICU stay, nine variables were finally included in the multivariable analysis. Following backward logistic regression, three variables remained in the final prediction model, which were age <6 months, PICU stay >48 hours and open sternum >48 hours. This model had an AUC-ROC of 0.78 (95% CI 0.73 - 0.83) with an acceptable Hosmer-Lemeshow goodness of fit of p=0.60.

Bootstrapping resulted in a shrinkage factor of 88%, suggesting some over-fitness of the model. Addition of other clinical variables, yielded similar ROC-AUC's (e.g. with addition of surgical complexity, the ROC-AUC remained identical). A complete cases analysis also resulted in the same predictive variables and ROC-AUC. Finally, when proven infections only were used, the final model was similar but with a somewhat reduced ROC-AUC of 0.72 (95%CI 0.65 – 0.79).

To develop the prediction rule, rule points were derived from the regression coefficients of the three final variables as shown in Table 4. One point was counted if

Table 2. Infection types and isolated micro-organisms

Infection type (CDC criteria)	n (% of all infections)	Gram-positive bacteria	Gram-negative bacteria	Viruses	Fungi/ yeasts	Micro-organism unknown
Surgical site infection	33 (25.9)					
Incisional	24	4	1	0	0	19
Organ/ space [a]	9	6	3	0	0	0
Blood stream infection	32 (25.1)					
Laboratory confirmed	16	12	4	0	0	0
Clinical sepsis	16	0	0	0	0	16
Urinary tract infection	15 (11.8)					
Symptomatic	12	0	12	0	0	0
Asymptomatic	3	0	3	0	0	0
Gastro-enteritis	18 (14.2)	0	0	10	0	8
Skin infection	6 (4.7)	1	0	0	2	3
Pneumonia [b]	3 (2.4)	0	3	1	0	0
Respiratory tract infection	9 (7.1)					
Upper	7	0	0	3	0	4
Lower	2	0	1	1	0	0
Other infections [c]	11 (8.7)	3	2	0	1	5
Total (% of all infections)	127	26 (20.5)	29 (22.8)	15 (11.8)	3 (2.4)	55 (43.3)

[a] Mediastinis (n=5) and pleuritis (n=4)
[b] In one pneumonia case, both Moraxella catarrhalis and Rhinovirus were isolated.
[c] Other infections: conjunctivitis (n=3), oral cavity infection (n=3), decubitus (n=1), endocarditis (n=1), epididymitis (n=1), infection not otherwise specified (n=2)

the child was <6 months of age at surgery, 4 points if the child had a PICU stay >48 hours, and 1 point if the sternum was open for >48 hours. For example, a child aged 6 years, already discharged from the PICU and with a closed sternum, will have score of 0, and thus an infection risk of 6.6%. In contrast, a neonate, still at the PICU at 48 hours and with an open sternum at that time (maximal score of 6) will have a risk of 57%. Negative and positive predictive values for different cut-off values are shown in **Table 5**. This shows that when a maximum number of rule points (6) is reached, the positive predictive value is 57.4%; hence, 57% of children with score 6 will likely encounter an infection, whereas 80% of all other children (score 5 or lower) will *not* become infected. In contrast, out of all children with a score of 1 or higher, 37% will encounter an infection, whereas 93% of all children not belonging to this category (so with a score of 0) will *not* become infected. Observed and predicted infection rates for all categories are depicted in **Figure 1**, where predicted infection risks are calculated for each category using the prediction rule.

Table 3. Univariable analysis of procedures without and with postoperative infection

	Procedures without infection (n=310)	Procedures with infection (n=102)	OR (95% CI)	*P* [c]
Pre-operative				
Age <6 months [a]	123 (40)	73 (72)	3.83 (2.35 – 6.23)	<0.001
Pre-operative admission PICU [a]	49 (16)	44 (43)	4.04 (2.46 – 6.64)	<0.001
Low complexity [a]	68 (22)	10 (9.8)	1.0	<0.001
Medium complexity [a]	228 (74)	74 (73)	2.21 (1.08 – 4.51)	
High complexity [a]	14 (4.5)	18 (18)	8.74 (3.34 – 23)	
Previous cardiac surgery	115 (37)	21 (20)	0.74 (0.46 – 1.20)	0.24
Intra-operative				
Surgery duration >3h	147 (47)	67 (66)	2.12 (1.33 – 3.38)	0.001
CPB duration >2h [a]	114 (37)	58 (57)	2.27 (1.44 – 3.57)	<0.001
Lowest nasal temp <25 °C [a]	80 (26)	49 (48)	2.66 (1.67 – 4.23)	<0.001
Postoperative				
Inotropes >48h	83 (27)	69 (68)	5.72 (3.52 – 9.29)	<0.001
Endotracheal tube >48h	66 (21)	61 (60)	5.50 (3.40 – 8.89)	<0.001
PICU stay >48h [a]	105 (34)	84 (82)	9.11 (5.20 – 16)	<0.001
Open sternum at 48h [a]	23 (7.4)	30 (29)	5.20 (2.85 – 9.49)	<0.001
Rethoracotomy	9 (2.9)	3 (2.9)	1.01 (0.27 – 3.82)	>0.999
RBC transfusion >50 ml/kg [a,b]	142 (46)	83 (81)	5.17 (2.99 – 8.92)	<0.001
Peak glucose >10mmol/L in first 24h [a]	151 (49)	65 (64)	1.85 (1.17 – 2.93)	0.009

OR odds ratio, *CI* confidence interval, *PICU* pediatric intensive care unit, *h* hours, *CPB* cardiopulmonary bypass, *RBC* red blood cell.
[a] These variables were used in multivariable analysis. [b] Seven patients had missing data for RBC transfusion and were imputated resulting in the above values. [c] P-values were calculated using Pearson Chi-Square or Fisher's Exact test, as appropriate.
Variables stated as n (% of infection group).

Table 4. Multivariable analysis and derivation of prediction rule points

	Univariable OR (95% CI)	Multivariable OR (95% CI)	p	Multivariable B	Points for rule
Age <6 months	3.83 (2.35 – 6.23)	1.53 (0.86 – 2.72)	0.15	0.44	1
PICU stay >48 hours	9.11 (5.20 – 16)	6.30 (3.35 – 12)	<0.001	1.84	4
Open sternum >48 hours	5.20 (2.85 – 9.49)	1.83 (0.95 – 3.51)	0.07	0.60	1
Total					6

OR odds ratio, *CI* confidence interval, *B* regression coefficient, *PICU* pediatric intensive care unit.

Table 5. Performance of various cut-offs for rule points

Number of rule points	n	True-positive (n=102)	Positive predictive value (%)	n (not in rule points)	True-negative (n=310)	Negative predictive value (%)
≥1	245	91	37.1	167	156	93.4
≥4	189	84	44.4	223	205	91.9
≥5	146	69	47.3	266	233	87.6
6	47	27	57.4	365	290	79.5

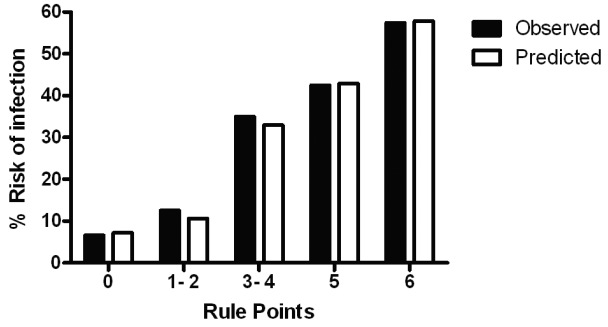

Figure 1. Calibration of the prediction rule, with observed and predicted infection risks. Score of 0 points, n=167; score of 1 or 2 points (pooled), n=56; score of 3 or 4 points (pooled), n=43; score of 5 points, n=99 and score of 6 points, n=47.

DISCUSSION

This study confirms that infections remain a common complication in children recovering from cardiac surgery. We propose a novel bed-side scoring system to assess the risk of a postoperative infection in children following cardiac surgery. Using three variables, namely age <6 months, postoperative PICU stay >48 hours and open sternum >48 hours, patients at high risk to develop an infection can be distinguished from those at low risk.

Recently, Barker and colleagues similarly developed a prediction rule, designed for clinical use[10]. The model included the variables age, complexity, genetic abnormality, previous cardiac surgery, pre-operative length of stay and pre-operative ventilator support. Although the model predicted as well as our study (ROC-AUC of 0.78), variables such as genetic abnormality and surgical complexity are not ideal in a bed-side prediction rule, as the former has often not yet been confirmed pre-operatively, and the latter demands a search through the extensive lists of Aristotle and RACHS-1 complexity scores. In addition, as the authors point out, many of the infection cases were diagnosed without the use of predefined specific criteria.

The current prediction rule is based on three simple variables; the age of the patient, PICU stay >48 hours and an open sternum >48 hours. Other studies have also shown

that these are important risk factors for postoperative infections. Neonates and young infants, are known to have an immature immune system lacking a proper innate immune response, likely responsible for the higher rate of infections in this group [2-4, 9, 11, 27]. Prolonged admittance (>48 hours) at the PICU is commonly associated with longer duration of mechanical ventilation and increased use of intravascular catheters [3, 5, 9, 11]. Furthermore, delayed sternal closure has not only been associated with a higher risk of surgical site infections, but also with an increased occurrence of blood stream infections[12]. As these two infection types make up the majority of the infections found in this study, this emphasizes the importance of the open sternum as risk factor.

As our results show, the incidence of postoperative infections remains high. This may be due to the inclusion of all infections, as defined by the CDC criteria (revised in 2008), where not all infections require a positive culture or PCR[15]. Comparing the specific infection incidences to those in literature, surgical site infections occurred after 6.4% of all procedures in this study, whereas this varies 0.0 to 9.9% in recent reports [1-4, 9, 10, 13]. Regarding blood stream infections, these also occurred after 6.4% of all procedures in our study, of which half were proven by cultures and the other half defined as 'clinical sepsis'. Recent reported incidences vary from 2.6% to 15%, where comparison of incidences is again difficult due to different criteria[1-4, 10]. The isolated pathogens in surgical site infections, are in accordance to the reports in literature, as *Staphylococcus aureus* was the most commonly found pathogen in our study [13, 14]. Similarly, blood stream infections are most often caused by gram-positive species, which our study confirms[28].

Regarding the clinical application of our prediction rule, various options may be possible. Firstly, the rule may be used to identify those patients that are at the highest risk of an infection, i.e. for use in a clinical trial assessing the effectiveness of antimicrobial interventions. Secondly, clinicians may wish to use the model to decide on the routine antibiotic prophylaxis. As the most prevalent infections in our cohort were surgical site infections and blood stream infections, mostly caused by gram-positive bacteria and with a median start of 1 week after surgery, a possibility may be to prolong the cefazolin treatment in high risk groups. This may be prolonged for a set number of days, or for example until drains and/ or central lines have been removed, as suggested in a recent study[29]. Overall, improving compliance of personnel to hygiene measures is likely one of the most important issues in infection prevention.

However, a preventive strategy specifically directed at one type of infection may be more effective. It was recently shown in adults that pre-operative screening and eradication of *Staphylococcus aureus* was an effective preventive measure[30]. Since the end of our study, this has become common practice in our hospital, the results of which have to be evaluated in future. Another preventive strategy against surgical site infections is the continuation of prophylactic antibiotics for 2-3 days after delayed sternal closure, with or without routine culturing of the sternum, which may or may not induce antimicrobial resistance [29, 31, 32]. Regarding blood stream infections, the use of lines coated with antibiotics, chlorhexidine wound dressings, routine culturing of incision sites, or the continuation of antibiotics are all possible measures to consider[33, 34].

Limitations of this study mainly apply to issue of heterogeneity in the infection cases. As we assessed all types of postoperative infections, we cannot define which risk factors specifically predict a surgical site infection, a blood stream infection, or an airway infection. This is due to the size of our cohort, which restricts multivariable analyses per subgroup. However, a (smaller) single centre study usually does result in a more homogeneous group as far as peri-operative strategies are concerned. Also, all data can be verified, which is crucial to the validity, especially in a study focussed on infections. Another limitation is that the prediction model has not been externally validated. The specific peri-operative management at our hospital may differ from others, although our infection cases are very similar to those in literature. Important characteristics of our peri-operative management are the choice of cefazolin as peri-operative prophylaxis and the use of dexamethasone during the induction of anesthesia (which may or may not influence susceptibility for infections[35, 36]). Finally, it is important to note that the prediction rule proposed in this study is based on categories of patients. Hence, the individual patient, who may already clinically show signs of a local infection, will likely have higher odds of infection than the predicted 'baseline' risk stated in this paper. However, aspecific clinical symptoms such as fever or a high C-reactive protein level after cardiac surgery fail to predict occurrence of an infection, which underlines the need for a reliable prediction rule[37].

In conclusion, postoperative infections remain common in this recent cohort of children undergoing cardiac surgery. Using important risk factors, we have developed a bed-side prediction rule, which estimates the risk of a subsequent infection during hospital stay. This tool may prove useful for directing preventive measures to those patients at the highest risk.

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8

LOW-FLOW ANTEGRADE CEREBRAL PERFUSION ATTENUATES EARLY RENAL AND INTESTINAL INJURY DURING NEONATAL AORTIC ARCH RECONSTRUCTION

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ABSTRACT

Objective

Deep hypothermic circulatory arrest (DHCA) and antegrade cerebral perfusion (ACP) are two cardiopulmonary bypass strategies mainly used in aortic arch reconstructions. It has been suggested that during ACP, abdominal organs are better protected than during DHCA, due to partial perfusion via collaterals. We tested this hypothesis using intra-operative near-infrared spectroscopy (NIRS), lactate measurements and biomarkers for early abdominal injury in neonates undergoing complex aortic arch repair.

Methods

Neonates scheduled for aortic arch reconstruction via median sternotomy between 2009 and 2011 were randomized to either DHCA or ACP. During surgery, regional oxygen saturations of the abdomen were monitored using NIRS. Immediately following DHCA or ACP, lactate concentrations from the inferior vena cava (IVC) were compared to those from the arterial cannula. Postoperatively, biomarkers for early abdominal organ injury were measured in urine.

Results

Twenty-five neonates were analyzed (n=12 DHCA, n=13 ACP). Procedures were performed at 18°C, and ACP flow was set 35-50ml/kg/min. Median abdominal NIRS value during DHCA was 31% (IQR 28-41), whereas during ACP it was 56% (IQR 34-64, $p<0.01$ between groups). Immediately after DHCA, median lactate from the IVC was 4.2 mmol/l (IQR 3.3-5.3), compared to 3.1 mmol/l (IQR 2.9-4.4) after ACP ($p=0.03$). Postoperatively, biomarkers for renal and intestinal damage (gluthatione s-transferase and intestinal fatty acid binding protein, respectively) were higher in the DHCA group compared to ACP ($p=0.03$, $p=0.04$, respectively).

Conclusions

These results substantiate earlier suggestions that ACP provides more abdominal organ protection than DHCA in neonates undergoing aortic arch reconstruction.

BACKGROUND

Deep hypothermic circulatory arrest (DHCA) and antegrade cerebral perfusion (ACP) are two cardiopulmonary bypass strategies applied in neonatal aortic arch repair. DHCA inherently results in full-body ischemia, whereas in ACP, the brain is selectively perfused at a low flow via the innominate artery. Most studies are focused on the neurological effects of either technique [1, 2]. However, abdominal organ functions also deserve attention as, for instance, postoperative renal function is closely related to general postoperative recovery[3]. Recently, we have shown that duration of postoperative recovery increases with longer duration of DHCA, whereas duration of ACP has no effect [4]. It is thought that this is due to partial perfusion of the viscera through collaterals during ACP [5-8]. Others have reported evidence of regional blood flow by abdominal near-infrared spectroscopy (NIRS) or blood pressure measurements in the abdominal or femoral arteries [5, 7, 8]. Still, to date, the protective effect of ACP has remained a speculation, as abdominal organ injury has not been directly assessed peri-operatively.

Various organ-specific biomarkers have proven useful to estimate the extent of abdominal organ injury at an early time point after cardiac surgery or other intensive care settings[9]. For renal injury, neutrophil gelatinase-associated lipocalin (NGAL) is the most widely reported marker, whereas others include kidney injury molecule-1 (KIM-1), liver fatty acid binding protein (L-FABP), gluthatione s-transferase (GST) and N-acetyl-β-D-glucosaminidase (NAG)[10-17]. For intestinal injury, intestinal fatty acid binding protein (I-FABP) is an established early biomarker [18, 19].

In this study, we assessed the above named biomarkers of early abdominal injury in neonates undergoing aortic arch reconstructions, before and after surgery. Furthermore, abdominal near-infrared spectroscopy (NIRS) and lactate measurements were performed intra-operatively to estimate abdominal perfusion. We hypothesised that ACP would provide more abdominal perfusion and consequently result in less renal and intestinal injury than DHCA.

METHODS

Patients and surgical procedures

The current study was performed as part of a prospective randomized controlled trial comparing the neurological effects of DHCA and ACP (clinicaltrials.gov number NCT01032876). In the subgroup reported here, data on abdominal perfusion were collected, which consisted of intra-operative abdominal NIRS monitoring and lactate measurements and pre- and postoperative urine analysis of biomarkers for early abdominal injury. The institutional medical ethics committee approved the study and all parents gave informed consent for enrolment. Patients suspect to have a congenital syndrome were not eligible for inclusion in the study.

In all patients, surgery was performed through a median sternotomy and standard cannulation techniques with double venous cannulation were applied. In case of interrupted aortic arch, double cannulation of the distal ascending aorta and the pulmonary trunk was

performed. CPB was performed using alpha-stat strategy, and patients were cooled for a minimum of 20 minutes to a nasopharyngeal temperature of 18°C. Antegrade cerebral perfusion was performed directly by advancing the arterial cannula into the innominate artery. In the case of a Norwood procedure with a modified Blalock-Taussig shunt, the shunt was anastomosed to the innominate artery prior to aortic arch reconstruction and the shunt was used to deliver ACP. A target flow rate of 25% (35-50 ml/kg/min) was used with a perfusate temperature of 18°C, and right radial pressures not exceeding 40 mm Hg. Depending on the individual anatomic situation, aortic arch reconstruction was performed with direct end-to-end or end-to-side anastomosis, reverse subclavian flap and patch plasty or patch plasty alone. All procedures were performed by a single surgical team.

NIRS measurements

Throughout the peri-operative period, regional saturations of the frontal cerebrum and the abdomen were estimated using NIRS. For somatic measurement, the electrode (size Pediatric, INVOS, Somanetics, Troy, MI, USA) was placed longitudinally on the left flank, just caudal of the costal margin. The electrode positioning was verified using ultrasound in the first 10 patients, all showing placement over the left kidney. Mean values of the following four periods were calculated: Pre-operative (minimum of 6 hours of monitoring), deep hypothermic full-flow CPB, during DHCA or ACP, and during the first 30 minutes after re-institution of normal CPB.

Lactate measurements

When aortic arch reconstruction was completed and full-body CPB reinstituted, blood samples were simultaneously drawn from the arterial cannula and the snuggered inferior venous cannula within the first minute of reperfusion. After temporarily clamping the inferior venous cannula and discarding the first 10ml of blood (as this approximates the dead space of the cannula), a sample of 0.5 ml blood was drawn from the inferior vena cava (IVC) for determination of lactate concentration.

Urine analysis for biomarkers

Urine samples were collected pre- and postoperatively. Pre-operatively, urine was collected before initiation of CPB, and the postoperative time point consisted of urine produced during the second hour after skin closure. Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), liver fatty acid binding protein (L-FABP) and intestinal fatty acid binding protein (I-FABP) were measured using ELISA. Gluthatione s-transferase (GST, total of α and π) and N-acetyl-β-D-glucosaminidase (NAG) were measured using colourimetric enzymatic assay. Biomarkers of organ injury were assessed at HaemoScan, Groningen, the Netherlands. To correct for dilution of urine, markers were additionally expressed as a ratio of urine production in ml/ kg/ hour [20].

Statistical analysis

Data in text are stated as median (interquartile range) or number of patients (percentage of group), unless noted otherwise. Differences in continuous variables between DHCA

and ACP groups were compared using the Mann-Whitney U test. SignalBase version 6.76 (University Medical Center Utrecht, the Netherlands) was used for calculation of NIRS values. SPSS version 15.0 (SPSS Inc., Chicago, Illinois) was used for statistical analyses and GraphPad Prism version 5.03 (GraphPad Software, La Jolla, California) was used for graphical figures.

RESULTS

Patients and surgical data

Specific diagnoses and surgical data are listed in **Table 1**. Eight neonates underwent univentricular repair, whereas the other 17 underwent biventricular repair. According to the randomization protocol, 12 neonates underwent surgery with DHCA, and 13 neonates with ACP.

One patient from the DHCA group died within 30 days postoperatively. This patient developed a low cardiac output syndrome and renal failure, which required peritoneal dialysis at 18 days postoperatively. The patient died one week later. None of the other patients had any abdominal complications.

Systemic saturation and cerebral NIRS

The pre-operative oxygenation status was similar in both groups. Before the start of CPB, the median systemic saturation in DHCA group was 97% (IQR 93-100) and for the ACP group this was 98% (IQR 95-99). Regarding pre-operative cerebral NIRS values, the DHCA group had a median of 59% (IQR 54-67), versus for ACP a median of 62% (IQR 54-67). At deep hypothermic full flow, the DHCA group had a median cerebral NIRS of 80% (IQR 70-92) which was 79% (IQR 72-85) in the ACP group. During DHCA a median of 51% was observed (IQR 49-54), versus 80% (IQR 70-88) during ACP. During reperfusion, saturations were 74% (IQR 71-79) for the DHCA group, and 73% (IQR 66-78) for the ACP group.

Abdominal NIRS

Abdominal NIRS measurements are depicted in **Figure 1**. Before surgery, abdominal NIRS values were similar in the two groups, with a median of 53% (IQR 49 – 59) in the DHCA group, and 56% (IQR 48 – 72) in the ACP group. At deep hypothermic full-flow CPB, the DHCA group had a median saturation of 89% (IQR 79 – 94), which was 77% (IQR 68 - 92) in the ACP group. During DHCA, abdominal NIRS values decreased to a median of 31% (IQR 28 – 41), which was significantly lower than during ACP (median 56% [IQR 36 – 64]; $p<0.01$). During the first 30 minutes after re-institution of normal CPB, the DHCA group had a median value of 74% (IQR 67 – 87) whereas this was 83% (IQR 76 – 91) in the ACP group.

Lactate measurements

Lactate measurements were collected in all but three neonates (n=11 DHCA, n=11 ACP), and are shown in **Figure 2**. Lactate concentrations from the arterial cannula and from the IVC were compared. Arterial lactate values were similar between DHCA

Table 1. Patient characteristics and operative data

	DHCA (n=12)	ACP (n=13)
Age at surgery (days)	9 (8-11)	9 (8 – 23)
Weight at surgery (kg)	3.6 (3.2 – 3.8)	3.6 (2.5 – 3.7)
Gestational age at birth (weeks)	39 (38.3 – 40.0)	39 (38.3 – 40.0)
Cardiac diagnoses, biventricular repair (n=17):		
Hypoplastic aortic arch/ CoA, plus:		
No intracardiac defects	0	1
VSD	1	3
ASD	0	1
cAVSD	1	0
TGA	1	0
Aortic valve stenosis	0	1
Double outlet right ventricle	1	1
Interrupted aortic arch, plus:		
VSD, ASD	0	1
Severe LVOTO (Ross-Konno procedure)	1	0
TGA	1	0
Hypoplastic left heart complex *	0	3
Univentricular repair (n=8):		
Hypoplastic left heart syndrome	4	1
Double inlet left ventricle	0	1
Tricuspid atresia	2	0
CPB data:		
Cooling duration (min)	35 (30 – 40)	30 (29 – 38)
Duration of DHCA or ACP (min)	33 (28 – 38)	39 (32 – 49)
Total CPB duration	167 (142 – 266)	161 (129 – 169)
ACP flow rate (ml/kg/min)		46 (41 – 49)
Nasopharyngeal temperature during DHCA/ ACP (°C)	17.3 (16.5 – 18.0)	17.7 (17.4 – 18.3)
Rectal temperature during DHCA/ ACP (°C)	20.7 (20.0 – 21.8)	20.7 (19.4 – 21.9)

Values shown in median (interquartile range) or number of patients. DHCA, deep hypothermic circulatory arrest; ACP, antegrade cerebral perfusion; CoA, coarctation of the aortic arch; VSD, ventricular septal defect, ASD, atrial septal defect; cAVSD, complete atrioventricular septal defect; TGA, transposition of the great arteries; LVOTO, left ventricular outflow tract obstruction. * Hypoplastic left heart complex was defined as an underdevelopment of the left heart with significant hypoplasia of the left ventricle and hypoplasia of the aortic and/ or mitral valve, ascending aorta and aortic arch, but in the absence of intrinsic valvar stenosis or atresia [25].

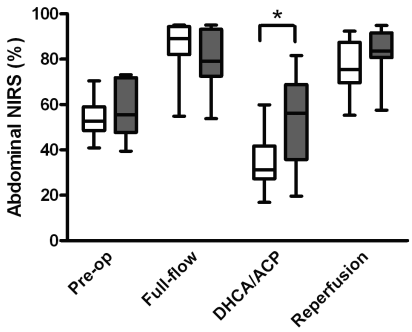

Figure 1 Abdominal NIRS values are lower during DHCA than during ACP (*p=<0.01). Values shown in medians and interquartile ranges (boxes) and minimum and maximum values (whiskers).

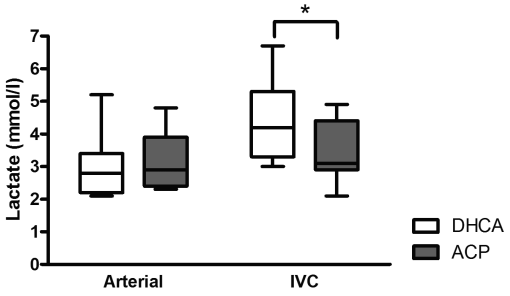

Figure 2 Arterial lactate concentrations are similar between DHCA and ACP, whereas lactate concentrations from the IVC are significantly higher after DHCA than after ACP (*p=0.03). Values shown in medians and interquartile ranges (boxes) and minimum and maximum values (whiskers).

and ACP (median DHCA 2.8 mmol/l (IQR 2.2 – 3.4) and median ACP 2.9 mmol/l (IQR 2.4 – 3.9]). Following DHCA, lactate from the IVC had a median of 4.2 mmol/l (IQR 3.3 – 5.3), which was significantly higher than the IVC lactate in the ACP group (median 3.1 mmol/l (IQR 2.9 – 4.4), p=0.03).

Abdominal biomarkers

Biomarkers of renal and intestinal injury were measured pre-operatively in 19 neonates (n=9 DHCA, n=10 ACP) and postoperatively in 23 neonates (n=11 DHCA, n=12 ACP). Results are listed in Table 2. The absolute concentrations of the renal marker GST and intestinal marker I-FABP were significantly higher postoperatively in the DHCA group compared to the ACP group (p=0.03 and p=0.04, respectively). Regarding the other markers of renal injury (NGAL, L-FABP, NAG and KIM-1) the difference of postoperative values between DHCA and ACP, did not reach statistical significance (p=0.07, p=0.61, p=0.50 and p=0.14, respectively). When correcting for urine output at the time of sampling, all markers except NAG and KIM-1 were significantly higher postoperatively in the DHCA group than the ACP group (NGAL p<0.01, GST p=0.02, L-FABP p=0.03, NAG p=0.11, KIM-1 p=0.05 and I-FABP p=0.01). The median postoperative urine output itself was 1.5 ml/kg/h in the DHCA group, versus 3.0 ml/kg/h in the ACP group (p=0.10). Stratified by uni- and biventricular repair, the median and ranges for the various biomarkers are listed in Supplementary table 1.

Serum creatinine and blood urea nitrogen (BUN) levels were available for a limited number of patients pre-operatively (n=12 DHCA, n=12 ACP) and postoperatively (n=7 DHCA, n=6 ACP). For BUN, a significant difference between postoperative levels was detected (p=0.03) whereas for creatinine this was p=0.05. Results are listed in Supplementary table 2.

Table 2. Abdominal biomarker results

	DHCA		ACP		
	Pre-op (n=9)	Post-op (n=11)	Pre-op (n=10)	Post-op (n=12)	p Post-op (DHCA vs. ACP)
Absolute urinary concentrations					
Renal injury					
NGAL	89 (4.6-149)	185 (92-235)	27 (9.2-60)	47 (24-117)	0.07
GST	5.2 (2.4-6.0)	7.2 (5.3-7.9)	5.4 (2.0-6.7)	3.8 (2.2-6.1)	0.03
L-FABP	128 (55-426)	262 (43-421)	83 (34-312)	132 (22-607)	0.61
NAG	8.6 (0.9-18)	39 (9.7-67)	8.1 (2.7-20)	24 (6.2-53)	0.50
KIM-1	2.7 (0.7-6.3)	1.5 (0.6-10.0)	1.1 (0.4-2.3)	0.6 (0.2-2.8)	0.14
Intestinal injury					
I-FABP	11 (6.7-90)	82 (30-180)	15 (7.9-38)	11 (0.0-101)	0.04
Results corrected for urine output					
Renal injury					
NGAL	19 (1.2-78)	119 (84-136)	11 (5.3-22)	14 (5.8-53)	<0.01
GST	1.2 (0.5-3.7)	4.7 (2.8-5.9)	0.9 (0.3-2.4)	1.4 (0.3-2.5)	0.02
L-FABP	83 (13-280)	271 (39-541)	37 (5.6-101)	19 (9.6-88)	0.03
NAG	3.8 (0.1-7.6)	26 (4.4-61)	2.3 (1.1-5.6)	5.1 (1.5-26)	0.11
KIM-1	0.5 (0.1-4.3)	1.4 (0.2-7.8)	0.4 (0.2-1.0)	0.4 (0.03-1.0)	0.05
Intestinal injury					
I-FABP	6.6 (1.5-65)	19 (59-134)	3.9 (0.00-13)	1.5 (0.00-39)	0.01
Urine output (ml/ kg/ h)	4.2 (2.4-6.8)	1.5 (0.8-3.1)	4.0 (2.4-5.8)	2.8 (1.4-6.3)	0.10

Values shown in median (interquartile range).
NGAL, Neutrophil gelatinase-associated lipocalin (in ng/ml); GST, gluthatione s-transferase (in U/ml); L-FABP, liver fatty acid binding protein (in ng/ml); NAG, N-acetyl-β-D-glucosaminidase (in mU/ml); KIM-1, kidney injury molecule-1 (in ng/ml); I-FABP, intestinal fatty acid binding protein (in ng/ml).

DISCUSSION

The results of the current study substantiate that in neonates undergoing complex aortic arch repair, abdominal organs are better protected by low-flow ACP than by DHCA. During DHCA, abdominal NIRS values were lower and lactate concentrations higher than during ACP, likely resulting in the increased concentrations of renal and intestinal organ damage markers after surgery.

The current findings confirm earlier suggestions in literature. Pigula et al were the first to measure evidence of flow in the abdominal aorta during ACP[8]. Since then, others have assessed abdominal NIRS trends during ACP, and have found that NIRS values rise with increasing ACP flow rate[5, 7, 21]. Recently, we have added to the

literature that DHCA duration during aortic arch reconstruction is linearly related to the duration of postoperative recovery, whereas ACP is not[4]. Taken together, these findings strongly point towards the phenomenon of abdominal perfusion during ACP.

We intended to assess markers of organ injury as close to the insult as possible. This explains the choice for the biomarkers used in this study, instead of the more well-known markers such as creatinine and urea for renal function. The latter markers are known to measure organ *function*, and are generally raised 1-3 days after surgery, whereas the current biomarkers in this study are able to detect early organ *injury*, making factors such as low cardiac output less likely to obscure results[9]. Renal injury was assessed by five different biomarkers. Of these, NGAL has generally been researched most extensively researched. Recently, two large studies validated the marker in the pediatric cardiac surgery population, and observed that acute kidney injury (AKI) could be detected by NGAL as early as two hours after surgery with mean values of 300 to 500 ng/ ml[10, 11]. Krawczeski et al specifically calculated a cut-off point for the neonatal population at 185 ng/ml. When this is applied to our cohort, after surgery, 5 of the 12 in the DHCA group would find themselves above this threshold, versus 2 of 13 in the ACP group. When comparing the concentrations of the current group of neonates to those reported for all five renal biomarkers used in this study, some of the organ injury markers are already elevated in the patients pre-operatively, likely reflecting the compromised cardiac situation. Postoperatively the values in the DHCA group indicate that substantial renal injury has occurred, whereas this is much less the case after ACP.

Previously published literature concerning renal biomarkers have mostly reported absolute urinary concentrations in their studies[10-17]. However, correction to the urine production at the time of sampling has recently been stated to be the most reliable way of assessing renal injury[20]. As differences between DHCA and ACP became especially apparent in the analysis corrected for urine production, this confirms that DHCA results in more renal injury than ACP.

Intestinal injury was estimated using I-FABP, which has been used both in adults and neonates to determine intestinal ischemia[18, 19]. Compared to these reports, the pre-operative values are acceptable, whereas the postoperative values after DHCA are amongst the highest reported, indicating that after DHCA, the intestinal injury is substantial. In contrast, after ACP, the values seem to be in the normal range.

Abdominal NIRS values during ACP showed a large variation between patients. This is likely due to both a varying degree of collaterals and a varying duration of ACP. Consequently, this does indicate that abdominal perfusion cannot be *guaranteed* in the individual patient during ACP, but at a group level, ACP does seem to provide protection from ischemic renal and intestinal injury. The lactate measurements support this notion, as these were generally increased in the DHCA group, whereas they remained unchanged in the ACP group. Neonates with aortic arch obstructions are probably ideal candidates for the advantageous effects of ACP, as their anatomy will have stimulated the growth of collaterals travelling from the aortic arch and the innominate arteries, via the mammary and epigastric arteries, to the abdominal viscera[22-24].

This study has important limitations which deserve consideration. First of all, apart from NGAL, the various biomarkers have not been validated in this specific population and the limited sample size in the current study prevents this. However, as these markers are known to show early injury, we chose to primarily assess these instead of the more traditional markers. These markers are currently as close to the injury as one can get. Ultimately, histological assays (in animal models) can only confirm specific organ injury, however, a significant limitation to the use of animals is the different collaterisation pattern. The second important limitation is the small sample size, which will have resulted in a lower power to detect statistical differences between the DHCA and ACP groups. Furthermore, despite the randomization for DHCA and ACP, the proportion of uni- and biventricular patients between the two groups is unbalanced. In order to minimize the impact of a low cardiac output state on biomarker results, urine samples were collected very shortly following surgery.

In conclusion, the results presented here show that during neonatal aortic arch reconstruction, ACP provides more abdominal perfusion than during DHCA. As a consequence, this likely has a protective effect on abdominal organs. The current results explain findings in previous studies showing an adverse effect of DHCA, but not ACP, on early postoperative recovery.

Supplementary table 1. Abdominal biomarkers: Uni- and biventricular repairs

		DHCA		ACP	
		Pre-op (2V n=5; 1V n=4)	Post-op (2V n=7; 1V n=4)	Pre-op (2V n=8; 1V n=2)	Post-op (2V n=10; 1V n=2)
Absolute urinary concentrations					
NGAL	2V	76 (4.0-222)	161 (26-797)	60 (21-168)	79 (27-126)
	1V	85 (22-149)	218 (202-235)	(8.7-11)	(358-560)
GST	2V	4.4 (0.00-5.8)	7.2 (6.4-7.9)	6.1 (4.2-11)	6.1 (1.7-11)
	1V	6.5 (5.9-7.0)	7.4 (6.8-8.0)	(1.7-3.2)	(2.2-2.2)
L-FABP	2V	128 (23-451)	371 (43-615)	236 (13-899)	313 (21-1093)
	1V	323 (97-549)	420 (29-811)	(79-86)	(21-612)
NAG	2V	9.3 (0.10-29)	31 (2.9-119)	13 (0.20-27)	22 (3.3-78)
	1V	15 (0.00-31)	42 (13-70)	(1.7-5.7)	(29-48)
KIM-1	2V	2.0 (0.22-6.5)	4.9(0.18-13)	1.6 (0.04-8.2)	1.9 (0.02-4.2)
	1V	2.5 (1.0-4.0)	9.3 (1.8-17)	(0.21-0.71)	(0.15-0.35)
I-FABP	2V	40 (6.9-150)	147 (30-205)	19 (0.00-50)	8.9 (0.00-165)
	1V	35 (0.00-70)	335 (79-592)	(32-36)	(6.3-198)
Results corrected for urine output					
NGAL	2V	19 (0.61-84)	119 (53-1139)	15 (5.3-42)	27 (4.0-84)
	1V	84 (3.2-165)	123 (112-134)	(2.0-5.5)	(23-373)
GST	2V	1.2 (0.00-4.8)	5.9 (4.8-14)	1.9 (0.67-2.8)	1.6 (0.24-5.8)
	1V	3.8 (1.0-6.6)	4.2 (3.8-4.5)	(0.74-0.85)	(0.14-1.5)
L-FABP	2V	51 (5.3-335)	374 (39-742)	45 (3.2-346)	57 (9.5-336)
	1V	312 (14-610)	277 (14-541)	(20-41)	(14-39)
NAG	2V	3.8 (0.02-7.8)	31 (2.6-88)	3.1 (0.05-6.5)	5.1 (1.2-36)
	1V	17 (0.00-34)	21 (8.9-33)	(0.40-2.9)	(1.9-32)
KIM-1	2V	0.5 (0.03-5.4)	5.8 (0.12-9.8)	0.5 (0.01-2.3)	0.8 (0.00-1.2)
	1V	2.3 (0.15-4.4)	4.6 (1.2-8.0)	(0.05-0.36)	(0.01-0.22)
I-FABP	2V	9.0 (1.0-125)	133 (22-294)	3.9 (0.00-19)	2.8 (0.00-75)
	1V	39 (0.00-78)	167 (53-282)	(8.8-17)	(0.40-132)
Urine output (ml/ kg/ h)					
	2V	4.8 (1.2-7.0)	1.1 (0.50-8.0)	4.5 (2.6-8.0)	3.0 (1.2-6.8)
	1V	2.6 (0.9-6.8)	2.6 (1.5-3.8)	(1.75-1.95)	(0.25-8.0)
Serum markers		(2V n=6; 1V n=6)	(2V n=4; 1V n=3)	(2V n=10; 1V n=2)	(2V n=4; 1V n=1-2)
Creatinine	2V	50 (36-78)	62 (47-70)	43 (24-59)	52 (41-59)
	1V	60 (56-92)	73 (73-75)	(43-68)	(61-63)
BUN	2V	3.1 (0.50-5.0)	3.1 (1.9-11)	1.8 (0.50-3.2)	2.3 (1.5-6.0)
	1V	2.9 (1.6-5.8)	4.1 (2.8-5.5)	(3.7-4.0)	4.0

Values shown in median (range). 2V, biventricular repair; 1V, univentricular repair; NGAL, Neutrophil gelatinase-associated lipocalin (in ng/ml); GST, gluthatione s-transferase (in U/ml); L-FABP, liver fatty acid binding protein (in ng/ml); NAG, N-acetyl-β-D-glucosaminidase (in mU/ml); KIM-1, kidney injury molecule-1 (in ng/ml); I-FABP, intestinal fatty acid binding protein (in ng/ml); creatinine in μmol/l; BUN in mmol/l.

Supplementary table 2. Serum markers of renal function

	DHCA		ACP		
	Pre-op (n=12)	Post-op (n=7)	Pre-op (n=12)	Post-op (n=6)	p Post-op (DHCA vs. ACP)
Creatinine	58 (46 – 69)	70 (54 – 73)	43 (39 – 55)	55 (45 – 60)	0.05
BUN	2.9 (1.4 – 4.2)	3.6 (2.9 – 5.2)	2.0 (1.4 – 3.1)	2.3 (1.4 – 3.1)	0.03

Values shown in median (interquartile range). Creatinine in µmol/l; BUN in mmol/l.

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9

INCREASING DURATION OF CIRCULATORY ARREST, BUT NOT ANTEGRADE CEREBRAL PERFUSION, PROLONGS POSTOPERATIVE RECOVERY FOLLOWING NEONATAL CARDIAC SURGERY

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ABSTRACT

Objective

Deep hypothermic circulatory arrest (DHCA) and antegrade cerebral perfusion (ACP) are two cardiopulmonary bypass techniques applied in aortic arch repair. In recent literature, cerebral effects of both techniques have received most attention, whereas the consequences for other organs have not been thoroughly investigated. Therefore, in this study, the impact of duration of DHCA and ACP on postoperative recovery was analyzed in a cohort of neonates undergoing aortic arch reconstruction.

Methods

All consecutive neonates who underwent aortic arch reconstruction from 2004 to 2009 were included in this retrospective study. Length of stay on the intensive care unit (ICU-LOS), duration of mechanical ventilation, inotrope score and areas under the curve (AUC) for lactate and creatinine were compared with respect to durations of DHCA and ACP, respectively. Correction for confounders was performed using multivariable linear regression.

Results

Eighty-three neonates were included, with a 30-day mortality of 4.8%. Longer duration of DHCA was associated with longer ICU-LOS both in uni- and multivariable analyses. Similarly, duration of mechanical ventilation and lactate and creatinine AUCs increased with duration of DHCA. Inotrope score was only associated with DHCA duration in univariable analysis. Duration of ACP did not affect any of the outcome parameters.

Conclusions

Increasing duration of DHCA, but not ACP, during neonatal aortic arch reconstruction prolongs short-term postoperative recovery. This suggests all efforts should be made to reduce the duration of DHCA to the shortest period possible, which may be achieved by exclusive use of ACP or a combination of the two perfusion techniques.

BACKGROUND

As mortality rates are steadily decreasing in pediatric cardiac surgery, focus is shifting towards improving associated morbidity and outcome. Large studies have identified neonates with complex congenital heart defects as being most at risk for a prolonged recovery on the intensive care unit following surgery[1, 2]. Therefore, it is important to identify modifiable peri-operative strategies to improve outcome in this group.

Controversy still exists on the use of deep hypothermic circulatory arrest (DHCA) versus antegrade cerebral perfusion (ACP). Initially, DHCA was frequently used for various intracardiac procedures in small infants. Nowadays, it is used less often; one of the most common indications is during aortic arch reconstructions, where standard CPB techniques are not feasible. ACP is a more recent technique, which aims to selectively perfuse the brain during aortic arch reconstruction. A combination of the two techniques is also possible to shorten the duration of DHCA. In recent literature, the neurological consequences of both techniques have received most attention, although this has not resulted in consensus on the superiority of DHCA or ACP [3-5].

Little is known about the impact of DHCA or ACP on other vitals, i.e. heart, lungs and abdominal viscera. In ACP, the CPB cannula is either advanced into the innominate artery, or perfusion may be provided via a shunt sewn to the innominate artery, at flows lower than full-flow CPB. It is perceived that an additional advantage of this technique may be that other organs also receive partial perfusion via collaterals. Various studies have found indications of this phenomenon during ACP, either by near infrared spectroscopy or arterial flow in abdominal regions[6-8]. Studies investigating whether this indeed has a favorable effect on postoperative morbidity either suffer from homogeneous but small cohorts, or adequately sized but heterogeneous groups, where the use of DHCA merely marks the most complex cases[2, 9]. Others have intended to compare perfusion techniques between groups but ultimately are testing the change in management protocols over the years[10, 11]. Studies in adult cardiac surgery are less hampered by these issues and report that ACP, compared to DHCA, results in substantially shorter postoperative length of stay on the intensive care unit (ICU-LOS) with less renal dysfunction[12]. However, as the degree and form of collateralisation may differ between adults and infants, the results cannot simply be translated into the current population.

In this study, we retrospectively analyzed a recent cohort of neonates undergoing aortic arch reconstruction and investigated the relationship between duration of DHCA and ACP, respectively, with regard to postoperative recovery on the ICU. We hypothesized that increasing duration of DHCA would be associated with a longer ICU length of stay, whereas ACP duration would have less impact.

PATIENTS AND METHODS

Patient population

Retrospectively, data were collected by medical chart review from all consecutive infants under the age of 2 months who underwent aortic arch reconstruction with

the use of CPB between July 2004 and October 2009, at the Wilhelmina Children's Hospital, University Medical Center Utrecht, the Netherlands. Patients with both uni- and biventricular physiologies were included. Patients with biventricular physiology and concomitant intracardiac anomalies in which primary correction was not possible (i.e. pulmonary artery banding) were excluded from this study. The local institutional review board waived the need for informed consent.

Surgical procedures

In all patients, surgery was performed through a median sternotomy and all patients received 1 mg/ kg of dexamethasone before CPB. Standard cannulation techniques were applied, except for cases of interrupted aortic arch, where double cannulation of the distal ascending aorta and the truncus pulmonalis was performed. CPB was initiated with a minimum of 20 minutes of cooling to a nasopharyngeal temperature of 18°C. Regarding pH strategy, $PaCO_2$ was kept between 45-55 mm Hg measured at 37°C and a target hematocrit of 24-26% was used. Depending on the individual anatomic situation, aortic arch reconstruction was performed with different techniques, i.e. direct end-to-end or end-to-side anastomosis, reverse subclavian flap plasty or patch plasty. Antegrade cerebral perfusion was performed directly by advancing the arterial cannula into the innominate artery. In the case of a Norwood procedure with a modified Blalock-Taussig shunt, a Gore-tex shunt was anastomosed to the innominate artery prior to aortic arch reconstruction and the shunt was used to deliver ACP. A target flow rate of 25% (35-50 ml/kg/min) was used, with a perfusate temperature of 18°C, and right radial pressures not exceeding 40 mm Hg. NIRS was used in the majority of cases to monitor cerebral oxygenation. All procedures were performed by a single surgical team.

Pre- and intraoperative data

Demographic data and data concerning the pre-operative clinical situation were recorded. Total surgery duration was timed from first incision until skin closure, and total CPB duration included ACP and/or DHCA durations. Surgical complexity was estimated with the Basic Aristotle Score[13], using the following modifications for unlisted procedures: Biventricular repair of hypoplastic left heart complex[14], or the Yasui (Norwood-Rastelli) operation, were classified as biventricular repair of hypoplastic left heart syndrome. Hypoplastic aortic arch repair without intracardiac procedures was scored as an extended end-to-end coarctation repair.

Postoperative data

The primary outcome in this study was postoperative length of stay on the intensive care unit (ICU-LOS). Secondary outcomes included mortality, duration of mechanical ventilation, inotrope score and lactate and creatinine AUCs.

ICU-LOS was regarded as the time period from admittance to the ICU after surgery until discharge to the cardiology ward. The same period was used for all other outcome variables. Patients who died or required a cardiac reoperation within this period were

excluded from analyses for ICU-LOS and duration of mechanical ventilation. Those who died within a week post surgery were excluded from renal and hemodynamic function analyses.

Hemodynamic function was estimated using the area under the curve (AUC) of lactate in mmol/l x days. Arterial lactate values above a threshold of 2.5 mmol/l were multiplied by the number of days that values exceeded this threshold, using the highest daily value[15]. For inotrope scores, the highest score during the postoperative ICU-stay was used. Scores were calculated using the following formula (μg/kg/min): dopamine + dobutamine + (15 × milrinone) + (100 × epinephrine) + (100 × norepinephrine)[16]. Renal function was estimated by the AUC of postoperative creatinine in μmol/L x days[17]. This was calculated by subtracting daily maximum creatinine values from the preoperative value, and subsequently multiplying this by the number of postoperative days that the daily value exceeded the preoperative value.

Statistical Analyses

Data are shown as median (interquartile ranges) or number of patients (percentage of group). Uni- and multivariable linear regression analyses were performed with lCU-LOS, duration of mechanical ventilation and AUCs of lactate and creatinine as outcome variables. All outcome variables were log-transformed to attain a normal distribution. Pre- and intra-operative confounders were identified for each of the analyses, based both on literature as well as those showing a linear relationship with the outcome variables in univariable linear regression ($p<0.1$). Postoperative variables were not included in the analyses, because they cannot influence duration of DHCA. Age at surgery, univentricular repair, Aristotle score and total CPB duration were considered important confounders. Weight at surgery was omitted from the analyses as it showed a weaker association than age with the outcome variables. Relationships between independent and outcome variables were verified for assumptions of linearity, normality and homogeneity of variances using residual plots. Associations between outcome variables are described using Pearson's correlation.

SPSS Version 15.0 (SPSS Inc., Chicago, Illinois) was used for statistical analyses.

RESULTS

Patient characteristics and intra-operative data

A total of 87 consecutive infants underwent aortic arch repair between July 2004 and October 2009. Four patients were excluded due to palliative procedures in biventricular physiology (i.e. pulmonary artery banding), which left 83 infants available for analysis. Eight patients (9.4%) had a genetic syndrome, including DiGeorge (n=3), Down (n=2) and Pierre Robin, Ivemark or Kabuki syndrome (all n=1). Twenty-four patients had non-cardiac comorbidities, which included small for gestational age (n=5), gestational age under 37 weeks (n=2), great vessel thrombosis (n=4), renal abnormalities (n=4), congenital hypothyroidism (n=3), infant respiratory distress syndrome (n=2), clavicular fracture (n=2), hemolytic disease of the newborn (n=1),

nitric oxide for pulmonary hypertension (n=1), third degree congenital heart block (n=1) and duodenal atresia (n=1). Patient characteristics and intraoperative data are depicted in Table 1. Specific diagnoses and surgical procedures with median DHCA and ACP durations are listed in Table 2.

Regarding choice of perfusion technique, 35 (42%) of the infants underwent the procedure with ACP only, and 14 (17%) infants with DHCA only. The remaining 34 (41%) infants received a combination of the two perfusion techniques.

Table 1. Patient characteristics, intra- and postoperative data

Patient characteristics	All patients (n=83)
Male sex	54 (65)
Gestational age (weeks)	39.6 (38.7 – 40.1)
Genetic syndrome	8 (9.6)
Non-cardiac comorbidity	24 (29)
Pre-operative mechanical ventilation	48 (58)
Age at surgery (days)	12.0 (9.0 – 18.5)
Weight at surgery (kg)	3.4 (3.0 – 3.8)
Intra-operative data	
Univentricular repair	30 (36)
Aristotle score	14.5 (10.0 – 14.5)
Surgery duration (min)	332 (298 – 379)
Total CPB duration (min)	167 (137 – 193)
Myocardial ischemia duration (min)	71 (53 – 92)
DHCA duration (min)	13 (0 – 29)
ACP duration (min)	34 (24 – 49)
Lowest nasal temperature (°C)	17.0 (16.0 – 18.0)
Postoperative data	
Mortality (<30 days)	4 (4.8)
ICU-LOS (days)	7 (5 - 11)
Hospital length of stay (days)	17 (11 – 27)
Mechanical ventilation (days)	5 (4 – 9)
AUC lactate (mmol/l x days)	2.8 (0.9 – 6.8)
Inotrope score	28.5 (19.6 – 67.4)
AUC creatinine rise (µmol/l x days)	29 (11 – 69)
Dialysis	3 (3.6)

Values expressed as number of patients (% of group) or median (interquartile range).
Kg, kilograms; *min*, minutes; *CPB*, cardiopulmonary bypass; *DHCA*, deep hypothermic circulatory arrest; *ACP*, antegrade cerebral perfusion; *ICU-LOS*, length of stay on intensive care unit; *AUC*, area under the curve.

Table 2. Cardiac diagnoses and procedures

	n	Median DHCA duration	Median ACP duration
Biventricular repair			
Hypoplastic aortic arch/ CoA, plus:			
No intracardiac procedures	4	0	37
VSD	11	0	34
ASD	2	22	22
cAVSD	3	0	24
TGA	6	0	31
Aortic valve stenosis	1	0	25
Ebstein's malformation	1	6	50
Double outlet right ventricle	2	11	24
Interrupted aortic arch, plus:			
VSD, ASD	4	28	5
Severe LVOTO (Norwood-Rastelli procedure)	4	8	69
Common arterial trunk	1	0	77
TGA	1	0	53
Aortopulmonary window	1	5	29
Hypoplastic left heart complex[14]	12	14	33
Univentricular repair			
Hypoplastic left heart syndrome	19	28	32
Double inlet left ventricle	4	0	56
Tricuspid atresia	6	4	59
DORV, IAA, severe LVOTO	1	49	0

DHCA, deep hypothermic circulatory arrest; *ACP*, antegrade cerebral perfusion; *CoA*, coarctation of the aorta; *VSD*, ventricular septal defect; *ASD*; atrial septal defect; *cAVSD*; complete atrioventricular septal defect; *TGA*, transposition of the great arteries; *LVOTO*, left ventricular outflow tract obstruction; *DORV*, double outlet right ventricle; *IAA*, interrupted aortic arch.

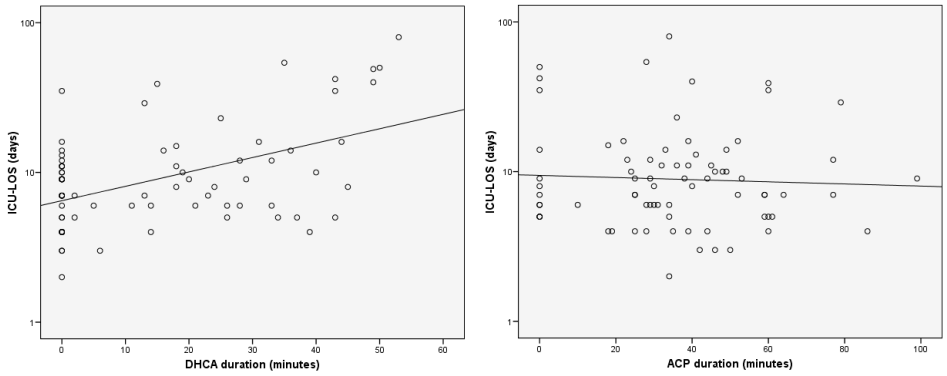
Postoperative results

Data concerning the postoperative recovery of the cohort are listed in Table 1. The 30-day mortality rate was 4.8%. Two infants died due to sepsis and multi-organ failure 3 weeks post surgery (after 23 minutes of DHCA and no ACP, and 6 minutes DHCA and 20 minutes ACP, respectively), one infant died 3 days after surgery due to low cardiac output syndrome and multi-organ failure (with no DHCA and 34 minutes ACP) and one due to a myocardial infarction on postoperative day 11 (with 18 minutes DHCA and 30 minutes ACP). Two patients required a cardiac reoperation during their ICU stay, one for a residual-VSD and one for a revision of a modified Blalock-Taussig shunt. Extracorporeal membrane oxygenation was not used in any of the patients.

ICU-LOS. The results of the linear regression analyses for log-transformed IC-LOS are shown in **Figure 1**. In univariable analysis, there was a significant relationship between DHCA duration and ICU-LOS. After adjusting for confounders (age at surgery, pre-operative ventilation, Aristotle score, CPB duration, and presence of non-cardiac comorbidities or a genetic syndrome[1]), the relationship between DHCA-duration and IC-LOS remained significant. The percentage of variation ('R-squared') of ICU-LOS explained by DHCA duration alone was 24%, which increased to 50% in the multivariable analysis.

In univariable analysis, no significant relationship between ACP duration and ICU-LOS was determined, was not attempted.

Results of the multivariable analysis can be interpreted as follows: when DHCA duration increases with 10 minutes ICU-LOS increases with 13% (=exp[0.012x10]). Therefore, for example, in an infant who would normally have an ICU-LOS of 7 days, and in whom 30 minutes of DHCA had been used, would then have an ICU-LOS of 7 + (7 x 13% x 3) = 10 days. In contrast, duration of ACP would not have any effect on ICU-LOS.



Linear regression results of log-transformed ICU-LOS

	Univariable b (95% CI)	b (95% CI) adjusted for confounders[a]	Univariable R	Univariable R^2	R^2 adjusted for confounders
DHCA duration	0.022 (0.012; 0.030)**	0.012 (0.003; 0.022)*	0.489	0.239	0.496
ACP duration	-0.002 (-0.009; 0.006)[n.s.]	n.t.	0.049	0.002	n.t.

[a]Adjusted for age at surgery, pre-operative ventilation, univentricular repair, Aristotle score, CPB duration, non-cardiac comorbidities and genetic syndromes.

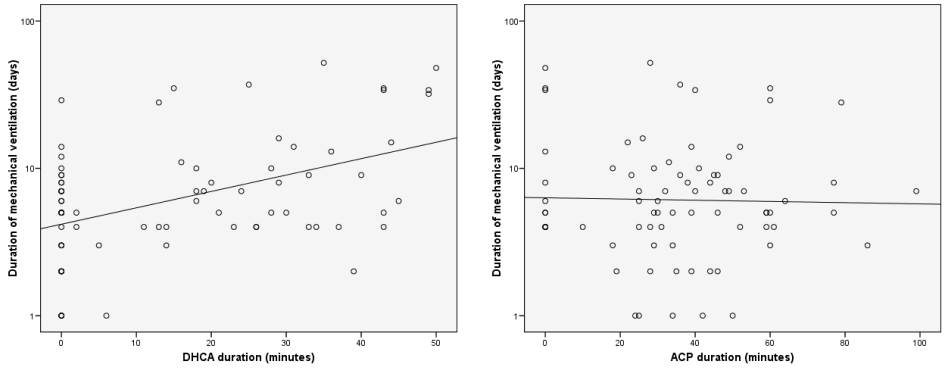
Figure 1. Linear regression of log-transformed ICU-LOS, n=76. *ICU-LOS*, length of stay on intensive care unit; *DHCA*, deep hypothermic circulatory arrest; *ACP*, antegrade cerebral perfusion; *b*, regression coefficient; *CI*, confidence interval; *R*, correlation coefficient, R^2, determination coefficient, *CPB*, cardiopulmonary bypass; *n.s.*, not significant; *n.t.*, not tested; *p <0.05; **p <0.005.

Duration of mechanical ventilation. The results of the linear regression analyses for log-transformed mechanical ventilation duration are shown in **Figure 2**. In univariable analysis, there was a significant relationship between DHCA-duration and mechanical ventilation duration. This remained the case after adjustment for age at surgery,

pre-operative ventilation, univentricular repair, Aristotle score, duration of CPB and presence of a congenital syndrome[18, 19]. ACP duration was not associated with duration of mechanical ventilation in univariable analysis.

Pearson's correlation coefficient between duration of mechanical ventilation and ICU-LOS was 0.9.

Interpretation of the multivariable analysis results in a 19% longer mechanical ventilation duration when duration of DHCA increases by 10 minutes. Therefore, in an infant who would have 5 days of postoperative mechanical ventilation and who had undergone surgery 30 minutes of DHCA, this would result in a mechanical ventilation duration of 5 + (5 x 19% x 3) = 8 days. If ACP had only been used, the duration would remain 5 days.



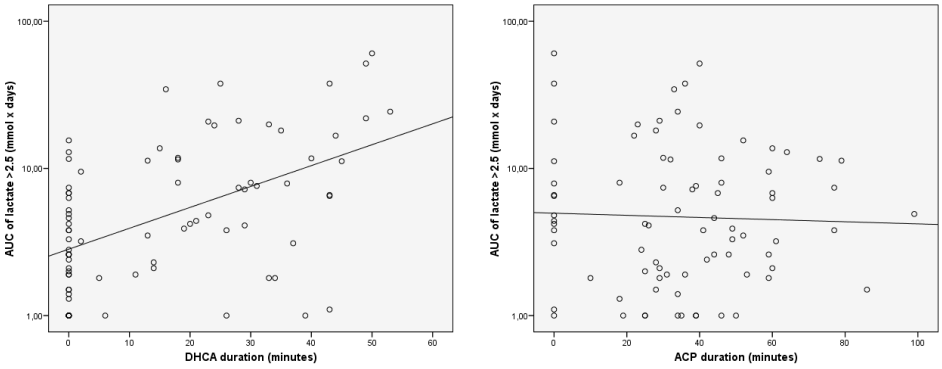
Linear regression results of log-transformed duration of mechanical ventilation

	Univariable b (95% CI)	b (95% CI) adjusted for confounders[a]	Univariable R	Univariable R^2	R^2 adjusted for confounders
DHCA duration	0.026 (0.015 – 0.037)**	0.016 (0.006 – 0.025)**	0.466	0.217	0.607
ACP duration	-0.002 (-0.011 – 0.008) n.s.	n.t.	0.040	0.002	n.t.

[a]Adjusted for age at surgery, univentricular repair, Aristotle score, pre-operative ventilation, duration of CPB and congenital syndromes.

Figure 2. Linear regression of log-transformed duration of mechanical ventilation, n=78. *DHCA*, deep hypothermic circulatory arrest; *ACP*, antegrade cerebral perfusion; *b*, regression coefficient; *CI*, confidence interval; *R*, correlation coefficient, R^2, determination coefficient, *CPB*, cardiopulmonary bypass; *n.s.*, not significant; *n.t.*, not tested; ***p*<0.005.

Lactate AUC. As a parameter for hemodynamic function, the AUC for lactate higher than 2.5 mmol/l was investigated against duration of DHCA and ACP, respectively. Results are shown in Figure 3. Linear regression of DHCA duration revealed a significant relationship with log-transformed lactate-AUC, both before and after adjustment for confounders (age at surgery, univentricular repair, Aristotle score and intra-operative myocardial ischemia duration). ACP duration was not associated with lactate-AUC. Pearson's correlation coefficient of lactate AUC with ICU-LOS was 0.8.



Linear regression results of log-transformed lactate-AUC

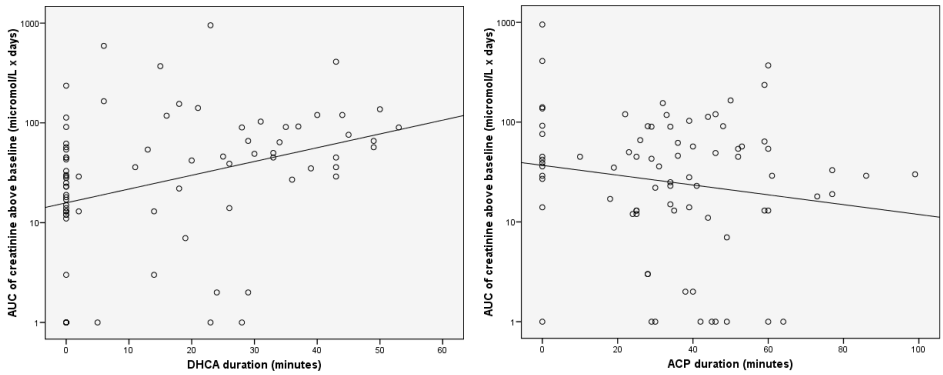
	Univariable b (95% CI)	b (95% CI) adjusted for confounders[a]	Univariable R	Univariable R^2	R^2 adjusted for confounders
DHCA duration	0.033 (0.020; 0.045)**	0.017(0.005; 0.028)**	0.512	0.262	0.574
ACP duration	-0.002 (-0.012; 0.009) n.s.	n.t.	0.035	0.001	n.t.

[a]Adjusted for age at surgery, univentricular repair, Aristotle score and myocardial ischemia duration.

Figure 3. Linear regression of log-transformed AUC of lactate >2.5 mmol/l, n=81. *AUC*, area under the curve; *DHCA*: deep hypothermic circulatory arrest; *ACP*, antegrade cerebral perfusion; *b*, regression coefficient; *CI*: confidence interval; *R*, correlation coefficient, R^2, determination coefficient, *n.s.*, not significant; *n.t.*; not tested; ***p*<0.005.

Inotrope scores. Univariable linear regression showed a significant relationship between DHCA duration and log-transformed inotrope score (b 0.008 [95%CI 0.001 – 0.016]). However, this did not remain the case in the multivariable analysis (using the same confounders as for lactate AUC). ACP duration was not associated with log-transformed inotrope score in uni- or multivariable analysis. Pearson's correlation coefficient between inotrope score and ICU-LOS was 0.6.

Creatinine AUC. Three patients required peritoneal dialysis due to poor renal function postoperatively (after 43 minutes of DHCA and no ACP, 53 minutes DHCA and 34 minutes ACP and 23 minutes DHCA and no ACP, respectively). Due to this low incidence, the AUC of creatinine above baseline values was used to compare renal function between patients, and results are shown in **Figure 4**. Linear regression of DHCA duration again showed a significant relationship with log-transformed creatinine AUC, both before and after adjusting for confounders (age at surgery, congenital syndromes, univentricular repair and CPB duration[18, 20] Although the relationship between ACP duration and creatinine AUC in the graph gives the impression of a slightly inverse relationship, linear regression showed no significant relationship. Pearson's correlation coefficient between creatinine AUC and ICU-LOS was 0.4.



Linear regression results of log-transformed creatinine-AUC

	Univariable b (95% CI)	b (95% CI) adjusted for confounders[a]	Univariable R	Univariable R^2	R^2 adjusted for confounders
DHCA duration	0.032 (0.012; 0.052)**	0.031 (0.010; 0.053)**	0.337	0.113	0.208
ACP duration	-0.011 (-0.027; 0.005) n.s.	n.t.	0.160	0.026	n.t.

[a] Adjusted for age at surgery, congenital syndromes, univentricular repair and duration of CPB.

Figure 4. Linear regression of log-transformed AUC of creatinine above baseline, n=80. *AUC*, area under the curve; *DHCA*: deep hypothermic circulatory arrest; *ACP*, antegrade cerebral perfusion; *b*, regression coefficient; *CI*: confidence interval; *R*, correlation coefficient, *R2*, determination coefficient, *CPB*, cardiopulmonary bypass; *n.s.*, not significant; *n.t.*; not tested; **$p<0.005$.

DISCUSSION

In this cohort of neonates undergoing aortic arch reconstruction, we observed that increasing duration of DHCA, but not of ACP, results in a longer postoperative recovery on the ICU. This is likely due to an adverse effect on various organ functions, essential for successful recovery. The results remain significant after adjustment for important confounders such as univentricular surgery. As duration of DHCA is a potentially modifiable intraoperative factor, these results suggest all efforts should be made to reduce the duration of DHCA to the shortest period possible, which may be achieved by the use of ACP.

Known risk factors for prolonged ICU-LOS following pediatric cardiac surgery are neonatal age, pre-operative ventilation, non-cardiac comorbidities, higher surgical complexity, longer CPB duration and occurrence of postoperative complications[1, 2]. This underlines the importance of investigating the current cohort of patients, which represents the most complex cases of pediatric cardiac surgery. In our study, only DHCA and CPB duration remained significantly associated with ICU-LOS in multivariable analysis. However, only 50% of the variation in ICU-LOS (R-squared) could be explained by our multivariable model, indicating other factors (most likely postoperative complications) also have an effect on ICU-LOS. As may be expected, the correlation between parameters of hemodynamic function and ICU-LOS was high. This means that the corresponding regression analyses can not be considered independent of each other. Renal function, however, showed only a modest correlation with ICU-LOS.

Our results suggest that increasing duration of DHCA negatively impact respiratory, hemodynamic and renal function, shown by an increase in the duration of mechanical ventilation and increased lactate and creatinine AUCs. In contrast, even 'prolonged' ACP (>60 minutes) did not seem to affect the outcome variables. A number of mechanisms may play a role in the vast contrast in postoperative organ function between the two techniques. As has been suggested before, in ACP there may be partial perfusion via collaterals to organs other than the brain[6-8]. DHCA inherently induces whole-body ischemia and reperfusion, possibly either directly leading to damage or at least putting cells at greater risk for a 'second hit'. Furthermore, the systemic inflammation, triggered by surgery and the use of CPB, seems enhanced by the use of DHCA[21, 22]. Especially in the presence of endothelial dysfunction post-DHCA, this can importantly impair end-organ perfusion[21-23]. The combination of these factors may have led to the declining hemodynamic and renal function with increasing DHCA duration. Recent literature confirms our findings, as in adult aortic arch repair with DHCA, longer duration of DHCA results in more renal failure postoperatively[24]. The washout of inflammatory mediators and metabolites after widespread ischemia may also have a direct cardiac depressant effect[25].

Although duration of mechanical ventilation and renal and lactate AUC (regarded as 'proxy's' for hemodynamic function) increased with DHCA duration, inotrope scores did not. These were only associated with DHCA duration in univariable analysis and not in multivariable analysis. Although we can only speculate about the reason for this finding, it may be due to the fact that unlike other organs like lungs and kidneys, the myocardium cannot receive partial perfusion via collaterals during ACP.

Limitations of this study are mainly due to the retrospective design and the heterogeneity of the cohort. To minimize the influence of any bias towards use of DHCA or ACP which may exist, we used multivariable analyses to adjust for important confounding variables as age at surgery, surgical complexity and uni- or biventricular repair. However, we cannot exclude that other factors may play a role. Another consequence of a retrospective study design is that one is limited to the data collected for clinical purposes. Consequently, we could not assess other abdominal function than renal function (i.e. intestinal or hepatic). Furthermore, a larger cohort may have permitted additional multivariable analyses for secondary outcomes as mortality or need for dialysis. On the other hand, an advantage of a (smaller) single centre study is that there are no differences in surgical and intensive care protocols between patients. Finally, it should be noted that this study does not directly compare DHCA only to ACP only, as in many patients a combination of the two techniques was used. However, by investigating whether the duration of either perfusion technique influences postoperative recovery, we have shown that efforts made to minimize DHCA, for instance by using ACP, are worthwhile in these patients.

CONCLUSION

This study indicates that increasing duration of DHCA during neonatal aortic arch reconstructions adversely affects postoperative recovery, whereas even prolonged duration of ACP is apparently without consequences. Although differences in neurological outcome of both techniques still need to be clarified, these results suggest that the use of ACP to reduce DHCA duration improves short-term outcome in this high-risk patient group in pediatric cardiac surgery.

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10

GENERAL DISCUSSION

PART I: CEREBRAL INJURY IN NEONATAL AORTIC ARCH OBSTRUCTIONS

In this thesis, the pre-, intra and postoperative phases which a patient with complex CHD faces, are discussed. There are many risk factors along the way which can have deleterious effects on the brain.

Some advocate that predominantly patient-related factors are decisive in whether a child will have an adverse neurodevelopment after cardiac surgery. Examples are socio-economic factors, type of congenital heart defect, birth weight, ethnicity, chromosomal abnormalities or specific genotypes[1, 2]. Others conclude that there are modifiable risk factors which can be tackled to decrease the risk of cerebral injury. Moreover, perhaps 'patient-specific factors' are not always fixed; although chromosomal make-up and diagnosis cannot be changed, the specific handling of a neonate with a certain type of CHD can be optimized to minimalize the chance of cerebral injury. This thesis is focused on the modifiable factors. Although there are many preemptive neuroprotective strategies in use, very few of the currently employed peri-operative practices are truly evidence-based [3]. So, there is work to be done to improve the prospects of the neonates who face complex cardiac surgery.

Pre-operative injury

Cerebral injury is thought to start already in fetal life in cases of complex CHD. Using magnetic resonance imaging (MRI), in cases of HLHS and TGA identified prenatally, it has been shown that the cerebral perfusion of these fetuses is suboptimal and high lactate values can be seen[4]. Although the altered cerebral perfusion is an ongoing situation during fetal life, it is still recommended to await delivery until at least 39 weeks of gestation[5, 6]. The reason is the immature aspect of the brain of a neonate with CHD, which already appears approximately 4 weeks younger than its actual age. So, premature or near-term birth would add more immaturity to the situation. This can be harmful as a more immature brain often shows more WMI, and is also more susceptible to new peri-operative injury[7]. Furthermore, the additional problems associated with prematurity (i.e., increased need for mechanical ventilation and higher susceptibility for infections) may also have an adverse effect on the brain.

Increased antenatal detection of cases of complex CHD has enabled a more prepared perinatal course, as the delivery may be planned at a tertiary centre where intensive care is available. Regarding delivery mode, it is unknown whether a caesarian section carries a different risk for cerebral injury than spontaneous delivery, but in our study in Chapter 2, we did not see any effect of the type of delivery on the presence or absence of WMI. As the immediate perinatal course is often unremarkable in neonates with ductal-dependant circulation, it seems plausible that no specific delivery mode is currently advised.

Although the cardiac condition does not put the neonate at risk of cerebral injury immediately after birth, antenatal diagnosis can provide the chance to optimize neonatal care in the period up to cardiac surgery. However, as described in Chapter 2; an antenatal diagnosis does not guarantee an injury-free brain. Comparing two pre-

operative management protocols of different centres, it became clear that there are substantial differences in rate of pre-operative cerebral injury, especially WMI. It seems that admittance of the neonate to the intensive care unit (ICU) in the period while awaiting surgery, is beneficial. Also, the later surgery is planned and a suboptimal hemodynamic situation persists, the more chance there is of pre-operative injury. Both these factors may mark a state of cerebral hypoperfusion, which is known to be a causal factor for WMI. Indeed, in other studies markers of circulatory insufficiency such as the need for cardiopulmonary resuscitation, mechanical ventilation and high lactate values have been seen to occur more frequently in patients with pre-operative cerebral injury[8, 9].

To exactly determine which pre-operative management strategy is best to prevent cerebral injury, studies should be focused per specific type of CHD as much as possible*. For example, in the case of HLHS, monitoring for pulmonary overcirculation is vital, to prevent cerebral hypoperfusion from occurring. On the other hand, reduced pulmonary circulation, which results in hypoxia, may also lead to injury. In contrast, in case of an isolated coarctation of the aortic arch, there is more concern of cerebral hyper- , than hypoperfusion due to the often observed hypertension proximal to the coarctation[10]. Hence, even within the aortic arch obstruction population, there are likely different hemodynamic situations which may be associated with a specific type of injury. Only a thorough analysis of both systemic and cerebral monitoring, together with pre-operative imaging, can enable definite conclusions.

Apart from preventing cerebral hypoperfusion, prevention of pre-operative infections can also help to reduce WMI. This is not surprising, as in premature neonates, sepsis is also associated with an increased burden of WMI, although the type of pathogen defines the exact risk[11-13]. Such detailed analyses could not be performed in our study due to the small numbers of patients. In future, it should be investigated which measures should be taken to prevent infections from occurring, especially in neonates whom are known to be less capable of dealing with an infection successfully*.

Intra-operative injury

DHCA versus ACP

There has been a longstanding debate as to which perfusion technique during aortic arch reconstructions, is the preferable one with regard to cerebral outcome. As increased duration of deep hypothermic circulatory arrest (DHCA) was initially associated with more cerebral injury, antegrade cerebral perfusion (ACP) gained interest due to the intuitive assumption that perfusion of the brain is better than none[14]. However, there had been no direct evidence in humans which indeed showed a beneficial effect of ACP compared to DHCA[15-17].

In our RCT, we found that new lesions in postoperative MRI, especially WMI, were just as common after ACP as after DHCA (**Chapter 3**). Moreover, after ACP, central infarctions were seen in a number of patients, where these were not seen at all

* future prospects

after DHCA. The infarctions were seen on the right side, in the regions of thalami, basal ganglia and internal capsule. We propose that these may be related to air or particulate emboli from the CPB apparatus. As the arterial CPB cannula was placed in the right brachiocephalic artery during ACP, the emboli could travel directly into the carotid artery and from there into the middle cerebral artery (being the largest) and lodge into the first branches, the lenticulostriate arteries, which perfuse the structures named above[18]. Emboli are indeed known to exit the CPB apparatus and consequently enter the cerebral circulation, as seen using Doppler ultrasonography[19]. This has been objectified in adults undergoing cardiac surgery, with a higher longer duration of CPB corresponding to a higher number of emboli[20]. Although a direct relationship between emboli load and postoperative cognitive defects has not been established in adults, this does not rule out an injurious effect in neonates. Apart from a different susceptibility of the (immature) brain, there may also be more consequences of the emboli due to a smaller size vasculature and corresponding cerebral structures. Furthermore, whereas in adults emboli are thought to be more due to atherosclerosis and fat emboli, in children emboli are presumed to be predominantly gaseous[21].

Central infarctions after ACP have not specifically been reported previously. McQuillen et al did report (bilateral) strokes exclusively after the use of ACP during the Norwood operation (in 5 of 9 ACP cases)[22]. Andropoulos et al have most extensively communicated their results after surgery with ACP, notably under strict NIRS-guidance and a high ACP flow (which is marginally higher than that of our centre)[23]. Although there were no specific reports of unilateral lesions, 20% did have new infarctions or hemorrhages, in a bilateral distribution. Hence, although the ACP technique at our centre is common practice, we cannot exclude that it is associated with an increased risk of specific cerebral injury. Further investigation of the different ACP methods which are used worldwide can shed light on whether different ACP techniques vary in their risk for emboli*. Animal models may prove most suitable to objectify the injury immediately after surgery.

We did not find a difference in WMI between use of DHCA and ACP, in contrast to the conclusion which was drawn recently in a large group of neonates undergoing various types of cardiac surgery, where use of DHCA was associated with more WMI[24]. However, in such cohort studies, DHCA may merely mark the most complex surgeries. Interestingly, besides the direct ischemic effect of DHCA, there are also other reasons for more injury after DHCA. Reduced cerebral perfusion has been observed in both humans and animal models, in the immediate period after surgery which included a period of DHCA[25, 26]. Increased cerebral edema has also been reported, which may also impede cerebral perfusion[27]. This has not been assessed in neonates undergoing hypothermic cardiac procedures. Repeated Doppler ultrasonography of the cerebral arteries in the hours and days after cardiac surgery could however shed light on the possibility of increased cerebral vascular resistance*.

The incidence of new injury, especially WMI, was high in our RCT (61% of patients), compared to the reports in literature of 23-47%[7, 8, 23, 24, 28]. We do not know the exact reason for the high rate of WMI. Notably, in our cohort, only one of the 37 included

patients died during hospitalization (2.7%) and could not undergo a postoperative scan. The estimated mortality risk for the current population ranged between 10.1% for aortic arch obstruction with VSD closure, and 23.7% for the Norwood procedure[29]. Due to the low mortality in our cohort compared to that in reports of similar patient groups at other centres, one might hypothesize that the low fall-out rate lead to the inclusion of the most complex cases in our cohort, whereas these patients may have been left out in other, often retrospective, studies.

Alternatively, there are other possible reasons for the high WMI burden; these include the routine use of dexamethasone administered pre-operatively at our centre; the exact combination of anesthetics; the alpha-stat strategy during CPB, or alternatively, during and after surgery, the absence of specific cerebral goal-directed regulation for instance of NIRS, blood pressure or pCO2. These possible risk factors are discussed in the following sections.

Other intra-operative risk factors

During surgery, there are also other perfusion-related issues in which choices need to be made. Amongst others, these include the ideal hematocrit, choice of alpha or pH-stat strategy, and the temperature during CPB.

Regarding hematocrit, a randomized controlled trial assessing low (20%) versus high (30%) hematocrit found a better neurodevelopmental outcome in the higher hematocrit group, but a trial comparing 25% to 35% did not show a clear difference. Hence, it is now pragmatically recommended to keep the hematocrit above 25% during all phases of CPB (where the protocol at our centre dictates to keep the hematocrit between 24 to 28%) [30, 31].

The alpha-stat (allowing pCO2 to remain at approximately 40 mm Hg during deep hypothermia) versus pH stat strategy (effectively, keeping pCO2 above approximately 70 mm Hg at the same temperature) is also a matter of constant debate. In adults, the pH-stat strategy is avoided as it may lead too much cerebral blood flow, resulting in cerebral edema, as well as loss of cerebral autoregulation [32]. In contrast, some centres advocate the use of pH-stat in pediatric cardiac surgery due to a lower perceived risk of hypoperfusion injury. The evidence for pH-stat, however, is not strong. The only study in children is a randomized controlled trial which showed that pH-stat was associated with a 9-minute earlier recovery of normal brain activity on EEG during rewarming[33]. Neurodevelopmental outcome however was not different between the two, and no other groups have pursued the search for the superior strategy [34]. There have been no studies using imaging directly postoperatively, which may be able to resolve this issue*.

Another contemporary issue is the temperature at which ACP is best performed. In our study, this was set at 18°C, in an effort to minimize cerebral metabolic rate. However, some centres have communicated their preference for a more moderate hypothermia, of approximately 25-30°C, mostly due to fewer perceived problems with hemostasis, systemic inflammation and a shorter CPB duration[35]. In adults, cerebral

* future prospects

and somatic outcomes are encouraging with no differences between deep and mild hypothermia, but it remains to be ascertained in the pediatric population[36]. Ly et al did retrospectively assess neonates who underwent arch repair at varying degrees of hypothermia and found no clinically apparent neurological differences, but this outcome parameter lacks sensitivity to reliably assess the cerebral status [35]. More detailed research is needed to assess whether higher temperatures during ACP are safe for the brain*. The high incidence of WMI in our study, which is assumed (partially) to be caused by hypoperfusion, suggests that energy demands should be minimalized as much as possible, which may be attained by use of deep hypothermia[37].

The CPB in itself initiates a profound systemic inflammatory response, due to the contact of blood with foreign material. It was long thought that the inflammation as such was injurious to the brain, but studies in adults have shown that there is no direct relationship between the severity of inflammation and subsequent cognitive decline[38]. Corticosteroids are nevertheless often administered before the start of CPB, to reduce the general systemic inflammatory response. It is unknown whether this has an effect on the brain, but one study which compared different dosages of methyprednisolone, did observe less cerebral edema with a higher dose[39]. In our study, neonates received a dose of 1 mg/kg of dexamethasone before surgery. Although this may dampen systemic inflammation, it may also be neurotoxic. This has been suggested in premature neonates, where repeated postnatal dexamethasone was associated with impaired neurodevelopment and restricted brain growth[40]. Hence, the widespread use of corticosteroids should be evaluated for their possible neurotoxicity*.

Postoperative injury

From literature it is known that in the postoperative period, there are various potential risk factors for injury. It is possible that this is a more dangerous period than that during surgery, as substantial periods of suboptimal perfusion may occur in the course of several days.

There is no doubt that the systemic perfusion must be optimal to deliver blood to the brain. A successful surgical repair is inevitable for sufficient cardiac output. However, periods of relatively low cardiac output especially in the first 24 hours are unfortunately common, resulting in low blood pressure and suboptimal tissue perfusion.

The type of cardiac repair which the neonate is recovering from, is essential to bear in mind when assessing hemodynamic issues and their subsequent risk for injury. The greatest difference in hemodynamic state lies between a uni- or biventricular repair. In contrast to a 'normal' biventricular repair of an aortic arch obstruction (i.e., for aortic coarctation or interruption), a Norwood procedure, performed in cases of HLHS, results in a univentricular circulation. In the latter, there is a constant cyanotic state after surgery (with oxygen saturations approximately between 70 and 85%). The perfusion of pulmonary and systemic beds must constantly be balanced, as when the pulmonary bed receives more perfusion there is a high risk of cerebral hypoperfusion. On the other hand, a diminished perfusion of the lung results in a low oxygen saturation which may also be injurious to the brain. Although we know that the HLHS group generally has the highest rates of brain injury postoperatively and worst neurodevelopment, it

is unknown whether this is due to hypoxia, hypoperfusion, intra-operative need for either DHCA or ACP, or a combination of these factors[28, 41].

Elaborating on the exact type of repair, different types of shunts used for instance during the Norwood procedure may have different effects on cerebral blood flow. For instance, in the case report in **Chapter 4**, we describe a specific case where the Blalock-Taussig shunt (BT-shunt) is used. This shunt connects the pulmonary artery to the brachiocephalic artery, so as to perfuse the lungs. In the described patient, after the procedure, a consistent asymmetry in frontal cerebral saturation values was observed, the right side being 20% lower than the left. Indeed, the postoperative MRI scan revealed an absence of flow in the right carotid artery. The circle of Willis also appeared to be incomplete, so that the left carotid artery was apparently left with the task of perfusing the largest part of the brain, a dangerous situation especially considering the unstable first postoperative days. This infant fortunately did not show an asymmetry in cerebral injury, and neurological follow-up was also normal. Later, results of a multi-centre RCT comparing the BT-shunt to a right-ventricle-to-pulmonary-artery shunt were published, which did not reveal any large differences in neurological outcome[1]. This means that patients with a BT-shunt apparently are not at dangerous risk for cerebral injury, which is reassuring, but this case does illustrate that cardiac shunts can disturb the normal cerebral blood flow pattern.

Bearing the increased burden of new WMI in our RCT cohort in mind, we searched for clinical factors in the postoperative period which may have played a role. As known risk factors for WMI in the current population, low blood pressure, low NIRS values and infections have been described previously, and these are similar to those seen in premature neonates[8, 42, 43]. As described in **Chapter 3**, we also found a low arterial pCO2 to be a risk factor for new postoperative WMI. Generally, the range of pCO2's in our cohort was lower than in other studies, which may contribute to the high presence of WMI. pCO2 level directly affects cerebral perfusion, as was shown in a study by McQuillen in which there was a direct linear relationship between CO2 concentration and cerebral NIRS[44]. Also in premature neonates, hypocarbia is associated with increased occurrence of WMI[45].

pCO2 may be important to counteract a naturally constrictive state of the cerebral arteries after surgery with deep hypothermia. As stated above, it is unknown whether this is due to constriction of the cerebral vasculature, or perhaps cerebral edema[27]. If vasoconstriction is the problem, an increase in pCO2 may have the potential to overrule this and help ensure adequate cerebral perfusion and help prevent hypoperfusion injury (i.e., WMI) from occurring.

When the phase of vasoconstriction after surgery has passed, cerebral autoregulation is likely still not fully functional. This has often been seen in premature neonates or otherwise 'ill' neonates, which places the current population at high risk for a suboptimal cerebral autoregulation[46, 47]. Cerebral autoregulation has been thoroughly investigated during surgery, but not in the postoperative period[48]. However, especially during low systemic perfusion states after surgery, absence of autoregulation may place the brain at important risk for injury.

Although postoperative infections do carry a higher risk of WMI, the exact role of systemic inflammation on peri-operative cerebral injury in these infants is yet to be clarified[43]. In adults undergoing cardiac surgery, the CPB-induced inflammation is generally not regarded (anymore) as detrimental[38]. Animal models report contradictory evidence, as initiation of systemic inflammation, results in more WMI, but in models with CPB with DHCA, inhibition of the inflammation using various compounds does not always limit neurological damage[49-51, 50]. In children, let alone neonates, the relationship between systemic inflammation and subsequent cerebral injury remains has not been assessed.

The role of coagulation and peri-operative cerebral injury has received the least of attention in the current literature. However, the abundance of cerebral sinovenous thromboses (CSVT) in our cohort is concerning. This became clear once magnetic resonance venography (MRV) was routinely included in the scanning protocol. In the 15 patients in whom MRV was performed postoperatively, 7 had partial or complete CSVT. Of note, three patients already had an abnormal signal in the sinus on the conventional sequences before surgery (MRV was not available pre-operatively due to scanning time constraints). So, we do not know exactly when, how, or how often, these CSVT occur. However, multiple pro-coagulative factors are often administered peri-operatively to prevent bleeding, as hypothermic CPB causes trombopathy[52]. Further research seems indicated, to establish whether CSVT indeed occurs at a higher rate than may be expected in a neonatal intensive care population, and subsequently the role of hemostatic practices should be investigated*.

Bed-side cerebral monitoring

NIRS

Cerebral near-infrared spectroscopy (NIRS) measures the oxygen saturation of the cerebral tissue (the regional saturation; rSat), which is predominantly composed of venous blood. The rSat is a result of the difference between oxygen delivery (so, arterial oxygen saturation, cardiac output, blood pressure, hematocrit and pCO2) and oxygen demand, which depends on brain metabolism. With this in mind, one would expect that there is a clear relationship between NIRS-values and subsequent injury, especially WMI, as hypoxia and hypoperfusion are presumed to be important causal factors. Indeed, in literature, NIRS values between 45 and 55% have emerged as thresholds below which there may be a higher risk of cerebral injury[7, 9, 42]. Some centres use these thresholds for bed-side interventions to increase cerebral perfusion[23], such increasing cardiac output, hematocrit, pCO2 and decreasing the level of basal metabolism (by for instance sedation).

Theoretically, this seems an effective way of preventing brain injury, but there is no direct evidence that this is indeed the case. The relationship between NIRS and injury has both been confirmed and refuted in literature. A large study in neonatal cardiac surgery showed a significant association between lower NIRS directly after CPB, and delayed neurodevelopment at one year of age[53]. Also in a smaller but more specific

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study with only HLHS cases, longer duration of NIRS under 45% was also associated with more injury on MRI[9].

We did not observe a relationship between cerebral NIRS values and patterns of injury on MRI (**Chapter 5**). Similarly, in a thorough study of 57 neonates undergoing uni- or biventricular cardiac surgery, Andropoulos did not observe any association between NIRS and new lesions on postoperative MRI[23]. One reason may lie in the likely multi-factorial etiology of cerebral injury, especially WMI. NIRS may only emerge as a significant risk factor if other factors are kept constant and therefore cannot disturb the relationship. The best way to assess benefit of NIRS may be to investigate whether intensive NIRS monitoring, with subsequent intervention to keep cerebral saturation above a minimum value, can reduce the burden of postoperative injury*. With the high incidence of WMI in our population, this seems a worthwhile exercise.

NIRS level may however not be important throughout the whole peri-operative period. It may for example, prove most important during periods of absent cerebral autoregulation immediately after surgery. This may be the reason why only NIRS values directly after CPB, were significantly associated with outcome, in the above named study of Kussman et al[53]. Loss of cerebral autoregulation is known to occur frequently during surgery, especially during hypothermia and rewarming. The effect on the brain has not been ascertained, but when blood pressure drops below a certain threshold, and autoregulation is absent, there is presumably a risk of ischemic brain injury. Autoregulation can be estimated by cerebral NIRS, where a NIRS trend which exactly follows that of blood pressure (hence, a direct 1:1 correlation) suggests a pressure-passive situation and thus loss of autoregulation[54]. The bed-side monitoring of this correlation, which is now possible using the BedBase system, holds important promise for future monitoring*.

aEEG

Whereas monitoring of NIRS may help to prevent brain injury, aEEG can make the end result of cerebral injury apparent to the clinician. It is however not yet used as widely as NIRS, possibly due to the less straight-forward interpretation of the aEEG which requires experience. However, bearing the the impressive prognostic capacities in neonates after birth asphyxia in mind, it has the potential to become of similar importance in cardiac surgery[55]. Both the background pattern of the aEEG, as well as the presence of subclinical seizures are informative parameters at the bed-side.

During surgery, the background pattern is especially useful during the deep hypothermic phase. Of note, cooling to 18°C did not result in the same activity in all patients; some showed a burst suppression pattern and in others, a flat trace was observed. Intuitively we assume that brain activity should be minimalized to prevent injury during low or absent perfusion states, but it is unknown whether indeed a flat trace during hypothermia is preferable to a burst suppression. However, we did not see a relationship between background pattern at this point and MRI injury (**Chapter 5**).

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Once rewarming has started, the background pattern will recover and at normothermia a discontinuous (DNV) or continuous normal voltage (CNV) is seen. We observed that after DHCA, it took longer to recover to a CNV pattern, which was also seen in the DHCA group of the Boston Circulatory Arrest Study[56]. The mechanisms underlying this are unknown, but neuronal activity may need longer to recover after an ischemic period than if there is still some cerebral perfusion during surgery.

In general, we observed a much faster recovery of the background pattern than in other studies, for instance the study of Gunn et al[57]. We do not know the reason for this, as sedative protocols seem similar and we do not expect inter-observer differences to play a role due to the use of standardized criteria for classification of background patterns. Also, we did not find a relationship between the recovery of the background pattern and injury as seen on MRI or neurodevelopmental outcome, as the study of Gunn et al did[58].

In contrast, we did however see a relationship between the occurrence of seizures and injury on MRI and cognitive and motor outcome, as has also been often seen after birth asphyxia. This is important, as this implies that aEEG can help to identify those cases at highest risk for injury. Patients often do not show any signs of clinical seizures in the days after surgery as they are heavily sedated. Of note, we also saw a high burden of seizures (31%), which may be related to the high incidence of new injury in our cohort. We strongly suggest the routine use of aEEG during and after surgery, to identify the cases at highest risk for injury at the bed-side, who then deserve further evaluation by for instance MRI and follow-up*.

Serum biomarkers

Serum biomarkers are appearing increasingly in literature, for a wide array of conditions in which there is a wish to monitor disease activity, or the response to therapy. As the neurological state of these neonates undergoing cardiac surgery is clinically difficult to assess, biomarker concentrations such as s100b and neuron-specific enolase measured at the bed-side would be ideal. After birth asphyxia, although not used in clinical practice yet, these markers have shown to correlate well with cerebral injury and outcome[59, 60]. However, the predictive capacity of s100b and NSE had not been assessed yet in pediatric cardiac surgery, especially not in relation to injury objectified by imaging or later neurodevelopment. We assessed this and discussed the findings in **Chapter 5**, where we found that at 4 hours after surgery, both a higher s100b and NSE were associated with worse neurodevelopment. Although of interest, it is difficult to explain why we did not observe the same trend in MRI lesions. We conclude that larger studies are needed to provide conclusive results on the usefulness of specific serum biomarkers to estimate the extent of cerebral injury*

MRI issues

There is no doubt that currently, MRI is the most sensitive modality to objectify cerebral injury. It is known that the lesions on MRI do represent actual injury, as histopathological studies of expired HLHS cases show clear patterns of periventricular WMI[61].

The question however remains, as to how clinically relevant the postoperative injury detected by MRI is. Most experience lies in the premature and post-asphyctic neonates, where MRI is the mainstay of neurological assessment and helps to guide treatment and neurodevelopmental follow-up[62]. In cases of perinatal asphyxia, mostly damage to the thalami and/ or basal ganglia is seen, as well as watershed injury. The severity of the injury to these regions predicts neurodevelopment well. We know from the premature population, in which similar patterns of WMI injury are seen as in the CHD population, that there is a strong relationship between cystic periventricular leukomalacia (PVL), hence severe WMI, and neurodevelopment. However, the relationship between milder injury, such as punctate WMI, and later neurodevelopment is not as clear.

Recently in a large group of neonates with CHD and MRI's performed peri-operatively, the extent of injury seen on MRI peri-operatively, was not predictive of early neurodevelopment. There was however a significant relationship with brain *maturity* scores and outcome[24]. This may not rule out a relationship between *injury* and outcome, as the overall burden of injury was relatively low: pre-operatively, 20% had WMI, and postoperatively 44% had new WMI, and most were classified as mild injury. However, generally the neonates had very immature aspects of the brain: median total maturational scores (TMS) of 10 were found, whereas our cohort had a median of 13 (which corresponds to a normal term brain])[63]. Andropoulos et al have also tested the relationship between MRI and neurodevelopment at two years of age and have found varying results: in the aortic arch obstruction group, they found no relationship between pre- or postoperative MRI and neurodevelopment, but in the arterial switch group, extent of injury on the pre-operative MRI was predictive[23, 64].

Whichever best predicts outcome on MRI, be it injury on MRI or markers of immaturity, consistently, a strong correlation between these two is reported. Hence, in an immature brain, more WMI is seen[7, 65]. This may be a chicken-and-egg effect; a more immature brain may have more oligodendrocytes which are susceptible to damaging influences, or perhaps the maturation process is delayed in an injured brain[65].

Although it remains a speculative issue, it seems plausible that the mild cases of WMI, similar to the punctate lesions in the premature population, will not result in overt adverse sequelae. In contrast, cases of severe WMI, perhaps resembling cystic PVL in the premature group, are more likely to have an effect. It is yet to be seen what the impact is of the grey area which lies in between these extremes. We are currently awaiting the neurodevelopmental status of the rest of our RCT participants, which is assessed at 2 years of age, so this may then shed light on the above issue.

Timing of scans

The difficulty with postoperative scans is that we do not know exactly when the injury developed. Did the injury occur during surgery, or during a period of low cardiac output postoperatively, or during the transportation from the operation room to the ICU? Or perhaps it is a continuum where a primary 'hit' predisposes to more injury during a second hit? Repeated imaging could help to answer these questions. However, 'only' performing pre- and postoperative scans is already a complicated and

expensive task. Furthermore, not only does the neonate need to be hemodynamically stable enough to safely endure the transport to, from and during the MRI facility, these procedures demand great flexibility and time from intensive care doctors and nurses who are to accompany the infant. Hence, even with the development of MRI-compatible incubators with functions to support the instable infant, more frequent MRI scans are not really a feasible option[66].

In contrast, in future, it is hoped that scanning facilities will be closer to the patient (i.e. to the ICU and operating facilities), which will provide more possibilities for repeated imaging. The total duration of scanning protocols can then be reduced to a strict selection of imaging sequences. Diffusion-weighted imaging (DWI) is a very promising candidate in this sense, as it is a short sequence, which can identify cerebral regions of ischemia within hours after the event. In an ideal research setting, immediately after surgery, DWI could be performed to assess whether injury has occurred intra-operatively. This way, in the first postoperative days, the possible additive effect of for instance a compromised cardiac output could be monitored for instance daily. This would help to shed light on exactly which peri-operative phase is most injurious to the brain and so where the (research) attention should go out to first*.

Other interesting possibilities in MRI include magnetic resonance spectroscopy (MRS), which shows the metabolic characteristics of the tissue. Lactate concentrations, for instance, can be measured to assess evidence of hypoxia-ischemia, and *N*-acetylaspartate can be used to estimate the neuronal organization of the tissue[67]. This information can help to unravel the relationship between tissue maturation and subsequent injury, for instance[65]. Also, arterial spin labeling (ASL) has recently shown to be applicable to the neonatal brain[68]. This technique visualizes blood flow in different cerebral regions, so hypo- and hyperperfusion phenomena can be assessed. Especially in the current population, this technique could help to ascertain whether hypoperfusion is as important as we suppose for the development of WMI. Furthermore, the effect of physiological variables (blood pressure, pCO2) and medication on the (distribution of) cerebral blood flow could be investigated*.

In the meantime, animal models could play an important role in unraveling the etiology and timing of peri-operative cerebral injury. However, these need to closely reflect the clinical situation. Performing surgery with CPB and hypothermia, while incorporating for instance, ACP, fluctuating blood pressures, cardiac output and blood gas management [i.e. pCO2] can prove a difficult task in an animal model. Also, one has to take into account that the animal brain unfortunately has a very different reaction to damaging circumstances, than the human brain. A relevant example is the pattern of injury seen in animal models after DHCA (thalami and basal ganglia), which is very different from the predominant WMI seen in our RCT cohort and in other studies[69, 70]. Another example is seen in investigation of the question whether pre-operative cerebral injury predisposes to more injury during surgery. In human

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neonates, Block et al found that no evolution of the already present lesions, whereas in a piglet model, lesions did clearly increase with surgery[28, 71].

*General suggestions for further research**

It is clear that the abundance of cerebral injury in these already high-risk neonates obligates further research into the exact incidence and mechanisms underlying this injury. Although there is increasing attention for the neurological effects of cardiac surgery, many studies do not use appropriate measures to test cerebral injury. Established imaging techniques such as MRI should be used before and after surgery, or neurodevelopment at a minimum of two years of age should be tested (and preferably, both). Many other short-term outcomes, especially those performed during or shortly after surgery, using bed-side monitoring or biomarkers, have not been validated enough to suit the purpose.

The type of lesion which was most common in our population was WMI. As the etiology is likely complex and multifactorial, investigation of the factors leading to this injury should be rigorous. Hemodynamic data should be continuously collected, as well as markers of cerebral perfusion (for example NIRS, and perhaps Doppler measurements of the carotid arteries) and function (i.e., aEEG). A system which collects all these data simultaneously at the bed-side, such as the SignalBase system, should be used. Only then, can we truly assess the interaction of various factors. Importantly, studies should focus on one type of diagnosis and procedure, as the hemodynamic consequences of various cardiac lesions can be very different and each may have its own set of risk factors for injury.

Focusing on one type of cardiac lesion means that sufficient numbers of patients need to be available to adequately answer the research questions. Even though there is currently a tendency for centralization of cardiac care, single-centre trials may not be able to reach sufficient numbers and multi-centre collaborations should be encouraged. This also allows for the comparison of different clinical approaches, and their consequences for the brain. As highlighted in **Chapter 2**, there are clear differences between centres in their peri-operative management protocols and these can help to assess which is most of benefit for the brain.

The use of alternative, but validated, statistical techniques should be encouraged. Especially in these groups with a relatively low incidence, especially if we focus on one type of lesion as is proposed above, the data which is collected should be used as efficiently as possible and no more patients included than strictly needed[72]. Sequential analysis, as applied in our RCT (**Chapter 3**) is an example of a non-conventional statistical technique, but there are others available, especially for randomized controlled trials. Although RCT's are still regarded as the gold standard to assess the superiority of one clinical practice over another, there are other reliable retrospective ways to assess this. **Chapter 9**, is an example, where all relevant variables thought to influence the

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outcome variables, were accounted for using multivariable analysis. Whichever the type of study, close collaboration with statistical experts can be of great benefit, to most efficiently and reliably answer the research question at hand.

Experimental therapies

There are a number of promising therapies which may be able to provide neuroprotection after cardiac surgery. The fact that the insult to the brain is in this case a planned event, provides a window of opportunity to intervene and prevent (further) injury. Especially WMI is hoped to be responsive due to the hypoxia and inflammation which underlie this type of injury. However, also in patients with stroke, these therapies may also help in tissue recovery and prevention of further ischemia.

In the immediate period after cardiac surgery, in analogy to birth asphyxia, therapeutic hypothermia may be an option which deserves attention. There are however some important side-effects which may have prevented the use thus far; importantly the heart rate is generally decreased which can impair the capacity of the neonate to increase cardiac output when needed[73]. Also, there may be different pharmacodymanics and – kinetics due to hypothermia, which especially may impact inotrope administration.

Also, ischemic pre-conditioning is described, where protective effects of pre-conditioning using ischemia, are attained by activating hypoxia-inducible factor-1α, which protects against injury[74, 75]. This would imply that repeated ischemia is not detrimental; although there may be an exhaustive effect and a continued hypoxic situation may have a different effect than short, repeated episodes.

A number of anti-inflammatory and/ or anti-apoptotic agents, commonly derived from animal models of perinatal asphyxia, are possible candidates for the cardiac surgery population[76]. Allopurinol has been one of the very few which was tested in neonates undergoing cardiac surgery[77]. Although the anti-oxidative stress effect was promising, it failed to show a reduction of cerebral lesions on MRI after surgery. Similarly, erythropoietin has also been assessed due to its putative anti-inflammatory and anti-apoptotic effects, but interim results have not been able to show a difference in cerebral injury[78]. Finally, the fact that xenon inhalational gas was beneficial in a number of perinatal asphyxia models, but worsened the outcome in a model of neonatal CPB due to expansion of air emboli, illustrates the discrepancy in the etiology between the two populations[79, 80] . In any case, even if the above agents are unsuccessful in the prevention of injury, efforts could also be directed towards restoration of the cerebral function, i.e. neuronal networks, after the injury has occurred. In this sense, mesenchymal stem cells have shown very promising results and may also prove to be effective after surgery[81, 82]*.

Finally, the use and choice of anesthetics is currently receiving much interest due to its potentially neurotoxic, but also perhaps neuroprotective effects. This is especially important in the group of CHD patients as there is repeated exposure to anesthetics and sedatives before, during and after surgery, while on the ICU, and also during re-operations in the years thereafter. Also, the routine use of systemic corticosteroids before CPB in an effort to decrease systemic inflammation should be

assessed for its cerebral effects. Especially dexamethasone is of concern, due to the injurious effects on the brain which are seen premature population, which may be less when hydrocortisone is used. Again, animal models may prove to be suitable for a preliminary assessment during cardiac surgery*.

PART II: INFLAMMATION AND SOMATIC EFFECTS OF PERFUSION TECHNIQUES

Systemic inflammation and infections

Cardiac surgery with the use of cardiopulmonary bypass (CPB) is known to initiate a robust systemic inflammatory response syndrome (SIRS). A leukocytosis is seen, with high levels of inflammatory markers such as C-reactive protein[83, 84]. In our cohort of neonates undergoing aortic arch reconstruction, we saw marked in both the cellular and soluble inflammatory compartments (**Chapter 6**). Neutrophils and monocytes showed an activated profile intra-and shortly postoperatively, and similarly cytokine concentrations (IL-6, 8 and 10) peaked. This is in accordance with other studies in neonates and infants and is likely due to a relatively long CPB time and multiple periods of ischemia and reperfusion (pulmonary, myocardial, somatic and in the case of DHCA, also cerebral)[85-87].

However, instead of investigating the postoperative changes, we focused on the intra-operative inflammation; more specifically, on the additive effect of cerebral ischemia during surgery. We observed that DHCA clearly resulted in more intra-operative inflammation than when ACP was used. The approximately 30 minutes of cerebral ischemia elicited a response immediately, even at the deep hypothermic temperature. From blood in the vena cava superior, which had just exited the ischemic brain after DHCA, we noted that neutrophils and monocytes had a markedly activated phenotype. Also, cytokines took on a pro-inflammatory profile. This shows that cerebral ischemia and reperfusion is indeed a robust and immediate trigger for systemic inflammation, and that even a low temperature cannot prevent this from occurring. Although inflammation is thought to be of major importance for the outcome after stroke[88], the immediate effects on systemic inflammation had never been investigated previously, as clinical cases of cerebovascular accidents are usually assessed at least hours after the ischemic insult has occurred.

After surgery, the result of systemic inflammation becomes clinically apparent by a general capillary leak. The peripheral edema is most clear, but pulmonary edema can also occur, and renal insufficiency is also thought to have an inflammatory component[83, 89, 90]. To decrease the burden of SIRS, corticosteroids are widely administered before the initiation of CPB. For example, at our centre, 1 mg/kg of dexamethasone is routinely used. Although corticosteroids have a clear effect on laboratory measures of SIRS (i.e., cytokine levels), clinically, they have not been found to be beneficial. Moreover, a pooled analysis of 38 North-American centres showed that use of corticosteroids in pediatric cardiac surgery

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was associated with a longer postoperative length of stay, with more infections and need for insulin therapy[91]. Also, in the neonatal population, especially dexamethasone may have injurious effects on the brain, as described in Part I of this Discussion.

The other side of SIRS is the hypo-inflammatory phase. Immune cells show a diminished response to infectious challenges in vitro. In our RCT cohort, besides high IL-10 concentrations, we observed a depressed phenotype and also functionality in monocytes and neutrophils after 24 hours postoperatively (unpublished data, Groeneveld et al). In another cohort of older children at our centre, monocytes were also seen to be hyporesponsive in vitro[84]. Similar postoperative changes in innate cell profiles have been associated with a higher burden of infections in previous literature[92, 93]. Especially neonates, who already are known to have an immature immune system, have a high susceptibility for infections during this state of immune paralysis[94].

Indeed, in our study of postoperative infections after pediatric cardiac surgery (**Chapter 7**), infants younger than 6 months encountered more infections. Other risk factors for infections were an open sternum and a length of stay on the intensive care unit (ICU) for longer than 48 hours. These variables together were able to reliably predict the chance of a postoperative infection at 48 hours postoperatively. This allows for additional measures to be taken at this time point, such as a prolonged period of antibiotic prophylaxis. The exact risk factors however, may vary between centres and even within centres, as peri-operative management is often subject to heavy change, which calls for validation of the prediction rule in another cohort*.

We assume that the length of ICU stay was the most important risk factor for infections, due to the associated increased exposure to mechanical ventilation and indwelling lines and catheters. This is supported by the relatively large proportion of blood stream infections. Surgical site infections, hence located at the sternum, made up the other large category of infections. Just after the study period, a pre-operative eradication protocol for S. Aureus was implemented for all cardiac surgery procedures, of which the end result on infection burden is yet to be investigated.

Antibiotic medication is currently still the mainstay of infectious prophylaxis in this population, as other approaches to support the immune system in its 'paralyzed' state have not yet reached the clinical setting. The administration of immunoglobulins is unfortunately not a promising candidate as previous studies in adult cardiac surgery, and neonatal sepsis, have shown discouraging results[95, 96]. More targeted approaches to promptly restore the function of specific components of the immune system, (for example, stimulation of phagocytosis in neutrophils or monocytes), have not been developed, but may prove effective in future*.

Other effects of perfusion techniques

It has become clear that cardiopulmonary bypass (CPB) is not a fixed entity, and that there are many variations on the original bypass system which was designed to support the circulation, enabling surgery to be performed on a still heart. As surgical techniques have changed and improved over the years, 'regular' full-flow (whole body) CPB has become possible for an increasing number of cardiac diagnoses. For example,

where originally procedures such the arterial switch, or even ASD or VSD closures were performed using DHCA, these surgeries can now be performed with full-flow CPB. For aortic arch reconstructions unfortunately, full-flow CPB is not a feasible option. Although during ACP, at least the brain is perfused, placement of an extra cannula in the descending aorta so as to perfuse the rest of the body is not a widely used technique, due to the constraints of a small surgical field in the neonate[97].

However, even without directly perfusing the lower body, ACP has proven to provide some flow to these organs as well. In our study assessing the intra-operative differences in somatic ischemia between DHCA and ACP, we observed better abdominal oxygenation and less organ damage in the ACP group. During ACP, renal NIRS values were higher, and in the inferior vena cava, lactate values and biomarkers of intestinal and renal damage were lower, than during DHCA. The protective effect of ACP is likely due to a partial perfusion of the abdominal tissues, via a network of thoracic collaterals. Others have also found signs of somatic perfusion during ACP both in adults and children, but the end result on organ injury had not been ascertained[98, 99]

In contrast, in DHCA, there is a whole-body circulatory arrest. In animal models, this has been seen to initiate an endothelial activation and to a certain degree, a capillary leak with edema[27, 100]. Similarly, the mucosa in the intestine has an increased permeability, resulting in an influx of endotoxin systemically[101]. Together with the direct ischemic effect of DHCA on tissues, it is not surprising that there will be substantial tissue damage. Indeed, when we assessed postoperative recovery in 83 neonates from our hospital undergoing aortic arch repair, we saw that with increasing duration of DHCA, length of stay on the ICU also increased linearly. The same applied to markers of myocardial function (inotrope score and lactate values), and duration of mechanical ventilation and creatinine values. So, we propose that with longer duration of DHCA, there was a cumulative injury to various organs which resulted in a prolonged postoperative recovery. Of note, with increasing duration of ACP, no trends in the outcome variables were seen, which underscores the above described protective effect of ACP.

Furthermore, there is an increasing tendency to use milder hypothermia (approximately 25-30°C) than deep hypothermia during ACP (18°C). In adults undergoing aortic arch procedures, results have been reassuring, but in children there have been very few reports[35, 36]. A drawback is the higher metabolism at a higher temperature, which may not have as many consequences for somatic tissues, but may be undesirable for the brain, as discussed in Part I.

*Suggestions for further research**

Regarding systemic inflammation, especially the hypo-inflammatory phase after surgery, resulting in infections, has proven to be burden for the postoperative recovery, as well as the possible negative effects on cerebral injury. Therefore, studies should be focused on prevention of infections, especially the youngest patients undergoing the

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most complex types of cardiac surgery. Translational research can shed light on the cellular and molecular mechanisms which make these infants prone to an infection, and may provide targets to enhance the function of the immune system.

However, the best way to prevent infections may be to prevent the overwhelming systemic inflammation from occurring. Limiting tissue ischemia, so, providing perfusion to *all* organs as is the case in a normal physiological state, may have the benefit of less systemic inflammation*. Similar to the use of ACP to perfuse the brain, the specific perfusion of somatic, cardiac and pulmonary vascular beds will hopefully all become feasible options, even in the smallest patients which present for surgery.

There is an opportunity to use NIRS during and after cardiac surgery, to monitor somatic oxygenation. During aortic arch surgery, somatic NIRS can be used to monitor the abdominal oxygenation, especially during ACP. There may also be an important clinical place before and after cardiac surgery, as a warning sign for a low cardiac output state. The benefit of this monitoring should be assessed in an intervention study, where the effect of interventions to keep the somatic NIRS above a certain threshold, can be compared to the current situation*. Similarly, the ratio between cerebral and somatic NIRS may have the potential to warn the clinician of a low-perfusion state, during which the brain is thought to receive more perfusion than the body.

'PART III': IN CONCLUSION

To perfuse or not to perfuse the brain?

In our randomized controlled trial comparing DHCA and ACP in neonatal cardiac surgery, we did not find a difference in the number of patients with new cerebral lesions after surgery. In both groups, WMI was clearly the most common type of new lesion. However, there was a striking difference in infarctions between the two techniques, most notably in the central regions of the brain (thalami, basal ganglia and internal capsule), with these in one-third of ACP patients, versus none in the DHCA group. We hypothesize that due to the placement of the arterial cannula in the brachiocephalic artery, CPB-related emboli, are directly released into the brain, and thereby obstruct the small capillaries perfusing the central regions. We do not know if this is due to the specific CPB apparatus or the ACP technique at our centre, but this urgently calls for further investigation and comparison with other centres.

The somatic side of the issue, is that ACP provided a beneficial effect for abdominal organs during surgery, which translated into a shorter postoperative recovery. This is thought to be due to the partial perfusion of the thoracic and abdominal regions during ACP, via a network of collaterals. In contrast, during DHCA, with increasing duration of the full-body ischemia, organ damage seems to accumulate and thereby the postoperative recovery is proportionally lengthened. So, for the general recovery process after neonatal cardiac surgery, ACP is clearly better.

As a successful recovery from surgery is also beneficial for the brain (i.e., lower risk of hemodynamic instability, thrombo-embolic and infectious complications), we propose that the technique of ACP should be optimized so that the risk for emboli is no longer an issue, and ACP will hopefully prove to be as good for the brain as it is for the body.

SUMMARY OF FUTURE PROSPECTS

Part I

General

- Further insight into the mechanisms underlying peri-operative brain injury is greatly needed.
- Studies should be as (cardiac) lesion-specific as possible.
- Use of alternative, but validated, statistical techniques should be encouraged to save patients, time and money.
- Centres should compare their pre, intra and postoperative clinical practices and cerebral outcome to those of others to gain information on potential risk factors for injury.
- Cerebral injury outcome should be assessed either using MRI or neurodevelopment at at least two years of age, and preferably both.
- All hemodynamic data should be prospectively collected continuously before, during and after surgery to thoroughly assess the impact of, and interactions between, these variables on cerebral injury.
- Imaging facilities should be more accessible for the intensive care patient. Then, it will be feasible to perform repeated imaging and gain more information on the timing of peri-operative cerebral injury.

Details

- Due to a high embolic load, the current ACP technique needs to be thoroughly investigated and improved.
- The possible beneficial effect of pH-strategy during cardiac surgery, as opposed to alpha-stat, needs to be assessed using imaging or neurodevelopment as outcome measures. The same applies for mild, instead of deep, hypothermia during ACP.
- Repeated Doppler flow measurements of the cerebral arteries may provide information on the increased vascular resistance after hypothermic CPB and the possible influence of pCO2 in overcoming this.
- The neurotoxic effects of corticosteroids, especially dexamethasone, and anesthetics should be assessed in neonates. On the other had, possible neuroprotective measures such as postoperative hypothermia or infusion of mesenchymal stem cells should be looked into.
- Incidence of pre- and postoperative cerebral sinovenous thrombosis should be ascertained.
- A randomized controlled trial of NIRS monitoring, with an intervention algorithm, is needed to investigate whether this can prevent especially peri-operative white matter injury.
- aEEG should be standard of postoperative care in neonates with aortic arch obstructions to identify those at high risk for cerebral injury.
- The predictive capacity of serum biomarkers for cerebral injury should be further assessed in a large cohort.

– Imaging techniques to assess cerebral blood flow, such as arterial spin labeling, can help to shed light on the effect of hypoperfusion on the neonatal brain.

Part II

– Research on systemic inflammation after cardiac surgery should focus on the postoperative infections, as these are a common complication which needs to be tackled, especially in neonates. Translational research may help to find suitable targets to support the immune system.
– The effect of the pre-operative eradication of S. Aureus on incidence of postoperative infections should be assessed. Also, the bed-side prediction rule should be re-tested and externally validated.
– CPB techniques should be developed further so that all organs receive perfusion during cardiac surgery, while still being technically feasible, even in neonates.
– Monitoring of cerebral and/ or somatic NIRS can be of help to assess postoperative cardiac output and organ perfusion, but this needs to be tested in an intervention study.

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SUMMARY

Neonates with complex congenital heart disease (CHD) are at high risk for cerebral and other organ injury. This is related to their underlying cardiac defect with the compromised hemodynamic situation, but also to injury occurring during surgery. In this thesis we investigate various aspects of peri-operative care and assess their role in cerebral and organ injury. In this thesis we focus on neonates undergoing aortic arch reconstructions, where specific cardiopulmonary bypass (CPB) techniques are an important issue.

Part I of this thesis is concentrated on the cerebral effects, before, during and after cardiac surgery. In **Chapter 2** the pre-operative burden of injury is investigated. In our cohort of neonates with aortic arch obstructions, who were compared to a combined cohort of the University of California, San Francisco (UCSF) and University of British Colombia (UBC, Vancouver) it became apparent that there is a large variation in occurrence of white matter injury (WMI) between centres, especially in those infants with a prenatal diagnosis of their aortic arch obstructions. In Utrecht, 11 of 22 cases (50%) who were prenatally diagnosed, had evidence of WMI, whereas this was seen in only 4 of the 30 (13%) at UCSF/UBC ($p<0.01$). Searching for reasons for this difference, it became clear that Utrecht patients underwent surgery at a later age, spent less of their pre-operative time on the intensive care unit (ICU), received more diuretic medication, less total parenteral nutrition (all $p<0.01$) and had more infections before surgery ($p=0.01$). Of note, patients who were not identified by prenatal screening, had a similar incidence of WMI in both centres (Utrecht 46%, versus UCSF/UBC 50%; $p>0.99$). The results of this study suggest that the pre-operative management of neonates with aortic arch obstructions can make a difference for the burden of pre-operative WMI. Prenatal diagnosis has the potential to be protective, but postnatal care must be optimized to fully realize this and subsequently improve outcome.

In **Chapter 3**, we focus on the intra-operative cerebral injury in neonates undergoing aortic arch reconstruction. As in literature, increased duration of deep hypothermic circulatory arrest (DHCA) during surgery has been associated with a worse cerebral outcome, this has led to the practice of Antegrade Cerebral Perfusion (ACP), in which the brain is selectively perfused at a low-flow. In a randomized controlled trial, we assessed whether there was a difference between DHCA and ACP in terms of new cerebral injury, as seen on postoperative magnetic resonance imaging (MRI). We found a high incidence of new injury, which was comparable in both groups (14 of 18 DHCA patients [78%] versus 13 of 18 ACP patients [72%]; $p=0.66$). Although WMI was the most common type of injury in both groups, central infarctions were seen exclusively after the use of ACP, in 6 of 18 patients. We hypothesize that this is due to the introduction of CPB-related emboli, which are released into the cerebral circulation by ACP, and subsequently lodge into the small lenticulostriate arteries, perfusing the thalami and basal ganglia. We conclude that ACP cannot reduce the incidence of new postoperative cerebral injury compared to DHCA; moreover, there may be additional risks associated with this perfusion technique.

The role of bed-side cerebral monitoring techniques is investigated in **Chapter 4**. Postoperative MRI findings and early neurodevelopmental outcome

were used as outcome parameters, to assess whether amplitude-integrated electroencephalography (aEEG), near-infrared spectroscopy (NIRS) and cerebral biomarkers in serum can predict cerebral injury. We found that aEEG was most useful in this respect, as postoperative electrographic seizures were significantly associated with both MRI injury and motor and cognitive outcome ($p<0.01$, $p=0.04$ and $p=0.03$). These electrographic seizures were seen in 11 of 36 patients [31%]. Of note, these were mostly subclinical, as only two of these patients had clinical symptoms during the recording of the aEEG. NIRS was not associated with the outcome variables in any of the peri-operative phases. Finally, the cerebral injury biomarkers s100b and neuron-specific enolase, measured at 4 hours postoperatively, revealed a significant relation to motor and cognitive outcome ($p=0.02$ and $p<0.01$), but not to new MRI injury. In summary, in this cohort, aEEG had the most potential to identify important cerebral injury at the bed-side.

In **Chapter 5** we describe an infant where cerebral NIRS helped to identify an asymmetrical perfusion of the brain during postoperative recovery. In this neonate, who was recovering from a Norwood palliation for hypoplastic left heart syndrome, the regional saturation on the right (as measured by NIRS) was continuously approximately 20% lower than the left. A few days later, MRI showed a stalled perfusion of the right carotid artery. This was deemed to be caused by a 'steal' of blood by the Blalock Taussig shunt, which is placed on the right brachiocephalic artery and directs blood from there, to the pulmonary artery. As MRI also revealed an incomplete Circle of Willis in this infant, there was a potential risk for ischemic injury in the right hemisphere. This case highlights how NIRS can be used post operatively in the detection of an asymmetric cerebral perfusion, and also shows the potential effect of cardial shunts on the cerebral circulation.

In Part II, we discuss the systemic inflammation and other somatic effects of CPB during cardiac surgery. **Chapter 6** concentrates on the systemic inflammation which occurs during procedures performed with DHCA or ACP. In the cohort of the randomized controlled trial, blood samples were drawn immediately before and after DHCA or ACP, from the superior vena cava, representing the cerebral circulation. To gain information on the direct consequences of cerebral ischemia on inflammatory markers, we compared the local cerebral inflammation after DHCA (so, cerebral ischemia), to that after ACP (no ischemia). Immediately after approximately 30 minutes of DHCA, there was clear activation of neutrophils, monocytes and cytokines, compared to before DHCA. These changes were not observed in ACP. So, this study adds to the current literature that cerebral ischemia is accompanied by marked inflammation, and more specifically, that this effect is an immediate one, which can be observed in the first minutes after ischemia.

In **Chapter 7** we investigate the incidence of postoperative infections after pediatric cardiac surgery. Cardiac surgery with the use of CPB initiates a general systemic inflammation, which has a dual effect; there is a pro-inflammatory aspect which clinical symptoms such as fever, increased capillary leak and decreased cardiac, pulmonary and renal function. However, the anti-inflammatory effects may be more apparent. We found that as many as 25% of patients undergoing various types of cardiac surgery

with CPB, encounter an infection during their recovery in hospital. To identify those patients at the highest risk, we developed a simple prediction rule to be used at the bed-side. Using three variables, namely age under 6 months, a continued stay on the ICU for longer than 48 hours and an open sternum, a total 'score' per patient can be calculated. This score then translates into a risk for a subsequent infection, which ranged from 6.6% in the lowest-risk group, to 57% in the highest. The prediction rule can, for instance, be useful in identifying those patients in which additional prophylactic measures are necessary.

In **Chapters 8 and 9** the differential effects of DHCA and ACP on organs other than the brain, are described. In **Chapter 8**, in the randomized controlled trial cohort, we investigated whether there was evidence of indirect perfusion of thoracic and abdominal organs, during ACP. It was previously hypothesized in literature that during perfusion of the brain in ACP, there was some perfusion of the rest of the body via collaterals. We tested this using abdominal NIRS (with the sensor placed in the renal region), and by measuring lactate in the inferior vena cava before and after ACP. We compared this to DHCA, in which there is complete circulatory arrest. We found that during ACP, abdominal NIRS values did not decrease as much as they did during DHCA (median regional saturation during ACP 56%, versus 31% in DHCA, $p<0.01$), and lactate values after ACP were lower (3.1 mmol/l in ACP versus 4.2 mmol/l in DHCA, $p=0.03$). Also, to assess organ injury, biomarkers for renal and intestinal damage were measured in urine immediately after surgery (gluthatione s-transferase [GST] and intestinal fatty acid binding protein [I-FABP], respectively). These showed lower levels after ACP than DHCA (GST 3.8 versus 7.2 U/ml, p0.03; I-FABP 11 versus 82 ng/ml, $p=0.04$, respectively).

To further assess the clinical impact of the above named phenomena, in **Chapter 9**, the postoperative recovery after neonatal cardiac surgery using DHCA versus ACP were compared. Because an elaborate statistical analysis was necessary to correct for other factors which may also influence postoperative recovery, we included all neonates who had undergone cardiac surgery using DHCA and/ or ACP in a 5-year period. The relationship between duration of DHCA and ACP and various measures of recovery were assessed: postoperative length of stay on the ICU, duration of mechanical ventilation and lactate and creatinine concentrations. We observed a linear relationship between the duration of DHCA, and all these outcome variables, even after correction for confounders in multivariable analysis. However, with increasing duration of ACP, this association was not seen. We conclude that whereas longer DHCA does lead to a prolonged postoperative recovery, ACP can apparently be used for a longer time without these adverse effects.

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NEDERLANDSE SAMENVATTING

Er worden in Nederland jaarlijks plusminus 1250 baby's geboren met een aangeboren hartafwijking, van wie eenderde een operatie of hartkatheterisatie zal ondergaan. Een openhartoperatie is vaak levensreddend, maar kan ook ongewenste bijeffecten hebben. Dit proefschrift omschrijft de effecten van complexe hartchirurgie op pasgeboren baby's (met een leeftijd minder dan 1 maand), op organen anders dan het hart. Er wordt gefocust op baby's die zijn geboren met een onderbroken of vernauwde aortaboog, met of zonder een te kleine linker hartkamer ('hypoplastisch linker hart syndroom'). Het wordt steeds meer duidelijk dat mensen die op jonge leeftijd een dergelijke openhartoperatie hebben ondergaan, later problemen kunnen ervaren; hetzij op motorisch of cognitief vlak, of voor wat betreft gedrag.

Het proefschrift is opgebouwd uit 2 delen; waarbij het eerste deel chronologisch het ontstaan van hersenschade voor, tijdens en na de operatie bespreekt. Het tweede deel beschrijft de effecten op andere delen van het lichaam: onder andere de buikorganen en het algehele herstel na de operatie.

Hoofdstuk 1 biedt een introductie voor alle daarop volgende hoofdstukken.

Deel 1

In het **tweede hoofdstuk** wordt duidelijk hoeveel van de pasgeborenen vóór de operatie al in hersenschade hebben, in mindere of meerdere mate. Aangenomen wordt dat dit wordt veroorzaakt door een verminderde doorbloeding van de hersenen, zuurstoftekort en/of een verstopping van de bloedvaten. Dit kan zowel voor de geboorte plaatsvinden, als nadien; in de dagen dat de baby in afwachting van de operatie is.

In dit hoofdstuk wordt duidelijk dat er een groot verschil bestaat tussen verschillende centra in het percentage pasgeborenen dat al hersenschade heeft vóór de operatie. We hebben de casussen in Utrecht die gepland stonden voor een aortaboogoperatie, beoordeeld door middel van een MRI hersenscan, en deze vergeleken met gelijksoortige baby's van een gecombineerd cohort van University of British Columbia (Vancouver) en University of San Francisco, California. Hierbij zagen we dat er een groot verschil was tussen Utrecht en UBC/UCSF, waarbij in Utrecht 50% van de baby's hersenschade op de MRI hadden, vergeleken met 13% in UBC/UCSF ($p<0.01$ tussen centra). Ook werd duidelijk dat er in Utrecht, ten opzichte van UBC/UCSF: 1) later werd geopereerd (9 versus 6 dagen leeftijd); 2) minder tijd werd doorgebracht op de intensive care (en meer op de verpleegafdeling); 3) meer plasmedicatie werd voorgeschreven; 4) minder infuusvoeding werd toegediend, en meer normale voeding (allen $p<0.01$); en tot slot, 5) de baby's meer infecties hadden ($p=0.01$). Al deze factoren kunnen hebben bijgedragen aan het verschil tussen de centra.

Bovenstaande getallen gelden voor de gevallen waarbij de hartafwijking door middel van een prenatale screening was gedetecteerd. Deze pasgeborenen kunnen hier een voordeel bij hebben, omdat de geboorte in een academisch centrum gepland plaats kan vinden, en tijdens en na de geboorte continu gemonitord kunnen worden.

De groep waarbij de afwijking pas na de geboorte bekend wordt, kan een heel ander beloop hebben, omdat deze baby's in een levensbedreigende situatie terecht

kunnen komen buiten het ziekenhuis, soms met een reanimatie tot gevolg. Hierdoor is theoretisch de kans op hersenschade groter. Echter, voor de kans op hersenschade, bleek het voor de Utrechtse groep niet uit te maken of de hartafwijking wel of niet voor de geboorte ontdekt was (in beide groepen had 50% van de casussen hersenschade). Dit in tegenstelling tot UBC/UCSF, waarbij de prenataal ontdekte groep er duidelijk beter aan toe was vergeleken met de groep waarbij de hartafwijking zich pas na geboorte openbaarde: van de prenataal ontdekte groep had 13% schade ten opzichte van 50% in de andere groep ($p<0.01$). Kortom, alleen in UBC/UCSF werkte een prenatale diagnose beschermend voor de hersenen. Hieruit wordt duidelijk dat baby's waarbij de hartafwijking prenataal ontdekt is, een voordeel kunnen hebben ten opzichte van baby's waarbij dat pas na de geboorte duidelijk wordt, maar dan dient de zorg direct na de geboorte wel optimaal te zijn.

Hoofdstuk 3 beslaat het meest omvattende deel van dit proefschrift; hierin worden twee operatietechnieken met elkaar vergeleken, met als doel te onderzoeken welke techniek in de minste hersenschade resulteert. Pasgeborenen met een aortaboogobstructie werden gerandomiseerd om de operatie met Diep Hypotherm Circulatoir Arrest (DHCA) of Antegrade Cerebrale Perfusie (ACP) te ondergaan. Dit zijn twee hart-long machinetechnieken die specifiek tijdens de aortaboogoperaties worden gebruikt. De reden voor een alternatieve techniek van de hart-longmachine is omdat tijdens een aortaboogoperatie de hart-longmachine niet - zoals normaal - continu kan draaien (zie hoofdstuk 1, Fig. 1 voor voorbeeld van normale hart-longmachine). Dit omdat de cannule welke zuurstofrijk bloed naar het lichaam vervoert, normaliter geplaatst wordt in de aortaboog, exact waar de operatie in dit geval moet plaatsvinden.

Daarom zijn er twee technieken ontwikkeld om de operatie toch uit te kunnen voeren. De eerste is Diep Hypotherm Circulatoir Arrest (DHCA), ontwikkeld in de jaren '60, waarbij het lichaam met de hart-longmachine gekoeld wordt naar 18°C om de energiebehoefte te minimaliseren, en de cannules tijdelijk uit het chirurgisch veld te kunnen halen. Hiermee komt de bloedsomloop volledig tot stilstand ('circulatoir arrest'). In deze periode kan de chirurg de aortaboog repareren, waarbij als vuistregel geldt dat dit maximaal een uur mag duren. Hierna wordt de hart-longmachine weer aangesloten en de patiënt weer opgewarmd. De tweede en meer recente techniek (gebruikt vanaf de jaren '80) is Antegrade Cerebrale Perfusie (ACP). Hierbij wordt het lichaam naar eenzelfde temperatuur gekoeld, en wordt een kleine cannule van de hart-longmachine opgeschoven in de aanvoerende hersenvaten (zie hoofdstuk 1, Fig. 5). Op deze manier worden de hersenen nog wel voorzien van bloed tijdens het uitvoeren van de aortaboogingreep, maar ontvangt de rest van het lichaam geen bloed.

Ondanks het gevoelsmatige voordeel van ACP ten opzichte van DHCA, was nog niet duidelijk wat exact de gevolgen van beide technieken waren voor de hersenen. In twee eerdere studies is gekeken naar de ontwikkeling van kinderen op 1 of 2-jarige leeftijd, na gebruik van beide technieken bij de operatie die zij als baby hadden ondergaan. Beide groepen hadden echter een vergelijkbare ontwikkelingsachterstand en ook bij een soortgelijk onderzoek bij volwassenen, werd er geen verschil aangetoond.

Wij hebben de twee technieken met elkaar vergeleken door middel van MRI-scans van de hersenen, gemaakt vlak voor, en plusminus een week na de operatie. Hieruit bleek dat 14 van de 18 DHCA patiënten (78%) nieuwe hersenschade had op de scan na de operatie, ten opzichte van 13 van de 18 ACP patiënten (72%). Beide groepen verschilden daardoor niet van elkaar. Echter, er dient ook te worden gekeken naar de soort hersenschade. Hoewel in beide groepen zogenaamde witte stofschade de boventoon voerde (wat wordt gedacht te komen door te weinig zuurstof en/ of doorbloeding – zie hoofdstuk 3, 'Supplemental Figure 1'), bleek alleen de ACP groep nog bijkomende kleine infarcten te hebben, welke helemaal niet gezien werden in de DHCA groep. Wij vermoeden dat deze infarcten, vooral centraal-rechts gelokaliseerd in de hersenen, veroorzaakt worden door kleine partikels uit de hart-longmachine. Deze partikels zouden direct de hersenen worden ingestuurd bij de techniek van ACP (via de rechter zijde). Daarbij kunnen ze vervolgens vastlopen in de kleine vaten die de rechtscentrale gebieden van de hersenen van bloed voorzien (zie hoofdstuk 3, Fig. 3).

Hiermee concluderen wij dat er zowel na DHCA als na ACP een (te) groot percentage van de baby's nieuwe hersenschade heeft na de operatie; echter bij ACP is er nog een extra risico op centrale infarcten aan de rechter zijde van de hersenen.

In **hoofdstuk 4** wordt een opmerkelijke samenkomstloop van omstandigheden beschreven, waarbij bij een patiënt die in de gerandomiseerde studie van hoofdstuk 3 participeerde, een asymmetrische hersenperfusie (doorbloeding) voorkwam. De patiënt had een Norwood operatie ondergaan waarbij een BT-shunt geplaatst werd van de rechter arteria brachiocephalicus naar de pulmonaalarterieen. Na de operatie werd gezien dat de cerebrale zuurstofsaturatie (gemeten d.m.v. NIRS) rechts wel 20% lager was dan links, gedurende enkele dagen. Op de MRI scan die 1 week na de operatie werd vervaardigd, bleek er geen flow aanwezig in de rechter carotis. Ook werd gezien dat de Cirkel van Willis incompleet was (zie hoofdstuk 4, Fig. 2); wat samen waarschijnlijk maakte dat rechts frontaal een lage saturatie gemeten werd. Wij concluderen dat een BT-shunt risico geeft op onderperfusie van de rechter arteria carotis en dat een bijkomende incomplete Cirkel van Willis een verder gevaar vormt voor de rechter hemisfeer. Voor deze patiënt bleek het uiteindelijk gelukkig geen klinische gevolgen te hebben gehad.

Hoewel MRI scans de gouden standaard zijn om hersenschade vast te leggen, zou het nuttig zijn als in de dagen na de operatie, aan het bed al duidelijk is welke kinderen beschadigd zijn en dus meer zorg behoeven, en welke niet. Daarover gaat **hoofdstuk 5**; waar drie manieren van hersen-monitoring worden besproken: 1) aEEG (amplitude-intregrated electro-encephalography), oftewel de hersenactiviteit, 2) NIRS (near-infrared spectoscopy), wat de zuurstofsaturatie van de hersenen meet, en 3) biomarkers in bloed. De eerste twee apparaten werden bij de baby's in het onderzoek (het cohort van hoofdstuk 3) de dag voor de operatie aangesloten, waarbij de meting doorging tijdens de operatie, tot twee of drie dagen na de operatie (zie hoofdstuk 1, Fig. 6). Tijdens de operatie kon worden gezien dat de activiteit van de hersenen afneemt door de koeling, en deze weer toeneemt bij het opwarmen (zie hoofdstuk 1,

Fig. 7). Daarnaast registreerde het aEEG apparaat epileptische activiteit, welke vaak niet aan het kind te zien was door de sedatie. Ook de zuurstofsaturatie veranderde door de temperatuurverlaging vanwege minder zuurstofverbruik, maar nog meer door bloeddrukwisselingen of veranderingen in het zuurstofgehalte van het bloed.

We hebben geanalyseerd welke van de drie 'bed-side' monitoring technieken het beste waarschuwt voor hersenschade door te kijken naar de relatie met de MRI scan (na de operatie). Hierbij werd duidelijk dat epileptische activiteit op het aEEG de eerste dagen na de operatie, het beste overeen kwam met hersenschade op de scan (zie hoofdstuk 5, Fig. 2 voor een voorbeeld). Ook hebben we de ontwikkeling van de kinderen op tweejarige leeftijd bekeken en bleek deze achter te lopen bij de gevallen die epileptische activiteit hadden vertoond.

De andere twee manieren van hersenmonitoring voorspelden minder goed; hoewel in vele publicaties de NIRS (zuurstofsaturatie) een relatie heeft met hersenschade, was dit in ons cohort niet het geval. De resultaten van de biomarkers zijn complexer: hoewel er geen relatie was met MRI-afwijkingen, voorspelden hogere waarden van s100b en NSE (beiden schademarkers) wel een achterstand in de ontwikkeling.

Deel 2 van het proefschrift beschrijft de gevolgen van een openhartoperatie op andere aspecten, onder andere op de buikorganen en het algehele herstel van de operatie en de inflammatie ('ontstekingsreactie') die hierbij kan plaatsvinden.

Als eerste wordt ingegaan op de inflammatie. Het is bekend dat openhartoperaties, zeker degene die uitgevoerd worden met een hartlong machine, een massale reactie van afweercellen teweeg brengen, die een soort steriele ontsteking in het hele lijf initiëren ('inflammatie'). In **hoofdstuk 6** hebben we deze inflammatie - welke tijdens de operatie gebeurt - vastgelegd. We hebben met name het effect van ischemie (geen doorbloeding) van de hersenen beoordeeld. We vergeleken de inflammatie meteen na DHCA met een situatie waarbij de hersenen continu doorbloed worden (ACP). Dit kan model staan voor een situatie van hersenischemie zoals die bij een beroerte bij volwassenen of kinderen, of een circulatiestilstand bij een reanimatie.

Door de afweercellen (monocyten en neutrofielen) te typeren in het lab en circulerende inflammatiestoffen (cytokines) te meten in het bloed, werd duidelijk dat de hersenischemie zoals die bij DHCA plaatsvindt tijdens de operatie, meteen een duidelijke inflammatie teweeg brengt. Dit werd niet gezien na ACP. Dit betekent dat in het geval van hersenischemie, er meteen (binnen enkele minuten) een reactie van onder meer afweercellen is, wat veel eerder is dan de uren tot dagen die tot nu toe beschreven. Dit kon eerder niet zo spoedig na het ischemisch moment worden gemeten, omdat er nooit zo snel bloed kan worden afgenomen nadat patiënten een beroerte hebben gehad. Hiermee wordt duidelijk dat er misschien mogelijkheden zijn om de afweerreactie bij ischemie a la minute te dempen en hierdoor de uiteindelijke hersenschade te beperken.

Na deze inflammatie *tijdens* de operatie, zijn de afweercellen *na* de operatie juist weer minder reactief, waardoor de patiënten verhoogd gevoelig voor infecties zijn. In

hoofdstuk 7 is gekeken hoe vaak een infectie na de operatie ('postoperatief') voorkomt. In ons centrum bleek, van alle kinderen die met behulp van een hartlongmachine een operatie hadden ondergaan, in 25% een infectie op te treden. Hoewel alle kinderen standaard antibiotica krijgen, is er dus een groep die mogelijk baat heeft bij aanvullende (preventieve) maatregelen. Om die groep te identificeren, hebben we een 'prediction rule' ontworpen. Drie factoren beïnvloeden de kans op een infectie: 1) leeftijd jonger dan 6 maanden; 2) opname op de intensive care langer dan 48 uur na de operatie; en, 3) de noodzaak om de borstkas langer dan 48 uur open te houden (hiermee wordt zwelling van de weefsels na de operatie opgevangen). Hiermee kan een totale score per patient worden berekend, die gepaard gaat met een bepaalde kans op een infectie, en zo kan de meest kwestbare groep worden geidentificeerd.

Hoofdstuk 8 en 9 beschrijven de effecten van de eerder beschreven technieken van DHCA en ACP, op de buikorganen en het algehele herstel van de patiënt na de operatie, op de intensive care. Zoals gezegd, bij gebruik van DHCA tijdens de operatie, is er in die periode een volledige stilstand van de bloedsomloop ('circulatory arrest'), weliswaar bij een lage temperatuur zodat de weefsels hier zo weinig mogelijk schade van hebben. Bij de techniek van ACP is het de bedoeling om (enkel) de hersenen van bloed te voorzien. Echter, er wordt sterk vermoed dat ook de borst- en buikorganen ook nog wel enige mate van bloeddoorstroming ontvangen, waarschijnlijk via allerlei andere ('collaterale') zijvaatjes, die vanuit de hersenen naar de borstkas en buik toe lopen. Chirurgen kunnen dit ook waarnemen doordat er tijdens operaties met ACP, terugstroom van bloed kan worden gezien vanuit de buik.

In hoofdstuk 8 wordt beschreven dat wij tijdens het uitvoeren van DHCA of ACP tijdens aortaboogchirurgie, de zuurstofsaturatie in de nierregio hebben gemeten met NIRS. Hierbij was de gedachte dat enige doorbloeding van de buik bij ACP, een hogere zuurstofsaturatie zal geven dan bij de circulatiestilstand bij DHCA. Conform de verwachtingen, was de zuurstofsaturatie lager in de DHCA groep dan in de ACP groep. Verder hebben we lactaat in het bloed specifiek uit de buik gemeten, wat ook een maat is voor weefseldoorbloeding; alsmede schademarkers in het bloed, specifiek voor lever, darm en nierschade. Deze waren allen hoger bij DHCA dan bij ACP, wat verder ondersteunt dat er minder buikorgaanschade tijdens de operatie is bij ACP.

Natuurlijk moet dit verschil ook tot uiting komen bij de patiënt om klinisch relevant te zijn. Derhalve is in hoofdstuk 9 onderzocht of het gebruik van DHCA of ACP tijdens de operatie, verschil maakte voor het herstel direct na de operatie. Hierbij werd duidelijk dat een langere duur van DHCA , evenredig verband hield met een langere verblijfsduur op de intensive care na de operatie (wat mogelijk komt door cumulatieve schade aan verscheidene organen tijdens de stilstand van circulatie). Dit terwijl de duur van ACP geen enkele invloed had op de verblijfsduur op de intensive care. Wij hypothetiseren dat dit komt omdat de organen geen, of minder schade, oplopen tijdens ACP, omdat de organen nog wel wat doorbloeding ontvangen. Ook hebben we getest of dit verschil tussen DHCA en ACP ook gold voor de duur van de beademing na de operatie, de noodzaak voor stimulerende cardiale medicatie en de

nierfunctie. Dit was inderdaad zo. Hiermee concluderen we dat het gebruik van ACP tijdens de operatie beschermend werkt voor hart, longen en buikorganen, en hiermee leidt tot een vlotter herstel na de operatie.

Dus... Wel of niet doorbloeden van het brein tijdens aortaboogchirurgie; DHCA of ACP?

Zoals te lezen is in hoofdstuk 3, is gebleken dat beide hart-longmachine technieken helaas resulteren in even zoveel gevallen van nieuwe hersenschade. Echter, de ACP techniek lijkt een bijkomend risico te hebben voor herseninfarcten. Aan de andere kant, beschrijven we in hoofdstuk 8 en 9 een positief effect van ACP op andere organen dan de hersenen.

De algehele conclusie luidt daarom dat de huidige ACP techniek zo verbeterd dient te worden, dat er geen risico meer bestaat op herseninfarcten. Daarmee zal hopelijk deze techniek net zo goed blijken te zijn voor de hersenen, als het nu al is voor de rest van het kind.

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ADDENDUM

LIST OF OTHER PUBLICATIONS

Differential homeostatic dynamics of Treg cell subsets following neonatal thymectomy
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Journal of Allergy and Clinical Immunology, in press

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Netherlands Heart Journal, 2011

Minimizing circulatory arrest by using antegrade cerebral perfusion for aortic arch reconstruction in infants causes fewer postoperative adverse events
Selder J, Algra SO, Evens F, Freund M, Strengers J, Schouten ANJ, Haas F.
The Thoracic and Cardiovascular Surgeon, 2010

Montgomery RK, Carlone DL, Richmond CA, Farilla L, Kranendonk ME, Henderson DE, Baffour-Awuah NY, Ambruzs DM, Fogli LK, Algra SO, Breault DT.
Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells.
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Characterization of expression in mice of a transgene containing 3.3 kb of the human lactase-phlorizin hydrolase (LPH) 5' flanking sequence.
Digestive Diseases and Sciences, 2011

Breault DT, Min IM, Carlone DL, Farilla LG, Ambruzs DM, Henderson DE, Algra SO, Montgomery RK, Wagers AJ, Hole N.
Generation of mTert-GFP mice as a model to identify and study tissue progenitor cells.
Proceedings of the National Academy of Sciences, 2008

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Infectiologie Tom Wolfs, Louis Bont

Radiologie Rutger-Jan Nievelstein, Jeroen Hendrikse, Niels Blanken, Anneke Hamersma

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CURRICULUM VITAE

Selma Oeke Algra was born in 1982 in the small village of Boornbergum, situated in the North of Holland. At the age of 6, her parents decided to expand their horizons and moved the family to Hong Kong. This is where Selma and her sister grew up in an international community. At 13, their education was continued in the Netherlands and Selma attained her VWO degree in Doetinchem in 2000. While nearly having started the Hotel School, she chose to become a doctor and studied in the vibrant university town Groningen, while doing her clinical rotations (co-schappen) on the Caribbean island Curacao from 2004 to 2005. A year later, she went to Boston to perform research on mouse intestines at the Grand-laboratory at the Children's Hospital.

After receiving her Medicine degree in 2007, she started the PhD project under supervision of Prof. Felix Haas, Prof. Linda de Vries, Prof. Berent Prakken and Dr. Koos Jansen, of which this thesis is the end result. She gladly travelled to Vancouver and San Francisco to collaborate with the experts in the research niche of neonatal cardiac surgery and cerebral injury (Dr. Steven Miller, Dr. Ken Poskitt and Dr. Patrick McQuillen).

Wishing to practice medicine more from the clinical side, she started as a resident in Pediatrics in 2012, first in TerGooi Ziekenhuis in Blaricum and later at the UMC Utrecht. As medical imaging is the discipline she is most attracted to, she is eager to start training as a Radiologist at the Medisch Centrum Alkmaar in 2014.

Stellingen behorend bij het proefschrift

NEONATAL CARDIAC SURGERY: PREVENTING COLLATERAL DAMAGE

1. Cerebral brain injury is too common both before and after surgery in neonates with aortic arch obstructions and efforts should be made to reduce this burden.
2. Large differences between centres call for a further analysis on the best peri-operative strategy to prevent cerebral injury. But first, there must be consensus on the terminology of lesion types.
3. The use of alternative but validated statistical techniques, such as sequential analysis for randomized controlled trials, should be stimulated.
4. De toenemende chirurgische mogelijkheden zijn voor veel families een zegen, maar we moeten wel zorgen dat er wat van het kind overblijft.
5. Move by yourself (Donovan Frankenreiter)
6. Hoe meer zielen hoe meer vreugd geldt niet altijd voor onderzoeksprojecten.
7. Geen betere deadline dan een vakantie.
8. Less research, more thinking! (Trisha Greenhalgh)
9. Een goede werksfeer komt de patient, en de arts, ten goede.
10. Music keeps the researcher going.
11. A picture tells a thousand words.

Selma Algra, 13-12-13