Evidence-Based Pharmacotherapy in Pediatric Cardiac Surgery

From Bench to Bedside

Gerdien Zeilmaker

Evidence based pharmacotherapy in pediatric cardiac surgery; from bench to bedside

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Evidence-Based Pharmacotherapy in Pediatric Cardiac Surgery; from Bench to Bedside

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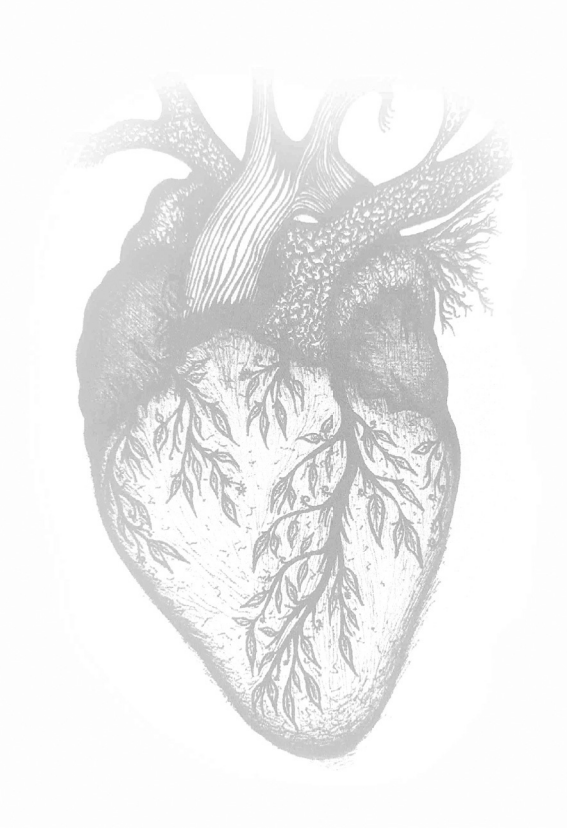

Chapter 1

Introduction

Introduction

Congenital heart defects

Birth defects are fairly common with an overall reported prevalence of 3-5% of all live births (1). Congenital heart defects (CHD) account for approximately one third of all birth defects, with a reported prevalence in Europe of 7.2 per 1000 births (2). Advances in prenatal detection and early treatment of CHD have increased survival of these children. However, surgical intervention in patients with CHD is needed within the first year of life in 58% or in the first three years of life in 67% (3).

Congenital cardiac surgery and influence of cardiopulmonary bypass and cell saver system on routinely used drugs

Cardiopulmonary bypass

Congenital cardiac surgery is often performed with use of the cardiopulmonary bypass (CPB). The CPB is connected to the patient during surgery to replace the function of the heart and lungs during surgery. The venous cannulas of the CPB is generally inserted in either the upper and lower caval vein, of the right atrium. The arterial canula is inserted in the ascending aorta. The blood from the patient is collected via the venous drainage line into the venous reservoir. The blood is transported by a pump through an, oxygenator and filter. The CPB has a continuous flow of blood from the patient, to the CPB and back to the patient. Different sizes of CPB systems are available, depending on the weight of the patient. Figure 1 givens a schematic interpretation of the blood flow in an adult CPB system.

Use of the CPB may have a profound effect on distribution and effects of drugs in the patient, therefore altering the pharmacokinetic (PK) parameters of drugs (4). There are several reasons for altered drug PK when using the CPB. First, there are hemodynamic changes due to change from pulsatile to non-pulsatile flow. This affects organ perfusion, and therefore organ function, with subsequent effects on organ drug metabolism. Second, at onset of the CPB, addition of CPB prime fluid to the patients' blood volume causes hemodilution.

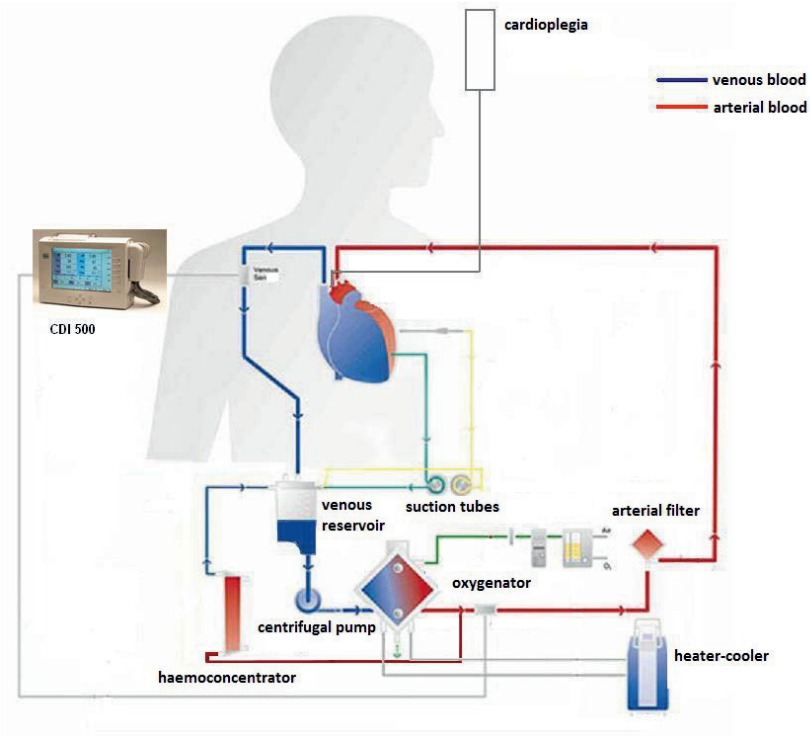

Figure 1: schematic overview of blood flow in a CPB system.

Especially in neonates, the total circulating volume may be doubled at onset of CPB. The impact of this effect is determined by drug properties in terms of protein binding, lipophilicity and volume of distribution. Blood pressure and blood flow rate are decreased during CPB, leading to decreased renal and hepatic perfusion and decreased clearance (Cl) of drugs from the patient. Hypothermia during surgery also decreases drug elimination, due to temperature dependence of metabolic enzyme function (5, 6). The use of CPB also stimulates a systemic inflammatory response by both the complex humoral and cellular reactions, leading to the "systemic inflammatory response syndrome" (SIRS). SIRS can vary from very mild to severe, mainly depending on duration of CPB (7). Finally, sequestration of drugs in the CPB system occurs, with mainly lipophilic drugs adhering to the tubing and oxygenator (8, 9). Also, the type of surgery may influence the PK of drugs, since this correlates with CPB run time, hemodynamic instability and SIRS. All summarized potential alterations in drug PK during cardiac surgery are investigated in adults. Almost no literature is available for routinely used drugs and CPB systems in neonates and children (4). This could lead to suboptimal treatment

of children undergoing cardiac surgery with use of CPB. Influence of CPB on drug PK may be more profound in neonates and children, since they have a lower bodyweight and circulating volume and are thus more vulnerable to the above described changes. To gain more insight on potential drug alterations, we aimed to investigate the influence of CPB on PK of routinely used drugs in neonates and children, aged 0-18, undergoing cardiac surgery. Since alteration on PK parameters are both patient and CPB dependent we also investigated different types of CPB systems *in vitro*. These *in vitro* studies ought to eliminate the variability within the patients and give more specific insight in the CPB systems used at least in our clinic and serve as a model for other institutions.

Cell saver

Allogenic donor blood is used in a majority of pediatric patients undergoing cardiac surgery with use of the CPB. Prime fluid in the CPB consists in part of donor blood to minimize the extent of hemodilution at onset of CPB. However, the use of allogenic donor blood increases the risk of postoperative morbidity, mainly infections (10). In order to minimize the need for allogenic donor blood during the operation an auto-transfusion devise is used, commonly known as a cell saver. The cell saver system collects blood from the operation site in a reservoir, after which it is processed, passed through a lipophilic filter and returned to the patient. Influence of the cell saver on removal of drugs per-operatively or on re-dosing drugs through the autotransfused blood is not known. However, this is of interest since cell saver blood is often returned to the patient on the Pediatric Intensive Care Unit (PICU). A clinically relevant drug dose of strong opioids or sedatives may lead to complications in this different setting. Also, gaining knowledge on influence of volume of distribution (Vd) and Cl of drugs by the cell saver adds to the sparse PK data that is available on routinely used drugs during pediatric cardiac surgery. To investigate the loss of drugs peri-operatively and the potential return of a clinically relevant drug dose postoperatively, we measured several commonly used drugs in various stages of the cell saver procedure.

Antibiotic prophylaxis in adult cardiac surgery

In the Netherlands all patients, adults and children, undergoing cardiac surgery receive antibiotic prophylaxis with cefazolin, a first-generation cephalosporin. Administration of cefazolin should prevent the development of postoperative deep sternal wound infections

(DSWI). DSWI have an incidence of approximately 2-3% and can lead to increased postoperative morbidity, such as prolonged hospital stay, subsequent reoperation and mortality (11). In adults, a national guideline from the Dutch Antibiotic Taskforce (Stichting Werkgroep Antibiotica Beleid, SWAB) is in place, advising on the optimal dosing and timing of cefazolin (12). Evidence for this guideline is limited, however this seems compensated by the liberal dosing regimen ensuring a cefazolin concentration above the Minimal Inhibitory Concentration (MIC). Upon reviewing the guideline in 2018, the recommended cefazolin dose is drastically decreased, for both peri-operative and postoperative administration. This new regimen is backed-up by evidence from non-cardiac surgery (13). Due to differences in PK parameters in patients undergoing cardiac surgery compared to non-cardiac surgery these new dosing recommendations may not obtain cefazolin concentrations above the MIC. We argue that more evidence should be available to ensure adequate cefazolin prophylaxis in adult cardiac surgery to optimally prevent the occurrence of DSWI. To investigate the new Dutch dosing guideline, cefazolin concentration during and after surgery was measured in 40 adult patients.

Pain treatment in children after cardiac surgery

Current guidelines

After the landmark paper by Anand and Aynsley-Green, adequate pain treatment in neonates and infants became an important focus in postoperative care using the concept of pre-emptive analgesia (14). In the average PICU population, postoperative pain has a mixed etiology, with different treatment approaches. Worldwide, opioids are the drug of first choice for pain treatment after cardiac surgery. Since the publication by Lynn et al., determining the adequate opioid dose after cardiac surgery based on respiratory adverse effects, limited research has been done to improve analgesic use in children after cardiac surgery (15). PK parameters of analgesic drugs are often not reported in studies, even though this is expected to be different in these children after cardiac surgery. A recent publication concluded that in children after cardiac surgery the Vd is increased and Cl decreased (16, 17). Perhaps due to this scarcity of data, specific international and national guidelines on optimal pain treatment in children after cardiac surgery are lacking. This is also reflected by the guideline from the Association of Pediatric Anaesthesists of Great Britain and Ireland published in 2012, that

recommends opioids, but does not provide dosing or PK specific information (18). To assess the clinical effect of the pain treatment, pharmacodynamic (PD) endpoints need to be considered. Several validated PD assessment tools are available for use in children, such as the COMFORT-Behavioral (COMFORT-B scale) score (19, 20), the Numeric Rating Scale (NRS), or the Nurses Interpretation of Sedation Scale (NISS). PD scores that take vital parameters, such as heart rate, into account cannot be used in children after cardiac surgery, because of the inevitable need for inotropes directly postoperative. Presumably the lack of uniform guidelines could results in a large variability in analgesic regimen per hospital. We aimed to clarify differences in local practice and preferences on use of analgesics and PD assessment tools by means of an international survey.

Morphine

Morphine is the opioid of first choice for pain treatment in children after cardiac surgery. Morphine elimination is mainly through glucuronidation by urine diphosphate glucuronosyltransferase (UGT) 2B7. Both morphine metabolites, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) are cleared through renal elimination. Decreased renal function could lead to accumulation of these active metabolites, and subsequent clinically important effects. Renal function is often decreased during and after cardiac surgery, and also the use of inotropes implies an impaired cardiovascular function. Also, morphine clearance has been shown to be significantly slower in children who need inotropic support after cardiac surgery (21).

A major concern about the use of morphine in neonates and infants are the potential negative effects of morphine on neurological development (22). In animal models and human fetal cells these negative neurological effects seem to be dose and duration dependent (23). Fortunately, but not necessarily reassuring, in our own institution follow-up studies in children who were born preterm and received morphine after birth, showed no negative long term effects (24, 25). A very small sample of the NEOPAIN study suggested that prematurity, opioid exposure and neonatal pain was significantly associated with brain volume, but not with major impairment in neuropsychological functioning (26). Other adverse drug reactions of morphine include hypotension and respiratory depression, which is a particular problem in hemodynamically unstable children after cardiac surgery (27). Recently several advances have

been made to reduce the morphine consumption in children. Population PK based studies of morphine dosing in neonates, infants and children have resulted in a dose reduction in children after non-cardiac surgery (28, 29). Replacement of morphine by a non-opioid analgesic after cardiac surgery could result in equally optimal analgesic effect and reduce adverse effects.

Paracetamol

The most preferable non-opioid analgesic to replace morphine is paracetamol, since NSAIDs are not used in children after cardiac surgery due to increased risk of bleeding and impaired renal function. Paracetamol is the world most frequent used analgesic, with additional antipyretic and weak anti-inflammatory effects. The working mechanism is not yet fully understood, but it is likely due to inhibition of prostaglandin synthesis in the central nervous system and in peripheral tissue, and interaction with the serotonergic system (30, 31). Paracetamol metabolism and PK has been described for children including newborns (32, 33). Paracetamol is metabolized by glucuronidation (50-60%), sulfation (25-44%), that both form inactive metabolites excreted by the kidney, and oxidation (2-10%). N-acetyl-p-benzo-quinone imine (NAPQI) is one of the end products of the oxidation pathway. NAPQI is toxic and interference in the conjugation of NAPQI could lead to an overdose causing mitochondrial dysfunction and centrilobular necrosis in the liver (34). However, when used in therapeutic doses in children without liver dysfunction, the safety profile of paracetamol is excellent (32).

The study by van der Marel et al. showed that rectal paracetamol does not reduce morphine consumption in young infants after major non-cardiac surgery (35). One of the main problems with rectal administration is the inadequate plasma concentration of paracetamol in 50% of patients. When comparing intravenous (IV) paracetamol to morphine as primary analgesic after major non-cardiac surgery in children aged 0-1 year, IV paracetamol proved to be equipotent to morphine (36). Also in the implementation of this regimen in another 100 patients this was proven, rendering IV paracetamol as the drug of first choice (37). Decreasing the postoperative morphine consumption resulted in less morphine related adverse effect. This may be especially preferable in children after cardiac surgery, who can be hemodynamically unstable and may be more prone to morphine related adverse effects. We investigated the equipotency of IV paracetamol and morphine in children after cardiac

surgery. We also aimed to investigate PK parameters of IV paracetamol and morphine in these children, to improve dosing recommendation. The efforts in conducting this multi-center randomized controlled trial are reported to offer advice to others who would want to engage in such an endeavor.

Aims and outline of this thesis

The aims of this thesis are:

- To study the influence of the CPB and the cell saver system on routinely used drugs in neonates and children undergoing cardiac surgery.
- To study the evidence for dose adjustments in cefazolin prophylaxis in adults undergoing cardiac surgery with the use of CPB.
- To study the use of IV paracetamol as primary analgesic after cardiac surgery in neonates and children aged 0-3 years, aimed to reduce postoperative morphine consumption.

Chapter 2 gives an introduction into treatment regimens and pharmacokinetic considerations for patients receiving analgesia in the PICU. Chapter 3 describes the potential influence of the cell saver system on drug concentration in auto-transfused blood. In vitro CPB systems spiked with routinely used drugs are studied in Chapter 4 and Chapter 5 to further understand the influence of the CPB system on Vd and drug absorption. Chapter 6 describes cefazolin time and concentration above the MIC before and after implementation of a new peri-operative protocol for cefazolin prophylaxis. An international survey on postoperative analgesia is reported in Chapter 7. Chapter 8 describes the study protocol investigating morphine versus IV paracetamol as primary analgesic after cardiac surgery in children aged 0-3 years (PACS study). The challenges concerning the design and implementation of the PACS study are discussed in Chapter 9.

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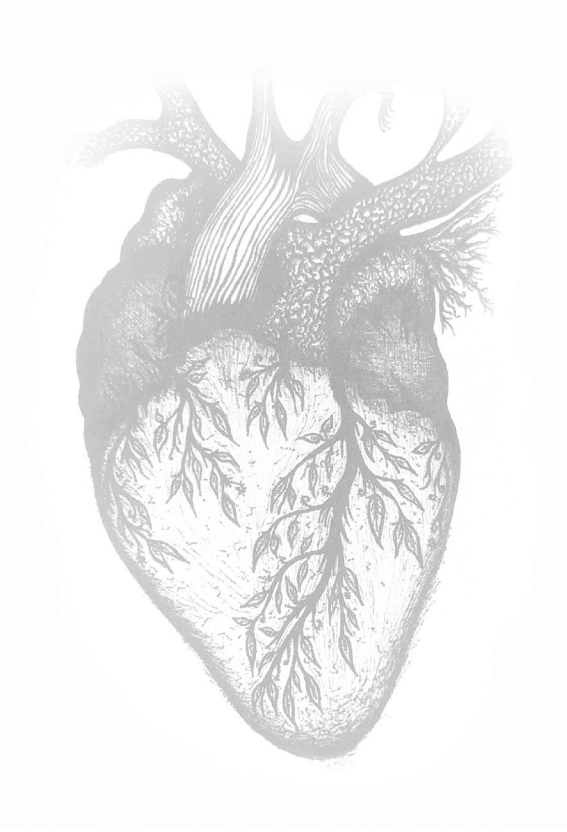

Chapter 2

Pharmacokinetic considerations for pediatric patients receiving analgesia in the intensive care unit; targeting postoperative, ECMO and hypothermia patients

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Expert Opinion on Drug Metabolism & Toxicology, 2018 Apr;14(4):417-428

Abstract

Introduction

Adequate postoperative analgesia in pediatric patients in the intensive care unit (ICU) matters, since untreated pain is associated with negative outcomes. Compared to routine postoperative patients, children undergoing hypothermia (HT) or extracorporeal membrane oxygenation (ECMO), or recovering after cardiac surgery likely display non-maturational differences in pharmacokinetics (PK) and pharmacodynamics (PD). These differences warrant additional dosing recommendations to optimize pain treatment.

Areas covered

Specific populations within the ICU will be discussed with respect to expected variations in PK and PD for various analgesics. We hereby move beyond maturational changes and focus on why PK/PD may be different in children undergoing HT, ECMO or cardiac surgery. We provide a stepwise manner to develop PK-based dosing regimens using population PK approaches in these populations.

Expert opinion

A one-dose to size-fits-all for analgesia is suboptimal, but for several commonly used analgesics the impact of HT, ECMO or cardiac surgery on average PK parameters in children is not yet sufficiently known. Parameters considering both maturational and non-maturational covariates are important to develop population PK-based dosing advices as part of a strategy to optimizing pain treatment.

Introduction

The importance of postoperative pain relief in neonates and infants became apparent after the landmark publication by Anand and Aynsley-Green (1). While untreated pain results in prolonged Pediatric Intensive Care Unit (ICU) stay and increases the children's stress responses (2, 3), overtreatment may result in prolonged artificial ventilation. Repetitive painful stimuli may induce hypersensitivity to pain and negative behavioral consequences in later life; longitudinal data on this issue in humans are limited (4-7).

The World Health Organization (WHO) has published guidelines on the pharmacological treatment of pain in children (2012) (8, 9). These general guidelines are also often applied to children admitted to specialized PICUs. Currently, approximately 50% of the current patient case mix in many PICUs concerns postoperative patients of various surgical specialties, including those admitted for surveillance of vital functions and/or undergoing hypothermia. Postoperative pain is hereby defined as pain within the first 48 hours after surgery. Standard systemic pharmacotherapy for mild postoperative pain consists of acetaminophen (paracetamol) and non-steroidal anti-inflammatory drugs (NSAIDS). For moderate and severe pain, opioids are recommended as part of multimodal analgesia (10-12). Although these WHO guidelines provide a framework with specific emphasis on the two-stage, multimodal approach (non-opioids to opioids) and on dosing suggestions for different analgesics, they still fail to catch the full spectrum of variability and heterogeneity, sticking to the concept of a one-dose to size or age-fits-all for analgesia (8, 9). Particularly, children undergoing hypothermia (HT); extracorporeal membrane oxygenation (ECMO) or after cardiac surgery) may benefit from fine tuning that takes into account the pharmacokinetics (PK), pharmacodynamics (PD), but also pharmacogenetics (PG) and disease characteristics (SIRS,HT, ECMO or cardiac surgery or renal impairment) to reach an optimal dosing regimen for postoperative pain relief. PK parameters such as volume of distribution (Vd) and clearance (CL) are expected to differ in maturational (e.g. size, weight, age) and non-maturational (HT, ECMO, cardiac surgery, disease severity, including renal impairment) variables. Validated PD assessment tools have been developed to assess the clinical effects of pain treatment (13, 14), but incorporating the endpoints in a dosing model remains challenging, while the assessment results may also be affected by the disease characteristics. While knowledge on PG is available for several genetic

polymorphisms involved in drug PK or PD, it is not yet routinely applied in clinical practice (15, 16).

Critically ill pediatric patients tend to display a large variability in PK and PD compared to the average pediatric ICU population or compared to patients after less extensive surgery. To illustrate this, due to mechanisms underlying cold-induced pain, the pain expression of children undergoing HT setting differs from that in children not exposed to HT (different cry, facial response, no shivering, no muscles tremor and decreased muscle contractions) (17, 18). Several studies have recently been undertaken to provide insight in the changes in PK in these specific patients groups (19, 20). Similarly, ECMO or cardiac surgery likely affects the PK (larger Vd, decreased CL) of several analgesics.

In this review, we aim to integrate both maturational (e.g. size, weight, age) and non-maturational characteristics that affect intra- and inter-patient variability in PK/PD, and to identify patient groups that are expected to differ in PK and PD compared to the general pediatric ICU population. We will first describe the general aspects of PK, focusing on drug absorption, distribution, metabolism and excretion (ADME) and PD of analgesics in neonates, infants and children (21). We will then focus on the earlier mentioned non-maturational characteristics and the expected variation in PK and PD. The magnitudes and effects of specific patient and disease characteristics on PK and PD parameters are described. However, a direction or magnitude of effect cannot always be given, since the direction may go both ways depending on covariates. Also the magnitude may vary depending on the patient and clinical circumstances.. Subsequently we explore the consequences of these variations, working towards a PK and PD based dosing recommendation for these specific patients. In the expert opinion section, we focus on tools for future research to close the current knowledge gap, including suggestions to integrate this knowledge in population PK/PD modelling.

Maturational and non-maturational related changes

Pharmacokinetics

In clinical pharmacology, drug-related effects are predicted from compound- and population-specific PK and PD. The general PK principles of drugs hereby apply, irrespective of population-specific characteristics. The absorption, distribution, metabolism and elimination (ADME) of

analgesic drugs in neonates, young infants and children are illustrated in *Table 1*. Absorption is described by the absorption rate constant (K*a*), the time to reach the maximum (peak) plasma concentration (T*max*) and drug bioavailability (F). F is the fraction of an administered dose of unchanged drug that reaches the systemic circulation and is typically 100% upon intravenous administration (22). Absorption can be affected by maturational and non-maturational changes (*Table 1*). Distribution is represented by the Vd of a central compartment and in some cases by the Vd of one or more peripheral compartments that are in equilibrium with the central compartment. For example, a peripheral compartment that is used as PK sample site for the central nervous system is the cerebrospinal fluid (CSF), which is in close equilibrium with the neurons in the brain, where the effect of opioids are expected to occur (23). More lipophilic opioids will diffuse faster across the blood-brain barrier – with faster onset of analgesia (24). Also, distribution can be affected by maturational and non-maturational changes (*Table 1*).

Table 1: General aspects of ADME of analgesic drugs on PK in neonates, young infants and children

PK covariates	Absorption	Distribution	Elimination (Metabolism/Excretion)
Maturation	↑ or ↓	Variable or ↓↑	Variable or ↓↑
Asphyxia	↓or variable	Variable NS or ↓↑	Variable or ↓↑
Sepsis (SIRS)	No data	Variable or ↓↑	Variable or↓↑
Hypothermia	Decreased ↓	Decreased↓	Decreased↓
ECMO	No data	Increased↑	Decreased↓

Abbreviation: NS, not significant. Updated and used with permission from P. Pokorna, 2015 (59).

Metabolic or primary renal elimination is commonly expressed as clearance (CL), while the elimination half-life ($t_{1/2}$) represents the time it takes to reduce by half the drug concentration. By definition, in a one-compartment model, the elimination half-life is influenced by both Vd and CL, since CL = k.Vd with $t_{1/2}$=0.693/k, with k = elimination rate constant) (25).

Simple extrapolation of PK or PD estimates from adults to pediatric patients is obsolete. Both PK and PD processes change with a child's growth and development, but these factors are collinear. Growth relates to the increases in weight, length and size with proportional or disproportional changes in body proportions, body composition and organ weights, and associated changes in activity or function (e.g. barrier functions, renal clearance, or hepatic drug metabolism) as reflected in the maturational changes in the ADME patterns. This is predominantly observed in infancy, and most notably in prematurely born children (26). Thus, within the pediatric-age range the inter-individual variability in PK and PD for almost all drugs is higher than that in adults (27, 28). Age, not weight, is key in the ontogeny of hepatic and intestinal drug transporters, with increases or decreases of specific transporters depending on age (29). These transporters are important to both drug absorption and elimination of drugs. Even when we only focus on PK, variability between and within a specific population occurs up to the level of clinical relevance, as ignoring the variability may result in concentrations below or above the therapeutic range. This variability is explored with the use of covariates, subdivided in maturational (size, weight, age) and non-maturational characteristics (disease and/or treatment dependent), together with pharmacogenetic (PG) characteristics (*Figure 1*). For these ADME related processes, maturational covariates have been reported, as summarized below.

Absorption: Following oral administration, absorption displays extensive maturation because of gastro-enteral maturation (e.g. anatomy, motility, drug metabolism or transporters). Also non-enteral routes (e.g. cutaneous, muscular size, inhalation and circulation) display age-related changes. *Distribution:* Although a 'theoretical volume', distribution volume depends on physical (e.g. extra- and intracellular water, lipophilic or water soluble compound, ionization and protein binding) and physiologic (protein binding, tissue uptake, permeation to deep compartments) processes. Consequently, the distribution volume is also driven by maturational changes and disease characteristics.

Metabolism: The drug metabolizing maturational activity of the different iso-enzymes is enzyme specific and determined by age (postnatal age, postmenstrual age) or size. The expression and activity of cytochrome p450 (CYP) iso-enzymes – proteins that catalyze phase I metabolism of many drugs – change dramatically from fetal life through adolescence. At birth, the overall CYP content and activity is 30-60% of adult activity when expressed per gram

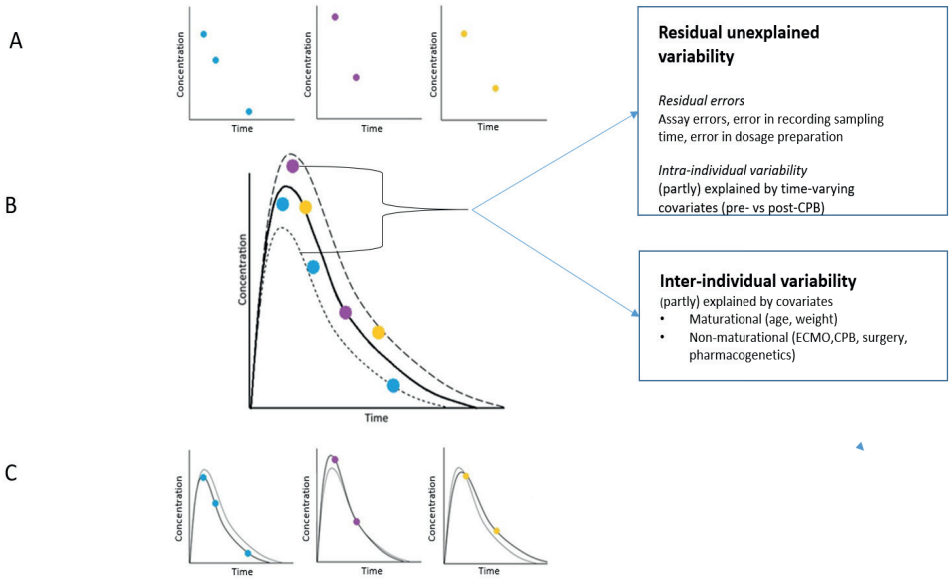
of liver (30). With advancing age, CYP-related metabolism increases, but the different isoenzymes of the P450 family show different developmental patterns (30). In addition to phase I metabolism, phase II metabolic pathways such as glucuronidation and sulfation also display maturational changes.

Excretion: the most relevant route is the renal route, both through glomerular filtration and renal tubular transport. These processes do not mature simultaneously and also relate to both postnatal age and weight. We refer the reader to some recent reviews on the maturational aspects of drug disposition throughout childhood s (26, 31, 32).

Obviously, these maturational changes have an important impact on drug disposition of analgesics, resulting in changes in PK parameters, as illustrated for acetaminophen, but this exercise can also be performed for opioids. Changes in acetaminophen absorption (gastric emptying) and Vd (body composition) have been quantified across neonates and adults (33). In addition, CL increases with age and/or size (27, 28, 34). The complex influence of these maturational covariates has also been illustrated for acetaminophen: Wang *et al*. documented that acetaminophen CL changes nonlinearly with bodyweight (33). Interestingly, the authors provided dosing guidelines for the age range from preterm neonates to adolescents, resulting in similar exposure across these age range (33). These changes in the clearance reflect both maturational changes in hepatic metabolism and subsequent renal elimination (35).

In addition to these maturational changes, non-maturational changes such as disease-dependent (asphyxia, sepsis, renal impairment, systemic inflammatory response syndrome (SIRS)) (36, 37), and multiple organ dysfunction syndrome (MODS) or treatment modalities (HT, ECMO) (38) can further influence PK parameters, leading to additional variability between and within patients. (*Table 1* and *Figure 1*). Acute kidney injury in critically ill neonates receiving ECMO warrants dosage adjustment or even avoidance of nephrotoxic drugs (39). At present, the numbers of evidence based observations and estimates on the impact of these covariates are limited. Moreover, the distinction between maturational and non-maturational covariates cannot always be made, as in the case of genetic polymorphisms or renal impairment.

Figure 1: concentrations-time profiles of individual patients (blue, purple, yellow) (a), concentration-time profile of population; including the variability (residual errors, intra- and inter-individual variability (b), individual profiles related to population concentration-time profile(c).



Genetic polymorphisms cannot simply be considered non-maturation related changes. The impact of polymorphisms in e.g. cytochrome-P-450 (CYP) enzyme activity can change throughout the childhood age range(maturational), as has been documented for CYP2D6 or CYP2C19 (40). Therefore, PG should be considered a potential covariate in individualized pain management in children of different ages. At present, dosing in clinical practice is not based on the information on PG differences amongst children explaining inter-patient variability is not yet taken into account for dosing in clinical practice (15, 41). However, it has been reported that PG can have an impact on outcome – including serious adverse reactions due to drug toxicity – (42, 43).

The frequency of genetic polymorphisms varies between racial and ethnic populations, with differences in allele functionality between European Caucasians, Asians and African-Americans of all ages (16). Polymorphisms in neonates receiving tramadol in a postoperative setting have been extensively described for CYP2D6 and organic cation transporter1 (OCT1). Implementation of a CYP2D6 activity score showed that inter-individual variability in

metabolism of tramadol to the tramadol metabolite CL could partly be explained by CYP2D6 polymorphism (15, 41). Furthermore, polymorphism in OCT1 (reuptake transporter in the hepatocyte) has demonstrated that the ratio metabolite/tramadol is higher with <2 functional copies. Tramadol also illustrates the impact of maturational and non-maturational changes in renal elimination clearance. The plasma tramadol metabolite concentrations will not only depend on their formation, but also on its subsequent renal elimination. This means that metabolite accumulation is more likely to occur in early infancy (renal elimination matures slower than hepatic metabolism) or in the context of renal impairment (41). A similar pattern has been described for morphine during HT, with accumulation of morphine metabolites (44).

Exploring the impact of covariates on the inter- and intra-individual variability in PK is relevant as these covariates can subsequently be used to develop PK-based dosing regimens for the subpopulations or even individual patients.

Pharmacodynamics

The clinical efficacy of any intervention should be established using validated PD endpoints (45). As with PK, there is a large inter and intra-variability in PD, with different PD endpoints being used, while a clear correlation between plasma concentration and for instance pain scores is often lacking (46).

Although integrated PK/PD studies have been published, PK data are usually reported separately or without integration with validated PD endpoints. PKPD modeling incorporating PD endpoints in a model is difficult and currently PKPD modeling studies are scarce for children. Moreover, validated PD assessment tools are highly tailored to specific age groups and circumstances as reflected by a mission statement paper from the European Society for Pediatric and Neonatal Intensive Care (ESPNIC) (45). For instance, the COMFORT-B scale is a validated postoperative pain instrument for children 0-3 years, including pre-verbal neonates and infants. It detects meaningful effects of pain treatment, but cannot be used during muscular relaxation (13, 14). The same holds true for the Face, Legs, Activity, Cry and Consolability (FLACC) scale. Another scale, the CRIES, consists of the following items: Crying; Requires increased oxygen administration; Increased vital signs; Expression; Sleeplessness, and was specifically developed for postoperative infants (47, 48).

Interestingly, the observed variability in PD can also in part be explained by polymorphisms. For polymorphisms are not limited to PK, but can also affect PD related outcome variables. This was illustrated for the link between combined opioid mu-receptor (OPRM1, opioid receptor) and Catechol-0-methyltransferase (COMT, intracellular signaling) polymorphisms and the need for rescue morphine in mechanically ventilated newborns (49, 50). To date the role of PG is still limited, but should at least be considered when an abnormal drug response is observed following surgery.

Considerations to treat postoperative pain in neonates, young infants and children: from one dose to size-fits-all towards patient tailored treatment

Pain treatment is warranted in case of operative procedures, for specific neonatal anomalies, underlying critical disease (51) and specific conditions (52). Optimal postoperative analgesia decreases stress and improves recovery and clinical outcome (53). However, associations between neuro-apoptosis, neurological outcome and exposure to analgesics have been observed in animal models, while the clinical implications for adults and children are still hard to interpret. The American Society of Anesthesiologists' 2012 Practice Guidelines for Acute Pain Management in the Perioperative Setting provided guidance on the prevention and treatment on pain. Designing a care plan tailored to the individual and the surgical procedure involved is recommended – with an emphasis on multimodal regimens and regular pain assessment (54). In children within the ICU setting, this commonly translates to intravenous administration of opioids and non-opioid analgesics.

Acetaminophen, morphine and fentanyl are the most widely used analgesic drugs. Multimodal analgesia in postoperative children (average age 4 year), acetaminophen combined with morphine, resulted in an opioid sparing effect (55). The PK data hereby resulted in the use of loading doses with patient-tailored maintenance dosages for IV morphine or acetaminophen (56), subsequently guided by validated assessment (PD) tools. This concept is strongly supported in the WHO guidelines on treatment of pain although the evidence for the use of multimodal analgesia in children is still limited (WHO guidelines 2012) (9). For other analgesics, the available data are even more limited (57, 58). Tramadol (59), nalbuphine (60)

and ketorolac (61) seem to be promising and safe alternative drugs for postoperative care, although pain relief was considered to be less effective for nalbuphine compared to other opioids. Sufentanil may be another alternative, but this has been hardly studied in children (62, 63) and neonates (64). The variability in PK of analgesics in neonates, young infants and children is further illustrated in *Table 2*. As discussed earlier, special conditions like HT, ECMO or cardiac surgery are likely to further affect PK, and likely also PD.

Table 2: Clinical studies on PK of selected analgesic drugs –opioids and NSAIDs (IV) in neonates, young infants and children

Drug	**Volume of distribution**	**Clearance**
Alfentanil (112)	Neonates: V_{dbeta} 1L/kg Children: 0.163-0.48 L/kg	Neonates: 2.2±2.4 ml/kg/min Infants and children: 5.9-11.1 ml/kg/min
Ketorolac (55)	4-8 years: 0.19 – 0.44 L/kg	2-18 months: S-enantiomer 4.4-5 ml/kg/min R-enantiomer 1-1.04 ml/kg/min
Morphine (19)	Vcentral : 46-81.2 L/70kg Vperipheral: 128 L/70kg	1.62 L/min/70kg
Acetaminophen (27)	0.179 - 17.2929 L (BW 0.5-50 kg)	0.047-13.422 L/h (BW 0.5-50 kg)
Sufentanil (57)	2.9 L/kg	30.5 (8.8) ml/kg/min
Remifentanil (77, 78)	0-2 years: 0.452(0.144) L/kg 2-6 years: 0.240 L/kg 7-12 years: 0.249 L/kg	0-2 years: 90.5 (36.8) ml/kg/min adolescents: 57.2 (21.2) ml/kg/min
Nalbuphine (54)	Vcentral: 210 L/70 kg Vperipheral: 151 L/70 kg	130 L/ h/ 70 kg, inter-compartment clearance 75.6 L/ h/ 70 kg
Tramadol (36, 53)	Neonates: fixed metabolite 224 L/70 kg 0-2 years: 0.2-0.3 L/kg 2-12 years: 2.5-3.0 L/kg	0-2 years: 90 ml/kg/min 2-12 years: 60 ml/kg/min

* Mean (SD) PK parameters reported from different studies in these populations. Abbreviation: BW: body weight, L: liter, h: hour, min: minutes, kg: kilogram

Hypothermia

Evidence-based guidelines on pain management for neonates and children who undergo HT are still lacking. However, analgesics (e.g. opioids, mainly morphine) are commonly combined with sedatives (e.g. midazolam) in term neonates exposed to HT after perinatal asphyxia (65, 66). Evidence based data on PK of drugs in neonates and children who underwent HT have been reviewed by van den Broek et al. 2010 (66) and Pokorna et al. 2015 (65), but only few data are available on PK of analgesics or on PK in cases when perinatal asphyxia indicated HT. PK changes in neonates and children who underwent HT are known in terms of reduction in absorption (due to low splanchnic flow) for non-parenteral administered drugs (for oral or rectal route of administered acetaminophen), changes in distribution (decreased Vd of lipophilic drugs) or in CL. The impact of HT on morphine (PK) in neonates undergoing HT on indication of perinatal asphyxia has been quantified as a 23% decrease in morphine CL (44).

The PHARMACOOL study illustrates how a pragmatic study design with opportunistic sampling can provide more insight in the effect of HT on the PK of frequently used drugs in these neonates. We therefore suggest to focus on short-acting and rapid-onset opioids (e.g. alfentanil, nalbuphine, remifentanil) or intermediate-acting drugs (tramadol) guided by validated PD scores. Once the PK aspects have been elucidated, we can explore PD related aspects since HT also affects pain expression by mechanisms related to cold-induced pain (17, 18) while thermal stimuli are initiated via thermoreceptors and the nociception may be sensitized under HT (17). In general, a surgical procedure will not be performed under hypothermia if the procedure is not considered lifesaving. The patient will be rewarmed first, undergo neurological evaluation, and surgery is considered once the neurological condition has stabilized.

Extracorporeal membrane oxygenation

ECMO provides temporary respiratory and cardiac support to critically ill neonates and children with Multiple Organ Dysfunction (MODS) if conventional treatment has failed. Optimal analgesia is essential during the ECMO run and during cannulation and decannulation (67). Similar to HT, the effects of ECMO superimpose on the underlying disease and thereby further affect the maturational and non-maturational PK changes of analgesics (68, 69). Compound specific drug losses in the ECMO circuit have been quantified in different *in vitro*

studies (68, 70). PK of analgesic drugs is hereby also affected by drug-dependent characteristics (drug lipophilicity, protein binding capacity and drug sequestration). *In vivo*, the final observed phenotypic disposition covers both maturational, disease related and equipment related aspects. For high lipophilic drugs like fentanyl or sufentanil (70), ECMO leads to an increased Vd and decreased CL. ECMO flow was found to have a small effect on M3G metabolite clearance. An increase in ECMO blood flow was associated with decreased clearance, possibly reflecting illness severity. Dopamine dose reflected decreased renal CL (71, 72).

Pain treatment after cardiac surgery

Current guidelines on pain treatment after cardiac surgery in children are not yet based on population tailored PK or PD. The main guidelines on this issue were provided by the association of Paediatric Anaesthetists of Great Britain and Ireland in 2012 but are based on small, non-randomized trials (73). Morphine is recommended as the analgesic of first choice, but very little PK and PD data on this drug are presented in this guideline, despite the fact that PK and PD based dosing guidelines have been established for non-cardiac surgery in children (19). Routinely used analgesics and sedatives in cardiac critical care are discussed in the recent consensus statement by Lucas et al. (74).

Several studies show differences in morphine serum concentration in children after cardiac surgery and children after non-cardiac surgery. Dagan et al. found that the use of inotropic support, e.g. epinephrine, dopamine or dobutamine, at more than 10 mcg/kg/min after surgery significantly decreased morphine CL from 1.5 ± 0.41 to 0.73 ± 0.3 L/kg/h. This is most likely due to changes in cardiovascular status, and a 50% reduction in morphine dosage was recommended for these children (75). Lynn et al. also found decreased morphine CL in children after cardiac surgery as compared to age-matched peers after non-cardiac surgery, but attributed this to slower maturation in the cardiac surgery patients (76). In contrast, Elkomy et al. more recently were unable to find differences of morphine CL in children after cardiac surgery and children after non-cardiac surgery (77). Morphine serum levels seemed to be dependent on age and renal function, with renal function being often impaired after cardiac surgery (78). Alternatively, Valkenburg et al. showed an increased Vd and a decreased morphine CL in children after cardiac surgery compared to non-cardiac surgery (79).

PK parameters in children after cardiac surgery have been reported for remifentanil, clonidine and dexmedetomidine. Two studies on remifentanil have been published in the last decade, both reporting maturational and non-maturational changes in children after cardiac surgery compared to non-cardiac surgery (80, 81). Due to an 2.4 times increase in Vd during CPB and post-CPB compared to pre-CPB values, a supplemental bolus dose of remifentanil of 25 microgram is proposed at the start of CPB to achieve the target concentration of 14 ng/ml in 50% of patients (81). While clonidine was initially used as a sedative, it is currently also used as an analgesic for its favorable hemodynamic profile and mild analgesic effects (82, 83). For intravenous clonidine administration, the Vd (central volume 123%, peripheral volume 126%) but not CL, was increased in neonates and children after cardiac surgery (84). The dosing recommendation for clonidine was only driven by maturational changes in neonates and infants, unrelated to cardiac surgery. , Su et al. recently published a PK-based dosing advice for dexmedetomidine in neonates and children after cardiac surgery (85). Both maturational (weight and age), and non-maturational (presence of intra-cardiac shunt and total bypass time) affected CL. Due to decreased CL during the first two weeks of life, a 30-40% dose reduction is required in neonates to achieve similar steady state plasma concentration when compared to infants. Alternatively, CL is increased by 24% in the presence of a right-to-left intra-cardiac shunt.

The PK in children after cardiac surgery can be expected to differ from that in children after non-cardiac surgery. This can be mainly ascribed to the perioperative use of CPB, which has profound effects on the PK because of 1) hemodynamic changes, 2) hemodilution, 3) HT, 4) SIRS, 5) sequestration of drugs in the CPB system. These effects change constantly during CPB and continue to have effects after weaning from CPB (78, 86).

1) Hemodynamic changes during CPB are due to a decrease in blood pressure and the related changes in blood flow. These changes can arise because of non-pulsatile flow, a decrease in peripheral vascular resistance and the use of inotropes in hemodynamically unstable patients (75, 87).
2) At the onset of CPB, priming fluid dilutes the patient's blood, to sometimes doubling of the circulating volume. Due to low protein concentration in the prime fluid, a shift in bound and unbound drug concentration may occur. Subsequent redistribution of

drugs from the peripheral to the central compartments depends on protein binding, lipophilicity and Vd.

3) HT induces changes in metabolism and organ perfusion. Decreased hepatic flow causes subsequent changes in drug metabolism such as CYP2D6, which lead to impaired CL and drug accumulation with increased risk of toxicity (66). This also potentially changes the child's response to the administered drug, thus the PD.
4) SIRS occurs in one third of patients during the use of CPB. Main risk factors are the duration of CPB and the amount of fresh frozen plasma used. SIRS is associated with organ dysfunction and prolonged stay at the intensive care unit (86, 88). Inflammation and organ dysfunction reduce CL of CYP3A-mediated drugs, such as midazolam (89). Therefore children may be at risk of increased plasma concentrations and associated toxicity.
5) Sequestration of drugs in the CPB system is a problem mainly for lipophilic drugs. The oxygenator has been identified as the main binding site of drugs. Coating of CPB tubing with heparin or biocompatible coating does not prevent absorption of drugs. Depending on the type of drug, up to 90% can be absorbed in the CPB circuit only minutes after administration (90, 91).

Based on what has been discussed until now, it seems essential to explore the PK and PD related differences in these specific subpopulations, and population PK modelling or PK/PD modelling is likely the ideal tool.

Tolerance, drug dependency and withdrawal

All three patient groups described above are at risk for tolerance, drug dependency and withdrawal after prolonged use of analgesics and sedatives. Tolerance is the decreased effect of a drug after prolonged exposure (92). Several subcategories are described, such as innate (genetic) and pharmacokinetic tolerance. However, pharmacodynamic tolerance is the most appropriate definition since it refers to alterations at the distal end of the receptor (93). The primary mechanisms of tolerance are receptor desensitization and upregulation of the cAMP pathway by various causes (92). Different types of opioids have different effects on these mechanisms, leading to a variable potential to cause tolerance. The risk of developing tolerance is higher with prolonged duration of therapy, early developmental stage of the

patient, male gender and the use of synthetic or short-acting opioids (92). Tolerance and withdrawal symptoms can occur after five days of continuous infusion of opioids or benzodiazepines. Validated PD tools to assess withdrawal are the Withdrawal Assessment Tool-1 (WAT-1) and the Sophia Observation withdrawal Symptoms score (SOS) (34, 45).

The clinical management of opioid tolerance and subsequent withdrawal is multimodal. Gradual weaning of opioids and replacing intravenous short-acting opioids with non-IV administered long-acting opioids is the recommended pharmacological therapy. Tolerance and withdrawal may be prevented or delayed by using daily interruption of sedation, nurse-controlled analgesia, or sequential rotation of analgesics (45, 92). Vet et al. showed that daily interruption of sedation in critically ill children decreased withdrawal symptoms, but did not improve clinical outcome (94). The influence of pharmacogenetics on the cellular changes that lead to tolerance is not yet clear (92).

Towards model-based dosing regimens using population pharmacokinetic modelling

Population pharmacokinetic modelling

Population PK modelling paves the way to estimate PK parameters such as CL and Vd (based on concentration-time profiles) and to translate them in model-based dosing regimens, which should take into account information on (before, during and after) ECMO, cooling and CPB. The development of such dosing regimens using PK data is illustrated in figure 2 and consists of a multi-step approach (95):

1) Optimal study design (based on preliminary data)

2) Development and internal validation of PK-model

3) External validation of PK-model

4) Prospective validation in clinical study

5) Proposed individualized dosing regimen

In the first step, previous PK studies must be evaluated on quality and the amount of data, such as clinical characteristics, drug concentrations in plasma, number of patients and time of sampling, retrieved from these studies (96). In addition, attention should also be given to the above-mentioned clinical characteristics when designing a new study. In the second step, a population PK model can be generated by using concentrations collected during studies or clinical practice. The development of a population PK-model is in three different steps: identification of the a) structural model; b) statistical model; c) covariate model (97). In the structural model, a population concentration-time curve is derived from the estimated parameters (*Figure 1b*) by pooling all concentrations obtained from all patients (*Figure 1a to 1b*), whereas the statistical model quantifies all levels of variability (inter-individual variability (between-subjects), intra individual variability (within subject) and residual variability (assay error, error in recording sampling time, error in dosage preparation (*Figure 2*). Thus, the model takes into account the differences among observations (*Figure 1c*). In the covariate model, the use of patient and treatment characteristics is aimed is to (partly) understand and explain the inter-individual and intra-individual variability in the model parameters. These characteristics are often subdivided in maturational (age, weight), demographic (sex, race, genotype) and treatment characteristics (HT, ECMO, CPB, surgery) but can also be time-varying covariates (for example pre- vs post-CPB) (*Figure 1*). Different methods are available to quantify the influence of maturational changes on PK parameters of drugs in children, including allometric scaling using bodyweight-based functions with fixed exponents (98). This can be combined with additional age-based maturation functions (99) or more data-driven approaches in which a systematic covariate analysis drives the definition of the statistically most appropriate relationship to describe maturational changes in observed data (97). For morphine, the maturation of clearance has for instance been described using different parameterizations, based on bodyweight, age or a combination of both (19, 100-102). PK covariates can then be used to determine if and how dosing can be individualized to yield similar drug exposure for different subpopulations or even individual patients. This is irrespective of whether the source of inter-individual differences is maturational or non-maturational and irrespective of the chosen parameterization for the influence of maturational changes on PK parameters, as long as the obtained relationships are properly validated (37, 103). For example, if a covariate, such as age, influences Vd, the loading dose needs to be adjusted based on age. Covariates can thus be used to determine if and how dosing can be individualized (37).

The advantage of the population approach is that an entire concentration-time curve does not need to be obtained for every patient. This enables sparse blood sampling, which is preferred for neonates and children. Another advantage is that samples taken from clinical care or even scavenged sampling can be used, instead of designing complete experimental studies. The last part of the second step is the internal validation, in which the stability of the PK parameter estimates and the final model robustness are assessed to estimate the predictive property in terms of general trends in the population and on variability.

Figure 2: multistep approach for development of dosing regimens using PK/PD data

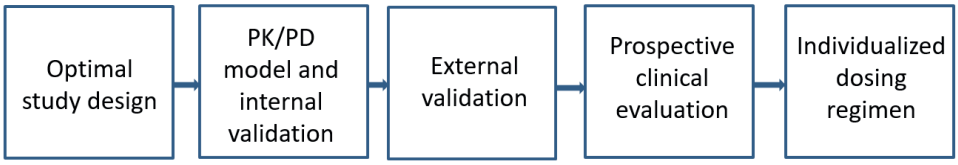


Figure adapted with permission from Ince et al. (95)

The third step is the external validation, which implies investigating the accuracy of the model in new patients from the same population that was used for model building. This step is often not performed, however, due to the lack of an external dataset even though historical or clinical data can be used for this purpose. Prospective validation in a trial is the fourth step. During this step the model is clinically tested to check whether the dosing advice derived from this model will reach the desired target range, and to identify patients in which the dosing regimen does not provide the correct concentrations or effects and must therefore be further elaborated. These might for instance include postoperative cardiac patients who have different PK parameters than postoperative non-cardiac patients (78). In the fifth step, individualized dosing regimens are achieved, and preferably incorporated in guidelines and formularies for use in clinical care.

Looking beyond morphine and acetaminophen for postoperative pain

Of the drugs commonly used to treat postoperative pain, morphine and acetaminophen have been extensively evaluated (34). Concerning morphine for postoperative pain, all five steps leading to PK-based individualized dosing regimens have been completed for neonates and children (range 0-3 years of age) (20). For both morphine and paracetamol, the PK has been identified and dosing guidelines have been proposed for the entire pediatric age range,

although the last step (prospective evaluation) still deserves more attention (33, 104). It should be realized, however, that the morphine and acetaminophen dosages are recommended for major non-cardiac surgery only (19). For other drugs used in the postoperative setting such as remifentanil (postoperative pain after cardiac surgery), diclofenac (105, 106), nalbuphine (107) and ketorolac (108), PK-parameters derived from population models are available for certain routes of administration and in various pediatric age-groups. For drugs such as ibuprofen, oxycodone or fentanyl, models have not yet been developed for neonates or children for postoperative pain treatment.

In all PK models developed for neonates, infants and children, maturational covariates (weight and/or age) are used to (partly) explain inter-patient variability in PK parameters. However, besides these maturational covariates, non-maturational covariates could also partly explain inter-individual as well as intra-individual variability. Both maturational and non-maturational covariates should be taken into account when developing a dosing regimen in the studied population. The pre- or post-CPB period are examples of a non-maturational time-varying covariate, emerging in the population PK model developed for remifentanil in infants and children undergoing cardiac surgery with CPB (81). Vd was increased during and post-CPB compared to the pre-CPB period. The authors concluded that following this increase, an extra bolus of 25ug must be administered at the start of CPB compared to the pre-CPB period to obtain an optimal target concentration of 14ng/ml during CPB and post-CPB (81).

Conclusion

PK studies that report on PK parameters and its covariates for morphine, fentanyl and acetaminophen are available, but data in specific subpopulations (HT, ECMO or cardiac patients) are limited. PK data are also lacking for other commonly used opioids (alfentanil, remifentanil, nalbuphine) and non-opioids (e.g. ketorolac) in critically ill neonates, young infants and children. PK parameters accounting for maturational and non-maturational covariates are important to develop population PK-based dosing advices for the pediatric age group. Besides disease characteristics and treatment modalities, PG should also be considered as a potential relevant covariate, but the impact of this covariate may depend on both maturational and non-maturational covariates. Irrespective of the compound, PD is often not investigated as endpoint in combination with PK parameters. PK models should be combined

with validated PD outcome data to establish optimal evidence based dosing regimens for specific populations and age groups.

Expert opinion

PK-parameters derived from population PK-models provide a first step towards individualized pain treatment in children. Besides maturational changes, non-maturational changes should be considered. In this review, this has been stressed for children undergoing HT, on ECMO, or cardiac surgery, including CPB. PK parameters for these subpopulations are considered to differ from those for other pediatric populations in an ICU setting. While PK-based dosing advices are certainly useful, they will be clinically relevant only if prospectively evaluated in proof of principle studies in which both the PK and PD are considered. In this section we will mainly focus on missing PK/PD data of routinely used analgesics and possibilities to turn this into personalized model-based dosing.

Most population PK-models report on maturational covariates (weight, age), mainly with respect to CL. The impact of non-maturational covariates (ECMO, CPB, disease state, inflammation) strongly depends on the type of patient group but can also vary within one and the same patient with time and disease progression. Given these differences in maturational and non-maturational covariates, attention should not only be paid to age and weight, but also to diagnosis and treatment modalities when developing PK-based dosing recommendations. For example, in critically ill children on ECMO, variation in PK parameters is extensive, with an increase in Vd between 5% and 400% for most drugs, and a decreased CL between 0% and 50% compared with children without ECMO (69). In this respect, physiologically-based (PB)-PK models can be considered to estimate the impact of non-maturational covariates on the disposition of specific compounds. PB-PK models use, besides PK information, also physiological information such as renal blood flow. These PB-PK models could be of interest, especially to explore whether results from one drug can be used to predict the PK for similar drugs; for example for drugs which are metabolized by the same route. An example is midazolam, which is mostly metabolized by CYP3A4. The PK parameters obtained from a PB-PK model of this drug could be evaluated for predictions for other drugs which are also CYP3A4 substrates. Physiological based models may be the future when dealing with large variability in maturational and non-maturational covariates. However, this will necessitate

collecting accurate data on the (patho)physiology in these cases, including but not limited to, trends in caloric needs and metabolism, renal and hepatic blood flow and function or body composition, including fluid shifts. Development of accurate biomarkers to predict disease state, real time organ function, body composition and fluid shifts is essential in furthering our efforts to individualize and optimize drug dosing in these patients. Until such data become available, semi-physiological function approaches can be considered, as have been used for e.g. primary glomerular filtration rate driven aminoglycosides CL throughout pediatric life (109).

Another approach to investigate PK in critically ill children is micro-dosing. Its feasibility in children has been demonstrated by Mooij et al. (110). Using a micro dose of C^{14} labeled acetaminophen, maturational changes -in drug metabolism were explored without additional risk and only limited burden to the critically ill child (111). As the feasibility of micro-dosing has been established, a similar approach can be considered in specific subpopulations like HT, ECMO or after cardiac surgery cases to quantify the impact of non-maturational covariates on drug disposition.

At present, PK parameters are not yet known for all routinely used analgesic drugs in children postoperatively or on ECMO. Regarding opioids, morphine and remifentanil have been well investigated in postoperative children (19, 20). Sufentanil and fentanyl are also often used after surgery, but the only report on sufentanil PK dates from 1987 (112). The reported changes in PK of sufentanil in infants and children undergoing cardiac surgery may no longer be applicable in current clinical practice due to changes in CPB systems and perioperative and postoperative care. No report could be retrieved for fentanyl PK after cardiac surgery in children. Considering the clinical use of both fentanyl and sufentanil in postoperative patients, we would urge to investigate PK parameters with validated PD assessment, applying population PK/PD modelling techniques. Regarding non-opioids, acetaminophen has been investigated in postoperative infants, although the derived models have not yet been implemented in clinical practice (33, 55). Acetaminophen has been less well investigated in children after cardiac surgery. Considering the equipotency of IV acetaminophen to morphine observed in non-cardiac patients (55), with the benefit of less adverse drug reactions, this is certainly worth investigating. We consequently suggest to use the earlier mentioned methods (population PK, including semi-physiologic functions or PB-PK, micro-dosing) when focusing

on these compounds because of the clinical need and the feasibility. In contrast, NSAIDs PK have not been investigated in children on ECMO. NSAIDs carry the risk of prolonged bleeding time and renal impairment, making physicians less inclined to use NSAIDs in these patients.

Once PK-models have been developed, PK/PD can be further investigated. The potential relevance of this PK/PD approach has recently been highlighted for morphine in ventilated preterm neonates undergoing endotracheal suctioning (113). Using the item response theory, an intra-individual relationship between morphine concentrations and pain reduction in preterm neonates was unveiled. While the effect of morphine was small, this report illustrates the strengths of a PK/PD analysis approach. For morphine, we can conclude that PK-models have been developed and validated and that individual dosing regimens have been derived for postoperative pain (19, 20). However, the number of PD-models is still small, so further research should focus on the PD across the pediatric age range; which could probably help explaining the remaining variability (20). One of the difficulties in PD-modelling is the use of multi-item scales (e.g. COMFORT) as an outcome variable. Different analgesics and sedatives are often administered simultaneously or a loading dose is administered, making it hard to link a dosing regimen to a pre-defined clinical endpoint, as other drugs than the one under investigation or the loading dose influence the outcome as reflected by the COMFORT score. Moreover, muscular paralysis, or the intervention itself (HT) may also affect the possibilities to evaluate the PD.

Article highlights box

PK-based dosing regimens are lacking for routinely used analgesics in specific groups of (preterm) neonates, infants or children in specific subgroups, like e.g. HT, ECMO, or cardiac surgery.

PK parameters depend not only on maturational, but also on non-maturational changes. These covariates, combined with intra-patient variability throughout the disease process further add to the variability in PK/PD of analgesics and the choice of the drug throughout childhood.

PD assessment is necessary but difficult given the diversity of the patient population and their disease characteristics. Its variability further adds to the intra- and interpatient variability observed in the PK/PD relationship.

New techniques in modelling, such as (semi-)physiologic models and PK/PD-based models, may better describe the clinical situation and estimate the extent of differences between specific subpopulations.

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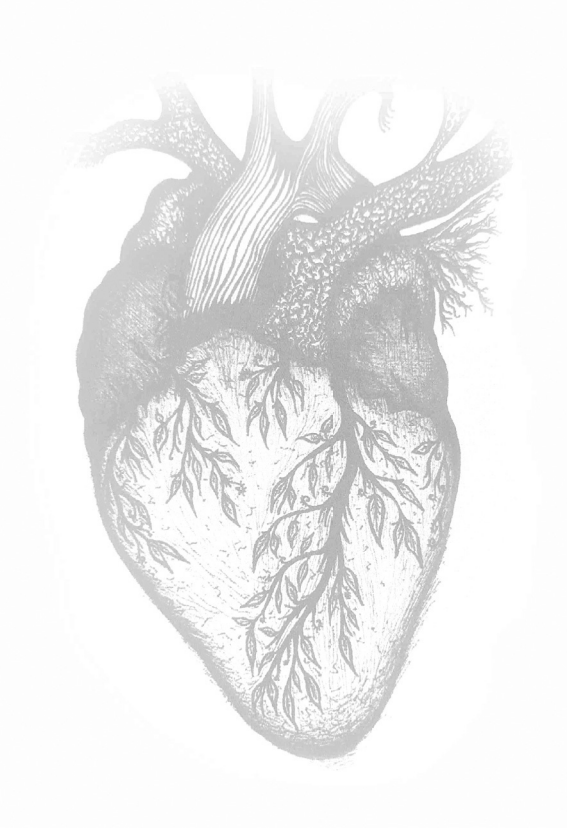

Chapter 3

Potentially clinically relevant concentrations of cefazolin, midazolam, propofol, and sufentanil in auto-transfused blood in congenital cardiac surgery

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Abstract

Background: use of donor blood in congenital cardiac surgery increases the risk for postoperative morbidity and mortality. To reduce the need for allogenic blood transfusion a technique for peri-operative mechanical red cell salvage is applied. Blood from the operation site is collected in a reservoir, processed, passed through a lipophilic filter and returned to the patient. Influence of this cell saver system on coagulation, fibrinolysis and inflammatory markers is known. To our knowledge no studies have been performed on the effects of autotransfusion on drug concentrations. A clinically relevant drug dose could potentially be returned to the patient through the auto-transfused blood, leading to unwanted drug reactions postoperatively. We aimed to measure drug concentrations in blood salvaged from the operation site and in the auto-transfused blood to determine if a clinically relevant drug dose is returned to the patient.

Methods: The study was performed at the Department of Cardiothoracic Surgery of a tertiary university hospital. Blood samples were taken from the reservoir, after processing before the lipophilic filter, the auto-transfused blood, and the waste fluid. Samples were stored at -80 Celsius and drug concentration for sufentanil, propofol, midazolam and cefazolin were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Drug concentrations measured in the reservoir and the auto-transfused blood were compared and the relative reduction was calculated for each patient.

Results: Blood samples were taken from 18 cell saver runs in 18 patients, age 0-13 years. Drug concentrations in the reservoir were comparable to concomitant concentrations in the patient. For sufentanil 34% (median, IQR 27-50) of drug concentration was retained from the reservoir in the auto-transfused blood, for midazolam 6% (median, IQR 4-10), for cefazolin 5% (median, IQR 2-6) and for propofol 0% (median, IQR 0-0) respectively.

Conclusion: Depending on the drug, up to 34% of the drug concentration salvaged from the operation site is returned to the patient through autotransfusion, potentially causing unwanted drug reactions postoperatively. Additionally, influence of a cell saver system should be considered in pharmacological research during and after congenital cardiac surgery and could result in dose adjustments in the postoperative phase.

Background

Allogenic donor blood is used in almost all small patients undergoing congenital cardiac surgery. This is mainly due to hemodilution caused by the use of cardiopulmonary bypass (CPB). The technique of mechanical red cell salvage is applied during congenital cardiac surgery. Equipment designed to undertake this task is routinely referred to as an autotransfusion or cell saver system. Using the cell saver system blood from the operation site is collected in a reservoir, processed, passed through a lipophilic filter after which it is returned to the patient. The use of donor blood during surgery increases postoperative morbidity, mainly infections (1). The cell saver system is used to reduce the need for allogenic blood transfusion and may therefore improve the outcome after surgery. The auto-transfused blood is usually returned to the patient postoperatively on the paediatric intensive care unit (PICU).

Influence of this cell saver system on coagulation, fibrinolysis and inflammatory markers is known (2, 3). In contrast, published literature on the effects of blood loss, volume replacement and use of a cell saver system on plasma drug concentration is limited. Sue et al. investigated the effect of surgical blood loss and fluid replacement on antibiotic pharmacokinetics in adult patients during cardiac surgery and determined the total cefazolin plasma concentration at several time points (4). In their study a cell saver system was used in six of eight patients. Reinfusion of cefazolin-containing blood did not appear to substantially effect postoperative plasma cefazolin concentration in this study. However, cell saver use was not specified per patient and fluid management during surgery will have changed since 1989. The authors state that major pharmacological effects may occur through infusion of auto-transfused blood in drugs that are highly protein bound or hydrophilic. Rohling et al. showed that plasma concentration of muscle relaxants were stable in autologous blood that was predonated after induction of anaesthesia and returned by the end of surgery without processing with a cell saver system. Recurarization occurred in two out of 18 studied patients (5).

To our knowledge, drug concentration in auto-transfused blood and influence of a cell saver system on plasma drug concentrations has not been investigated in infants and children. However, based on published literature, highly protein bound or hydrophilic drugs could persist in the auto-transfused blood. In our clinic, propofol, midazolam, sufentanil and cefazolin are routinely used anaesthetic agents that fit this risk profile. Our hypothesis was

that potentially relevant drug doses of propofol, midazolam, sufentanil and cefazolin could be returned to the patient through the auto-transfused blood. This influence could be important, because it may lead to unwanted drug reactions postoperatively.

We aimed to measure drug concentrations in blood salvaged from the operation site and in the blood processed by a cell saver system during congenital cardiac surgery to determine if a clinically relevant drug dose is returned to the patient.

Methods

The study was performed at the Department of Cardiothoracic Surgery of a tertiary university hospital. Induction and maintenance of anaesthesia was performed by the attending anaesthesiologist as per local protocol. Either inhalation induction was performed with sevoflurane, or intravenous induction with midazolam or propofol, sufentanil and pancuronium. Maintenance of anaesthesia was performed with continuous infusions of sufentanil, midazolam or propofol. Cefazolin was administered before, during and after the surgical procedure. CPB technique was dependent on patient weight and operation procedure, and will be described extensively in the CPB-PHARM study publications. No patient underwent deep hypothermia or circulatory arrest during the procedure. We used an Electa cell saver system (Livanova, München, Germany), with a 55 ml processing bowl. Cell saver processing was done as per local protocol, according to manufacturer's settings. Blood from the operation site was mixed in the suction tubes with heparinized normal saline to prevent clotting in the tubes. Washing of the cell saver blood was done with NaCl 0.9%. After processing, the cell saver blood was filtered with a Pall Lipiguard blood filter (Haemonetics S.A., Signy, Switzerland), resulting in the end product, the auto-transfused blood. All residual blood volume from the CPB system was also directly transferred to and processed by the cell saver system after decannulation. The auto-transfused blood is composed according to the standard of the manufacturer, with 55-60% erythrocytes in NaCl 0.9%.

Blood samples were taken from the reservoir, after processing of salvaged blood before the lipophilic filter, the auto-transfused blood, and from the waste fluid. Samples were collected at the end of surgery. If there was a need to run the cell saver system both during and after surgery, samples were taken after the first run for best comparison. Samples were stored at 4° Celsius until processing. Samples were centrifuged (10 min at 3600 rpm) and the plasma

transferred to polypropylene cryogenic vials with polypropylene screw caps (Sarstedt Aktiengesellschaft & Co, Nümbrecht, Germany). Samples were stored at -80° Celsius until analysis.

Drug concentration were measured at the pharmacological laboratories of the Erasmus MC. A certified research technician from the ISO certified pharmacy laboratory performed the FDA validated drug analyses. In all analyses quality control samples are included, as is obliged in FDA analyses and ISO and GCP certified laboratory. Drug concentrations for propofol (Fresenius Kabi Nederland BV, Zeist, the Netherlands), midazolam and midazolam metabolites (Actavis Group PTC ehf., Hafnarfjördur, Iceland) were measured using LC-MS/MS (Waters Corp., Milford, MA, USA). Drug concentration for sufentanil (Hameln Pharma Plus GmbH, Hameln, Germany) and cefazolin (Kefzol®, Eurocept BV, Ankeveen, the Netherlands) were measured using LC-MS/MS (Thermo Fisher Scientific, Waltham, MA, USA).

Lower and upper limits of quantification (LLOQ and ULOQ, respectively) were: sufentanil, LLOQ 0.25 mcg/L, ULOQ 50 mcg/L; propofol LLOQ 100 mcg /L, ULOQ 25000 mcg /L; midazolam LLOQ 2 mcg/L, ULOQ 2400 mcg/L; OH-midazolam LLOQ 3 mcg/L, ULOQ 2300 mcg/L, midazolam glucuronide LLOQ 10 mcg/L, ULOQ 3000 mcg/L; cefazolin LLOQ 1 mg/L, ULOQ 100 mg/L. Drug concentrations for propofol were not measurable after auto-transfusion and were treated as zero in the analysis.

Samples size calculation

The sample size was set at 18 patients. Due to a lack of published literature on this subject, a formal sample size calculation or power analysis was not considered feasible. The chosen sample size of 18 patients should be sufficient to estimate the median and the variability of the drug concentrations with reasonable precision.

Statistical analysis

The ratio between drug concentrations measured in the reservoir and drug concentrations measured in the auto-transfused blood was calculated for each patient. The distributions of the drug concentrations in the reservoir and in the auto-transfused blood and their ratios were summarized with the median and the interquartile range (IQR) per drug, to give an indication of the percentage of the drug concentration remains in the auto-transfused blood. The

correlation between the absolute drug concentrations measured in the reservoir and concentrations in the auto-transfused blood were calculated using Spearman's rank correlation coefficients. Drug concentrations did not follow a normal distribution and were log(10) transformed in order to calculate the relative reduction. Relative reduction per drug was calculated by subtracting the log(10) transformed concentration in the auto-transfused blood from the log(10) transformed concentration in the reservoir. The relative reduction per drug is plotted against the reservoir concentration to predict the relative reduction per starting concentration in figures 3a-d, together with a linear regression line. The R^2 was used to show the predictive value of the drug concentration in the auto-transfused blood based on the starting concentration in the reservoir, with a R^2 of 0 representing no correlation and a R^2 of 1 representing a perfect correlation. The reported R^2 and the 95% confidence interval (CI) in figures 3a-d are based on the linear regression of the log(10) transformed relative reductions on the log(10) transformed concentrations in the reservoir.

This study was a part of the CPB-PHARM study, investigating pharmacokinetics (PK) and pharmacodynamics (PD) of routinely used drugs in neonates and children during cardiac surgery with the use of cardiopulmonary bypass (approved by IRB Erasmus MC, protocol number 2011-400, Dutch Trial Registry NTR3579). Informed consent was obtained for all study participants according to Dutch law.

Results

Blood samples were taken from 18 cell saver runs in 18 paediatric patients. CPB was used in all surgical procedures. Patient characteristics are shown in Table 1. As shown in Table 1, the cell saver volume processed postoperatively is larger than the total blood loss during surgery because of addition of the residual volume of the CPB. The amount of washing fluid used is mainly dependent on the processed cell saver volume. Not all patients received all tested drugs (see Table 2).

Patient drug concentrations for sufentanil, cefazolin, midazolam and propofol were measured for the CPB-PHARM study (MEC2011-400). This study investigates the influence of the cardiopulmonary bypass on the PK and PD of routinely used drugs during and after congenital cardiac surgery. Patient drug samples taken from the arterial catheter during surgery were compared to drug concentrations measured in the reservoir. Drug concentrations measured

in the reservoir of the cell saver system before processing were comparable to concomitant concentrations in the patient, as was expected, and are thus clinically relevant doses. Drug levels for the different drugs and compartments are shown in Table 2. For all drugs, the decrease in drug concentration was largest after washing of the cell saver blood. The effect of the lipophilic filter further decreased drug concentrations in all drugs except sufentanil. Considerable concentrations of all drugs were measured in the waste fluid.

Table 1: Patient characteristics

Characteristic	**N (mean, minimum – maximum)**
Age	2 y 6 months (2 months -13 y 11 months)
Female /male	10 / 8
Surgical procedure Correction ASD type 2 TCPC Correction TOF Correction CAVSD Miscellaneous	 6 3 3 2 4
Total peroperative blood loss (ml/kg)	12.6 (1.6 – 62.7)
Cell saver volume processed (ml)	290 (135-910)
Cell saver product after processing (ml/kg)	12 (3.9 – 26)
Washing fluid used (ml)	783 (300-1200)

Kg: kilogram, ml: millilitre, y: years. Surgical abbreviations: ASD: Atrial Septal Defect, TOF: Tetralogy of Fallot, TCPC: Total Cavopulmonary Connection, CAVSD: Complete Atrioventricular Septal Defect. Miscellaneous: correction Partial Abnormal Pulmonary Venous Return (1), Chauvaud procedure (1), Mitral Valve Replacement (1), Correction Subaortic Stenosis (1).

Median sufentanil concentration in the reservoir was 0.27 mcg/L (IQR 0.18-0.35). Median sufentanil concentration in the auto-transfused blood was 0.10 mcg/L (IQR 0.09-0.10). Therefore 34% (median, IQR 27-50) of drug concentration was retained from the reservoir in

the auto-transfused blood. Detailed concentrations per sample site and percentage recovery are shown in Table 2 and Figure 1.

Median midazolam concentrations in the reservoir and the auto-transfused blood were 192.69 mcg/L (IQR 49.06-316.42) and 11.87 mcg/L (IQR 7.16-20.16) (6%, median IQR 4-10) respectively. Details of midazolam recovery are shown in Table 2. Biologically active midazolam metabolites, 1-hydroxy-midazolam and midazolam glucuronide, were also measured. Recovery of 1-hydroxy-midazolam and midazolam glucuronide was 11% (median, IQR 7-29) and 6 % (median, IQR 4-8) respectively. Details of 1-hydroxymidazolam and midazolam glucuronide recovery are shown in Table 2 and Figure 1.

Median cefazolin concentration in the reservoir and the auto-transfused blood was 228.44 mg/L (IQR 118.47-295.85) and 8.7 mg/L (IQR 5.13-11.44) (5%, median, IQR 2-6) respectively. Detailed concentrations per sample site and percentage recovery are shown in Table 2 and Figure 1.

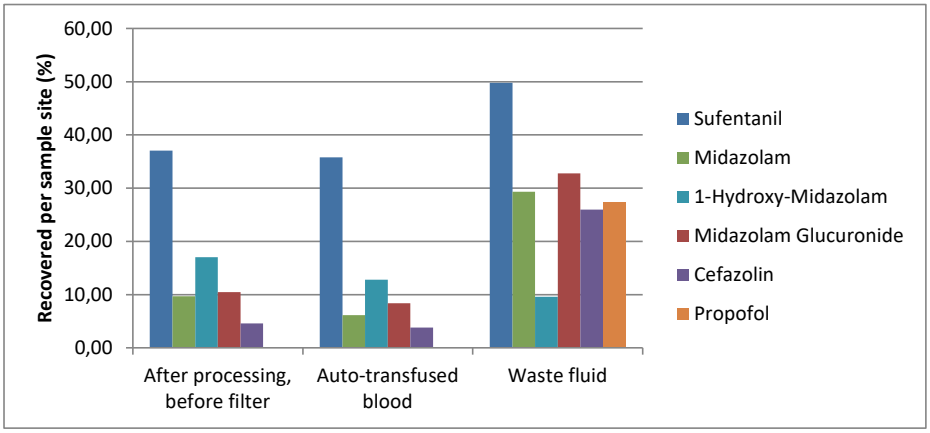
Median propofol concentration in the reservoir and in the waste fluid was 0.65 mg/L (IQR 0.21-1.86) and 0.18 mg/L (IQR 0.11-0.25) respectively. Propofol concentration could not be measured after processing and in the auto-transfused blood. Detailed concentrations per sample site and percentage recovery are shown in Table 2 and Figure 1.

Table 2: Drug concentration per sample site.

Drug	**Patients (n)**	**Concentration (median, IQR)**				**Ratio reservoir vs autotransfused blood in % (median, IQR)**
		Reservoir	*After processing, before filter*	*Auto-transfused blood*	*Waste fluid*	
Sufentanil (mcg/L)	18	0.27 (0.18-0.35)	0.10 (0.09-0.12)	0.10 (0.09-0.10)	0.13 (0.10-0.16)	34 (27-50)
Midazolam (mcg/L)	18	192.69 (49.06-316.42)	18.68 (4.91-29.88)	11.87(7.16-20.16)	56.48 (17.25-75.18)	6 (4-10)
1-hydroxy-midazolam (mcg/L)	18	36.56 (9.45-62.74)	6.23 (4.39-11.52)	4.67 (3.82-5.95)	3.51 (2.72-3.84)	11 (7-29)
Midazolam glucuronide (mcg/L)	18	348.21 (236.63-592.21)	36.41 (15.83-57.13)	29.22 (21.40-41.82)	114.07 (75.33-189.71)	6 (4-8)
Cefazolin (mg/L)	18	228.44 (118.47-295.85)	10.50 (6.63-17.47)	8.70 (5.13-11.44)	59.34 (40.68-77.80)	5 (2-6)
Propofol (mg/L)	6	0.65 (0.21-1.86)	0 (0-0)*	0 (0-0)*	0.18 (0.25-0.11)	0 (0-0)

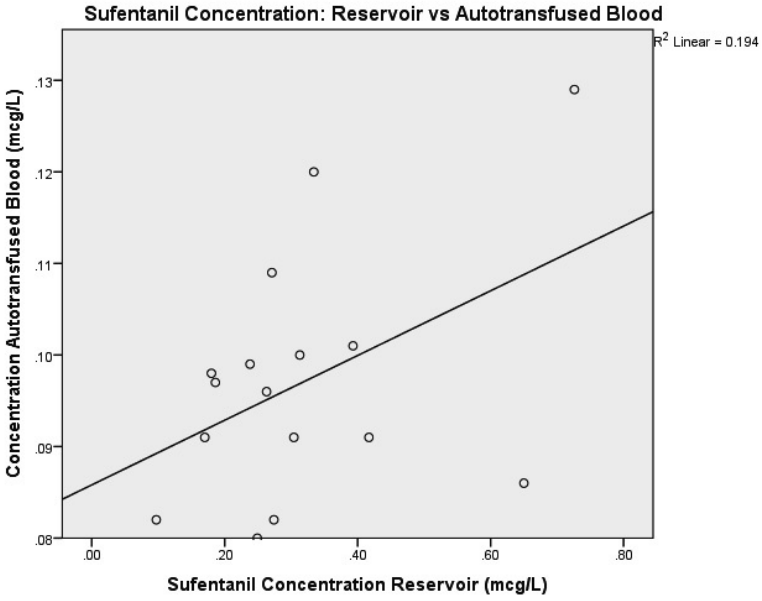
IQR: interquartile range, L: liter, Mcg: microgram, mg: milligram, n.a.: not applicable *Drug concentrations for propofol were not measurable after autotransfusion and were treated as zero in the analysis.

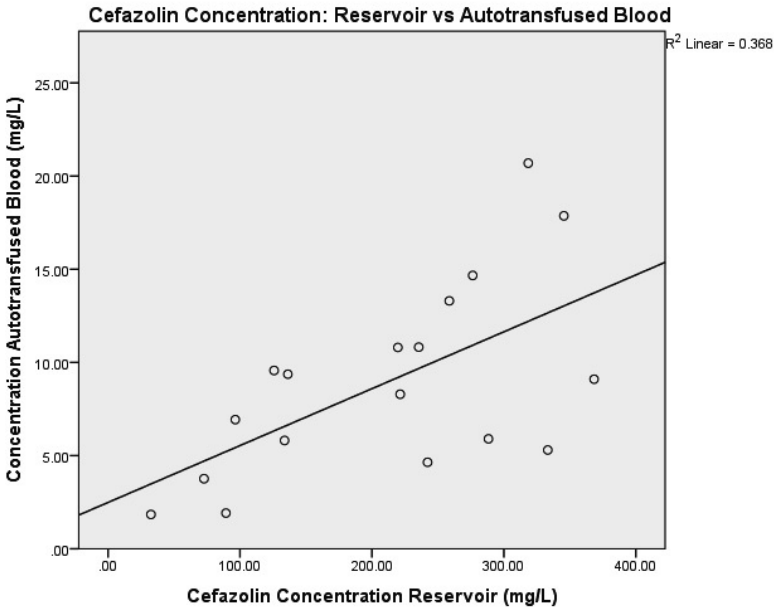
Figure 1: drug recovery per sample site (%). Drug concentration in the reservoir is set as 100%.

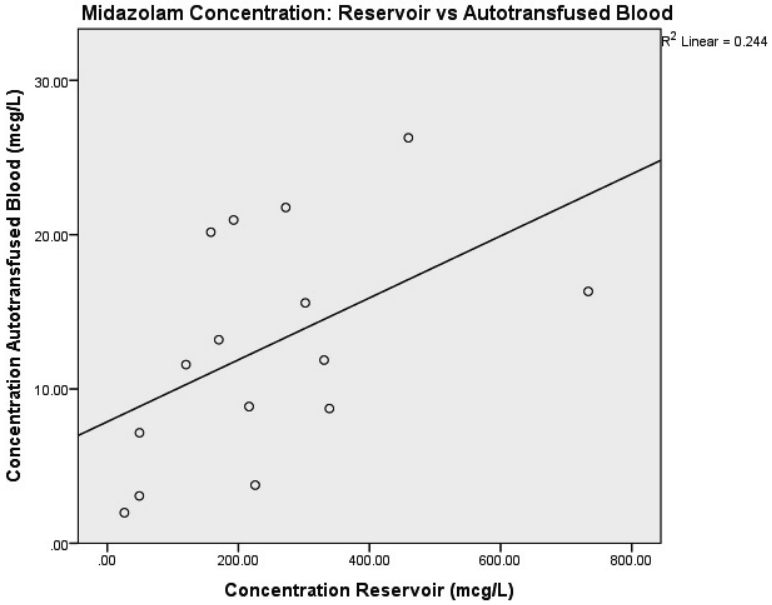


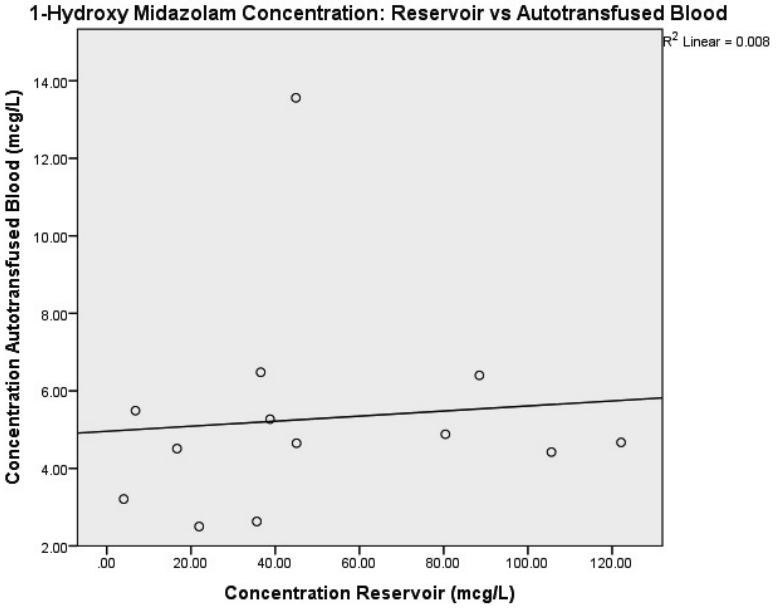
The correlations between drug concentrations measured in the reservoir and concentrations in the auto-transfused blood are shown in figures 2a-e. The relative reduction per drug is plotted against the reservoir concentration to predict the relative reduction per starting concentration in figures 3a-e.

Figure 2a-e: correlation of drug concentration in the reservoir and the auto-transfused blood. (a Sufentanil, b) cefazolin, c) midazolam, d) 1-hydroxy midazolam, e) midazolam glucuronide.




Cefazolin Concentration: Reservoir vs Autotransfused Blood
R2 Linear = 0.368
Concentration Autotransfused Blood (mg/L)
25.00
20.00
15.00
10.00
5.00
.00
.00
100.00
200.00
300.00
400.00
Cefazolin Concentration Reservoir (mg/L)


Midazolam Concentration: Reservoir vs Autotransfused Blood
R2 Linear = 0.244
Concentration Autotransfused Blood (mcg/L)
30.00
20.00
10.00
.00
.00
200.00
400.00
600.00
800.00
Concentration Reservoir (mcg/L)


1-Hydroxy Midazolam Concentration: Reservoir vs Autotransfused Blood
R2 Linear = 0.008
Concentration Autotransfused Blood (mcg/L)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
.00
20.00
40.00
60.00
80.00
100.00
120.00
Concentration Reservoir (mcg/L)

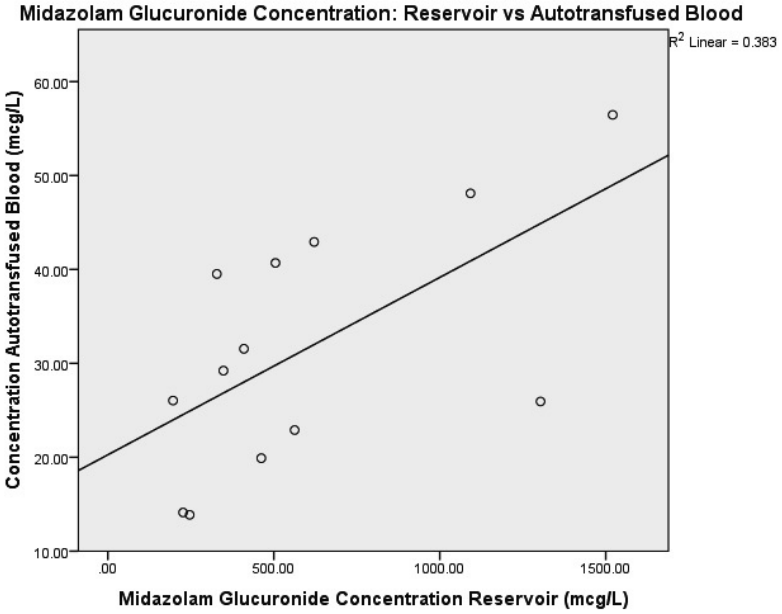
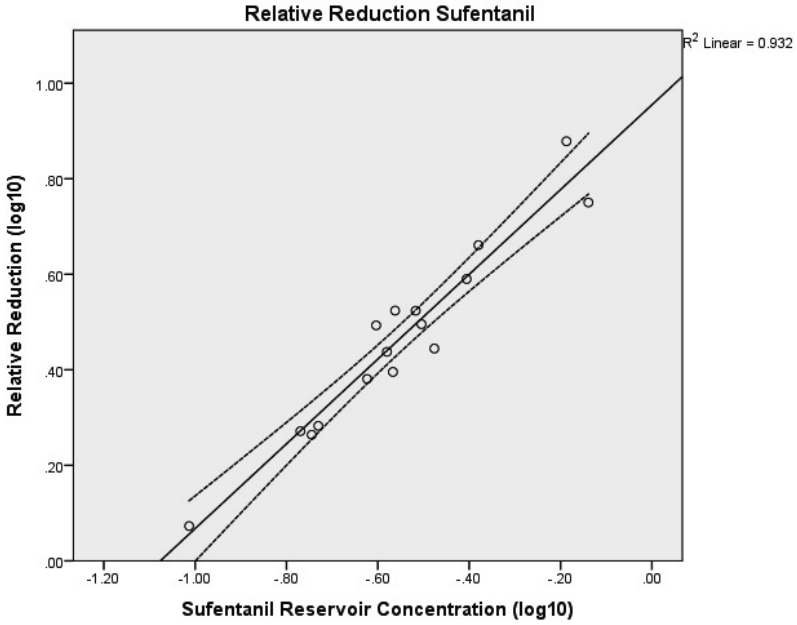
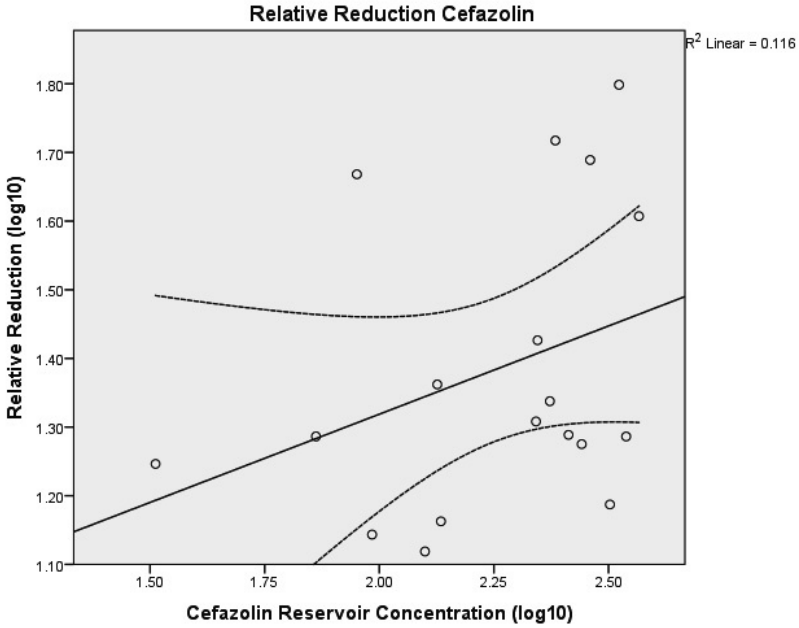
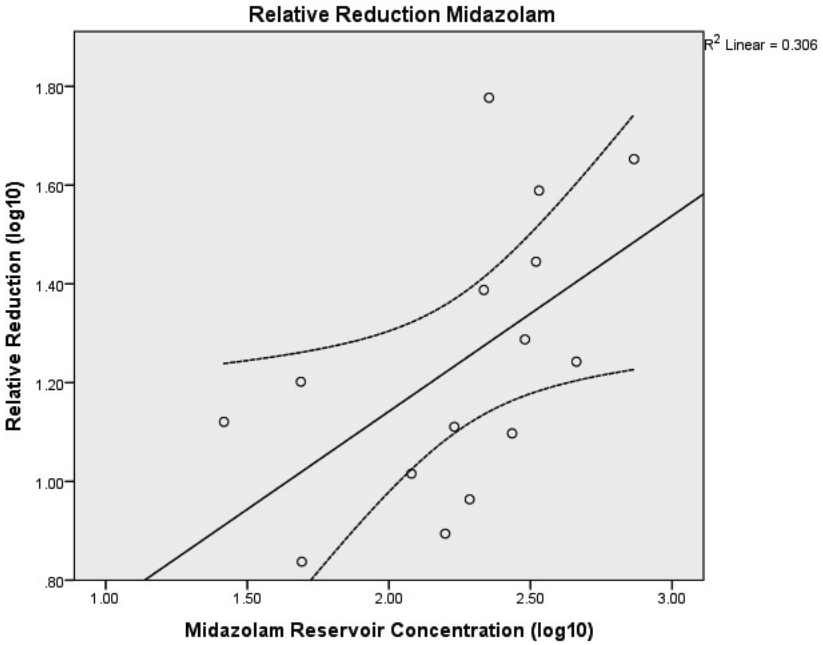
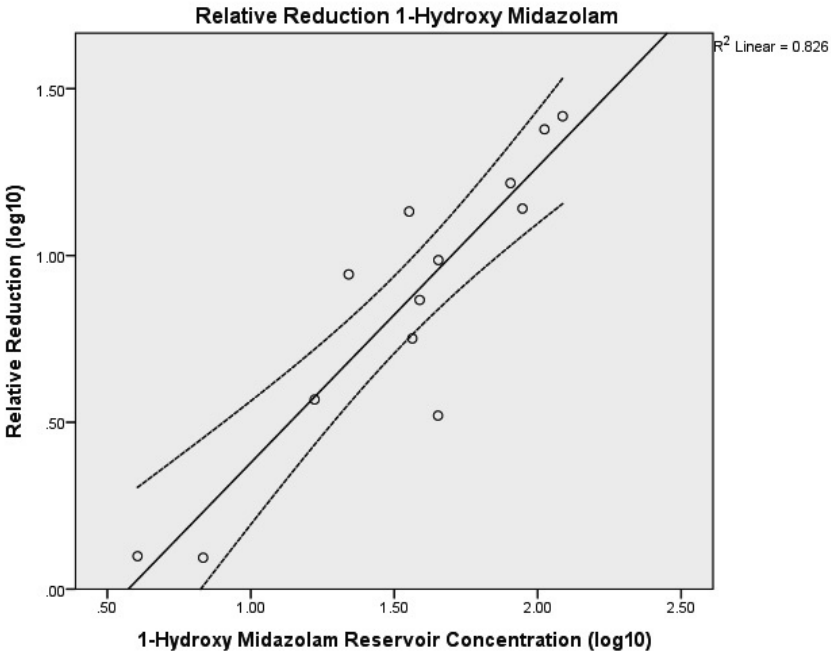

Midazolam Glucuronide Concentration: Reservoir vs Autotransfused Blood
R2 Linear = 0.383
Concentration Autotransfused Blood (mcg/L)
60.00
50.00
40.00
30.00
20.00
10.00
.00
500.00
1000.00
1500.00
Midazolam Glucuronide Concentration Reservoir (mcg/L)

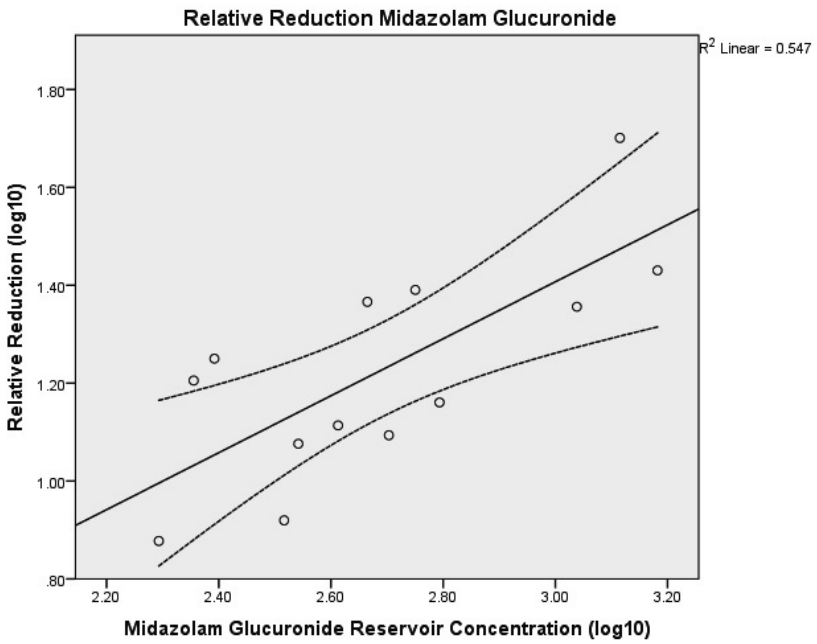
Figure 3a-e: relative reduction predicted by concentration in reservoir (with dotted line 95% CI). a) sufentanil, b) cefazolin, c) midazolam, d) 1-hydroxy midazolam, e) midazolam glucuronide.






Relative Reduction Midazolam
R2 Linear = 0.306
Relative Reduction (log10)
1.80
1.60
1.40
1.20
1.00
.80
1.00
1.50
2.00
2.50
3.00
Midazolam Reservoir Concentration (log10)


Relative Reduction 1-Hydroxy Midazolam
R2 Linear = 0.826
Relative Reduction (log10)
1.50
1.00
.50
.00
.50
1.00
1.50
2.00
2.50
1-Hydroxy Midazolam Reservoir Concentration (log10)



Discussion

To our knowledge this is the first study to measure drug concentrations in auto-transfused blood after paediatric cardiac surgery. Drug recovery varies between drugs in the auto-transfused blood and not all drugs are found in clinically relevant concentrations. For sufentanil 34% (IQR 27-50) of drug concentration was retained from the reservoir in the auto-transfused blood, for midazolam 6% (IQR 4-10), for cefazolin 5% (IQR 2-6) and for propofol 0% (IQR 0-0), respectively. For midazolam mainly the 1-hydroxy-midazolam metabolite was recovered in the auto-transfused blood. 1-hydroxy-midazolam is at least as active as midazolam and may contribute to the overall activity of midazolam (6). The potential influence of the return of auto-transfused blood processed in a cell saver system on plasma drug concentration in infants and children has not previously been investigated.

Based on the literature by Sue et al (4) and our own experience with extra corporeal membrane oxygenation (ECMO) circuit characteristics (7), the expectation was that lipophilic drugs would be sequestered in the synthetic components of the cell saver system and the lipophilic filter and thus be prevented from returning in the auto-transfused blood whereas more hydrophilic drugs could be retained in the auto-transfused blood.

Any lipophilic compounds that are left in the auto-transfused blood after washing should be removed by the lipophilic filter. According to the manufacturer proteins and lipids should be completely washed from the end product so that it contains only erythrocytes and NaCl 0.9%. We have shown that washing of the cell saver blood is the most effective step in clearing drugs from the auto-transfused blood. However both protein bound as well as lipophilic drugs were recovered in the end product. Interestingly sufentanil concentrations were markedly higher compared to the other lipophilic drugs. Drug characteristics of sufentanil, propofol and midazolam regarding protein binding and lipophilicity are fairly similar. With a logP of 3.95, sufentanil is highly lipophilic, with 93% protein binding, mainly to albumin. Propofol and midazolam are also both highly lipophilic with logP's of 3.79 and 3.89 respectively, with a slightly higher protein binding than sufentanil, of 95-99% and 97% respectively. Therefore lipophilicity or protein binding do not seem to predict drug concentrations in the auto-transfused blood.

Redistribution of lipophilic drugs from erythrocytes into the auto-transfused blood could explain why lipophilic drugs are recovered. Redistribution may occur because of a shift of drugs from the erythrocyte to the NaCl 0.9% solution, or because of haemolysis of the erythrocyte. Also, measuring propofol by the precipitation method instead of LC-MS/MS may have resulted in measurable propofol concentrations after processing. Overall, the recovered absolute drug concentrations of the tested drugs where low. Therefore the absolute differences in plasma drug concentration in the patient and drug concentration in the auto-transfused blood may not be substantial.

The hydrophilic drug, cefazolin, was almost entirety washed from the auto-transfused blood. With a logP of -0.58 cefazolin was the most water-soluble drug we have measured. Our results are probably explained by the washing of the cell saver blood with NaCl 0.9%. It is likely that cefazolin dissolved in the NaCl 0.9% solution and was washed from the auto-transfused blood, even though cefazolin concentrations in the waste fluid were low.

Unwanted drug reactions due to auto-transfused blood may not be clinically relevant in all patients. Most at risk for clinical effects are small patients who have had major cardiac surgery with the use of CPB, where the volume of returned cell saver blood is relatively large compared to the patient's own circulating volume. Also, due to long CPB time, organ perfusion will be

decreased, resulting in a lower clearance of drugs from the body (8). Returning a large volume of auto-transfused blood to a small patient with a decreased drug clearance could lead to an accumulation of drugs, resulting in adverse effects and toxicity especially for sufentanil. Also, the cell saver blood is generally administered as a bolus, rather than a continuous infusion. This may be particularly problematic in patients who are not on mechanical ventilation when the auto-transfused blood is administered. However, the concentration of sufentanil in the auto-transfused blood is 0.1 mcg/L. In a worst case scenario, when 1 litre of auto-transfused blood is returned to a patient with a bodyweight of 3 kg, the sufentanil dose administered with the auto-transfused blood would be 0.033 mcg/kg. The target sufentanil dose at induction of anaesthesia is 0.6 mcg/kg (9). Therefore, if the auto-transfused blood is returned to the patient during or very shortly after surgery, the sufentanil concentration in the patient is higher than the sufentanil concentration in the auto-transfused blood and dilution of plasma sufentanil concentration will occur. If the auto-transfused blood is given in a bolus sometime after sufentanil is stopped, a peak in plasma concentration may still occur.

Influence of the auto-transfused blood on adverse drug reaction is therefore dependent on weight of the child, plasma drug concentration at the time of cell saver processing, and amount, speed and timing of administration of the auto-transfused blood.

The influence of cell saver systems should be accounted for when performing pharmacological trials after cardiac surgery. Optimizing drug dosing in neonates, infants and children during and after cardiac surgery is important to improve clinical care, especially in an era where fast track recovery is becoming more important. Ideally, potential effects of the CPB- and cell saver systems on drug concentrations should be incorporated in dosing advices (8). The current study provides insight into the potential return of drugs through auto-transfused blood. Population pharmacokinetics can be used to determine subsequent dose adjustments of the investigated drugs (8, 10).

Future research should focus mainly on lipophilic anaesthetic drugs that could cause a potential adverse reaction when given to patients postoperatively through the auto-transfused blood. Also, future endeavours should aim to incorporate the results of the CPB-PHARM trial and the results of this trial into a new dosing regimen for routinely used drugs for

neonates, infants and children during and after cardiac surgery. This new dosing regimen will take into account the influence of the CPB and the cell saver system.

Conclusion

Depending on the drug, up to 34% of the drug concentration salvaged from the operation site is returned to the patient through autotransfusion, potentially causing unwanted drug reactions postoperatively. Additionally, influence of a cell saver system should be considered in pharmacological research during and after congenital cardiac surgery.

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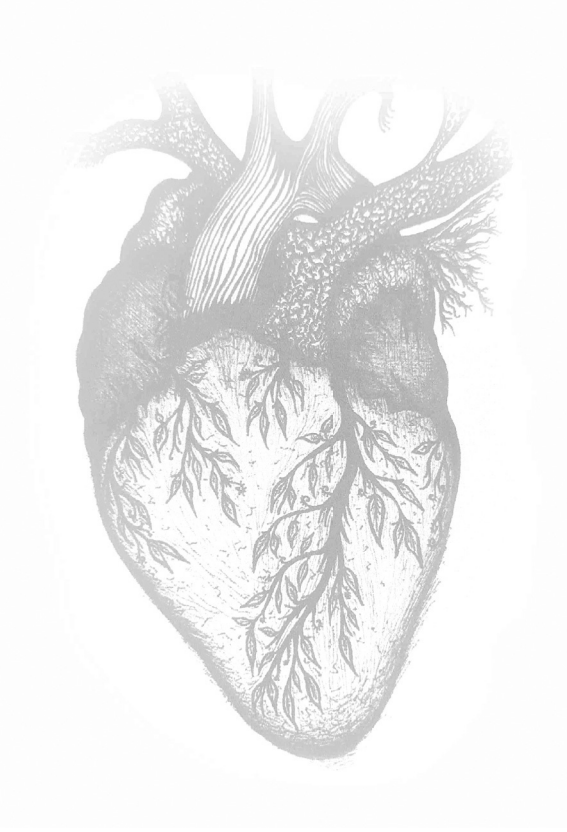

Chapter 4

Recovery of cefazolin and clindamycin in *in vitro* pediatric CPB systems

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Abstract

Objective: cardiopulmonary bypass (CPB) is often necessary in congenital cardiac surgery, but CPB can alter drug pharmacokinetic parameters resulting in underdosing. Inadequate plasma levels of antibiotics could lead to postoperative infections with increased morbidity. The influence of pediatric CPB systems on cefazolin and clindamycin plasma levels is not known. We have measured plasma levels of cefazolin and clindamycin in *in vitro* pediatric CPB systems.

Design: we have tested three types of CPB systems. All systems were primed and spiked with clindamycin and cefazolin. Samples were taken at different time points to measure recovery of cefazolin and clindamycin. Linear mixed model analyses were performed to assess if drug recovery was different between type of CPB system and sampling time point.

Setting: the experiments were conducted at a tertiary university hospital.

Measurements and results: 81 samples were analyzed. There was a significant difference in the recovery over time between CPB systems for cefazolin and clindamycin ($p < 0.001$). Cefazolin recovery after 180 minutes was 106% (95%CI; 91-123) for neonatal, 99% (95%CI; 85-115) for infant, and 77% (95%CI; 67-89) for pediatric systems. Clindamycin recovery after 180 minutes was 143% (95%CI; 116-177) for neonatal, 111% (95%CI; 89-137) for infant, and 120% (95%CI; 97-149) for pediatric systems. Clindamycin recovery after 180 minutes compared to the theoretical concentration was 0.4% for neonatal, 1.2% for infants, and 0.6% for pediatric systems.

Conclusion: recovery of cefazolin was high in the neonatal and infant CPB systems and moderate in the pediatric system. We found a large discrepancy between the theoretical and measured concentrations of clindamycin in all tested CPB systems.

Introduction

During pediatric cardiac surgery the use of the cardiopulmonary bypass (CPB) is often necessary. However, CPB has several effects that can alter drug pharmacokinetic parameters leading to potentially increased or decreased plasma drug concentration in the patient. This may be a problem when dosing antibiotics to prevent surgical site infections (SSI), since inadequate antibiotic plasma concentration may lead to an increased risk to develop SSI and subsequent morbidity and mortality (1).

The most profound changes CPB systems induce on drug concentrations are due to hemodilution, causing changes in drug distribution and clearance, changed hemodynamic status and protein binding, hypothermia and occasionally hemofiltration. The influence of these changes on plasma drug concentration is known for some routinely used drugs, such as midazolam and propofol (2).

The CPB system itself also has an effect on drugs administered during surgery. Mainly lipophilic drugs are known to sequester in the CPB system (3, 4). Factors that can contribute are type and coating of tubing (5, 6) and type of priming fluid (2). Studies on effects of CPB on drugs plasma levels have not been performed recently and may no longer be comparable to current practice, since CPB systems and peri-operative management may have changed. Data on pediatric CPB systems is lacking, since published literature on *in vitro* assessment of the CPB systems investigates only adult CPB systems. The size of the system, added volume and composition of priming fluid are different in adult CPB systems compared to pediatric CPB systems and data from adult CPB systems cannot simply be extrapolated to pediatric systems.

We have designed the CPB-PHARM study to investigate the influence of CPB on routinely used drugs during and after pediatric cardiac surgery (MEC2011-400). We have added an *in vitro* sub-study to investigate the effects of the CPB without the influence of surgery or patients. This *in vitro* study describes the influence of different pediatric CPB systems on plasma levels of cefazolin and clindamycin, our first and second choice antibiotic prophylaxis. We did not receive funding for these studies.

Material and methods

We have investigated three types of pediatric CPB systems used at the department of Cardio-Thoracic Surgery of the Erasmus MC, Rotterdam, The Netherlands. Since all experiments were *in vitro* and no patients were involved, there is no issue of informed consent in accordance with Dutch law. Circuits that would soon expire were made available free of charge by Terumo Europe NV, Leuven, Belgium and Sorin group, Mirandola, Italy. Terumo Europe NV and Sorin group had no influence on study design and protocol, data collection and analyses or publication.

CPB systems

We have tested three CPB systems used for congenital cardiac surgery in our institution. The neonatal, infant and pediatric CPB systems are used for patients with bodyweights of under 10 kg, 10-20 kg, and 20-40 kg respectively. An overview of the components of these CPB systems is given (Table 1).

Table 1: CPB systems characteristics

	Oxygenator	Reservoir	Arterial filter	Venous filter cardiotomy	Defoaming sponge	Silicone tubing	PVC tubing	Priming volume
Neonatal Roller	Capiox® FX05, Terumo Europe NV, Leuven, Belgium Hollow fiber Polycarbonate housing, polypropylene membrane 0.5 m^2, priming volume 43 ml, X-coating™	Open hardshell polycarbonate, minimum capacity 15 ml, maximum capacity 1000 ml	Integrated polyester screen type Surface area 130 cm^2, pore size 32 µm	Polyester screen type, pore size 47 µm	Polyure-thane	Sorin® Kids neonate set, custom made, Sorin Group, Mirandola, Italy Diameter ¼ inch, length 1.10 m, 0.02 m^2 contact surface area, Phisio coating	Sorin® Kids neonate set, custom made, Sorin Group, Mirandola, Italy Diameter ¼ inch, length 2.95 m, 0.069 m^2 contact surface area, Phisio coating	260 ml
Infant Roller	Sorin Kids D101, Sorin Group, Mirandola, Italy Hollow fiber	Open hardshell, polycarbonate, minimum capacity 30 ml,	Sorin Kids D131 stand-alone arterial filter, Sorin	Polyester, pore size 51 µm	Polyure-thane	Sorin® Kids, custom made, Sorin Group,	Sorin® Kids neonate set, custom made,	430 ml

	Polycarbonate housing, polypropylene membrane 0.61 m^2, priming volume 87 ml, Phisio coating	maximum capacity 1500 ml	Group, Mirandola, Italy Polycarbonate housing, phosphorylchloride screen type membrane Surface area 27 cm^2, pore size 40 µm, priming volume 28 ml			Mirandola, Italy Diameter ¼ inch, length 1.05 m, 0.02 m^2 contact surfacearea, Phisio coating	Sorin Group, Mirandola, Italy Arterial part diameter ¼ inch, length 1.88 m, Venous part diameter ⅜ inch, length 1.51 m, Total 0.08 m^2 contact surface area, Phisio Coating	
Pediatric Centrifugal Revolution, Sorin Group, Mirandola, Italy.	Capiox® FX15, Terumo Europe Europe NV, Leuven, Belgium Hollow fiber	Open hardshell polycarbonate, minimum capacity 70 or 200 ml, maximum	Integrated polyester screen type Surface area 360 cm^2, pore size 32 µm	Polyester screen type, pore size 47 µm	Polyure-thane		(Sorin® Kids Pediatric set set, custom made, Sorin Group, Mirandola, Italy)	683 ml

Pump casing polycarbonate priming volume 57 ml	Polycarbonate housing, polypropylene membrane 1.5 m^2, priming volume 144 ml, X-coating™	capacity 3000 or 4000 ml					Diameter ⅜ inch, length 4.87 m, 0.15 m^2 contact surface area, Phisio coating	

In summary, all systems consist of a hollow-fiber oxygenator and an open hard-shell reservoir. The oxygenator of the neonatal and pediatric systems are coated with X-coating™ (poly(2-methoxyethylacrylate) (PMEA)), a non-heparin biocompatible polymere with hydrophilic and hydrophobic properties. The neonatal and pediatric systems have an integrated arterial filter, whereas the infant system has a stand-alone arterial filter. The arterial filter is a polyester screen type filter in the neonatal and pediatric systems and a phosphorylchloride screen type filter in the infant system, with varying surface areas. The venous reservoirs are made of polycarbonate with capacities of respectively 1000 ml, 1500 ml and 3000 to 4000 ml for the neonatal, infant and pediatric CPB systems. A roller pump is used in the neonatal and infant systems. In the pediatric system a centrifugal pump is used, with a pump casing of polycarbonate. A combination of silicone an PVC (polyvinylchloride) tubing is used in the neonatal and infant systems. In the pediatric system only PVC tubing is used. The tubing in all systems is coated with Phisio coating: a non-heparin, biomimatic layer consisting of a phosphorylcholine polymere.

Three system were assembled for each category and placed on a mast mounted, remote pump head console (Stöcker S5 Perfusion System, Sorin Group, Mirandola, Italy), with a pediatric configuration. A ¼- ¼ or a ¼ - ⅜ connection piece was used to make the CPB systems continuous.

All systems were primed according to hospital protocol (Table 2). Priming fluid in all systems consist of fresh frozen plasma (FFP), Gelofusine (B. Braun, Melsungen, Germany), red blood cells (RBC's), human albumin (Sanquin Plasma Products BV, Amsterdam, the Netherlands) and sodium bicarbonate 8.4% (Fesenius Kabi Nederland BV, Zeist, NL). Heparin was added to avoid clotting of blood in the system. Hematocrit was aimed to be 28% during CPB. FFP's and RBC's those were just expired were obtained from the local blood bank.

CPB systems were kept running at a temperature of 36°C for six hours, since this is the maximum runtime for which the quality is guaranteed by the manufacturers. Before start of the experiments the pCO2, pO2, and pH were measured with iStat (Abbot BV, Hoofddorp, The Netherlands). These parameters were kept within physiologic ranges during the experiments using sweep gas flow, gas composition and if needed, additional of sodium bicarbonate 8.4%.

Table 2: Prime fluid composition

	Neonatal	**Infant**	**Pediatric**
Priming volume (ml)	263.4	430	683
RBC (ml)	135	235	365
FFP (ml)	30	40	50
Gelofusine (ml)	30	40	50
Albumine 20 % (ml)	40	50	100
Mannitol 15 % (ml	20	50	100
NaHCO3 8.4 % (ml)	3	15	18
Heparin (ml)	0.4	0.5	1
Flow (l/min)	0.5	1.5	3
Temperature (°C)	36	36	36
Line pressure (mmHg)	100	100	100

RBC: red blood cell, FFP: fresh frozen plasma, ml: milliliter, L: liter, min: minutes

Flow rates were kept at 0.5 L/minute, 1.5 L/minute and 3 L/minute for the neonatal, infant and pediatric CPB systems. Using the venous clamp postmembrane pressures were kept constant at 100 mmHg.

Drug administration

Drug doses were standardized based on the average weight for a patient for the neonatal, infant and pediatric CPB system. Drugs for the neonatal system were based on a 5 kg patient, for the infant system on a 15 kg patient and for the pediatric system a 30 kg patient. Drugs were dosed according to our institutional guidelines for the induction for general anesthesia, i.e. cefazolin at 30 mg/kg and clindamycin at 12 mg/kg for neonates and 6.7 mg/kg for children with a body weight of above 10 kg (see Table 3).

Drugs were injected into the CPB systems at the venous side of the system via a manifold sample port. After each drug a 2 ml physiological saline solution (0.9%) flush was injected to prevent pooling or crystallization of drugs in the sample port. Drugs were administered in the same order for all systems and all experiments.

Table 3: drug concentration administered to the CPB systems

	Neonate	**Infant**	**Pediatric**
Cefazolin (Kefzol®, Eurocept BV, Ankeveen, the Netherlands, 100 mg/ml)	250 mg	750 mg	1500 mg
Clindamycin (Dalacin®, Pfizer BV, Capelle aan den IJssel, the Netherlands)	60 mg	100 mg	200 mg

Samples

From each CPB system four ml blood samples were taken at different time points. The first samples was immediately after injection of the drugs (T1), thereafter samples were taken at 2, 5, 7, 10, 30, 60, 180 and 300 minutes. Samples were taken from the arterial (postoxygenator) side of the CPB system via a manifold sample port. Polypropylene EDTA tubes were used (7.2 mg EDTA, BD Vacutainer®, BD Life Sciences, Plymouth, UK).

Samples were stored at 4^0C until centrifugation (10 minutes at 3600 rpm). The supernatant serum was transferred to polypropylene cryogenic vials with polypropylene screw caps (Sarstedt Aktiengesellschaft & Co, Nümbrecht, Germany) and stored at -80^0C until further analysis.

Assay methods

Liquid chromatography mass spectrometry (LC-MS/MS) was used to measure total plasma drug concentrations of cefazolin and clindamycin.

Drug concentrations for clindamycin were measured using LC-MS/MS in a Thermo TSQ Quantiva triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) at the pharmacy laboratory of the University Medical Center Groningen, the Netherlands.

Drug concentrations for cefazolin were measured using LC-MS/MS in an AB SCIEX QTRAP® 5500 triple quadrupole mass spectrometer (AB SCIEX, Concord, Ontario, Canada) and Analyst® software version 1.6.2 (AB SCIEX, Concord, Ontario, Canada) at the IBMP Institute for Biomedical and Pharmaceutical Research in Nürnberg-Heroldsberg, Germany.

Limits of quantification are specified in Table 4. A certified research technician from the ISO certified laboratories performed the FDA validated drug analyses. In all analyses quality control samples are included, as is obliged in FDA analyses and ISO and GCP certified laboratory.

Table 4: Limits of quantification

Drug	Lower Limit of Quantification (LLOQ)	Upper Limit of Quantification (ULOQ)
Cefazolin	209.3 mg/L	2009.23 mg/L
Clindamycin	0.6 mg/L	57.00 mg/L

Mg: milligram, L: liter

Statistical analysis

We performed linear mixed model analyses with drug concentration as dependent variable, and type of CPB system and sample time point as independent variables. We used mixed models analysis because a correlation can be expected between multiple measurements of the same variable (drug concentration) in the same subject (CPB systems). Given the non-normal distribution of the drug concentrations, the dependent variable in the linear mixed model was defined as the log-transformed concentration of cefazolin and clindamycin. The independent variables were treated as categorical variables, and an interaction effect between the independent variables was included in the model. We used a random intercept and assumed a first-order autoregressive error covariance matrix to correct for within-system correlations between time points. This model specification was chosen by comparing values of the Akaike information criterion between different structures for the random effects and the error covariance matrix.

The predicted values for the log-transformed concentration for each CPB system and at each time point were provided by the linear mixed models. We calculated the differences between the predicted values for each time point and each CPB system and T1, including the 95% Confidence Interval (95% CI) of this difference. We exponentiated this difference and the 95% CI to obtain drug recovery (percentage of drugs still present in the system) for T2 to T300. We calculated the maximum expected concentration (MEC) of drug in the CPB systems by dividing the amount of drug added to the CPB systems by the total priming volume in the systems. We

used the MEC since it was unclear whether the drug was mixed completely with the prime fluid at T1.

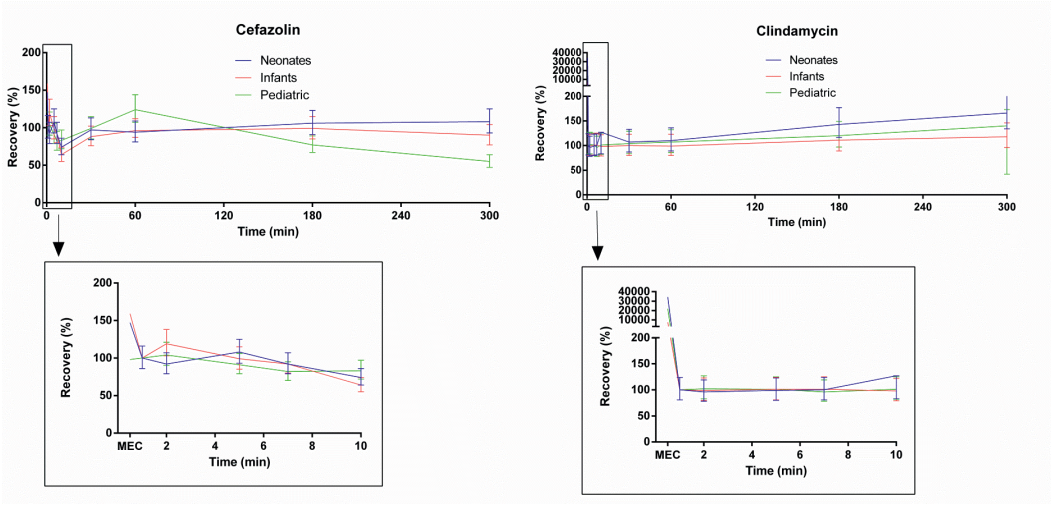
Statistical analyses were performed using IBM SPSS Statistics 24, and all statistical tests used a two-sided significance level of 0.05.

Results

A total of 81 samples, 27 for each CPB system category, was analyzed. We encountered no technical problems during execution of the experiments.

Figure 1 shows predicted drug recovery (based on the estimated marginal means of the linear mixed models) versus time for cefazolin and clindamycin, for each CPB-system category. Based on the interaction effects in the mixed models, there was a significant difference in the pattern of recovery over time between systems for both cefazolin and clindamycin ($p < 0.001$). For cefazolin, the lowest drug recovery was in the pediatric systems, while the highest drug recovery was in the neonatal systems. In the pediatric system, cefazolin concentration seems to show an initial decline, but then increased again to the starting drug concentration. This maximum in recovered concentration was found after two hours of CPB runtime. After these first two hours cefazolin concentration decreased to approximately 50% recovery at the end of the experiment.

Figure 1: Predicted recovery of cefazolin and clindamycin (in % of theoretical concentration) versus time



For clindamycin, the highest recovery was observed in the neonatal systems, whilst the lowest recovery was in the infant systems. The clindamycin concentration was relatively stable during the experiment, with a mild increase in drug recovery in all systems starting at 60 minutes to the end of the experiment.

Mean recovery for cefazolin after 60 and 180 minutes, compared to T1, was 94% (95% CI; 81-109) and 106% (95% CI; 91-123) for the neonatal systems, 96% (95% CI; 83-112) and 99% (95% CI; 85-115) for the infant systems, and 124% (95% CI; 107-144) and 77% (95% CI; 67-89) for the pediatric systems.

Mean recovery for clindamycin after 60 and 180 minutes, compared to T1, was 110% (95% CI; 89-136) and 143% (95% CI; 116-177) for the neonatal CPB systems, 99% (95%CI; 80-123) and 111% (95% CI; 89-137) for the infant systems, and 107% (95% CI; 86-132) and 120% (95% CI; 97-149) for the pediatric systems.

For cefazolin the MEC was in the range of the measurements of T1 and T2, indicating complete mixing of cefazolin after injection in the CB system. For clindamycin MEC was much higher than all measurements, indicating a steep decline after injection. Mean clindamycin recovery

after 60 and 180 minutes compared to the MEC was 0.3% and 0.4% for neonatal systems, 1.1% and 1.2% for infants systems, and 0.5% and 0.6% for pediatric systems.

Discussion

Influence of pediatric CPB systems on plasma drug concentration is largely unknown. However, use of CPB is inevitable in most congenital cardiac surgery procedures. For adult patients, *in vivo* and *in vitro* studies have been published (4, 7-9). We have investigated cefazolin and clindamycin plasma concentration in three pediatric CPB systems that are used in our institution.

For cefazolin, there are no previous *in vitro* publications for adult or pediatric CPB systems. However, cefazolin was investigated *in vitro* in pediatric extracorporeal membrane oxygenation systems (ECMO) (10). In these experiments recovery of cefazolin was high for both neonatal and pediatric ECMO systems, with an average cefazolin recovery at 2 minutes of 87%, and recovery at 180 minutes of 84.3%, with almost no difference between systems. After approximately 10 minutes plasma cefazolin concentration reached steady state. These results are much in line with our findings. Our findings indicate that cefazolin mixes within minutes in the CPB systems and concentration moderately decreases throughout the experiments. Only in our pediatric systems the cefazolin concentration decreased by 50% by the end of the experiment. We cannot fully explain this decrease in only the pediatric systems. The components of the neonatal and pediatric CPB systems are similar, and cefazolin plasma concentration is stable in the neonatal systems. The type and length of tubing could make a difference, with a larger contact surface area in the pediatric CPB systems. Absorption of drugs to PVC tubing with various coatings have been shown for fentanyl and morphine. This may also effect cefazolin plasma concentration and account for the more profound decrease in the larger CPB system. Recently, de Cock et al. published *in vivo* data on cefazolin plasma concentration during and after pediatric cardiac surgery (11). The authors collected blood samples from children undergoing cardiac surgery and concentration-time profiles were analyzed using population pharmacokinetic modelling (12). The authors estimated that the volume of distribution of the CPB compartment in the pharmacokinetic model was equal to the priming volume of the CPB system. This means there should be no sequestration of cefazolin in the CPB system. However, duration of surgical procedure and subsequent CPB use

was the primary factor for not achieving the optimal cefazolin plasma levels. Considering the apparent lack for sequestration in the CPB systems, other factors may be responsible for the moderate decrease in cefazolin plasma levels that are not yet understood.

For clindamycin, the measured plasma concentrations remained stable over time, but the difference between the MEC and measured plasma concentration was large. These results were comparable for all tested CPB systems.

There are no previous studies published on clindamycin use during cardiac surgery in adults or children. Our results therefore are the first to present these plasma concentrations. Measured clindamycin concentration remains stable during the CPB run, with an increase in concentration towards the end of the experiment. This mild increase may be due to recirculation of clindamycin from tubing or other CPB components. The most striking result is the large difference between the MEC and the first measured plasma concentration. This large difference may have several reasons. The first reason may be incomplete mixing of clindamycin with the prime fluid after injection in the CPB systems. This may lead to a preferential flow in the reservoir causing clindamycin to precipitate without circulating in the entire CPB system. An open hard-shell reservoir is used in all CPB systems and this may be the compartment to cause clindamycin precipitation. However, we cannot explain the cause of precipitation other than incomplete mixing. Another potential cause for the discrepancy between MEC and measured concentration, is sequestration of clindamycin to the plastic components or the defoaming area in the CPB system. Hydrophilic drugs such as clindamycin are not known to sequester in CPB systems. Sequestration in these systems is expected for lipophilic drugs, such as sufentanil or midazolam (13). As shown by Shekar et al (14) in ECMO circuits, drugs with a LogP greater than 2.3 have a significantly higher decrease in concentration compared to less lipophilic or hydrophilic drugs. As both cefazolin and clindamycin have a lower LogP they are not expected to sequester in the CPB systems (see Table 5).

Table 5: Drug characteristics

Drug	Blood/plasma ratio	LogP	Protein binding (%)	Vd (l/kg)	pKa
Cefazolin	1.21	-0.4	74-86	12L	3.03 (strongest acidic)
Clindamycin	Unavailable	1.59	92-94	70L	6.74 (strongest basic)

Vd: volume of distribution, pKa: acid dissociation constant at logarithmic scale, L: liter, Kg: kilogram, LogP: logarithm of partition coefficient

Highly protein bound drugs (>80% protein bound) also sequester significantly more in ECMO systems, and this could be a possible cause for sequestration of clindamycin, since clindamycin is strong protein bound. The Vd of clindamycin is mainly influenced by albumin and alpha-1 acid glycoprotein (15). Albumin, but not alpha-1 acid glycoprotein was added to the prime fluid and may have caused a shift in clindamycin protein binding resulting in a higher unbound fraction. However, we have measured total serum concentration and we would not expect this concentration to change so dramatically due to changes in protein binding. The acid dissociation constant (pKa) could make a difference in mixing and recovery, with cefazolin being a strong acid compared to clindamycin. To our knowledge no influence of pKa on sequestration of drugs in CPB or ECMO circuits has been published before. During the experiments clindamycin concentration seems to gradually increase to above 100% of the T1 concentration, but not to the MEC values. No previous results have been published on a potential reversible binding of hydrophilic drugs to CPB compartments. However, considering our results this may be a potential explanation for the increasing clindamycin concentration, or we may have an incomplete mixing of clindamycin that gradually evolves. We cannot completely exclude an error in medication administration or analysis. However, the CPB systems were run on several days and medication was always double-checked.

Our experiments show the effects of pediatric CPB systems on plasma concentration of cefazolin and clindamycin. Adequate concentration of cefazolin and clindamycin are imperative to prevent SSI. In children after cardiac surgery SSI reportedly occur in 1.9-8% of patients (16). Correct plasma concentrations of antibiotics are a relatively easily modifiable factor to prevent SSI, and are therefore important to investigate *in vitro* and *in vivo*. As shown by Shah et al. incorrect dosing of antibiotics gives a 1.7-fold increased risk of developing a SSI

in pediatric patients (17). Sub-optimal dosing due to influence of the CPB system, may affect the occurrence of SSI. To date, no uniform guidelines for dosing of antibiotic prophylaxis in children is available. Also, potentially increased risks of SSI after cardiac surgery are not incorporated in current dosing advices (11). Several procedural risk factors, such as the use of a bladder catheter, longer duration of CPB or prolonged surgery time, may increase the risk of SSI in pediatric cardiac surgery patients (1). Our results show that plasma cefazolin concentration only moderately decreases during CPB in the pediatric system. Cefazolin concentration remains stable over time in the neonatal and infant systems. The increased risk of SSI with longer CPB runtime may be associated with other surgery and patient related factors, such as systemic inflammatory response syndrome (SIRS) and increased volume of distribution with subsequent lower plasma concentration. The most important moment to obtain adequate plasma concentrations is at the time of wound closure. This should be investigated *in vivo*, with the added patient related influence on plasma drug concentration. The loss of clindamycin in the CPB systems, with the recovery of only 0.3-1.2% compared to the MEC, should be further investigated. If this indeed holds true, antibiotic prophylaxis with clindamycin is not effective for children undergoing cardiac surgery.

Our study has several limitations. Firstly, we have not corrected for the blood/plasma ratio of the measured drugs. For clindamycin the blood/plasma ratio is not known. The blood/plasma ratio for cefazolin could potentially slightly change the measured plasma concentration. However, the measured concentrations are relatively high compared to clinically relevant values and therefore the blood/plasma ratio was not considered a large influence. Also, the blood/plasma ratio remains constant over time and does therefore not influence the increase or decrease of drug concentration. Secondly, we did not correct for spontaneous degradation. Spontaneous degradation may introduce a potential bias in the measured plasma concentration, since this is a cause for drug loss. However, a study on spontaneous degradation of cefazolin by Donnelly showed that cefazolin remains stable over 30 days (18). To our knowledge no data have been published on clindamycin stability. From personal communication with our laboratory we know clindamycin to be stable for two years when kept at -20^0 Celsius between processing and analysis, as is in line with good laboratory practice. Finally, during cardiac surgery, antibiotic administration is often repeated at start of the CPB system. In contrast, we spiked the CPB systems at the beginning of the experiments,

and did not administer a re-dose during the experiment. This timing of administration only resembles short cardiac surgical procedures, without redosing at onset of CPB. However, this method is often used in *in vitro* studies and most accurately reflects the influence of CPB runtime on drugs.

Conclusion

In conclusion, our study shows high recovery of cefazolin in the neonatal and infant CPB system, and moderate recovery of cefazolin in the pediatric CPB system. We found a large discrepancy between the MEC and measured concentrations of clindamycin in all tested CPB systems. Our results do not explain this discrepancy, and this should be further investigated to ensure adequate antibiotic prophylaxis in children undergoing cardiac surgery.

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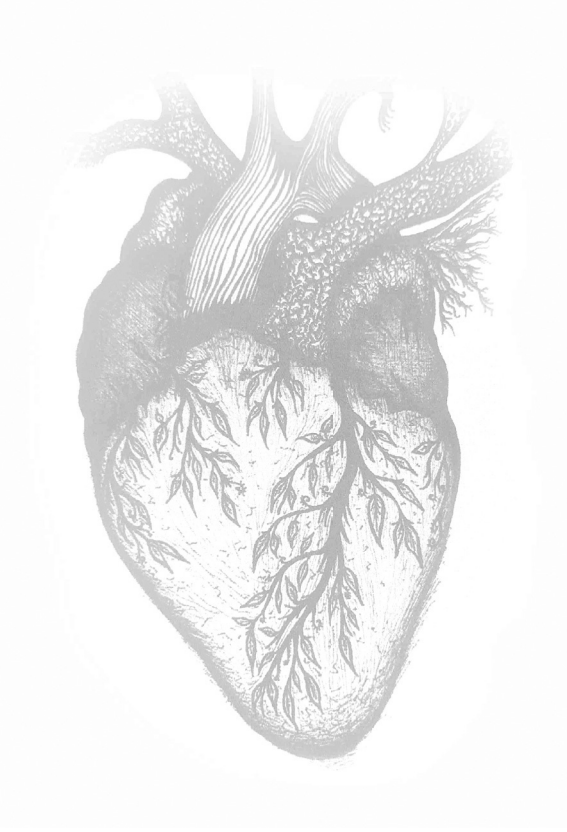

Chapter 5

In vitro recovery of sufentanil, midazolam, propofol and methylprednisolone in pediatric cardiopulmonary bypass systems

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Abstract

Objectives: To evaluate in vitro drug recovery in cardiopulmonary bypass (CPB) systems used for pediatric cardiac surgery.

Design: Observational in vitro study.

Setting: Single-center university hospital.

Participants: In vitro CPB systems used for pediatric cardiac surgery.

Interventions: Three full neonatal, infant, and pediatric CPB systems were primed according to hospital protocol and kept running for 6 hours. Midazolam, propofol, sufentanil, and methylprednisolone were added to the venous side of the systems in doses commonly used for induction of general anesthesia. Blood samples were taken from the postoxygenator side of the circuit immediately after injection of the drugs and after 2, 5, 7, 10, 30, 60, 180, and 300 minutes.

Measurements and Main Results: Linear mixed model analyses were performed to assess the relationship between log-transformed drug concentration (dependent variable) and type of CPB system and sample time point (independent variables). The mean percentage of drug recovery after 60 and 180 minutes compared with T1 was 41.7% (95% confidence interval [CI] 35.9-47.4) and 23.0% (95% CI 9.2-36.8) for sufentanil, 87.3% (95% CI 64.9-109.7) and 82.0% (95% CI 64.6-99.4) for midazolam, 41.3% (95% CI 15.5-67.2) and 25.0% (95% CI 4.7-45.3) for propofol, and 119.3% (95% CI 101.89-136.78) and 162.0% (95% CI 114.09-209.91) for methylprednisolone, respectively.

Conclusions: The present in vitro experiment with neonatal, infant, and pediatric CPB systems shows a variable recovery of routinely used drugs with significant differences between drugs, but not between system categories (with the exception of propofol). The decreased recovery

of mainly sufentanil and propofol could lead to suboptimal dosing of patients during cardiac surgery with CPB.

Introduction

CPB is necessary to facilitate most cardiac surgery in children. The effect of CPB on in vivo drug concentrations in patients can be profound and is attributed to hemodilution, altered hemodynamic status, hypothermia, systemic inflammation, changes in acid-base status, exclusion of the lungs from the circulation and hemofiltration (1). Furthermore, the plastic components of the CPB-system themselves have been shown to absorb drugs (2-7).

In our institution multiple experiments have taken place to determine in vitro drug recovery in ECMO-systems (8, 9). Drug recovery has been defined as the concentration of drug present in the priming fluid after a certain amount of time has passed since addition of the drug to the CPB-system (8, 9). Previous publications have used the term absorption to indicate the decrease in concentration of drug in the priming fluid. In our institution, recovery has been deemed a more precise definition, since not all drug is actually absorbed by the system components. Drugs are also subject to spontaneous degradation, for example, providing an altogether different reason for a decrease in drug concentration than absorption of drug to components of the CPB-system (8). There is a lack of data in the literature concerning pediatric CPB-systems. As part of our CPB-PHARM study, aiming to measure and model drug concentrations during CPB for pediatric cardiac surgery (registered at the Netherlands Trial Register no NTR3579), the current in vitro experiments were undertaken. The ultimate goal is to incorporate these data into in vivo population pharmacological models.

Methods

The study was conducted at the Department of Cardiothoracic Surgery of a tertiary teaching hospital. No human subjects were involved in the study, so the need for medical ethical review board approval was waived according to Dutch law.

Soon to be expired CPB-systems were made available free of charge by Terumo Europe NV (Terumo Europe NV, Leuven, Belgium) and Sorin Group (Sorin Group, Mirandola, Italy). This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. There was no role for Terumo Europe NV or Sorin Group in the design of the study, collection, analysis and interpretation of data, writing of the report or the decision to submit the report for publication.

CPB-systems

Table 1 shows the composition of the different CPB-systems used. All systems contained a hollow-fiber membrane oxygenator with a PMP membrane. For the neonatal and pediatric systems an arterial filter was integrated in the oxygenator, for the infant system a stand-alone arterial filter was used. Silicone- and PVC-tubing with different lengths and diameters were used in the neonatal and infant roller-pump systems. In the pediatric system a centrifugal pump was used, and the silicone tubing was discarded. Tubing was made continuous via a ¼-¼ or a ¼-⅜ connection piece. A venous reservoir completed the systems. Terumo components of the systems were coated with X-coating™ (PMEA): a non-heparin biocompatible polymer with hydrophilic and hydrophobic properties. Sorin components of the systems were coated with Phisio coating: a non-heparin, biomimatic layer consisting of a phosphorylcholine polymer.

All systems were placed on a conventional mast mounted, remote pump head console (Stöcker S5 Perfusion System, Sorin Group, Mirandola, Italy) with a specific pediatric configuration.

Three full systems were assembled for each category (neonatal, infant and pediatric) and primed according to hospital based protocol (see Table 2). Priming fluid contained FFP and Gelofusine (B. Braun, Melsungen, Germany). RBC's were added to the priming to achieve a hematocrit of 28%. Recently expired RBC's and FFP's obtained from our local blood bank were used for priming. The priming fluid was completed with human albumin (Sanquin Plasma Products BV, Amsterdam, The Netherlands) and 2-5 ml sodium bicarbonate 8.4% (Fresenius Kabi Nederland BV, Zeist, The Netherlands). Heparin was added to the system according to hospital protocol to prevent clotting.

The CPB-systems were kept running for 6 hours. This is the maximum runtime with guaranteed quality by the manufacturers. The temperature was maintained at 36°C. pO2, pCO2 and pH were measured with an iStat™ handheld device (Abbot BV, Hoofddorp, The Netherlands) and maintained within physiologic ranges by titration of sweep gas flow, gas composition and addition of sodium bicarbonate 8.4 % if needed.

A flow rate of 0.5 l min^{-1} was maintained for the neonatal circuits, 1.5 L min^{-1} for the infant circuits and 3 L min^{-1} for the pediatric circuits. Post membrane pressures were kept at 100 mmHg by adapting the resistance using the venous clamp.

Drug administration

For the neonatal system we used a standardized body weight of 5 kg, for the infant system 15 kg and for the pediatric system 30 kg. Drugs were added to the venous reservoir via a manifold sample port in a dose that would normally be used for the induction of general anesthesia according to our institution's guidelines: midazolam (Actavis Group PTC ehf., Hafnarfjördur, Iceland, 1 mg ml^{-1}) 0.2 mg kg^{-1}, propofol (Fresenius Kabi Nederland BV, Zeist, The Netherlands) 10 mg ml^{-1}) 2 mg kg^{-1}, sufentanil (Hameln Pharma Plus GmbH, Hameln, Germany, 50 mcg ml^{-1}) 2 mcg kg^{-1} and methylprednisolone (Pfizer BV, Capelle a/d IJssel, The Netherlands) 30 mg kg^{-1}. Drugs were injected in the same order for all systems. Between each drug and after the last drug the sample port was flushed with 2 ml of saline 0.9% solution to prevent crystallization or pooling of drug. Midazolam, propofol, sufentanil and methylprednisolone were used because these drugs are commonly used in pediatric cardiac anesthesia in our institution.

Table 1: CPB systems characteristics

	Oxygenator	Reservoir	Arterial filter	Venous filter cardiotomy	Defoaming sponge	Silicone tubing	PVC tubing	Priming volume
Neonatal Roller	Capiox® FX05, Terumo Europe NV, Leuven, Belgium Hollow fiber Polycarbonate housing, polypropylene membrane 0.5 m^2, priming volume 43 ml, X-coating™	Open hardshell polycarbonate, minimum capacity 15 ml, maximum capacity 1000 ml	Integrated polyester screen type Surface area 130 cm^2, pore size 32 µm	Polyester screen type, pore size 47 µm	Polyure-thane	Sorin® Kids neonate set, custom made, Sorin Group, Mirandola, Italy Diameter ¼ inch, length 1.10 m, 0.02 m^2 contact surface area, Phisio coating	Sorin® Kids neonate set, custom made, Sorin Group, Mirandola, Italy Diameter ¼ inch, length 2.95 m, 0.069 m^2 contact surface area, Phisio coating	260 ml
Infant Roller	Sorin Kids D101, Sorin Group, Mirandola, Italy Hollow fiber	Open hardshell, polycarbonate, minimum capacity 30 ml,	Sorin Kids D131 stand-alone arterial filter, Sorin	Polyester, pore size 51 µm	Polyure-thane	Sorin® Kids, custom made, Sorin Group,	Sorin® Kids neonate set, custom made,	430 ml

	Polycarbonate housing, polypropylene membrane 0.61 m^2, priming volume 87 ml, Phisio coating	maximum capacity 1500 ml	Group, Mirandola, Italy Polycarbonate housing, phosphorylchloride screen type membrane Surface area 27 cm^2, pore size 40 µm, priming volume 28 ml			Mirandola, Italy Diameter ¼ inch, length 1.05 m, 0.02 m^2 contact surfacearea, Phisio coating	Sorin Group, Mirandola, Italy Arterial part diameter ¼ inch, length 1.88 m, Venous part diameter ⅜ inch, length 1.51 m, Total 0.08 m^2 contact surface area, Phisio Coating	
Pediatric Centrifugal Revolution, Sorin Group, Mirandola, Italy.	Capiox® FX15, Terumo Europe Europe NV, Leuven, Belgium Hollow fiber	Open hardshell polycarbonate, minimum capacity 70 or 200 ml, maximum	Integrated polyester screen type Surface area 360 cm^2, pore size 32 µm	Polyester screen type, pore size 47 µm	Polyure-thane		(Sorin® Kids Pediatric set set, custom made, Sorin Group, Mirandola, Italy)	683 ml

Pump casing polycarbonate priming volume 57 ml	Polycarbonate housing, polypropylene membrane 1.5 m^2, priming volume 144 ml, X-coating™	capacity 3000 or 4000 ml					Diameter ⅜ inch, length 4.87 m, 0.15 m^2 contact surface area, Phisio coating	

Table 2: Priming fluid composition

	Neonatal	**Infant**	**Pediatric**
Priming volume (ml)	263.4	430	683
RBC (ml)	135	235	365
FFP (ml)	30	40	50
Gelofusine (ml)	30	40	50
Albumine 20 % (ml)	40	50	100
Mannitol 15 % (ml	20	50	100
NaHCO3 8.4 % (ml)	3	15	18
Heparin (ml)	0.4	0.5	1
Flow (l/min)	0.5	1.5	3
Temperature (°C)	36	36	36
Line pressure (mmHg)	100	100	100

RBC: red blood cell, FFP: fresh frozen plasma, ml: milliliter, L: liter, min: minutes

Samples

Four ml blood samples were taken from the arterial (post oxygenator) side of the circuit via a manifold sample port in a PLP EDTA tube (7.2 mg EDTA, BD Vacutainer®, BD Life Sciences, Plymouth, UK). Samples were taken immediately after injection of the drugs (T1) and after 2, 5, 7, 10, 30, 60, 180 and 300 min.

Samples were stored at 4°C until processing. After centrifugation (10 min at 3600 rpm) the supernatant serum was transferred to PLP cryogenic vials with PLP screw caps (Sarstedt Aktiengesellschaft & Co, Nümbrecht, Germany) and stored at -80°C until analysis.

Assay methods

Drug concentrations for sufentanil, midazolam, propofol and methylprednisolone were measured using liquid chromatography mass spectrometry (LC-MS/MS). Methods were validated according to U.S. FDA guidelines for bioanalytical method validation (10). All analyses included quality control samples, as is obliged in FDA analyses and ISO and GCP certified laboratories, and were performed by a certified research technician.

Drug concentrations for sufentanil were measured using a Thermo TSQ Vantage triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) at the pharmacy laboratory of the Erasmus MC.

Drug concentrations for midazolam were measured using a Quattro Premier mass spectrometer from Waters Corp (Waters Corp., Milford, MA, USA) at the pharmacy laboratory of the Erasmus MC.

Propofol was measured using a Thermo TSQ Quantiva triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) at the pharmacy laboratory of the University Medical Center in Groningen, the Netherlands.

Drug concentrations for methylprednisolone were measured using a SCIEX Triplequad™ 6500+ mass spectrometer (AB SCIEX, Concord, Ontario, Canada) and Analyst® software version 1.7 (AB SCIEX, Concord, Ontario, Canada) at the IBMP Institute for Biomedical and Pharmaceutical Research in Nürnberg-Heroldsberg, Germany.

LLOQ was 0.25 mcg l^{-1} for sufentanil, 2.0 mcg l^{-1} for midazolam, 100.0 mcg l^{-1} for propofol and 0.01 mcg ml^{-1} for methylprednisolone. ULOQ was 50.0 mcg l^{-1} for sufentanil, 2400 mcg l^{-1} for midazolam, unknown for propofol and 30.1 mcg ml^{-1} for methylprednisolone.

Statistical analysis

We assessed the relationship between log-transformed drug concentration (dependent variable) and type of CPB-system and sample time point (independent variables) with linear mixed model analyses. Linear mixed model analyses were used because a correlation can be expected between repeated measurements of the same variable (i.e. drug concentration) in the same subject (i.e. individual CPB-systems). Both independent variables were treated as categorical variables and a two-way interaction effect between the type of CPB-system and sample time point was included in the model. To correct for within-system correlations between time points we used a random intercept and assumed a first-order autoregressive (AR1) error covariance matrix. This model specification was chosen by comparing values of the Akaike information criterion between different structures for the random effects and the error covariance matrix.

For each time point and each CPB-system we calculated the difference between the predicted log-transformed estimated marginal means(11) at T1 and the predicted log-transformed concentration at the different time points and the 95 % CI of this difference. Finally, this difference and the 95 % CI were exponentiated to obtain the percentage drug recovery (the percentage of drug still present in the system) for T2-T300. We calculated MEC (the maximum expected concentration of drug in the CPB-systems) by dividing the amount of drug added to the CPB-systems by the total priming volume used, because it was unclear whether mixing of drug with the priming fluid would be complete at T1.

Spearman correlations were calculated to assess the relationship between drug recovery at 60 and 180 min and logP, protein binding and pKa among the four drugs (i.e. with a sample size of n = 4 drugs). For each drug, the recovery used for the calculation of this correlation was based on the estimated marginal means of the linear mixed model.

Statistical analyses were performed using IBM SPSS Statistics 24. All statistical tests used a two-sided significance level of 0.05.

Results

We encountered no technical problems during the experiments. A total of 81 samples (27 for each CPB-system category) were analyzed. No loss of drug samples occurred.

Figure 1 shows predicted drug recovery (based on the estimated marginal means of the linear mixed models) versus time for propofol, for each CPB-system category. There is a sharp and significant decline in recovery of propofol compared to T1 in all the system categories in the first 60 min, to 41.3 % (95 % CI 15.5-67.2, based on the estimated marginal means of the linear mixed models), meaning that approximately 59 % of the added drug has been lost from the circulating prime fluid at this time. After that, recovery continues to decrease slightly but significantly throughout the study period to 25 % (95 % CI 4.7-45.3) after 180 minutes and 19 (95 % CI 5.2-32.8) after 300 min. Based on the interaction effects in the mixed models, there was a significant difference in the pattern of recovery over time between systems for propofol ($p < 0.001$). Recovery is highest in the infant system, followed by the pediatric system. The neonatal systems appear to absorb the largest amount of propofol to their system components.

Figure 1: Drug recovery versus time based on estimated marginal means for propofol, for each cardiopulmonary bypass system category, expressed as means and 95% confidence interval based on the linear mixed models. MEC, maximum expected concentration in case of perfect mixture of drug. *p < 0.002.

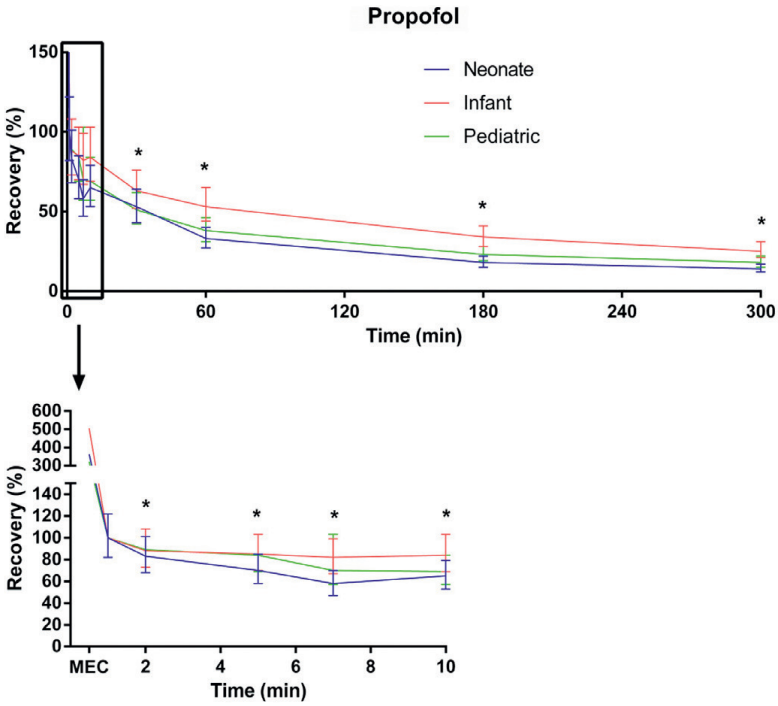
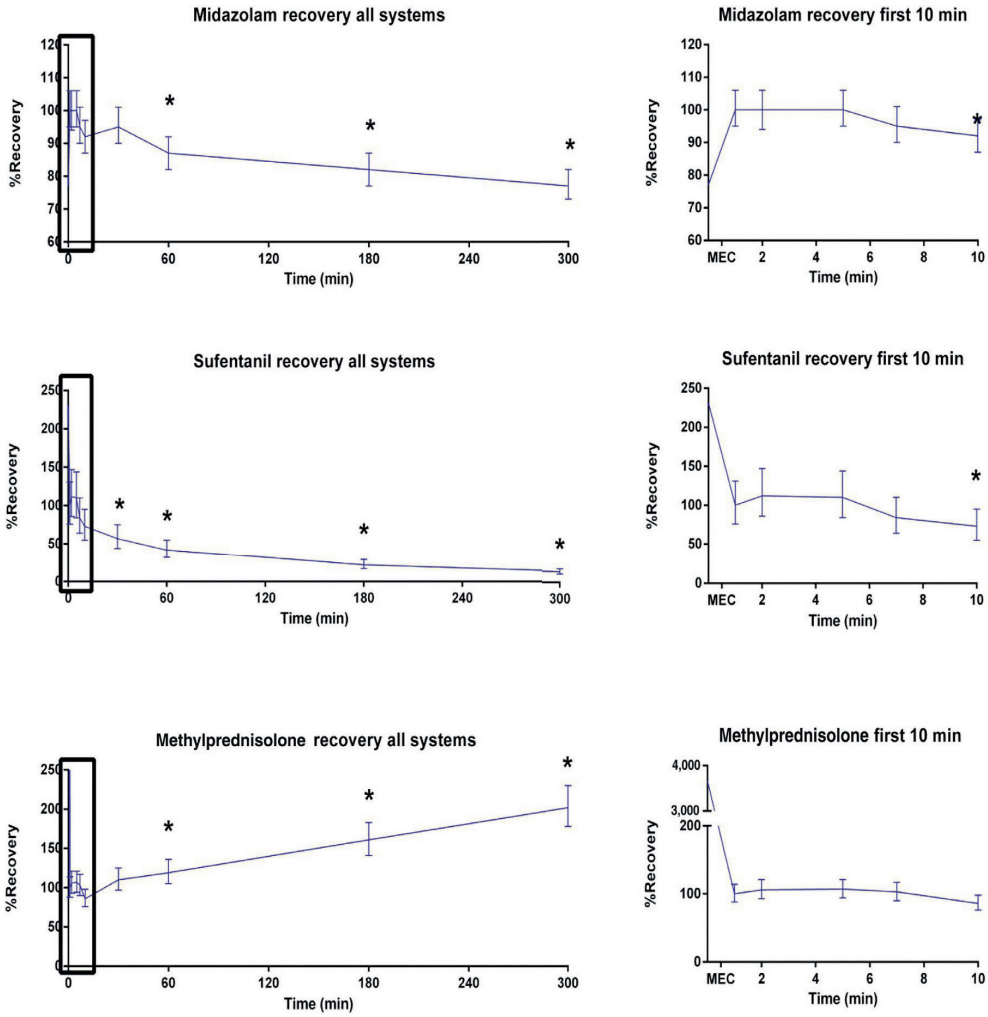


Figure 2 shows predicted drug recovery (based on the estimated marginal means of the linear mixed models) versus time for sufentanil, midazolam and methylprednisolone. Because there was no significant interaction effect between the type of CPB-system and sample time point for sufentanil (p = 0.111), midazolam (p = 0.213) or methylprednisolone p = 0.829), a single graph is used to depict decrease of drug recovery over time for all three systems. The pattern of decline in recovery of sufentanil shows stable drug concentrations in the first 7 minutes, with a significant decline compared to T1 from T10 onward. Drug recovery is 41.7 % (95 % CI 35.9-47.4, based on the estimated margical means of the linear mixed models) at 60 minutes. After 60 minutes recovery continues to decrease slightly but significantly throughout the study period. For sufentanil recovery is 23.0 % (95 % CI 9.2-36.8) after 180 minutes and 15 % (95 % CI -1.3-31.3) after 300 min.

Figure 2: Drug recovery versus time based on estimated marginal means for midazolam, sufentanil, and methylprednisolone, expressed as means and 95% confidence interval based on the linear mixed models. MEC, maximum expected concentration in case of perfect mixture of drug. *p < 0.05



For midazolam there is also stable drug recovery in the first 7 minutes. The decline in drug recovery compared to T1 reaches significance at T10 and from T60 forward. Drug recovery is 87.3 % (95 % CI 64.9-109.7) after 60 minutes and 82.0 % (95 % CI 64.6-99.4) after 180 min. For

methylprednisolone there is stable recovery of drug in the first 30 minutes. After that, recovery increases significantly compared to T1 to values much higher than 100 %.

We found no significant correlation between log P and percentage recovery of the four drugs at 60 and 180 min (ρ -0.324, p = 0.304). We did find a decreased recovery of highly protein bound drugs (ρ = -0.822, p = 0.007). The third factor correlated to percentage recovery at 60 and 180 min in our study was pKa (ρ = 0.822, p = 0.007).

Discussion

This in vitro experiment investigated drug recovery in three different pediatric CPB-systems used in our center. Decrease of drug concentration in the circulating prime fluid for propofol and sufentanil is fast in the first 60 minutes. Since 60 minutes is a relatively common bypass time in pediatric cardiac surgery this period is clinically very relevant. The decreasing speed of decrease in drug concentration after 60 minutes may be an indication of near complete saturation of binding places on the different components of the CPB-systems. It is, however, unknown if there is a finite amount of binding places and if complete saturation of these binding places is possible. Hammaren (12) and Myers (13) have shown that for propofol there appears to be no maximum saturation of binding places in complete adult CPB-systems even at very high propofol concentrations. Binding of propofol may be concentration dependent (12). In contrast, complete saturation of oxygenator membrane fragments has been shown for fentanyl in in vitro studies (5).

In our study midazolam recovery is remarkably large in both centrifugal and roller pump systems. Unfortunately, there are no in vitro studies of pediatric CPB-systems to compare our results with. An ECMO study performed in our hospital showed a recovery pattern similar to our experiment in systems with a centrifugal pump(8). In roller pump systems there was just 7.5 % recovery after 2 min and 0.6 % recovery after 180 min. For midazolam we have to take into account, however, that the concentrations measured in our study were far above the ULOQ. We believe that this may at least partly be the reason that the MEC is lower than the measured concentrations. This introduces an unknown amount of bias, but we do not expect that this measurement bias would explain the high recovery rates. An error in the addition of medication to the system or a lab error are also not expected, since the experiments were

performed on different days for the different systems and all the percentage recovery versus time curves show a similar pattern.

Methylprednisolone concentrations in the first hour are much lower than would be anticipated from the MEC. This is most likely caused by a problem with the mixing of methylprednisolone with priming fluid. Another explanation would be very fast binding of methylprednisolone to components of the CPB-system and release of drug from those binding sites after 60 minutes. It is however unknown if binding of drug to components of a CPB-system is a reversible process.

The substantial differences in recovery we found between drugs suggest that drug characteristics influence the interaction with components of the CPB-system (Table 3). From previous studies in ECMO-systems, recovery of drugs seems highly dependent on lipophilicity (8, 9, 14, 15). In our study we found no correlation between log P and recovery percentage. This is likely caused by the recovery profile of midazolam. Significantly decreased recovery of highly protein bound (> 80 %) drugs was also shown (15). We did find a generally decreased recovery of highly protein bound drugs, which may be caused by binding of drug to protein adhered to system components (15). In contrast, midazolam is also a highly protein bound and highly lipophilic drug but shows relatively high recovery in our study. The similar recovery patterns of propofol and sufentanil suggest there is a common physicochemical property of both drugs causing this effect. We can however not explain why the recovery pattern of midazolam is different, since the physicochemical properties known to influence recovery percentage are very similar to propofol and sufentanil. Another factor correlated to percentage recovery at 60 and 180 min in our study was pKa. To our knowledge no correlation between pKa and recovery has been described previously. The surface coated CPB-systems are negatively charged, making electrostatic attraction of positively charged molecules a possible mechanism for absorption of drugs to CPB-system components. Drugs with a high pKa are unlikely to be dissociated at normal pH, which was maintained during the study period.

We found a significant difference in drug recovery between the different types of CPB-system only for propofol. In general one would expect a larger system to have more binding sites for drugs and thus a lower recovery for drugs with similar properties. The surface area of the

oxygenator and the PVC-tubing in our pediatric systems are much larger than that in our neonatal and infant systems. In our study midazolam and methylprednisolone do indeed show lower recovery in larger systems, though not significantly so. For propofol there is however higher recovery in larger systems. The differences are smaller than would be expected if system size were the only factor involved.

Different components of the CPB-system are capable of absorbing drugs to their plastics. Differences in drug recovery between different types of oxygenator have been extensively described (5, 16). With the new PMP and PLP membranes the oxygenator does not appear to be a factor of considerable interest in drug recovery anymore. Based on a study by Preston et al 80 % of drug is lost to PVC tubing, with a small additional amount of drug (of just 5 %) lost to the oxygenator (17). Silicone tubing has been shown to decrease the recovery of drugs compared to PVC tubing (2, 8). The effect of different surface coatings on both oxygenator and tubing on drug recovery has been investigated by several authors (7, 12, 13), suggesting different effects for different coatings for different types of drugs. Addition of an arterial filter may also lead to decreased drug recovery (13). Many factors are thus at play and interact with each other.

It is unclear which differences in system composition play a role in our study. The interplay of differences in surface area, coating, tubing type and pump type makes it difficult to draw firm conclusions about the influence of individual system components on drug recovery. In an earlier study by Preston et al a Terumo Baby Rx oxygenator was used (17). We used the Terumo Capiox® Fx05 in our neonatal systems, which is the same oxygenator with the same membrane, the same coating and the same surface area, but with an integrated arterial filter. The Baby Rx oxygenator absorbed 3 % of fentanyl added to the system in the study by Preston et al, which amounts to 0.6 ng/cm². Raffaeli et al have shown that sufentanil absorption is similar to fentanyl absorption in their ECMO systems (9). Assuming the above holds true the amount of drug absorbed in our neonatal systems just by the tubing and the arterial filter would be 4.2 mcg of sufentanil at 180 minutes for our systems (total uptake of sufentanil 72 % at 180 min of 10 mcg added to the neonatal systems minus the amount absorbed by the oxygenator). Because our neonatal systems use silicone tubing, this relatively low amount of absorption seems unlikely.

A similar calculation for our pediatric systems is possible. The Capiox® Fx15 oxygenator would absorb 9 mcg in total, due to its larger surface area. After 180 min 76 % of the 60 mcg of sufentanil added to the system would be absorbed. Further calculation shows that this would mean that the Sorin® tubing would absorb 25.1 ng/cm² of sufentanil. This would however mean that all the sufentanil in our neonatal system would have to have been absorbed, which is not the case. For a more extensive calculation we refer to the supplemental materials.

Hynynen (18) describes propofol recovery of 25 % after 120 min of circulation in an adult system. Hammaren and Myers (12, 13) describe recovery of 37 and 43 % respectively after 60 minutes. These values are remarkably similar to ours, even though completely different CPB-systems were used. Given the calculations performed above and the results by Hynynen, Hammaren and Meyers it appears that it is very difficult to translate research performed in individual centers to one's own clinical practice, given the amount of factors and interaction at play. It is clear that not all factors influencing absorption to different components of CPB-systems are known. A possible lack of generalizability may thus be seen as a limitation to our study, as well as other studies already performed in this field.

Several authors have found no influence of temperature management on the recovery of drugs in their systems (16, 19, 20). Therefore we did not attempt to simulate a cooling protocol.

Despite the significant decrease in recovery of drug from the priming fluid of the CPB-system found in our in vitro studies, clinically patients do not wake up on initiation or during CPB. A clinical study in adult patients has shown no change or even a decrease in BIS values during CPB (21). This is most likely caused by an increase in unbound drug concentration caused by decreased protein concentration on initiation of CPB (21-23), causing a greater amount of drug available for end organ effect.

Our study has several other limitations. We only tested complete systems, thus we have no information as to which amount of drug was absorbed by which system component. Unfortunately this type of research is costly, due to the costs of systems, blood products for priming and the cost of drug concentration measurements. Also, for our purposes, namely the integration of these in vitro results with the results of the in vivo part of the CPB-PHARM study, we wanted to mimic every day practice in our hospital as closely as possible, making testing

of individual components less useful. For the same reasons, we have not performed isolated drug studies. It is not known if there is competition for binding sites to components of the CPB-system for different individual drugs. Although theoretically this is an interesting topic, in clinical practice patients receive multiple medications at the same time.

We used just three full systems in each category. This may seem like a small number, but our sample size is comparable to that of other publications performed in both CPB- and ECMO-systems and cited in this article.

Spontaneous degradation may produce bias in our study because it causes a decrease in drug concentrations not caused by adherence of drug to components of the CPB-system. Previous studies in our hospital have shown that spontaneous degradation over 24 hours for midazolam is 11,4 % and for sufentanil is 0 % (9). For propofol spontaneous degradation in glass bottles with daylight and room temperature is around 5 % after 6 hours (24). For methylprednisolone no references were found for spontaneous degradation. Since degradation is usually calculated over 24 hours, the effect on our results would be constant over time.

For the calculation of MEC we did not correct for blood-plasma ratio. In previous studies in our hospital midazolam was shown to have a blood-plasma ratio of 75 % (8). Since our MEC value is calculated in blood, but drug concentrations are measured in plasma MEC would be underestimated for midazolam. Since propofol is highly bound to red blood cells in vivo a high blood-plasma ratio would be expected, and our MEC would be overestimated.

We did not aim for a similar drug concentration in the different types of CPB-systems. Instead we added the dose of drug that would be administered to a typical patient connected to a neonatal, infant or pediatric CPB-system in our daily clinical setting, since the goal of this study was to mimic our everyday practice as closely as possible, so that in the future we will be able to incorporate CPB-system recoveries in a larger population pharmacokinetic model of the influence of cardiopulmonary bypass on drug concentrations in children.

Due to the high doses of drug added to our systems, drug concentrations were above the ULOQ for some drug assays, necessitating additional dilution before quantification. The high

doses might also have resulted in potential differences in absorption rates, as has been shown for propofol (12).

In our study we found indications that mixing of drug with the priming fluid is not always complete. This may have clinical consequences if drugs are added to the CPB-system during CPB, rather than given directly to the patient.

Despite these limitations our study is the first comprehensive in vitro testing of CPB-systems used in pediatric congenital cardiac surgery.

In conclusion, our in vitro experiment with neonatal, infant and pediatric CPB-systems shows a variable recovery of routinely used drugs with significant differences between drugs, but not between system categories, except for propofol. We have also shown that the generalizability of this type of research may be limited. The clinical consequences of our research will have to be further investigated. The decreased recovery of sufentanil and propofol could lead to suboptimal dosing of patients during cardiac surgery with the use of CPB, even though clinically this doesn't show. It is thus important that these findings are integrated with the results of in vivo studies into population pharmacokinetic models, to further investigate the clinical relevance of our findings and the implications for perioperative patient care.

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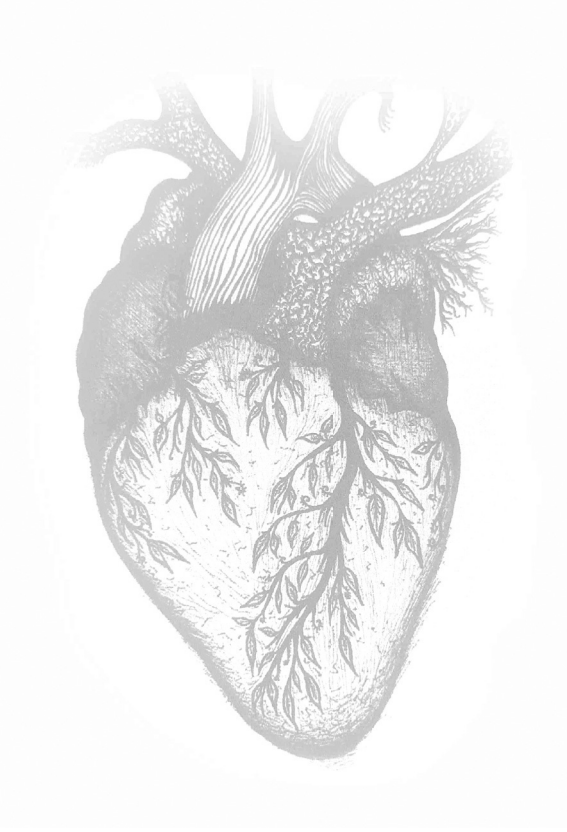

Chapter 6

The current cefazolin dosing regimen for peri-operative prophylaxis in cardiac surgery is adequate

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Submitted

Summary

Introduction: Cefazolin is the first choice antibiotic prophylaxis during cardiac surgery and is widely used to prevent deep sternal wound infections (DSWI). Recently, the Dutch guideline has been changed where the cefazolin dose given at the onset of cardiopulmonary bypass (CPB) has been removed and the postoperative dosage are both halved. The aim of the study is to assess if adequate cefazolin plasma levels are obtained with the new Dutch guideline.

Methods: Twenty-four adults undergoing cardiac surgery with cardiopulmonary bypass (CPB), receiving cefazolin were studied. The main goal is 100% $fT_{>MIC}$ during surgery and epidemiological cutoff is 2 mg/L. During the postoperative phase the goal is 40% $fT_{>MIC.}$ Per patient 9 to 11 blood samples were collected to measure plasma cefazolin concentrations.

Results: During surgery 100% of the measured concentrations were above the ECOFF of 2mg/L. BMI was not significantly associated with unbound cefazolin concentrations. Sex, age, albumin levels preoperative and duration of CPB were also not significantly associated with cefazolin unbound concentration. Redose was significantly associated with higher total plasma concentration. In the postoperative phase 85% were above the ECOFF of 2mg/L.

Conclusion: This study shows that the unbound cefazolin concentration during surgery is 100% above the ECOFF of 2mg/L. In the postoperative period more than 85% was above the ECOFF of 2mg/L. We concluded that the cefazolin dosing regimen according to the current guideline is adequate.

Introduction

Cefazolin is the first-choice antibiotic for peri-operative prophylaxis during cardiac surgery. This first-generation cephalosporin is widely used to prevent deep sternal wound infections (DSWI) due to *Staphylococcus aureus*. DSWI is a severe complication occurring in 0.5 to 3.4% of adults undergoing cardiac surgery (1, 2) and is associated with increased morbidity and mortality (3).

Both European and American guidelines advise cefazolin as antibiotic prophylaxis until 24 hours after surgery (4, 5). The American guideline gives no specific advice on timing of dosing or the need for redosing during surgery (4). The recent guideline from the European Association for Cardio-Thoracic Surgery (EACTS) advises to redose cefazolin if the expected duration of the surgical procedure exceeds two half-lives of cefazolin or in case of extensive blood loss (5).

Recently, the Dutch guideline for use of cefazolin as peri-operative prophylaxis has been changed. The cefazolin dose given at the onset of cardiopulmonary bypass (CPB) has been removed (6). However, onset of CPB has been shown to decrease cefazolin drug concentrations, most likely due to hemodilution (7, 8). Also, the peri-operative dose administered four hours after the initial dose is reduced from two to one gram, if the remaining duration of surgery is expected to be under three hours, two grams otherwise will be administered. If surgery is completed within four hours, no redose is given during surgery. This decrease in cefazolin dosing could potentially lead to a decreased unbound cefazolin plasma concentration and values under the targeted minimum concentration during surgery and therefore inadequate prophylaxis.

Approximately 80% of cefazolin is protein bound and thus biologically inactive. In this study unbound plasma cefazolin concentrations were measured in 24 adults undergoing cardiac surgery after implementation of the new Dutch guideline. The aim is to compare the peri-operative and postoperative unbound cefazolin values with the minimum target concentration of 2 mg/L (SWAB guideline) (6) to assess if adequate cefazolin plasma levels are obtained with the new Dutch guideline.

Methods

Patients

Twenty-four adult patients who were scheduled for cardiac surgery with the use of CPB were included after written informed consent was obtained. Inclusion criteria were: age above 18 years, cardiac surgery with the use of CPB, cefazolin as antibiotic prophylaxis, BMI under 40. Exclusion criteria were: known allergy to cefazolin, treatment for active infection with antibiotics prior to surgery, use of minimal invasive extracorporeal system, eGFR < 35ml/min or ASAT/ALAT > 3 times normal values. In our institution, almost no patients scheduled for cardiac surgery have a BMI over 40. Therefore, we considered these patients outliers and excluded them from the study to ensure a more homogenous population. The study was approved by the Institutional Review Board (IRB) of the Erasmus MC (MEC 2018-1519).

Study design and procedures

All patients were treated according to standard anesthesiologic and surgical procedures, including the placement of an arterial catheter. According to Dutch protocol, two grams of cefazolin (Eurocept international, Netherlands) were administered as an intravenous bolus at induction of anesthesia, i.e. between 0 and 60 minutes prior to incision. An additional dose was given after four hours if the surgery was expected to last longer; one gram cefazolin in case the remaining time of the procedure was less than 3 hours. If the remaining time was more than three hours, two grams of cefazolin were administered.

The blood sampling scheme of two milliliters samples, is shown in Figure 1. In case additional perioperative dosing was necessary, samples five and six were taken just before redose and 10-20 min after administration of the dose. Sample number eight was taken during wound closure. In the postoperative phase three samples were collected.

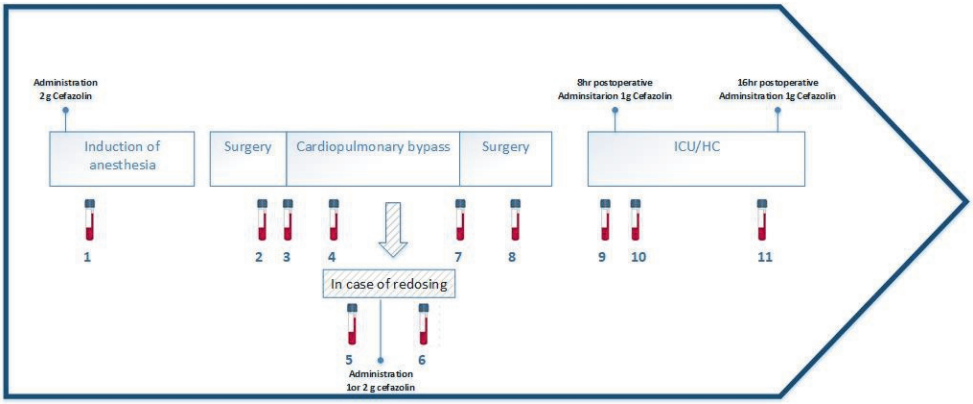


Fig 1: Study overview with timing of cefazolin administration and blood sampling. Blood sampling: 1-10 min after administration of 2 grams cefazolin (1), before cannulation of CPB (2), right after start CPB (3) and 30-40 min after start CPB (4). In case surgery duration was longer than 4 hours, a redose was given and sample 5 (predose) and 6 (10-20 min after dosing) were drawn. Other samples: during weaning from CPB (7) and during sternal wound closure (8), in the postoperative phase 15 minutes before (9) and 10-60min after 8-hours postoperative dose (10) and 15 min before 16-hours postoperative dose (11).

Equilibrium dialysis and bioanalytical method

Blood was collected in ethylenediaminetetraacetic acid (EDTA) coated tubes and stored at 4°C with until processing, with a time range between sample collection and processing from 30 min-12 hours at the research laboratory at the Erasmus MC. Blood was centrifuged (15 min at 3000 rpm) at ambient laboratory temperature and the plasma was transferred to polypropylene cryogenic vials with polypropylene screw caps (Sarstedt Aktiengesellschaft & Co, Nümbrecht, Germany). Samples were stored at -80°C until analysis.

To determine the unbound/free concentrations of cefazolin in the patient plasma samples, plasma samples were first subjected to *in vitro* equilibrium dialysis in the bioanalytical laboratory of BioNotus (Niel, Belgium). The equilibrium dialysis was carried out on a HTD96b (HTDialysis, USA) using dialysis membranes with a molecular weight cut-off 12–14 kDa. The dialysis experiments were conducted at 37°C against phosphate buffered saline (PBS) for 3 hours (sufficient to reach the equilibrium as indicated by a preliminary experiment). Spiked control samples were included for 50 and 150 µg/mL in triplicate. Upon completion of the equilibrium dialysis, solutions from both compartments (plasma and PBS) were aliquoted. Equal amounts of blank human plasma and blank PBS were added to PBS and plasma aliquots,

respectively, in order to process the samples and subsequently analyze them as described below.

The cefazolin concentrations in the samples obtained after dialysis were determined by LC-MS/MS on a Shimadzu UPLC-MS/MS system consisting of a Nexera X2 ultra-fast liquid chromatography (UPLC) system coupled to a Shimadzu LCMS-8050 triple quadrupole mass spectrometer (MS/MS) (Shimadzu, Japan). The bioanalytical method was developed and pre-validated according to the Food and Drug Administration (FDA) and European Medicines Agency (EMA) (9, 10) guidelines, and according to the European Bioanalysis Forum (EBF) recommendations. This method was in accordance with the acceptance criteria: the lower limit of quantification (LLOQ) within 20% of the nominal concentration and for other concentrations within 15% of the nominal concentrations. The calibration range of this method was 0.5 - 200 µg/mL. Seven calibration curve standards (CCS) were included (0.5, 1, 5, 25, 50, 100 and 200 µg/mL). Quality control (QC) samples were included in triplicate for 0.5 (LLOQ), 1.5 (LQC), 50 (MQC) and 150 (HQC) µg/mL. CCS, QC and study samples were processed by protein precipitation and flucloxacillin was used as the internal standard. Prior to each study samples analysis, the seven CCS and four QCs were injected. Afterwards incurred sample reanalysis (ISR) was performed and the criteria of less than 20% difference was met for 2/3 of the samples that were re-analyzed. The cefazolin concentrations measured in the PBS samples obtained after dialysis represent the free plasma cefazolin concentrations.

Sample size

Due to lack on literature on this topic a formal sample size calculation or power analysis was not considered feasible. Fifty percent had a BMI under 25 (kg/m^2), and the other fifty percent over 25 (kg/m^2), to give a representative sample from the general population.

Targeted minimum concentration

In published literature, a variation of minimal inhibitory concentrations (MIC) were reported. In general a concentration of 2 mg/L is accepted to prevent wound infections with the main pathogen *S. aureus* (6, 11, 12). During surgery the concentration needs to be above the targeted concentration of 2mg/L 100% of the time (100% $fT_{>MIC}$) and in the postoperative phase 40% is sufficient. The lowest *in vitro* concentration that prevents visible growth

(observed by the unaided eye) in a standardized medium over 18 +/-2 h, at 34-36 °C using a standardized inoculum (13) is defined as the MIC. These concentrations are 2-fold dilutions from 1 mg/L and thus the MIC represents an endpoint measurement that results from growth and bacteriostatic/bactericidal effects over time. A routine MIC measurement cannot be compared directly with any *in vivo* concentration. The accuracy for measurements of an MIC is not very high due to biological variation within one strain, between different strains and variation in assay used in different laboratories. Given previous mentioned challenges, MICs need to be considered a value that is a member of a probability distribution and are presented as the epidemiologic cutoff (ECOFF) (14). The ECOFF is the upper end of the wild-type distribution (i.e. highest MIC for isolates devoid of phenotypically detectable resistance)(15, 16). The EUCAST website provides a list of these ECOFF values. The minimum target for *S. aureus* is therefore 2mg/L (17, 18).

Statistical analysis

SPSS version 25 *(IBM corporation, 2017*) was used for the statistical analysis. Patient characteristics were summarized using descriptive statistics. Categorical data were compared using the X^2 or Fisher's exact test when appropriate. Normally distributed continuous variables were compared using the Independent samples T-test.

R *(Rstudio, version 3.6.1, 2019)* was used to do an additional analysis for continuous repeated measurements using mixed-models with random intercepts and time slope for patients. A more elaborate explanation of mixed models is provided in supplementary text 2.

Results

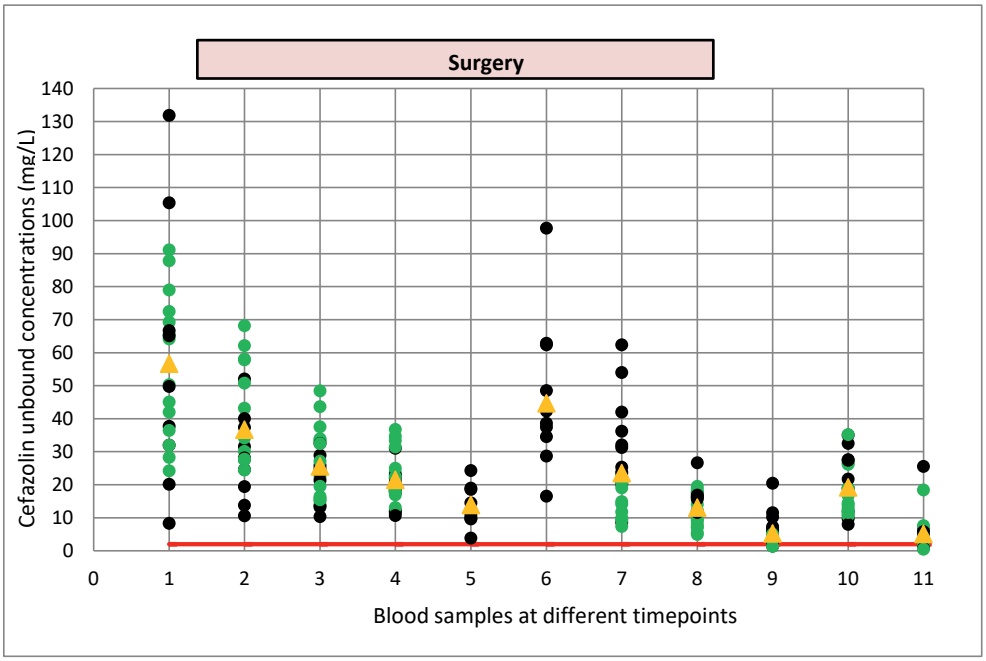
Twenty-four patients were included from March until April 2019. A summary of the patient characteristics is shown in Table 1.

A total of 227 samples were collected for the unbound cefazolin concentrations. Concentration versus time profiles are presented in Figures 1 and 2.

Table 1. Patient characteristics.

Patient characteristics (n)	**BMI <25 (kg/m2) (13)**	**BMI >25 (kg/m2) (11)**
Sex (male) (n, %)	10 (77)	8 (73)
Age (years, mean, sd)	64 (15)	63 (16)
Ethnicity (n, %)		
Caucasian	11 (84)	10 (91)
African	1 (8)	
Asian	-	1 (9)
Other	1 (8)	-
Weight (kg, mean, sd)	69.8 (15.9)	89 (20.9)
BMI ((kg/m2) , mean, sd)	22.4 (2.5)	28.3 (2.7)
Medical history (n, %)		
Hypertension	5 (38)	5 (45)
Angina	3 (23)	1 (9)
Diabetes mellitus	-	1 (9)
Hypercholesterolemia	1 (8)	2 (18)
Previous cardiac surgery	1 (8)	1 (9)
Surgical procedure (n, %)		
CABG	3 (23)	3 (27)
Valve repair	4 (30)	5 (46)
Arch replacement	2 (15)	-
ASD 2 closure	1 (8)	-
Combined procedure	3 (23)	3 (27)
Extra dose administered (n,%)	4 (33)	6 (50)
Duration of surgery (minutes, mean, sd)	249 (91)	252 (93)
CPB runtime (minutes, mean, sd)	127 (38)	146 (73)

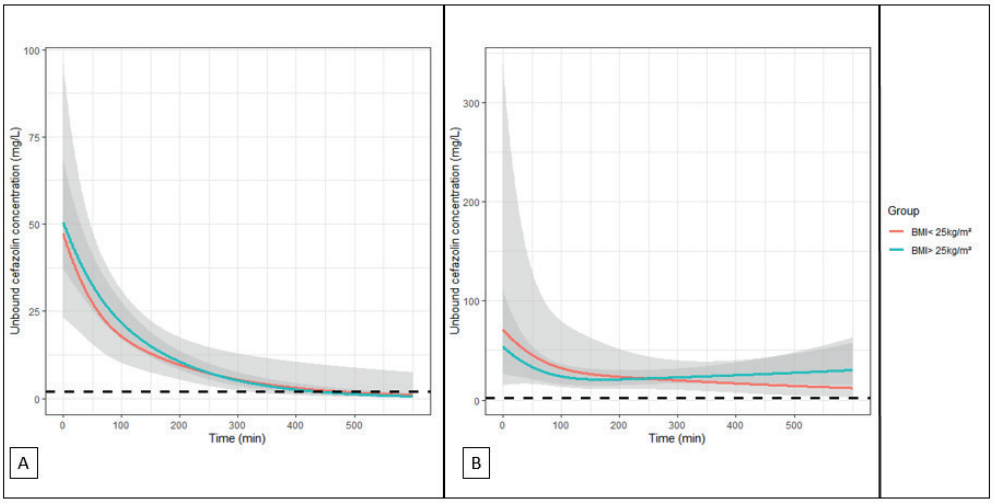
Figure 1: Unbound cefazolin serum concentrations at different time points. Blood samples were taken at 9 to 11 time points. Black dots denote the patients who did receive a redose intra-operative and sample 5 and 6 were taken. The green dots denote the patients, whom did not receive a redose intra-operative. Yellow triangles denote the median values, the red line denotes the ECOFF of 2 mg/L. Intervals between time points on the x-axis are not uniform. The intra-operative period is marked.



The concentrations were further analyzed using a continuous repeated measurements analysis using mixed-models with random intercepts and time slope for patients for the peri-operative and postoperative period. Model assumptions were checked and valid after log transformation.

The unbound cefazolin concentrations were measured in 24 patients. During surgery trough unbound cefazolin plasma concentrations were all above the ECOFF of 2 mg/L. BMI (*P= 0.8)* was not significantly associated with unbound plasma concentrations). Sex (*P= 0.8*), age (*P= 0.2*), albumin preoperative (P= *0.8*) and duration of CPB (*P= 0.8*) were also not significantly associated with cefazolin unbound concentrations in two different models. Redose (*P<0.001*) was significantly associated with higher cefazolin concentrations.

Figure 2: A) Unbound Cefazolin serum concentrations over time during surgery for 14 patients who did not receive a second perioperative dose. B) Unbound Cefazolin serum concentrations over time during surgery for 10 patients who did receive a second perioperative dose. Stratified per BMI below 25 and above 25. The grey ribbon denotes 95% confidence interval. The dashed line denotes the ECOFF of 2mg/L



In the postoperative period the unbound cefazolin concentrations were above the ECOFF of 2mg/L 85% of the time. The cefazolin unbound concentration is significant above the 2mg/L till 497 minutes after end of surgery. On average 775 minutes after end surgery the concentrations reaches levels below the targeted minimum concentration of 2mg/L. BMI (*P= 0.8)* was not significantly associated with unbound plasma concentrations. Sex (*P= 0.7*), age (*P= 1*) and albumin postoperative (P= *1*) were also not significantly associated with cefazolin unbound concentrations.

The unbound cefazolin concentration was below 2 mg/L 10 times in 9 patients, all in the postoperative phase. There was no significant association between covariates and a concentration below the 2mg/L. (Table 2).

Table 2. Univariate Poisson analysis of subject and surgery related variables for unbound cefazolin concentration during study period

Variables	**Unbound cefazolin concentrations <2mg/L**	
	Estimates	*P*
BMI group	-0.41 (-1.76, 0.85)	*0.5*
Pre-operative albumin level	0.11 (-0.08, 0.36)	*0.6*
Duration surgery	-0.009 (-0.02, 0.0006)	*0.1*
Duration CPB	-0.02 (-0.04, -0.003)	*0.06*
Age	-2.34 (-2.46*10^-15, 2.38*10^-15)	*0.8*
Sex	-1.17 (-4.08, 0.50)	*0.3*

Discussion

This study evaluates the new Dutch protocol for cefazolin prophylaxis during cardiac surgery in adults. Our primary question was if the changed dosing scheme decreased the cefazolin concentration to below the minimum target value. Current antibiotic regimen according to the recently implemented quidelines resulted in adequate cefazolin concentration in all patients.

From literature, the ECOFF for common pathogens in cardiac surgery is often considered to have at least a value of 2 mg/L. The American guideline on peri-operative antibiotic prophylaxis states that the antimicrobial agents should be administered at the right time to provide adequate serum and tissue concentration exceeding the ECOFF for the probable organism associated with the procedure, at the time of incision and the duration of surgery (4). The EACTS guideline states that most pathogens isolated from patients with SSI are gram positive bacteria(5). Calic et al. used a MIC of 8 mg/L corresponding with a total concentration of 40 mg/L assuming the targeted minimum concentration needs to be 4 times the MIC of 2mg/L for maximal bactericidal effect. The authors reported total concentrations and

observed that almost 10% of samples taken at closure were below 40 mg/L, as were almost 40% of intraoperative trough levels at the time of redosing(12). Lower body weight and shorter duration of surgery were significantly associated with cefazolin concentration <40 mg/L at wound closure, probably due to decreased cefazolin administration at time of redosing. This lack of redosing, or decreased cefazolin dosage could also account for our results. In the current protocol, a redose is administered at 4 hours after initial dose pre-operative of 2 grams, eg 1 gram if expected to last <3 hours, 2 grams if expected to last over 3hours. In our cohort duration of CPB was not significantly (*P= 0.06*) associated with cefazolin unbound concentrations below the ECOFF of 2mg/L and all concentrations during surgery were above 2mg/L.

We also expected to see a difference in the cefazolin concentrations between patients in the different BMI groups (< 25 vs 25-40 kg/m2). In literature, results on interactions between bodyweight and cefazolin concentration are inconsistent. Calic et al showed that lower body weight is significantly associated with lower cefazolin concentrations at wound closure. Others report that bodyweight is the strongest predictor of volume of distribution and unbound cefazolin concentration reduces with increasing body weight (19). Van Kralingen et al showed that unbound cefazolin concentrations at 240 min of surgery were not correlated with total bodyweight. These studies were performed in morbidly obese patients. We did not include patients with an BMI >40 kg/m^2 , which could account for the differences between our findings and the literature.

Measuring unbound cefazolin concentration in plasma only gives an indication of the efficacy. The actual targeted minimum concentration needs to be achieved in the tissue. Howard et al showed that the unbound fraction of cefazolin in plasma correlated well with the interstitial fluid (20). Brill et al showed that tissue concentration of cefazolin was decreased in morbidly obese patients, requiring adjusted dosing scheme (19). Based on this we can assume that the patients in our cohort (BMI < 40 kg/m^2) attained sufficient tissue concentrations.

It is often stated that the target concentration needs to be maintained until 24 hours after surgery. It has already been shown that prophylaxis up to 48 hours is not necessary (5). The most important moment for adequate tissue and serum cefazolin concentration is during wound closure (19). From microbiologic point of view 100% $fT_{>MIC}$ is not necessary in the

postoperative period because manipulation of the surgery is finished and a beginning infection is treated. As treatment 40% $fT_{>MIC}$ should be sufficient. This is based on effectivity studies in mice where 30 to 40% 100% $fT_{>MIC}$ to produce a net bacteriostatic effect(21, 22) All of our patients maintained adequate plasma levels during wound closure, all were above the ECOFF of 2mg/L. This could be sufficient to prevent surgical site infection. In the postoperative period 85% was above the ECOFF of 2 mg/L for *S.aureus*. However there are more bacteria that can cause a surgical site infection. The second most common bacteria to cause DSWI after cardiac surgery are Gram-negative bacilli (23-25), e.g. *E.coli* which has a higher ECOFF of 4mg/L. However this is based on only 285 observations. Furthermore, if an infection occurs with gram-negative bacilli, cefazolin is not the choice of antibiotic for treatment. In our study we used the ECOFF of 2mg/L, because this is the most common pathogen *S. aureus*. This confirms that the current guideline is not adequate for other bacteria that can cause surgical site infections. Our study was not adequately powered to detect an increase in surgical site infection in our population.

Conclusion

Our study investigated unbound cefazolin concentration in adult patients undergoing cardiac surgery, after introduction of the new Dutch protocol for antibiotic prophylaxis. We found that cefazolin concentration remained above the ECOFF of 2mg/L during surgery. In the postoperative phase the cefazolin concentration was above the ECOFF 85 % of the time. The current guideline is adequate. In the near future we aim to identify patient and surgery specific factors that influence cefazolin total and unbound concentration during cardiac surgery and go more in depth in the protein binding using population pharmacokinetics.

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Supplementary 1: Elaborate explanation mixed-models

Continuous repeated measurements are analyzed using mixed-models with random intercepts and time slope for patients. The models are visualized by effect plots.

Spline function

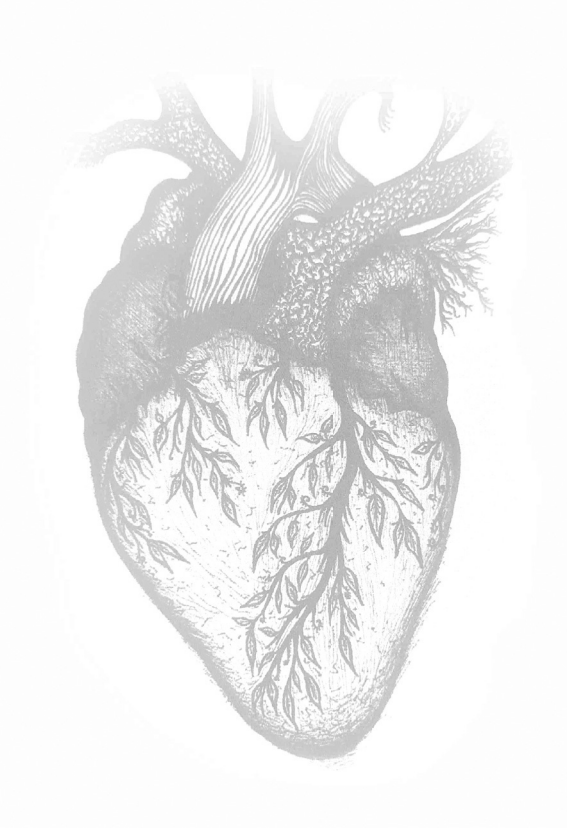
A spline is a function with knots (cut points) that cut data in several pieces, or in others words: A piece-wise polynomial. Using this function one can compute a regression coefficient for e.g. the first part of the data and de second part, using 1 knot.

Models with only the time effect

Natural splines as fixed and random effects for time were added to establish flexibility, starting with 2 knots to zero knots. In order not to lose interpretability of splines in random and fixed effect, the statistical models always contained the same number of knots in random as fixed effects. Model performance for different number of splines were compared using AIC and BIC, and the model with the lowest AIC and BIC was chosen. In case of disagreement of AIC and BIC; estimated longitudinal evolution was plotted in 20 random patients and the most suitable evolution was chosen, taking into account smoothness of the line. Effect plots are truncated when <10% of the data/patients remained in the study, or when remaining patients dropped below 5. QQ-plots of standardized residuals were inspected to determine if model assumptions were violated.

Models with time, another covariate and their interaction

All models contained time, the covariate of interest and their interaction term as independent variables. The interaction term was included in order to asses difference courses over time. The method of choosing the number of knots in the spline function for time is described above



Chapter 7

An international survey of management of pain and sedation after paediatric cardiac surgery

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Abstract

Objective: The mainstay of pain treatment after paediatric cardiac surgery is the use of opioids. Current guidelines for its optimal use are based on small, non-randomized clinical trials, and data on the pharmacokinetics (PK) and pharmacodynamics (PD) of opioids are lacking. This study aims at providing an overview of international hospital practices on the treatment of pain and sedation after paediatric cardiac surgery.

Design: A multicentre survey study assessed the management of pain and sedation in children aged 0-18 years after cardiac surgery.

Setting: PICUs of nineteen tertiary children's hospitals worldwide were invited to participate. The focus of the survey was on type and dose of analgesic and sedative drugs and the tools used for their pharmacodynamic assessment.

Results: Fifteen hospitals (response rate 79%) filled out the survey. Morphine was the primary analgesic in most hospitals and its doses for continuous infusion ranged from 10-60 mcg kg^{-1} h^{-1} in children aged 0-36 months. Benzodiazepines were the first choice for sedation, with midazolam used in all study hospitals. Eight hospitals (53%) reported routine use of sedatives with pain treatment. Overall, type and dosing of analgesic and sedative drugs differed substantially between hospitals. All participating hospitals used validated pain and sedation assessment tools.

Conclusion: There was a large variation in the type and dosing of drugs employed in the treatment of pain and sedation after paediatric cardiac surgery. As a consequence, there is a need to rationalize pain and sedation management for this vulnerable patient group.

What is known about the subject

- There is large variability in choice and dosing of analgesics and sedatives after cardiac surgery in children worldwide.
- Validated pain and sedation tools were used extensively.

What this study adds

- Insight into clinical protocols on use of analgesics and sedatives in children after cardiac surgery, showing large variability in choice and dosing of analgesics and sedatives.
- Morphine is the first choice analgesic, midazolam the first choice sedative. Dosing of both drugs differ considerably between hospitals.
- Use of validated PD assessment tools is not standard in clinical practise. Lack of a validated PD assessment tool could result in oversedation.

Introduction

Congenital heart disease (CHD) accounts for almost one third of all congenital defects (1). Adequate postoperative sedation and pain management is important in these patients because untreated pain can lead to a delayed recovery, prolonged adverse behavioural consequences and negative physiologic responses (2-4).

Morphine is widely used for analgesia after major surgery in neonates and children. Several studies on morphine PK/PD have resulted in age specific dosing algorithms in children after non-cardiac surgery. Ceelie et al. showed equipotency of paracetamol as primary analgesic as compared to morphine in neonates and children < 1 year of age after major non-cardiac surgery. It is currently unclear if these data can be extrapolated to children after cardiac surgery (5, 6).

Postoperative analgosedation in children after cardiac surgery is mainly achieved with opioids combined with sedatives. The most common opioid used is morphine, with doses ranging from 5-80 mcg kg^{-1} h^{-1} (7). Morphine is recommended as drug of first choice by the Association of Paediatric Anaesthetists of Great Britain and Ireland (8). However, this guideline is based on small, non-randomized clinical trials. PK data are available for the routinely prescribed analgesics and sedatives, but combined PK/PD data are scarce.

Lynn et al. described adequate pain relief after cardiac surgery with continuous morphine infusions of 10-38.5 mcg kg^{-1} h^{-1} (9, 10). Even though adequate analgesia can be achieved, plasma morphine concentrations above 20 ng ml^{-1} have been associated with adverse effects, such as hypotension and respiratory depression (9, 11). Patients with cyanotic heart defects showed lower morphine requirements and higher plasma concentrations compared to patients with non-cyanotic heart defects, indicating that type of defect or type of surgery may be associated with altered PK/PD necessitating different dosing regimens (12).

A recent review by Lucas et al. (13) on pharmacotherapies in paediatric cardiac critical care provides an extensive overview of PK of analgesic and sedative drugs used in children after cardiac surgery but focused less on their use in protocols for clinical practice. Changes in clearance and volume of distribution (PK) and/or PD due to the use of cardiopulmonary bypass (CPB), disease processes, low cardiac output syndrome, surgical procedure and age may alter

optimal way of dosing analgesics and sedatives in children after cardiac surgery (14). These expected PK/PD differences are not incorporated in existing guidelines and it is unclear if they are introduced into local protocols.

While current dosing is commonly titrated to effect (sedation or pain score), quantification of that effect can be difficult as pain and sedation scores are not always validated for different patient groups. A one size fits all dosing regimen may lead to over or under sedation resulting in less efficacy or increased toxicity. As clear individualized evidence based dosing guidelines are lacking a wide variety can be expected in clinical practice.

Our primary objective was to ascertain international analgosedation practices after paediatric cardiac surgery with a self-reported survey. Our main focus was the use of local protocols, choice and dosing range of analgesics and sedatives, use of pain and sedation scores and the use of treatment algorithms.

Methods

Design

A self-designed web-based survey (Monkey Survey, https://nl.surveymonkey.com/) was circulated to medical specialists in tertiary cardiac care hospitals who are responsible for the treatment of children after cardiac surgery. Hospitals were selected based on expertise and yearly conduct of more than 150 paediatric on-pump cardiac surgical procedures. The survey was designed by a small focus group consisting of a congenital cardiothoracic surgeon, three paediatric intensivists and a paediatric cardiac anaesthesiologist because of the absence of validated questionnaires. The potential respondents were instructed that this survey aimed at collecting data on the current treatment strategies concerning the use of analgesics and sedatives, as well as the tools used for the measurement of pain and sedation, according to their local protocols and not their personal preference. The survey has been provided in the appendix.

The survey focused primarily on the choice and dosing regimens of analgesic and sedative drugs prescribed in the surveyed institutions as well as the PD assessment tools that were used in these circumstances. Additional questions related to the characteristics of the unit.

Potential participants initially received a letter asking for their involvement in the survey. If they agreed details of the survey as well as the link to enter their answers were provided by email. If necessary an additional email to remind the participants about the survey was sent two and four weeks after the initial letter. Data were collected between June and August 2014.

Ethical approval was not needed for this study, since no patients are involved neither person related questions are raised to the individual hospitals.

Results

Hospital characteristics

A total of 19 hospitals on three different continents were willing to participate; 15 (response rate 79%) hospitals completed the survey in full. Twelve respondents (80%) were from European hospitals. Three respondents were from non-European hospitals based in New-Zealand, Australia and Canada. Non-respondents were based in the USA (1), United Kingdom (2) and China (1). Respondents were physicians, mainly paediatric intensivists or paediatric cardio-anaesthesiologists who work in paediatric cardiac critical care units. Two paediatric intensivists reported that they had consulted a paediatric cardio-anaesthesiologist on questions relating to the perioperative management.

The participating hospitals perform a total of over 3000 on-pump paediatric cardio-surgical procedures annually with a postoperative ICU stay ranging between 2 and 7 days.

The number of procedures per age category varied between hospitals, however about two third of the procedures were performed in children under the age of 1 year.

Medication

Table 1 shows the type and dosing of reported analgesics. Table 2 shows the type and dosing of reported sedatives. Both tables show the results for treatment protocol in neonates and children until the age of two years.

There was a wide range of choices and dosing regimens of drugs reported for both analgesics and sedatives. Moreover, polypharmacy is often used to accomplish the desired effects for

both analgesia and sedation. Eleven different analgesics and eight different sedatives were reported.

None of the hospitals based analgosedation according to protocol on cardiac diagnosis, severity scores or type of surgery. One hospital added fentanyl for analgesic therapy in patients returning from the operating room with an open sternum.

Analgesics

Opioids were the preferred analgesic; morphine was the opioid of choice in 13 (87%) of the hospitals. Dosing ranged from 10-60 mcg kg^{-1} h^{-1} for a continuous infusion and from 50-500 mcg kg^{-1} for a bolus dose. Morphine was supplemented with a second analgesic in 73% of hospitals, either as standard practice or rescue therapy. The primary choice of analgesics varied between hospitals but did not differ between age groups within hospitals. Most drugs were dosed according to weight. However, overall dosing ranges in neonates tended to be lower compared to infants and children. Furthermore in children over two years of age more alternative analgesic drugs were reported as used per protocol in some hospitals; namely oxycodone, nalbuphine and diclofenac.

Dexmedetomidine could be considered a sedative but was reported as an analgesic in the survey and therefore reported as such.

Table1: Results of the international survey for type and dose of analgesics in children after cardiac surgery

Medication	**Neonates 0-28 days**		**Infants 29 days – 2 years**	
	Use in hospitals (n)	*doses*	*Use in hospitals (n)*	*doses*
Morphine IV bolus mcg kg^{-1}	9	50-200	9	50-500
Morphine IV mcg kg^{-1} h^{-1}	12	5-40	12	10-60
Piritramide IV bolus mg kg^{-1}	1	0.2-1.2	2	0.05-0.4

	Neonates 0-28 days		Infants 29 days – 2 years	
Piritramide IV mg kg^{-1} day^{-1}	n.a.	n.a.	1	1.2
Fentanyl IV bolus mcg kg^{-1}	1	1-2	1	1-2
Fentanyl IV mcg kg^{-1} h^{-1}	3	1-6	3	1-6
Remifentanil IV bolus mcg kg^{-1}	1	1	1	1
Remifentanil IV mcg kg^{-1} min^{-1}	1	0.1-0.2	1	0.1-0.2
Sufentanil IV mcg kg^{-1} h^{-1}	1	1- 2	1	1- 2
Dexmedetomidine IV bolus mcg kg^{-1}	n.a.	n.a.	1	50
Dexmedetomidine IV mcg kg^{-1} h	n.a.	n.a.	2	0.5-1.5
Paracetamol IV mg kg^{-1} Q6h	5	7.5	7	7.5-15
Paracetamol PO/PR mg kg^{-1} day^{-1}	5	45-90	7	45-90
Metamizol IV mg kg^{-1}	1	40	1	40
Diclofenac IV/PR mg kg^{-1} day^{-1}	n.a.	n.a.	3	1-3
Ibuprofen PO bolus mg kg^{-1}	n.a.	n.a.	2	5-10
Dexketoprofen IV mg kg^{-1}	1	0.5-1	1	0.5-1

Hospitals represented in the survey: Erasmus MC-Sophia, Rotterdam; LUMC, Leiden; UMC Utrecht; UMC Groningen; Our Lady's Children's Hospital, Crumlin; Children's Hospital Bambino Gesù, Rome; Royal Brompton Hospital, London; Royal Children's Hospital, Melbourne; University Hospital, Leuven; University Hospital La Paz, Madrid; Starship Children's Hospital, Auckland; Hospital for Sick Children, Toronto; German Heart Centre, Munich; Queen Silvia Hospital Gothenburg, Memorial Hospital – Child Health Centre, Warsaw. IV: intravenous, mcg: microgram, kg: kilogram, h: hour, Q: each, min: minute, PO: per oral, PR: per rectum, n.a.: not applicable

Sedatives

Midazolam was the primary sedative in 100% of hospitals, either as a bolus or a continuous infusion. Eight hospitals (53%) reported routine use of sedatives with pain treatment. The other hospitals only started sedatives in response to discomfort. Of eight hospitals who routinely use sedatives with pain treatment, six used morphine as primary analgesic in an average dose of 10-30 mcg kg^{-1} h^{-1} with one outlier using morphine from 30-60 mcg kg^{-1} h^{-1}, in neonates and infants, respectively. Two other hospitals who routinely use sedatives with pain treatment used piritramide (dose 1.2 mg kg^{-1} d^{-1}) and fentanyl (dose 5 mcg kg^{-1} h^{-1}) as primary analgesics. Average dosing of morphine in hospitals without routine sedation was 5-40 mcg kg^{-1} h^{-1}. Overall morphine dosing in hospitals that use standard sedatives are comparable or lower than hospitals that do not use standard sedatives.

Sedatives were used as per protocol or at the attending physician's discretion. Treatment strategies between neonates and infants varied less for sedatives than for analgesics. There were no reported differences in choice of drugs and dosing between infants 29 days-2 years and children older than 2 years.

Table 2: Results of the international survey for type and dose of sedatives in children after cardiac surgery.

Medication	**Neonates 0-28 days**		**Infants 29 days – 2 years**	
	Use in hospitals (n)	*doses*	*Use in hospitals (n)*	*doses*
Midazolam IV bolus mg kg^{-1}	12	0.05-1.5	12	0.05-1.5
Midazolam IV mg kg^{-1} h^{-1}	15	0.06-4	15	0.06-0.5
Clonidine IV bolus mcg kg^{-1}	3	0.5-2	3	0.5-2
Clonidine IV mcg^{-1} kg^{-1} h^{-1}	7	0.5-2	7	0.5-2
Lorazepam PO mg kg^{-1}	3	0.05	3	0.05
Propofol IV bolus mg kg^{-1}	3	1	3	1

	Neonates 0-28 days		Infants 29 days – 2 years	
Propofol IV mg kg^{-1} h^{-1}	3	1-6	3	1-6
Esketamine IV bolus mg kg^{-1}	1	0.5-1	1	0.5-1
Esketamine IV mg kg^{-1} h^{-1}	1	0.5-1.5	1	0.5-1.5
Chloral hydrate IV mg kg^{-1}	3	10-50	3	10-50
Chloral hydrate NG mg kg^{-1}	1	12.5-25	1	12.5-25
Promethazine mg kg^{-1}	1	0.5-1.5	1	0.5-1.5
Chlorpromazine mg kg^{-1}	2	0.5-1.5	2	0.5-1.5

Hospitals represented in the survey: see table 1. IV: intravenous, mg: milligram, mcg: microgram, kg: kilogram, h: hour, PO: per oral, NG: nasogastric

Sedation scores

All hospitals used a validated pain and sedation score. Table 3 shows the different scores used. Pain and sedation was assessed using a total of six different paediatric pain and four different sedation scores. Eleven (73%) of the 15 hospitals used the COMFORT-B scale and Numeric Rating Scale (NRS) for pain and sedation. Frequency of pain and sedation assessment varies between hospitals. Reassessment after an intervention, either medical or non-medical, was reported by two respondents.

Each centre reported the use of a local protocol to guide analgosedation after cardiac surgery.

Table 3: PD tools reported in the survey.

Scale	Validated age range	Number of centres	How often assessed first 72 hours after surgery? (Minimal and maximal)
Pain assessment			
FLACC[33]	2 months- 7 years	2	n.s.
CRIES[34]	0-28 days	1	n.s.
COMFORT-B scale[35-37]	0 - 3 years	1	n.s.
VAS pain obs	0-3 years	7	8 hourly, after bolus
NRS pain obs	0-3 years	4	2-4 hourly, after bolus
LLanto scale		1	n.s.
Sedation assessment			
NISS[38]	0-18 years	2	8 hourly, after bolus
COMFORT-B scale[38]	0-18 years	11	4-8 hourly, after bolus
Brussels Sedation Scale [39]	Adults	1	n.s.
Ashworth scale [40]		1	n.s.

FLACC: acronym for Face, Legs, Activity, Cry, Consolability, CRIES: acronym for Crying, Requires O2 for SaO2 <95%, Increased vital signs (Blood pressure and Heartrate), Expression, Sleepless, COMFORT-B: COMFORT-behavioural scale, VASobs: Visual Analogue Scale observation, NRS: Numeric Rating Scale pain observation, LLANTO SCALE: acronym for llanto, actitud, normorrespiración, tono postural y observación (crying, attitude, respiratory pattern, muscle tone and facial), NISS: Nurses' Interpretation of Sedation Score, N.S.: not specified, Mo: month, Y: years.

Discussion

The choice and dosing regimens of analgesics and sedatives after cardiac surgery in children varied extensively across the globe. Opioids were the analgesics of choice. Morphine was the preferred analgesic drug, with a wide range of doses, both for continuous infusion and bolus administration in both the neonatal age group and in older infants and children. Morphine was supplemented by a second analgesic drug in 73% of the surveyed hospitals. Differences between local protocols were evident in all age groups, however more variation in analgesics and sedatives was found in infants and children as compared to neonates. The underlying cardiac diagnosis, severity score or type of surgery did not result in different treatment algorithms or dosing regimens. Eight hospitals routinely used a sedative in combination with pain treatment, all other institutions started sedatives only in response to a clinical need for sedation.

The reported use of drugs are comparable with those described by Wolf in 2011 and reflect in part the guidelines from the Royal College of Paediatrics and Child Health (United Kingdom) as well as the guidelines from the Association of Paediatric Anaesthetists of Great Britain and Ireland (7, 8). The recent consensus statement by Lucas et al., describes the pharmacotherapies currently available to manage pain and sedation in paediatric cardiac critical care patients and summarizes dosing recommendations from available literature (13). Lucas and colleagues conclude that a more individualized analgesic and sedative treatment strategy is necessary to provide optimal care without adverse effects resulting from pharmacotherapy.

This need for individualised dosing is possibly reflected in the reported wide range of dosing for morphine with the highest morphine infusion rate of 60 mcg kg^{-1} h^{-1} and largest bolus of 500 mcg kg^{-1}in the participating centres as well as the use of adjuvant analgesics and sedatives. However, doses mainly differed between hospitals, not within hospitals. Differences in morphine dosing could also reflect differences in local practices and preferences between hospitals rather than individualised dosing regimens based on clear PD endpoints.

Ideally we would like to predict individual morphine requirement beforehand and better categorise the efficacy of adjuvant or alternative analgesics to minimize adverse effects. Advances towards precision medicine have been made for morphine in non-cardiac surgery

patients mainly focusing on the patients' size, maturation and organ function (6, 15, 16). By using information from PK/PD studies on morphine consumption after cardiac surgery we aim to individualize and assess treatment effect by regular pain and sedation assessment and tracking of adverse drug reactions. However, PK parameters of analgesics and sedatives, or potential PK alterations in children after cardiac surgery are currently incomplete. Changes of clearance and volume of distribution would be expected in this cohort, dependent on the use of the CPB, age and underlying pathology. For remifentanil (17, 18), dexmedetomidine (19, 20), clonidine (21) and ketamine (22), studies have been published within the last 10 years with PK parameters in neonates and children after cardiac surgery. However, these studies show conflicting results on PK alterations and most lack PD endpoints to assess efficacy, making it difficult to implement dose recommendations in clinical practice.

Due to polypharmacy it is difficult to assess the efficacy and safety of individual drugs. Our survey showed that a multimodal drug approach is often used for analgesics and sedatives. The challenge is to determine how these drugs interact (23, 24). The combination of sedatives and opioids may contribute to oversedation, which is highly undesirable and could lead to longer PICU stay, longer ventilation times, drug tolerance and dependence (25).

PD aspects after cardiac surgery are rarely described in literature, making interpretation of PK knowledge clinically limited. Validated PD scoring tools were used in our survey hospitals; mainly the Comfort-B scale (73%), VAS (47%) and the Numeric Rating Scale (26%). Interpretation of some scores can be problematic, because of poor validation in neonates and infants after cardiac surgery. Moreover, items for rises in blood pressure and heart rate are less useful in children after cardiac surgery because of the use of inotropic agents.

This study has several limitations. Clinical practice may deviate from protocol which might not be reflected in the survey. Also, the participating hospitals are all based in developed countries, mostly in Europe. Although the data from our study seem to reflect the day-to-day practice of analgosedation after cardiac surgery in children, we cannot rule out that some selection of the hospitals that were approached and that responded may have an effect on the diversity of the findings. A larger survey might increase the amount of variability or show more consensus within countries.

Conclusion

This survey shows that there is large variability in both dosing and choice of analgosedative drugs used in pediatric post cardiothoracic surgery patients especially between hospitals. This large variability reflects the complexity of analgosedation in these vulnerable patients and highlights the need for clinical studies combining PK with validated PD outcomes. Such studies are necessary to understand specific changes in this population and permit evidence-based and personalised treatment protocols.

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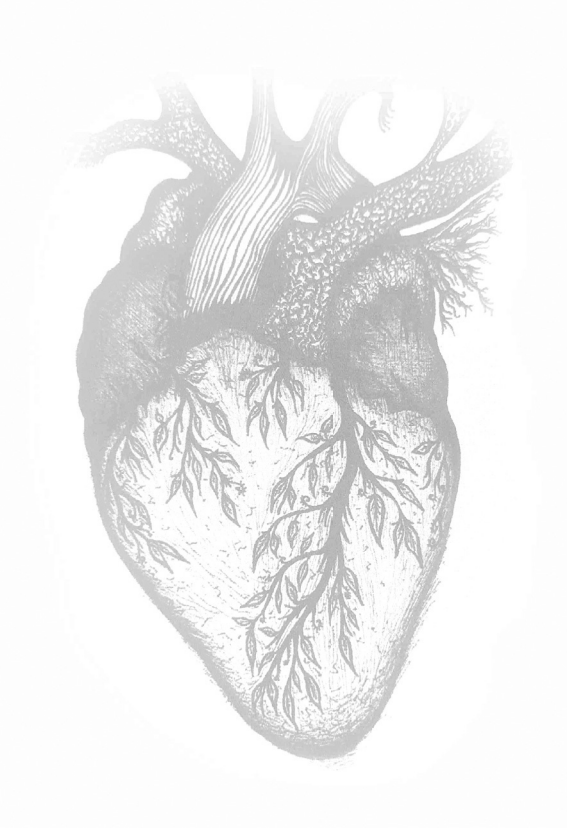

Chapter 8

Intravenous morphine versus intravenous paracetamol after cardiac surgery in neonates and infants; a randomized controlled trial

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Abstract

Background: Morphine is worldwide the analgesic of first choice after cardiac surgery in children. Morphine has unwanted hemodynamic and respiratory side effects. Post cardiac surgery patients may therefore potentially benefit from a non-opioid drug for pain relief. A previous study has shown that intravenous (IV) paracetamol is effective and opioid sparing in children after major non-cardiac surgery. The aim of the study is to test the hypothesis that intermittent IV paracetamol administration in children after cardiac surgery will result in a reduction of at least 30% of the cumulative morphine requirement.

Methods: A prospective, multi-center, randomized controlled trial at four level 3 pediatric ICUs in the Netherlands and Belgium. Children aged 0-36 months will be randomized to receive either intermittent IV paracetamol or continuous IV morphine up to 48 hours postoperatively. Morphine will be available as rescue medication for both groups. Validated pain and sedation assessment tools will be used to monitor patients. The sample size (n=208, 104 per arm) was calculated in order to detect a 30% reduction in morphine dose, with a two-sided significance level of 5% and a power of 95%.

Discussion: This study will focus on the reduction, or replacement, of morphine by IV paracetamol in children aged 0-36 months after cardiac surgery. The results of this study will form the basis of a new pain management algorithm and will be implemented at the participating ICUs, resulting in an evidence-based guideline on postoperative pain after cardiac surgery in infants aged 0-36 months.

Background

Congenital heart disease (CHD) accounts for almost one-third of all congenital anomalies, with a reported total prevalence in Europe of 8.0 in 1000 births (1, 2). Surgical intervention is necessary within the first year of life in 55% and in 67% during the first three years of life (3).

The importance of adequate postsurgical pain relief in neonates and infants became apparent after findings that untreated pain results in increased stress hormone levels and prolonged behavioral consequences (4). These findings have resulted in an increased use of morphine as the world-wide standard for pain relief after major surgery in neonates and children (5-9). However, morphine can cause unwanted hemodynamically and respiratory reactions and therefore patients could potentially benefit from a non-opioid analgesic.

In a recent randomized controlled trial iv paracetamol was compared to morphine as a primary analgesic drug in non-cardiac postoperative children up to 1 year. IV paracetamol was equally effective in pain relief and no difference in rescue analgesics was shown between groups (10). The iv paracetamol group had a lower cumulative morphine dose the first 48 hours after surgery and less adverse drug reactions. Whether these results also apply to neonates and children after cardiac surgery is unclear. Pharmacokinetic (PK) parameters are assumed to be different in patients during and after cardiac surgery compared to non-cardiac surgery and changes in pharmacodynamics (PD) and pharmacogenetics (PG) concerning pain perception need to be taken into account.

Cardiothoracic Surgery

Based on the general anesthesia guidelines, opioids are considered standard of care in children to prevent and treat postoperative pain after cardiac surgery even though clear PK data is lacking (11). There are reasons to assume that children after cardiac surgery have different PK and/or PD compared to adults or children after non-cardiac surgery. Valkenburg et al. describes a lower clearance of morphine and a higher volume of distribution in 38 children after cardiac surgery compared to non-cardiac surgery, possibly necessitating a different dosing regimen (12).

The use of cardiopulmonary bypass (CPB) is the main reasons to expect PK changes. CPB has a profound effect on the PK parameters because of hemodynamic changes, hemodilution,

hypothermia and systemic inflammatory reactions (SIRS). These effects change constantly throughout CPB and some continue to exert influence after the patient has been successfully weaned from CPB (13).

Hemodynamic changes affect organ perfusion and ultimately organ function. Hemodynamics may also be altered by the need for inotropic support during and after surgery. Children with a normal cardiovascular system undergoing surgery seem to clear morphine more efficiently than infants undergoing cardiovascular surgery (14). On initiation of CPB, prime fluid causes dilution of the patients' blood. This causes a shift in the bound and unbound fraction of the drug and a redistribution from peripheral to central compartments. Decreased renal and hepatic perfusion due to hypothermia, hypotension, decreased flow rate and hemodilution may result in decreased elimination of drugs (13).

Surgical correction of more complex congenital cardiac defects has an additionally influence on systemic circulation influencing PK of drugs itself, due to longer CPB run times with higher risk for severe SIRS and hemodynamic instability (13, 15, 16). However, there is currently no literature suggesting a difference in sedative or analgesic requirements based on type of congenital defect or surgical procedure.

Morphine

The elimination of morphine is mainly through glucuronidation by urine diphosphate glucuronosyltransferase (UGT) 2B7. Morphine clearance directly reflects the formation of its two major metabolites that are pharmacologically active, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Both metabolites are cleared through renal elimination, and a reduced renal function can lead to accumulation. Evidence suggests that clearance of morphine is significantly slower in children who need inotropic support after cardiac surgery (17).

The unwanted hemodynamically and respiratory effects of morphine are a particular problem in these hemodynamically unstable children and may lead to delayed recovery and prolonged PICU stay (18, 19). Other adverse effects of morphine are intestinal obstruction, which mainly occurs in younger children, whereas nausea, vomiting and itching mainly occur in older children (7).

Paracetamol

The analgesic effect of paracetamol is not yet fully understood but is likely due to interaction with the serotonergic system. Glucuronidation is the major pathway of paracetamol metabolism (50-60%), with an contribution of sulfation (25-44%) and oxidation (2-10%). Glucuronidation and sulfation result in inactive and non-toxic end products. The hepatic oxidation pathway forms NAPQI (N-acetyl-p-benzo-quinone imine) (20). NAPQI is toxic and in case of overdose causes mitochondrial dysfunction and centrilobular necrosis in the liver (21). Several studies show that, when used in therapeutic doses in children without liver dysfunction, the safety profile is excellent (20).

Enteral or rectal dosing is sufficient for mild to moderate pain. However, after major surgery rectal administration of paracetamol was shown to be insufficient to reach a therapeutic level for pain relief and does not reduce the morphine consumption (22-24). Potentially, intravenous paracetamol performs better in case of severe or acute pain. IV paracetamol rapidly penetrates an intact blood brain barrier in children, which contributes to the fast of the analgesic effect (25-27).

Pharmacogenetics

A large number of candidate gene studies has illustrated associations between genetic variants with opioid response (28, 29) and paracetamol efficacy (30). The genetic impact can arise from polymorphisms in genes that alter drug levels (PK) such as metabolizing enzymes and transporters. PK genes relevant for morphine are *UGT2B7, ABCC3* and *OCT1* (31-33).

Hypothesis

It is our hypothesis that intermittent IV paracetamol is effective as the primary analgesic drug in post cardiac surgery patients up to 3 years of age and that the use of IV paracetamol will reduce overall morphine requirements.

This hypothesis is currently being tested at three level 4 PICUs in the Netherlands and Belgium (Erasmus MC- Sophia Rotterdam, Wilhelmina Children's Hospital UMC Utrecht, Beatrix Children's Hospital UMC Groningen, UZ Leuven).

Methods

Trial design

This study is a multi-center, prospective, randomized, double blind study, with a non-inferiority design.

Study setting

The study will be conducted at 4 level 3 PICUs in the Netherlands and Belgium (Erasmus MC-Sophia Rotterdam, Wilhelmina Children's Hospital UMC Utrecht, Beatrix Children's Hospital UMC Groningen and UZ Leuven).

Interventions

All patients will receive a loading dose of morphine 100 mcg/kg at the end of surgery. After the loading dose, patients are randomized to receive either a morphine continuous infusion or intermittent intravenous paracetamol. A double dummy (intermittent or continuous infusion of NaCl0.9%) will be used in each group to ensure blinding. Patients in the intervention group receive intermittent iv paracetamol.

Justification of dosing

Howard et al. prospectively evaluated effectiveness, morphine requirements and safety of iv morphine in over 10,000 pediatric patients, including almost 1000 patients after cardiac surgery in a tertiary care hospital (34). Patients after cardiac surgery had the highest morphine requirements with an average dose of 23 mcg/kg/h (5-50 mcg/kg/h) but PK data was not collected and it is unclear whether morphine dosing was based on validated pain scores. Lynn at al. described morphine serum levels of 15-20 ng/ml in infants after cardiac surgery with continuous morphine infusions of 10-20 mcg/kg/h without describing analgesic efficacy (9). These morphine serum levels are typically associated with adequate pain relief (17). In 2009 Knibbe et al. proposed a novel morphine dosing regimen for neonates and children based on PK studies resulting in a significant dose reduction of morphine in neonates < 10 days post-natal age (35). This model was further validated with new data sets (36) and ultimately resulted in a prospective randomized controlled trial in which the proposed dosing regimen for morphine dosing was evaluated (37). This morphine dosing regimen was used in the

previous trial comparing continuous iv morphine with intermittent iv paracetamol after major non-cardiac surgery.

Several studies have determined paracetamol metabolism in children (20, 38). Pharmacokinetics of IV paracetamol in children until the age of 16 years have been described, however, no children undergoing cardiac surgery were included in this study (39, 40).

IV paracetamol will be dosed according to the Dutch pediatric formulary with a loading dose of 20 mg/kg, and maintenance dose of 10 mg/kg (<1 month of age) or 15 mg/kg (>1 month) (41).

Worldwide iv paracetamol is given in 3-4 dosages daily and not as continuous infusion. Even though continuous administration of iv paracetamol is possible, limited evidence in healthy adults shows that the analgesic effect of iv paracetamol is better with intermittent administration (42). Different delivery schedules for morphine (continuous) and paracetamol (intermittent) are therefore selected.

Control group

Patients in the control group will receive a continuous morphine infusion after the loading dose of morphine 100 mcg/kg. Based on our PK data on morphine in non-cardiac and cardiac pediatric patients (35, 37, 40) we developed a new morphine dosing regimen for neonates and children aged 0 to 36 months. The dosing algorithm is added in appendix A.

Use of co-intervention

Standard postoperative care is given to all patients with analgesic rescue medication. Short acting analgesics and sedatives are available during interventions, such as chest drain removal. All co-medications used during the first 48 hours after surgery are registered in the database.

Rescue analgesic medication

Rescue morphine will be administered with a maximum of three times per hour whenever the Numeric Rating Scale (NRS) is equal to or greater than 4. Standard additional dose of morphine is 10 mcg/kg for patients aged <10 days postnatal age and 15 mcg/kg for patients aged ≥10 days.

Ten minutes after each extra dose of morphine, pain is re-assessed. If there is no improvement in the scores (i.e. NRS ≥ 4) after three additional (rescue) doses, a morphine loading dose of 100 mcg/kg is given and a continuous morphine infusion is started at 10 mcg/kg/h in a separate pump (to ensure blinding). Whenever pain is not responding to the extra morphine boluses and the additional continuous morphine infusion in a maximum dose of 30 mcg/kg/h, fentanyl is started at 1-2 mcg/kg loading dose and 1-3 mcg/kg/h continuous infusion. At the start of fentanyl, morphine will be discontinued. In case of discomfort midazolam is started. Discomfort is determined as Comfort-Behavior (COMFORT-B) score >22 or COMFORT-B score between 11 and 22 but with the Nurse Interpretation of Sedation Score (NISS) suggesting undersedation. Standardized sedation will be part of the treatment protocol. Sedation protocols regarding the primary sedatives are already comparable between the participating ICUs, This treatment algorithm is similar with the one used in the recent study comparing morphine and paracetamol in non-cardiac patients (10).

In both groups, continuous morphine infusion (if started) will be decreased in the second 24 hours depending on the NRS and COMFORT-B score.

If discharge from the ICU occurs within 48 hours after surgery, the study medication will be continued on the ward. The arterial catheter will be removed at discharge from the ICU. PK sampling on the ward will only be done simultaneously with routine blood examination. At 48 hours after surgery, the study medication will be changed to open label paracetamol and rescue morphine if needed.

PD outcome measurements

PD outcomes will be measured using validated instruments. Both pain and under- and over sedation need to be assessed. Signs of pain and distress may overlap, making accurate assessment difficult. Therefore, the use of concomitant sedative drugs will be standardized in the participating ICUs. The COMFORT-B scale is mainly a distress assessment and to lesser extent a pain instrument that asks observers to consider the intensity of six behavioral manifestations: Alertness, Calmness, Respiratory response (for mechanically ventilated children) or Crying (for spontaneously breathing children), Body movements, Facial tension and Muscle tone. For each of these items, five descriptions, rated from 1-5, are provided reflecting increasing intensity of behavior in question. Summating the ratings of the six

behavioral manifestations leads to a total score ranging from 6-30. The COMFORT-B scale has been extensively validated in postoperative infants with and without Down syndrome and in infants after major cardiac surgery (43-46).

The Numeric Rating Scale pain (NRS) is a scale from 0 (no pain) to 10 (worst possible pain) and is used in conjunction with the COMFORT-B scale to represent the rater's expert opinion (47). The NRS focusses on pain, whereas the COMFORT-B mainly assesses discomfort. Any additional rescue morphine is given when the NRS score is four or higher. This dose escalation schedule is consistent with additional analgesic treatment in the normal clinical setting.

Parents will be able to participate in rating the pain in their children, using the NRS. This will be used alongside the nurses NRS and COMFORT-B scale. Parents will receive a very short instruction explaining the different factors that should be take into account when assigning the NRS.

Parents' participation will be on a voluntary basis. If parents indicate that active participations is not wanted anymore, they will be able to stop at any time. Variation in nurse and parent evaluation of pain or discomfort will be analyzed as a secondary endpoint.

The NISS has been validated for this age group and represents the caregiving nurse's expert opinion scored as 1= undersedation, 2= adequate sedation, or 3= oversedation (47).

Follow-up will consist of the "parents postoperative pain measurement-short form" (PPPM-SF) to complete after discharge from hospital. This tool includes 10 items asking parents about signs of pain and distress at home in children after surgical intervention (48). Parents will be called two days after discharge from the hospital to inform after the child's health and to take the questionnaire.

Patients after major cardiac surgery are also at risk for opioid withdrawal syndrome and pediatric delirium. Therefore, these two conditions were assessed as well. (33, 49).

The Sophia Observation withdrawal Symptoms-scale (SOS) has been validated to detect withdrawal syndrome in critically ill children. It contains 15 items that are scored either not present (0) or present (1). A score of 4 or higher suggests withdrawal syndrome (50, 51).

Pediatric delirium in our study will be assessed with the SOS-PD and Cornell Assessment of Pediatric Delirium (CAPD) (52). The SOS-PD consists of 17 items of which the maximum total sum score is 17 points. A total score of at least 4 is used as a cut-off for delirium or when the item “hallucination” is scored positive. The CAPD consist of 8 items, scoring the interaction of the nurse with the patient. The maximum total sum score is 32 points, with a cut-off for delirium at 9 points or greater. According to standard local protocol a pediatric psychiatrist will be consulted in case of delirium. Treatment of delirium will be done according to local protocol (53).

Severity of illness will be estimated with the validated Pediatric risk of Mortality-lll score (54) together with the more specific Risk Adjusted Classification for Congenital Heart Surgery (RACHS-1) score (55). All Dutch hospitals use the Aristotle score to classify the congenital cardiac surgery patients. This Aristotle score will also be used to estimate the severity of surgical procedure and to compare the two groups with respect to the surgical intervention (56, 57). The PELOD-2 score will be used to assess the severity of cases of Multiple Organ Dysfunction Syndrome in the PICU on day 0 (day of surgery), day 1 and day 2 (58).

PK analysis and blood sampling

Blood samples will be drawn for PKPD analysis, using an indwelling arterial catheter. Blood samples will be taken after the morphine bolus dose, directly after the start of the trial medication, 30-60 minutes after start trial medication, 3-4 hours after start trial medication, and at three standard moments during the day. The timing of the standard sampling moments is dependent on local clinical practice. Because of small timing differences this will create a diverse sampling scheme, which is very applicable for PKPD analysis. Also, samples will be taken before and after changes in the dose of analgesic medication. For ethical reasons not more than 5% of the total blood volume will be drawn from the patient. PK samples will thus be obtained by sparse sampling, with a minimal burden to the individual patients. Using population pharmacokinetics analysis, the data points from the individual subjects will be combined to form solid pharmacokinetic data on morphine and paracetamol and their metabolites, since these metabolites are biologically active. The population PK analysis will be done using non-linear mixed effect modelling (NONMEM).

DNA analysis will be performed to evaluate interindividual variability drug responses. Genetic variability of morphine and paracetamol will be the main focus.

Eligibility criteria & parental consent

Patients eligible to participate in the study will be infants and children aged 0-36 months admitted to the ICU after cardiac surgery with the use of the CPB. Information regarding the trial will be given to parents, or authorized surrogates, of potential trial participants at the out-patient clinic or at the ward. Information will be given by a researcher, either the doctor or research nurse. Written informed consent is obtained from all trial participants before surgery.

Inclusion criteria

- Informed consent,
- Neonate / infant aged 0-36 months,
- Cardiac surgery with the use of CPB.

Exclusion criteria

- No informed consent
- Known allergy to or intolerance for paracetamol or morphine,
- Administration of opioids in the 24 hours prior to surgery.
- Hepatic dysfunction defined as three times the reference value of ALAT/ASAT.
- Renal insufficiency defined as Pediatric RIFLE category - injury, defined as estimated creatinine clearance reduced by 50% and urine output <0.5 ml/kg/h for 16 hours.

In case patients develop renal or hepatic insufficiency after randomization they will be withdrawn from the trial.

Primary objectives

To test the hypothesis that analgesia with intermittent intravenous paracetamol will lead to a morphine sparing effect of at least 30% as compared to model-based continuous iv morphine infusion during the first 48 hours after cardiac surgery in infants aged 0-36 months.

The primary outcome measure is the weight-adjusted cumulative morphine consumption (mcg/kg) in the first 48 hours postoperatively.

The reduction of morphine has been chosen as the primary outcome, since this is directly related with the decrease of morphine related adverse drug reactions. The previous trial comparing iv paracetamol with morphine clearly showed a reduction of morphine related adverse drug reactions in the iv paracetamol group. Among these drug reactions are gastro-intestinal symptoms, which are also described as two out of six endpoints in the recent StEP recommendations for patient comfort (59).

Secondary objectives:

1. Incidence of adverse drug reactions
    a. hemodynamic: hypotension or bradycardia, with the need for intervention by means of medication or a fluid bolus.
    b. Decreased gastro-intestinal motility or intestinal obstruction not directly related to the underlying diagnosis and not previously existing, with the need for intervention.
    c. Vomiting.
    d. Number of re-intubations.
    e. Pediatric delirium as measured by the SOS-PD-scale or CAP-D score.
2. Non-inferiority analysis of comparing patients with one or more NRS pain scores ≥4 between groups
3. DNA analysis will be performed to evaluate the effect of gene polymorphisms on the PK of analgesic medication.
4. Concomitant use of sedatives.
5. The number of hours on ventilation.
6. The length of PICU stay.
7. Role of alarmins in the systemic inflammatory response (only at Wilhelmina Children's Hospital, UMCUtrecht). To develop a population PKPD-based postoperative pain management algorithm based on the results of this trial.

Sample size calculation

We estimate that the required morphine dose in the paracetamol group can be reduced by at least 30% compared to the morphine group in the first 48 hours after surgery. This is in line with the outcome of a previous study we conducted in a 0-1 year old patient group after major non-cardiac surgery with similar study medication (10).

The power analysis is based on a comparison of the primary outcome between groups using a Mann-Whitney test. A simulation study was done for this power analysis using data on the cumulative morphine dose from a previous study with comparable morphine dosing (10). Based on this data set, the median cumulative morphine dose was 357 mcg/kg (IQR: 220-605) in the control group, and we assumed that this morphine dose will be reduced by 30% in the intervention group. The simulation study showed that using a two-sided significance level of 5%, 86 patients per group will be required to obtain a power of 95%. To account for the effects of stratification by center and missing data, we will include 104 patients per study arm, 208 in total. We expect this sample size to also be sufficient to assess secondary outcomes.

As described above, the primary endpoint of the study is the total amount of morphine administered in the first 48 hours after surgery. Any additional morphine given will be based on NRS scores of 4 and higher. In a secondary, non-inferiority analysis, we will therefore also compare the percentage of patients with one or more NRS scores of 4 and higher between the two groups, using a non-inferiority margin of 20%, based on the previous trial and clinical experience. NRS-pain scores are assessed often per patient and should give a clear indication of number of painful moments per treatment group. Since NRS scores are recorded often per patient we set the non-inferiority margin on 20%, assuming this will reflect a clinically relevant difference. Non-inferiority will be assessed using a one-sided 97.5% confidence interval for the difference in the percentage of patients with at least one NRS score of 4 or higher between the paracetamol group and the morphine group, and non-inferiority will be proven if the upper limit of this confidence interval is lower than 20%. The confidence interval will be calculated using the method of Klingenberg , with adjustment for center. Using data from our previous study (10) on paracetamol and morphine it is estimated that 60% of all patients will have one or more NRS scores >3. Using a simulation study, we calculated that to detect non-inferiority with a power of 75%, 200 patients (100 per group) are required. Even though the

power is 75%, this is considered to be sufficient for this secondary endpoint. This means that the sample size of 104 patients per group is sufficient for the non-inferiority analysis.

Randomization, blinding and treatment allocation

Blocked randomization with randomly chosen block sized and stratification by center will be used. The randomization schedule will be kept in the local pharmacy at every center. The pharmacist is the only person to have access to the randomization schedule to ensure concealed allocation. The randomization schedules are made by the study's biostatistician. Study medication will be prepared at the participating centers. We will use standard morphine and paracetamol formulations.

In case of a medical emergency the pharmacists can be consulted what medication was administered to a patient.

Statistical methods

The non-parametric Van Elteren test with stratification by center will be used to compare the primary outcome of cumulative age-adjusted morphine between groups.

Analysis of secondary outcomes will include length of PICU stay, number of hours on ventilation and concomitant use of sedatives . These secondary outcomes will be compared using linear regression analysis with group and treatment center as categorical independent variables. Analysis using linear regression models will be performed with cumulative rescue morphine (first 48 hours) as outcome variable and group (morphine vs paracetamol) as predictor variable. Center, Down syndrome (yes/no) and cyanotic vs non-cyanotic cardiac defects will be added as covariates. Robust regression models will be used when necessary, i.e. when the outcome variable is non-normally distributed.

In a secondary, non-inferiority analysis, we will also compare the number of patients with one or more NRS scores of 4 and higher between the two groups, using a non-inferiority margin of 20%. Non-inferiority will be assessed using a one-sided 97.5% confidence interval for the difference in the percentage of patients with at least one NRS score of 4 or higher between the paracetamol group and the morphine group, and non-inferiority will be proven if the upper limit of this confidence interval is lower than 20%. The confidence interval will be calculated using the method of Klingenberg (Stat Med 2014), with adjustment for center.

Adverse drug reactions will be specified as hemodynamic, gastro-intestinal, respiratory reactions or pediatric delirium, as previously described. Adverse effects and other dichotomous outcomes, such as re-intubation, will be compared between groups using Fisher's exact tests, and the uncertainty in these estimated proportions will be assessed using 95% confidence intervals. The level of significance will be set at 5%, and all tests will be two-sided. Non-linear mixed effect modelling (NONMEM) will be used to perform population pharmacokinetic analysis.

Safety

Patients can be withdrawn from the study at any time by the investigator or the treating physician. The intention-to-treat analysis will include all subjects. The subjects that have been withdrawn during the study will be included only for the time period in which they have participated. The cumulative morphine requirement will be calculated for the time that the patient participated in the study.

An external data safety monitoring board (DSMB) is established composed of pediatric intensivists and cardio-anesthesiologist with extensive clinical and research experience in the field of analgosedation on the PICU. The study protocol does not contain an interim analysis. The secondary endpoints require more included patients then the primary endpoint. An interim analysis could terminate the trial prematurely based on a favorable primary outcome, while not having enough power to assess secondary endpoints. However, the DSMB can advise to stop the trial if necessary. The DSMB has advised the researchers during the study set-up and evaluated study proceedings after inclusion of the first 10 patients. The DSMB evaluated inclusion rate and safety of participants (need for rescue morphine in both groups) several times during the inclusion period, with the last meeting in October 2017. The DSMB advised to continue the study, without changes to the protocol.

An advisory board is established composed of representatives from two patient and parent associations (*Stichting Kind en Ziekenhuis, and Patientvereniging Aangeboren Hartafwijkingen*), a neonatologist and clinical pharmacologist and a pediatric cardiologist. The advisory board has been involved in the design of the study and parental participation.

Data management and monitoring

Data is collected through case report forms in OpenClinica, a web-based database (OpenClinica, LCC, Waltham, MA, USA), supported by the Erasmus MC. Range checks for data values were added in the CRF, if possible, to promote data quality. Data management is coordinated by the Erasmus MC researchers and trial support unit. All participants receive a trial ID, with the personal details only known to the researchers, pharmacy and attending physician. Double data entry and validation is done by all participating centers. Monitoring of the data and trial proceedings is coordinated by the Erasmus MC. Monitoring of the data and trial proceedings is done per research site before the start of trial, soon after start of the trial, and three times a year for the duration of the trial. A close-out visit is done after completion of the inclusion period per research site. Trial auditing is done only by invitation from the hospital board and its frequency is not specified.

The Erasmus MC, as the coordinating center and owns intellectual property. The investigators have unlimited access to the final dataset. A newsletter with the study results will be made available on the website of the patient and parent organizations and will be sent to participants upon request. Public access to the data and data sharing is in line with the guidelines of ZonMw (data management plan), the main funder of this trial. Data access is restricted to authorized use only, and access will be granted by the researchers upon reasonable request.

Discussion

PK-based dosing of analgesics in children after cardiac surgery is lacking. Intravenous paracetamol as primary analgesic after cardiac surgery could have an opioid sparing effect and therefore less opioid related adverse drug reactions. This study will also provide the necessary PK and PD parameters to establish a PKPD-based dosing regimen for analgesics after cardiac surgery. This will lead to a more individualized dosing regimen to guide clinician in providing the best analgesic therapy for their patients.

The results of this trial will by incorporated in an international guideline for pain treatment after cardiac surgery in neonates and children aged 0-36 months. This guideline will be endorsed by several scientific societies for pediatricians and anesthesiologists.

Trial status

The study was initiated in March 2016. On March 23, 2018, 124 patients had been enrolled in the study. Enrolment of all 208 patients is expected to be completed in the fall of 2018. The Spirit checklist and figure on the trial proceedings are added as appendices B and C, respectively.

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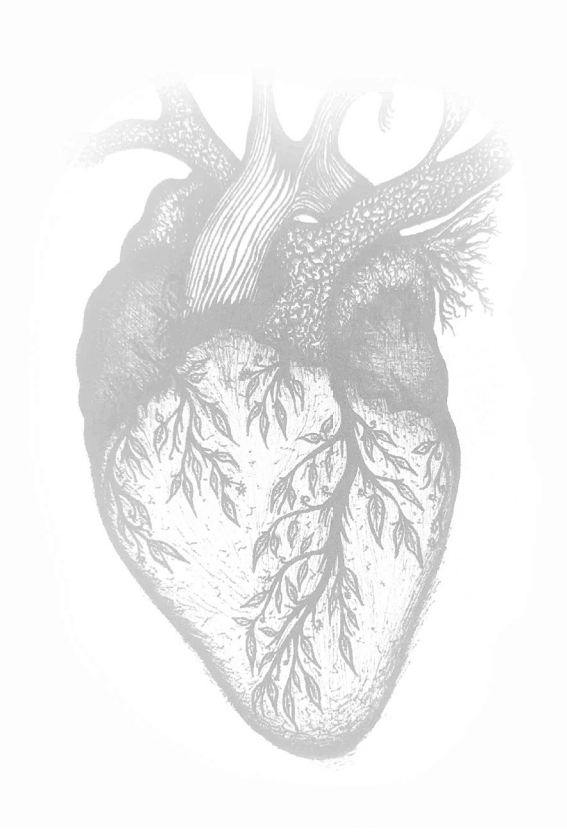
Appendix A: morphine dosing algorithm (adapted from Wang et al (40))

Weight of patient	dosis mcg/kg/h	dosis mcg/day	Continuous infusion (ml/h) (solution 100 mcg/ml)
2.5-2.9	3.9	257.4	0.1
3-3.4	5.3	413.4	0.2
3.5-3.9	7.1	639.0	0.3
4-4.4	9.3	948.6	0.4
4.5-4.9	11.4	1299.6	0.5
5-5.4	13.2	1663.2	0.7
5.5-5.9	14.5	2001.0	0.8
6-6.4	15.4	2310.0	1.0
6.5-6.9	16.0	2592.0	1.1
7-7.4	16.4	2853.6	1.2
7.5-7.9	16.6	3087.6	1.3
8-8.9	16.8	3427.2	1.4
9-9.9	16.9	3853.2	1.6
10-10.9	16.8	4233.6	1.8
11-11.9	16.7	4609.2	1.9
12-12.9	16.6	4980.0	2.1
13-13.9	16.5	5346.0	2.2
14-14.9	16.4	5707.2	2.4
15-15.9	16.0	5952.0	2.5
16-16.9	16.0	6336.0	2.6
17-17.9	16.0	6720.0	2.8
18-18.9	16.0	7104.0	3.0
19-19.9	16.0	7488.0	3.1
20-20.9	16.0	7872.0	3.3



Chapter 9

Lessons learned from designing and conducting a multi-center pediatric randomized controlled drug trial

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Submitted

Abstract

Background: Conducting a randomized multicenter drug trial in critically ill children is challenging. In recent years insight is gained on methods and reporting of randomized clinical trials (RCT) conducted at the pediatric intensive care unit (PICU). Also, facilitators and barriers in conducting RCT's in critically ill children have been investigated. However, when conducting a RCT applying these facilitators and avoiding the barriers can still remain difficult. Our goal is to apply the known facilitators to real-life examples from our study, and to share practical advice for future research.

Methods: The PACS (Pediatric Analgesia after Cardiac Surgery) study is a multi-center randomized controlled trial that started in 2016, comparing intravenous (IV) morphine with IV paracetamol after cardiac surgery in children aged 0-3 years. We will discuss the set-up and execution of the PACS study, with a focus on the barriers we came across, involving several stakeholders such as clinical staff, funding agencies and judicial departments. We explore the interests of the different stakeholders and its effect on the study, including the effects on the study timeline. We highlight the informed consent procedure in drug research in neonates and infants.

Results: funding, pharmaceutical licenses and judicial negotiations caused delays in our trial time line. Also, the unexpected withdrawal from one trial site required us to find a suitable replacement within our research network. Known barriers in literature are related to lack of funding, while known facilitators are related to cooperation within a research network and a dedicated study team. The discussion highlights our key learning points in relation to current literature.

Conclusion: completing a multicenter RCT in children within the predefined timeframe is extremely challenging. Involving all stakeholders, such as pharmacists, judicial advisors and clinical staff early in the preparations and logistics of a trial will prevent unnecessary delay.

PACS study: Trial registration at Dutch Trials Registry (NTR5448/Trial NL5339), EudraCT Number: 2009-014892-31.

Introduction

Publications in medical literature often focus on outcomes of research. However, authors hardly focus on the preparation and logistics of a multi-center cooperation that precedes the conduction of a successful trial. Already in 1998, Farrell wrote that researchers must often reinvent the wheel, because the knowledge on how to run randomized controlled trials (RCT) is not widely disseminated (1). Fewer RCT's are conducted in children compared to adults, with more challenges in obtaining funding and a higher risk of bias in the pediatric RCT's (2, 3). The Strategies for Trial Enrolment and Participation Study (STEPS) investigated factors for success and failure of multicenter clinical studies, but does not focus on children (4). Recently Duffet et al. highlighted several barriers and facilitators in performing RCT in pediatric critical care (5). The main barriers in both studies were related to lack of funding, whereas main facilitators were related to dedicated research time and collaboration within a research network. Applying this knowledge in clinical practice can be challenging. We aim to provide a practical overview of the management and conduct a multicenter drug trial in children with applying the good clinical practice requirements. We describe the steps we have taken in the preparation and logistics of our RCT in children after cardiac surgery and highlight the barriers and facilitators.

We set up the so-called PACS (Pediatric Analgesia after Cardiac Surgery) study, with enrollment of patients starting in 2016 (NTR5448/Trial NL5339, EudraCT Number: 2009-014892-31). The PACS study is a double blind multi-center RCT, comparing IV morphine with IV paracetamol in children, aged 0-36 months, after cardiac surgery. The primary aim of the study is to reduce the cumulative morphine consumption after surgery by 30%. The details of the PACS study are published previously (6). We will use examples from the study design, preparations and logistics as input for improvement and recommendations for the performance of future studies. We will also discuss the planned and actual study timeline as a result of the unexpected barriers between study design and execution.

Study design

Sponsors

Funding is one of the requisites when planning to conduct clinical research (5). In general, three types of funding are often requested, namely industry, government, or non-profit organisation, each with potential influence on study duration and report of results (2, 7). Clinical trials in children mainly rely on non-profit or government funding, whereas trials in adults are predominantly funded by industry (3, 8). The PACS study applied for funding from the government-related agency ZonMw (The Netherlands Organisation for Health Research and Development), and their national programme on Priority Medicines for Children (9). In order to convince the funding agency for the need of this trial an international survey was conducted (10). The results of this survey emphasized the need for more uniform and evidence-based guidelines. As an additional facilitator, all Dutch pediatric cardiac surgery hospitals signed an agreement to participate in a multicenter PACS study. One center refused to participate after IRB approval was obtained. New IRB approval was needed for the replacing center. Additional funding was obtained from the Scientific Review Board of the Erasmus MC-Sophia Children's Hospital to employ a pharmacist-researcher for population pharmacokinetic and pharmacodynamics modelling of the study results. Funding agencies often require that the study is completed within a predefined timeframe. We did not complete the study within the set timeframe due to several issues we encountered during preparation and execution of the PACS study. The following paragraphs will discuss these issues.

Key message: adequate sponsoring is essential and needs to be sufficient for the trial set-up. Before submitting the funding application, recruit participating centers and convey pilot data to back-up the application.

Parents and patient associations

Funding agencies in the Netherlands require the involvement of a patient association in designing a clinical trial. Representatives from patient associations can give input on preferred outcomes and increase the readability of the patient information brochure (11). Increased readability of the information brochure could decrease potential misunderstanding by parents on trials procedures (12). Representatives from 2 patient associations (*Stichting Kind en*

Ziekenhuis, and *Patientvereniging Aangeboren Hartafwijkingen*), were asked to participate in designing PACS. There was some concern from the research team that patient associations might be worried about children not receiving morphine after cardiac surgery. The assumptions of the study team proved wrong, since research on pain treatment and potential opioid sparing analgesics in these children was considered of high priority. This phenomenon is known as gatekeeping and is commented on in the discussion (13).

Key message: parents need to be involved in the design of a clinical trial and researchers have to keep an open mind towards this involvement.

Building the study team and identify potential pitfalls

Ideally, a study team should consist of representatives of all involved medical specialties and representative of each participating center. Our study team consisted of pediatric intensivists, a pediatric cardiac anesthesiologist and a congenital cardiac surgeon from the Erasmus MC, together with all local site investigators. We involved a dedicated research nurse with expert knowledge on implementation of trials and trial results. This may be of particular importance since most trials have soft or surrogate outcome measures that greatly depend on nurses interpretation and protocol adherence (2). A dedicated research nurse was appointed in each center. Local site investigators could identify potential differences in local clinical care that may lead to poor study protocol adherence. An informed account from all local investigators is essential to recognize potential pitfalls. In the preparation of the PACS study, it became apparent that there were differences in local protocol on the use of a morphine loading dose and the dose of continuous morphine infusion. In the PACS study, morphine dosing scheme was based on the pharmacokinetic-based morphine regimen designed by Knibbe et al. (14, 15). This evidence-based approach and its use in the study by Ceelie (16) was first introduced to all doctors, who agreed on it. Afterwards each site investigator introduced the scheme to the nurses on the clinical ward. The involvement of all clinical staff helped to increase the willingness to apply a different morphine dosing scheme for the study.

Key message: representatives of all involved clinical specialties and all trial sites need to be involved from the start. Using evidence based data in the design of the trial increases acceptance by clinical staff to defer from local daily practice.

Preparation of the study

Trial contract

Clinical Trial Agreements (CTA) were drawn and negotiated by the judicial department of the Erasmus MC. The newly implemented European General Data Protection Regulation act makes privacy and data protection issues easier to negotiate for trial sites within Europe. However, insurance and indemnity becomes a problem when cooperating with trial sites in different countries, since this is regulated by local law. In The Netherlands, no fault compensation is never used, whereas in Belgium, no fault compensation is standard. Because of these difficult negotiations, a liability expert from the judicial department was added to the judicial team. Some hospitals have Clinical Trial Centers (CTC) to advice on this type of cooperation. Unfortunately, when this is not a service from the local hospital, involving a CTC in the study can be expensive.

Key message: anticipate on legal issues, particularly when conducting a trial in different countries, and involve judicial expert advice on specific topics early in the negotiations.

Trial medication

Opioids require additional licenses for use in a clinical trial. The national Competent Authority (CA) needs to approve the use of opioids in clinical trials. Obtaining approval from the CA in multinational drug research can be expensive and time consuming, as described in detail by Osborne and O'Callaghan (17). Depending on the country, the CA varies from a review on pharmacovigilance to a very detailed investigation that can take months to years (18). Also, import and export licenses are required when shipping opioids to foreign trial sites. In Europe, drug trials need to be submitted to the European Clinical Trials Database (EudraCT). Experienced trial pharmacists are necessary to guide the submission process for shipping licenses, CA approval and EudraCT registration. We involved the trial pharmacist of the Erasmus MC early in the preparations for the PACS study to ensure the morphine handling license. To avoid delay by obtaining an import and export license for the non-Dutch trial site of UZ Leuven, this study site bought their study drugs directly from the same supplier as the Erasmus MC trial pharmacy.

Key message: anticipate on regulations involving multinational drug trials and required drug licenses, and consult a trial pharmacist early in the trial design.

Conducting a multi-center study

Engagement and training of clinical staff

We organized a kick-off meeting at the start of the PACS study with the study team, advisory board and data safety monitoring board (DSMB) to increase everybody's awareness of potential pitfalls in the enrolment of the study. The study coordinator from the initiating trial site was always available for all local investigators. This combined study team of local and coordinating investigators was the main facilitator for patient enrolment and protocol adherence of the PACS trial. Before the start at each trial site, local staff was trained in the study protocol. During patient enrolment educational moments with nurses and doctors were created to ensure protocol adherence and discuss specific inter-center variations. The main reported difficulty for the nurses on all trial sites was the double blind aspect of the study, and therefore not knowing if a patient was treated with opioids. In very few cases nurses made a negative comment about the study toward the parents. Despite the very occasional negative comments, parents endorsed the study. Only very few parents said that, in hindsight, given the comments of the nurses, they might reconsider participating in the study.

One general study coordinator to monitor trial proceedings in all centers is necessary. In the PACS study, the study coordinator changed during the trial. The challenge in this transition was to preserve the established study contacts and pass on insight in study proceedings and pressure points to the new coordinator. We have ensured the continued study quality by an extensive training period for the new coordinator.

Key message: involve all trial site investigators from the start of the study and use hospital-specific differences in educational moments to create an open mind towards the study. A dedicated research team per trial site is essential, coordinated by one central contact person.

Informed consent procedure

Informed consent can be difficult in young children. Several studies have reported discontinuation of studies partly due to failed informed consent (19). We focused on obtaining

an informed consent rate between 40-50%, as we have had in previous pain studies (16). The informed consent procedure differed per hospital depending on local logistics.

Three trials sites informed parents of eligible patients by sending them the informal PACS study flyer. The first contact with the researcher was after admission at the ward, one day before surgery. We encountered two problems in this approach. First, parents did not always read the flyer, reportedly due to time restrictions. Second, concerns parents might have about the study proceedings could not be addressed immediately. Most often parents had concerns about measuring pain and distress in their children, since the children were too young to express this themselves according to international consensus to use the gold standard of self-report.

Patients enrolled at the Wilhelmina Children's Hospital Utrecht received the informal flyer from the researchers at the out-patient clinic. At the Erasmus MC-Sophia children hospital, an out-patient clinic was set-up eleven months after start of enrolment of the PACS study. At the out-patient clinic, parents were informed about the PACS study and questions related to the study proceedings were answered. Before the start of the out-patient clinic informed consent was given by 39%. After start of the out-patient clinic, informed consent was given by 43%. The introduction of the outpatient clinic did not dramatically increase the informed consent rate. However, parents reported to appreciate the time to consider study participation. This was in line with previous studies showing that direct communication with the researcher and sufficient time to consider participation increased parental understanding of the study (12). Another advantage of the out-patient clinic was to allow parents time when they did not agree on participation. When parents had trouble reaching an agreement the researcher registered this as no consent. This did not happen often.

Parents reported two main reasons for participation. Firstly, the possibility of receiving less morphine after surgery was considered positive. Secondly, the confidence that the study analgesics were equally effective, regardless of a beneficial morphine-sparing effect of IV paracetamol. Parents who did not want to participate in the trial where mostly too stressed about the surgery to consider research. Another reason for not participating was the belief that IV paracetamol was not sufficient to provide pain relief after cardiac surgery. The surgical procedure, expressed in Risk Adjustment for Congenital Heart Surgery (RACHS) (20), and

cyanotic or non-cyanotic, did not seem to influence study participation. Age of the patient made a minor difference, with neonates being the most difficult patients to include in the study.

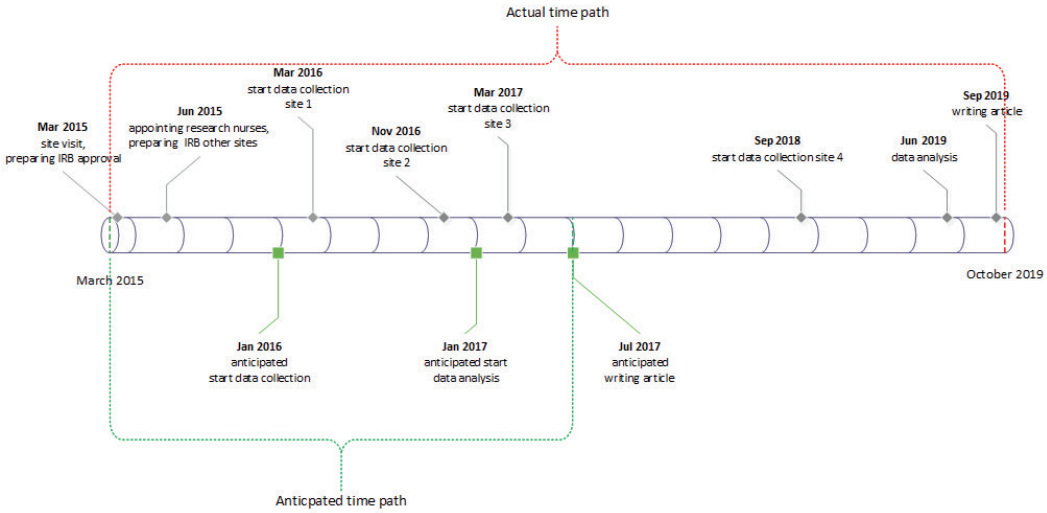
Patients undergoing emergency procedures or heart transplantation were excluded from the study because of insufficient time for informed consent. We did not use deferred consent, since the majority of patients undergo elective cardiac surgery.

Key message: sufficient time for parents to consider study participation does not increase patient enrolment much, but is highly appreciated by parents. It also provides time for parents to discuss participation and reach a mutual agreement.

Expected timeline of trial proceedings

The study time path is shown in Figure 1. Preparation of the trial started in March 2015 and was expected to take several months. Patient enrolment was planned to start beginning 2016. The main delays in the start of patient enrolment at all Dutch trial sites were either due to pharmacy regulations or judicial negotiations. The first patient was enrolled at the Erasmus MC in March 2016. A stepwise approach was taken to start patient enrolment in participating centers, to optimally support each site. After the Erasmus MC, the Wilhelmina Children's Hospital in Utrecht started preparations to start patient enrolment. Their first patient was enrolled in November 2016. Inclusion of patients started in spring of 2017 in the Beatrix Children's Hospital in Groningen.

Figure 1: Expected and actual time path of the PACS study.



IRB: institutional review board

The last center planned to start study proceedings withdrew because of understaffing. This was a major setback, since preparations for all four centers were done and IRB approval was obtained. Within our collaboration network, we searched for a pediatric cardiac surgery center as a substitute. Recently reported by Duffett et al. as a strong facilitator for research (5, 21), we aimed for a hospital that we successfully collaborated with before and that already had a strong research team. We had previously collaborated with the UZ Leuven on a randomized controlled trial in the PICU (22). Since UZ Leuven is also a pediatric cardiac surgery hospital, we asked them to collaborate on the PACS study. Preparation of study in UZ Leuven started in the spring of 2017. Judicial negotiations were difficult due to previously described differences between Dutch and Belgium law on privacy and liability. Judicial negotiations lasted until late summer of 2018. The first patient was included in September 2018.

Follow-up

The PACS study included follow-up after hospital discharge. Approximately two days after hospital discharge parents were called to inquire how their children were doing during the first days at home and the validated Postoperative Parents Pain Measurement-short form was completed (23). This short questionnaire with ten yes/no questions is aimed at detecting signs

of pain and distress in the child as assessed by parents. Parents appreciated the phone follow-up. Overall, parents highly appreciated the relationship with the researcher both during the hospital admission and after discharge. The main explanation was that it was reassuring for parents to have one familiar person that kept in touch during hospital admission.

Key message: contact with one researcher during the trial and follow-up will raise parental satisfaction and may allow sympathy for research and possible future trial participation.

Discussion

We have discussed the different phases of design and preparation of the PACS study. In this discussion, several key messages proposed in the previous sections will be discussed in relation to literature. We will also focus on the patient inclusion and discuss whether the expected rate of enrolment was realistic.

Financial aspects have an impact on trial design and progress. Especially funds for a research nurse and a dedicated trial team increase the success rate of a study (5).The PACS study needed two sponsors and resources from our own institution to execute the trial. Snowdon et al. identified three broad areas of financial activity throughout the course of a trial, namely, economic foundation work, maintenance work and resourcing completion (24). The foundation work refers to the preparation of the trial before the funds are granted, such as conveying pilot data to back-up the application and establishing a cooperation agreement. In case of the PACS trial, we conducted an international survey to convey the pilot data needed for the funding application (10). Resourcing completion, to ensure trials to complete their aim, was a problem in the PACS study, since our inclusion period was longer than anticipated due to the previously mentioned pharmacy and judicial obstructions. We therefore required an extension of the recruitment period, within the limits of the original funds. We used the DSMB to back-up the request, since they were convinced PACS should be completed with the required number of patients. To answer the primary research question and to prevent the risk of no answer due to under recruitment. This request for an extension is certainly not uncommon, since STEPS showed that an extension of trial grants was requested in 54% of trials to complete the original trial (4).

In 2001, European law aimed to simplify and harmonise the administrative provisions governing clinical trials in the European Union came into effect. After evaluation, this legislation resulted in the current EU Clinical Trials Directive (No 536/2014) (25). Unfortunately, introduction of this directive does not show an improvement in regulatory approval procedures, nor an increase in clinical drug trials (26, 27). Furthermore, liability is not included in the EU Directive and remains governed by local law. Therefore our problem in establishing a multinational cooperation is most likely not unique and may affect the time path of other multinational studies.

Complicated logistics and trial design decrease the success rate of a trial, which was also described by Baarslag with examples from the CloSed trial (18, 28). The PACS study was a two group comparison, and thus a fairly simple design. Trial site investigators and patient representatives were involved throughout all the stages of design and preparation of the PACS study, leading to a multidisciplinary cooperation. This multidisciplinary approach has been reported to increase patients enrolment (4). The involvement of patient representatives also avoided potential gatekeeping by clinicians. This phenomenon occurs when patients are not asked to participate in clinical research based on the judgement of the physician (13). This is not a new phenomenon since it was already introduced in 1984 by Taylor et al. (29). Gatekeeping can introduce bias in the study population and unjustly withholds parents and patients the chance to participate.

Prolonged duration or even discontinuation of trials due to failed enrolment or stringent exclusion criteria is not uncommon (30, 31). In children, a recent study showed that 19% of trials were discontinued, most often due to poor patient recruitment (19). We believe that the expected inclusion rates of PACS were realistic and the prolonged inclusion time was not due to overestimation of the inclusion rate. Previous studies into analgesia in our group obtained inclusion rates of around 50% (16). Overall, the inclusion rate of the PACS study is 44%, with minimal differences between centers. This slightly lower inclusion rate in the PACS study could be attributed to the extensive nature of the surgery, which was also mentioned as a causal factor of decreased parental consent by van Dijk et al. (32).

The presence of a research nurse has been shown to detect more eligible patients and to increase enrolment (33). Informed consent was done by the research nurse or coordinator.

The physician only asked informed consent when the research nurse was not available. We believed this would distinguish treatment from research, and thus prevent suspicion of conflict of interest. This informed consent procedure is in line with the GCP and often used in research (34). However, a recently published Delphi survey on consent in neonatal studies showed that parents liked to be informed by the attending physician (35). Also, parents may have difficulty with informed consent in a stressful or emergency situation (36). By informing parents about the PACS study at the out-patient clinic we have attempted to reduce the stress of the upcoming surgery. Parents had more time to consider consent and were able to consult other physicians about the PACS study, which was one of the recommendations by Neyro et al. (35). In our experience the key to successful inclusion of patients is early personal communication with parents. This early communication was also one of the outcomes from a survey amongst family care providers, conducted by Merritt et al. (11)

Conclusion

Many factors are involved in preparation and logistics of a trial and problems on any of these factors potentially compromises the quality of the trial and the anticipated timeline. Continued attention for all participating departments and all stakeholders is needed to prevent unnecessary delay. Involvement of all clinical departments and patient representatives in the design and preparation of the trial increases awareness for the successful execution of the trial.

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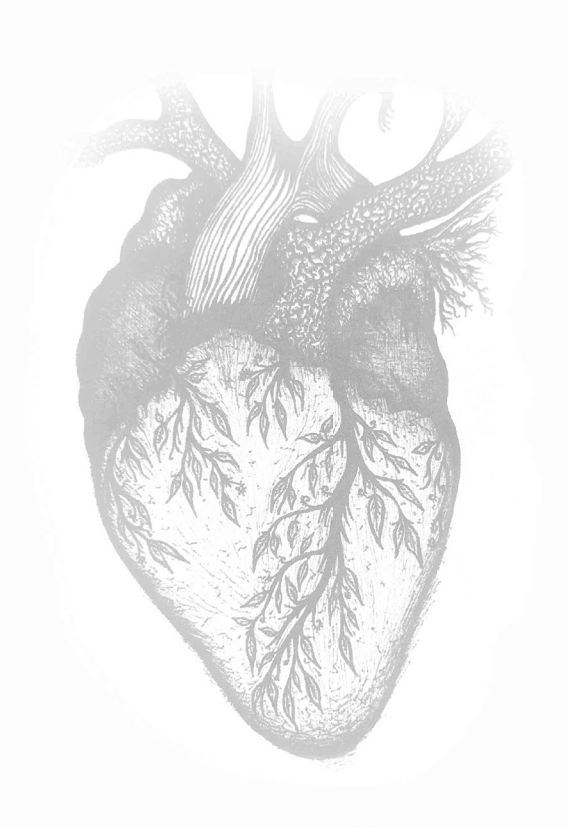

Chapter 10

General discussion

Congenital cardiac defects are the largest group of congenital defects, with a prevalence of 7.2 per 1000 live births (1). Surgical correction is often needed in the first year of life, and most patients will have undergone surgery in the first three years of life (1, 2). Little is known on preferred, evidence-based drug use during surgery and the recovery period during their stay in the pediatric intensive care unit (PICU) (3). This knowledge gap has resulted in a large variability in drug therapy in children during and after cardiac surgery.

In pharmacotherapy, the most important aspects are pharmacokinetics (PK) and pharmacodynamics (PD). PK follows the general principles of drug absorption, distribution, metabolism and elimination (ADME) (4, 5). Knowledge of ADME increases accuracy in drug dosing in patients, i.e. obtaining the desired target concentration. PD investigates the reactions of the body on drugs, i.e. the desired effect of drugs in the patient. Ideally, both PK and PD should be investigated in drug research, determining the optimal dose combined with the optimal drug effect in the patient. PK parameters can be determined by measuring drug concentrations in patients. To evaluate PD, validated assessment tools need to be used. Interpretation of these assessment tools and the effects of drugs on the outcome can be difficult to determine, especially in case of polypharmacy (6, 7). Equally important are the desired outcome parameters on long term effects, such as abnormal executive functions.

In the last decade, the different aspect of ADME, were increasingly investigated in children under a variety of conditions and patient populations (8, 9). Before these studies became available, drug doses from adults were repeatedly downsized to the pediatric population. However, children are not small adults when it comes to PK and simply extrapolating adult data to children has increasingly been recognized as inappropriate (4, 10-13). Even within the pediatric age group, PK is expected to be different between patient populations, warranting more accurate information on drug dosing in specific patients and age groups (4, 14-16).In particular considering patients with congenital cardiac defects, the various aspects of PK are very likely different from children without cardiac defects. Absorption, i.e. the time to reach peak plasma drug concentration, is variable and can be influenced by maturational, such as weight and age, and non-maturational changes. Non-maturational changes are disease and treatment specific and may include differences in systemic and pulmonary circulation due to shunt physiology, altered first pass effects in the liver, hemodynamic status and the effects of surgery. Distribution is expressed by the Volume of distribution (Vd) of drugs in the body. This

Vd consists of a fictional central compartment and sometimes one or more peripheral compartments that are in equilibrium with the central compartment. Vd is strongly dependent on drug characteristics, with lipophilic drugs having a larger Vd compared to hydrophilic drugs. Also, maturational and non-maturational changes affect Vd. An example is the development of systemic inflammatory response syndrome (SIRS) during and after surgery, leading to decreased clearance and potentially a larger Vd (17). SIRS is very common in children admitted to a PICU, and 21% of patients with SIRS do not have an infectious origin of SIRS (18). Elimination is influenced by hepatic and renal function and can therefore be affected by decreased renal or hepatic blood flow.

Traditional PK models incorporating all these different variables are based on measured drug concentrations in selected patient groups. The measured drug concentrations are combined in a time-concentration curve, showing the drug concentration in patients over time. The classical approach to determine a time-concentration curve requires frequent blood sampling in patients to reconstruct a reliable curve. In some patient groups, such as critically ill children, frequent blood sampling is not feasible. These special patients groups can be investigated using population PK (4, 19). Population PK combines data from sparse sampling regimens to a reliable time-concentration curve. Using population PK analyses, the burden to participate in drug research is negligible for individual patients. However, in combining the data from all patients, accurate predictions in drug concentration over time can be made.

This thesis investigates drug dosing in children with congenital cardiac defects undergoing cardiac surgery. Several factors related to the surgery influence PK, such as the use of the cardiopulmonary bypass (CPB) and subsequent hemodilution and changed organ perfusion. Therefore, PK in cardiac surgery patients is expected to differ substantially from non-cardiac surgery patients (15). PK parameters investigated in children without cardiac surgery cannot simply be extrapolated to cardiac surgery patients. When describing the factors that influence PK in children with congenital cardiac defects undergoing cardiac surgery, several phases of treatment and recovery need to be discussed. In all of these phases, different aspects of the cardiac defect and the treatment are expected to influence drug PK. In this thesis we have investigated these different phases and treatments in a systematic approach to gain insight in these PK changes. Validated PD assessment tools were used to determine the effects of drug therapy. This insight is needed to incorporate potential influences on PK and eventually PD of

these treatment phases in dosing regimens to improve clinical care for these children and eventually reach personalized- or precision- medicine

The first phase is the preoperative phase, since children undergoing cardiac surgery have a different hemodynamic condition due to the cardiac defect. Also, children can be critically ill before surgery and may therefore have different PK and PD responses compared to elective surgery in non-critically ill children. Second, the perioperative phase distinguishes itself from non-cardiac surgery because of the use of the CPB and an auto-transfusion system. Use of CPB increases the Vd and decreases clearance of drugs (20). An increased Vd could lead to sub-therapeutic plasma concentration of drugs. This has been shown in adult patients undergoing cardiac surgery, who had for instance a substantial decrease is plasma cefazolin concentration at onset of CPB (21). In contrast, decreased clearance of drugs could lead to overdosing and potential toxicity. Other factors, such as hypothermia and changes in the hemodynamic state due to surgical correction of the cardiac defect may also influence PK and PD due to changed enzyme activity. The final phase is the postoperative phase. Due to the correction of the cardiac defects with shifts in volume and pressure load, some children will have a changed hemodynamic condition after surgery compared to the preoperative state.

Preoperative phase

Even though this thesis mostly examines the peri-and postoperative phase, Chapter 2 highlights differences in PK in children on the PICU. Additional considerations on PK related changes in children with congenital heart defects are also discussed in Chapter 8. Many different types of cardiac defects are known, and they can roughly be classified in cyanotic or non-cyanotic. Data on the influence of cyanotic or non-cyanotic defects before cardiac surgery on PK is extremely scarce. Decreased clearance of alcuronium, a routinely used muscle relaxant, has been suggested during surgery, but no other data on this topic could be found (22). However, cyanosis in patients before surgery could result in an increased cardiac output, resulting in increased renal and hepatic perfusion. For high extraction ratio drugs, i.e. drugs that are liver flow dependent, such as morphine, this increased perfusion may lead to an increased clearance (23). Patients with cyanotic heart defects are at risk of developing renal impairment, leading to a decreased clearance with drug specific effect on the Vd (24-26). When considering drug dosing in children with cyanotic cardiac defects, dosing schemes do

not take this potentially changed hemodynamic state into account. This may lead to increased adverse drug events and patient morbidity. Given the large variability of type; dosing and concomitant use of drugs more data from large patient studies are needed to distinguish the different effects before, during and after surgery, comparing cyanotic and non-cyanotic children. Data from two clinical studies from our institution and partly described in this thesis, the CPB-PHARM and the PACS study, are currently being analyzed. These studies did not change treatment based on cyanotic or non-cyanotic hemodynamic state. However, PK parameters and treatment effects were analyzed comparing patients with cyanotic and non-cyanotic heart defects.

All children admitted to the PICU are critically ill, especially after extensive surgery like correction of congenital cardiac defects. Most children are not admitted to the PICU before cardiac surgery and the procedure is planned electively. However, these children can still have a cyanotic cardiac defect and therefore an altered hemodynamic state. Neonates with congenital cardiac defects may require drug therapy to maintain an open arterial duct to achieve an adequate systemic circulation, for instances in patients with hypoplastic right or left ventricles or transposition of the great arteries. These neonates are admitted to the PICU before surgery, and are treated according to local protocols that do not incorporate the open ductus and subsequent cardiac defects. However, a persistent ductus arteriosus causes a larger Vd potentially leading to underdosing of drugs (27).

Clinical PK studies in critically ill children are difficult to perform due to large inter-patient variability (28). However, it is known that critical illness effects PK of routinely used drugs. When comparing midazolam clearance between critically ill children admitted to the PICU, pediatric oncology patients and healthy children undergoing major craniofacial surgery, midazolam clearance was decreased by 86%-93% in PICU patients (29). When investigating midazolam in critically ill children in further detail, Vet et al. found that inflammation and organ failure strongly reduce midazolam clearance (30). This seems to implicate that midazolam is strongly affected by inflammation and thus by SIRS. Even though we suspect children with cardiac defects also have SIRS, data on the preoperative phase has not been published. SIRS, varying from limited to extensive, is very common in children after correction of cardiac defects, most likely due to the use of the CPB (15, 31, 32).

Variation in PD in children is even larger then variation in PK. PD parameters are changeable and vary over time, per age group, and disease and treatment modalities (33). Altered neurological state could be expected to influence PD in patients and may alter a patients response to certain drugs, however no clear examples of this could be found in literature. Until recently, complications in neurologic state and neurodevelopment in children with congenital cardiac defects where believed to be caused by the surgical procedure and concomitant CPB use (34). However, even before surgery 50% of patients with mainly complex congenital cardiac defects already has neurological injury (35). MRI's showed preoperative and postoperative neurological injury, with mainly new white matter injury after surgery. The potential effects that this brain injury may have on administered drugs has not been observed and quantified in current literature. Ideally for quantification and observation of PD, validated tools and endpoints need to be used. When investigating this in our international survey (see Chapter 7), very often validated PD assessment tools were not incorporated in clinical practice and an objective observation was unfortunately not reported.

Considering all the above mentioned factors, children undergoing cardiac surgery most likely already have a different PK and PD compared to children undergoing non-cardiac procedures even preoperatively. Due to the nature of the surgery these differences only become more extensive and reverse gradually after the procedure.

Key messages:

- Children with cardiac defects potentially have a different PK and PD already preoperatively.
- Types of cardiac defects, i.e. cyanotic or non-cyanotic, are not incorporated in current drug dosing guidelines, even though hemodynamic state and therefore PK could be different between patients.
- Brain injury that is already present before surgery may result in a different response of children with cardiac defects to administered drugs.

Perioperative phase

The most extensive change in perioperative PK parameters is due to the use of the CPB. The changes induced by the CPB have been described in detail in Chapter 2 (4). In summary,

hemodilution, sequestration of drugs and decreased organ perfusion are the main reasons for changes in PK and PD (15). Sequestration of drugs in pediatric *in vitro* CPB systems is described in Chapters 4 and 5. SIRS can also be induced by the use of CPB, and prolonged CPB use increases the SIRS reaction leading to potentially an ever larger Vd and decreased clearance (32). However, very little and contradictory data is published on the effects of SIRS on Vd and clearance (17, 36). Next to the influence of the CPB, general surgical and anesthesiology management such as hypothermia influences drug metabolism. During surgery, an autotransfusion system, commonly known as a cell saver, is often used to prevent blood loss and to decrease the need for allogenic donor blood. We have investigated the influence of the cell saver on drug loss from the operation site and drug administration through autotransfused blood in Chapter 3. Finally, the patient himself is a black box when it comes to drug PK and PD during cardiac surgery. Very little is known when it comes to hepatic or renal function during cardiac surgery, which is important for drug metabolism and clearance.

Investigating all the above mentioned factors together would require a large in vivo study, preferably measuring plasma drug concentrations before, during and after surgery to determine the effects of every step of the procedure. The disadvantage of such an approach could be that patient, CPB and cell saver influence on drug plasma concentration cannot be distinguished from one another. Therefore, we have investigated the different phases of the procedure, in an attempt to separate the patient, CPB and cell saver related factors (see Chapters 2, 3, 4 and 5).

Our study of autotransfused blood (Chapter 3) showed that certain drugs could potentially be autotransfused to the patient in therapeutic levels (37). The risk of autotransfusion of drugs seems to be the greatest for certain lipophilic drugs. Sufentanil, but not midazolam or propofol was measurable in clinical relevant concentrations in the autotransfused blood. The discrepancy between these lipophilic drugs could not fully be explained by our experiments. Propofol could be bound to erythrocytes and therefore not measurable in plasma, but this does not account for the low midazolam plasma concentration. Drug concentrations in autotransfused blood has not previously been published, and we have therefore no comparison for our results or an indication for potential source for loss of midazolam or propofol. The drug concentration that we found in the blood removed from the surgical site was comparable to concomitant drug concentrations measured in the patients. Therefore, the

cell saver could be a potential source of loss of drugs during surgery. This loss applies to all drugs used during surgery. However, the contribution of the cell saver as a source of perioperative drug loss is most likely relatively small, except perhaps in small patients undergoing extensive repair of congenital cardiac defects. Due to the limited amount of circulating volume in these patients, blood and drug loss may influence PK and PD more compared to older patients. Even though the drug loss may apply to all drugs, autotransfusion of drugs is most likely only relevant for lipophilic drugs. Caution may be necessary when administering autotransfused blood to neonates who are no longer mechanically ventilated. In neonates still on ventilatory support, the clinical effects on therapeutic sufentanil levels may have a negative hemodynamic effect but will most likely not cause serious problems. Adjustments of type and dose of drugs based on the use of the cell saver would not be recommended based on our findings. Also, in our institution sufentanil and remifentanil are the analgesics of first choice and both are lipophilic. Replacing these drugs by a hydrophilic analgesic could be most undesirable for optimal analgesic effect.

Our studies in *in vitro* CBP systems (Chapters 4 and 5) found that mainly lipophilic drugs sequester in the CPB system compared to hydrophilic drugs (38). This sequestration is different per type of pediatric CPB system, with higher losses in larger pediatric CPB systems. An increased foreign surface to blood ratio is most likely the reason for the decreased drug plasma concentration in larger compared to smaller pediatric CPB systems. For adult CPB systems, sequestration of lipophilic drugs is not uncommon, however *in vitro* data is sparse (15, 39). One of the main sites of sequestration seems to be the tubing (40). The tubing diameter and length is relatively large in pediatric CPB systems compared to adult CPB systems. Even though *in vitro* CPB data in pediatric systems has not been published previously, drug sequestration in extracorporeal circulation in children is not new. This topic has been investigated in pediatric extracorporeal membrane oxygenators (ECMO) (41-43). Even though ECMO experiments are focused on routinely used drugs in the PICU, similarities can be found between pediatric CPB and ECMO. In both CPB and ECMO use, patients can be considered critically ill with decreased organ perfusion. All children treated with ECMO and CPB have an increased Vd and decreased clearance, leading to potential differences in plasma drug concentrations compared to other critically ill children. In ECMO systems, drug loss was less in hollow fiber membranes compared to silicon-based membranes. All our tested pediatric

CPB systems had hollow fiber oxygenators, and we may therefore assume this was not the main site of drug sequestration. Different types of surface coating of ECMO tubing have been investigated *in vitro*, since this was previously identified as the primary source of sequestration (44). However, regardless of type of surface coating, drug loss in the tubing was considerably. So far, no solution seems to be available for decreasing the drug loss in CPB systems. To date, almost no data is available on drug regimens compensating for the drug loss due to the use of CPB. In adults undergoing cardiac surgery, redosing of cefazolin at the onset of CPB restores the plasma concentration to therapeutic range (45). This change in cefazolin plasma concentration is probably only due to an increased Vd, since cefazolin is hydrophilic and not expected to sequester rapidly in CPB systems. For lipophlic drugs, the increase in Vd may be accompanied by a sequestration in the CPB systems. This may potentially cause an even greater drop in plasma concentration than we found *in vitro*. In pediatric *in vitro* ECMO systems, midazolam and sufentanil were lost over 50% after 24 hours (41). Together with our results, this implies that longer duration increases the sequestration and a steady decline is observed during the entire run time. In part, the decrease in drug concentration may be less pronounced with administration by a continuous infusion. Often, analgesic and sedative drugs are titrated to effect with an increased heart rate or blood glucose levels indicating stress. However, with all confounding factors, such as hypothermia and co-medication, PD cannot be measured reliably. Solutions to this dilemma could be to increase drug dosing during surgery, but this may increase hemodynamic instability and prolong recovery after surgery. The use of a loading dose or an additional dose at the onset of CPB seems to be more adequate based on our findings. These assumptions need to be validated in *in vivo* studies with enough power and the use of modern ways of analysis such as popPK modelling (8).

The changes in Vd and clearance may be less pronounced in new CPB techniques, due to their smaller size. These smaller CPB systems need less prime fluid and have a smaller foreign body surface area, leading to better hemodynamic stability during surgery (46). As a consequence of these alterations, the increase in Vd that occurs at the onset of conventional CPB is likely to be less profound. Also, better hemodynamic stability could result in better organ perfusion influencing clearance of drugs. Therefore, the use of mini CPB could potentially limit the changes in PK parameters during cardiac surgery. The PK parameters in these children would then be more similar to the non-cardiac surgery patient. This could have implications for the

administration and dosing of drugs. However, no published literature could be found on the influence of mini CPB on PK of routinely used drugs. A first step towards investigating the influence of mini CPB systems on drugs could be performing *in vitro* experiments. As we have shown, sequestration of drugs is influenced by foreign body surface area and therefore sequestration should be less in mini CPB systems. The next step should be to investigate drug concentration during pediatric cardiac surgery with use of mini CPB systems. Combining this data could provide more insight into Vd and Cl of drugs during surgery with mini CPB systems and therefore the influence of mini CPB systems on PK parameters. Potentially, a more targeted approach in perioperative drug dosing is possible if variation in PK parameters during surgery is decreased, leading to less risk of overdosing or underdosing.

During surgery, hypothermia is often used to protect neurological and organ function. The most extreme hypothermia is complete circulatory arrest at a body temperature of 18^0C. If this deep hypothermic circulatory arrest is necessary, isolated cerebral perfusion is used. Due to the hypothermia organ function will decrease leading to changes in PK and PD. All aspects of ADME are influenced by hypothermia (47). Absorption is decreased, with a later onset of effect. Vd is altered with a preferential blood flow towards coronary and cerebral vasculature. Vd and clearance of drugs can be increased, decreased or unaltered depending on the drug. When investigating the effect of hypothermia on PD, results are highly variable and depend on the drug-target interaction and alteration in enzymatic processes. Inflammation does not seem to be affected by hypothermia (48). Other alterations in PD may be due to neurological injury caused by the underlying cardiac defect; the surgical procedure and CPB use (35).

As discussed, technical and procedural factors influence PK and PD during surgery. Also, patients themselves may be a reason for variation in drug PK and PD. Increasing age is often not an issue with children undergoing cardiac surgery for congenital defects. However, obesity is a major health problem in both adolescents and children. In Europe childhood obesity ranges from 15.3% to 25.6%, depending on the region (49). Considering recent literature, body mass index (BMI) may become an interesting patient related factor for PK alterations. Alterations in PK in obese patients may be because of more fat tissue and less lean body mass, increased blood volume, increased cardiac output and low grade inflammation (50). We already know that inflammation occurs in patients preoperatively and SIRS becomes more profound during and after the operation. In obese adults, liver abnormalities such as fatty

infiltration were present in 90% of patients. Liver abnormalities may result in altered enzyme activity in phase 1 and 2 metabolism, decreasing among others, cytochrome P450 subtypes (CYPs). A lower CYP3A4 activity is reported in obese compared to non-obese adults, indicating a lower CAP3A4 mediated clearance of drugs such as midazolam. A lower clearance could lead to accumulation of drugs and thus an increase in drug related adverse events. Our CefCheck study (Chapter 6) already stratifies patients depending on BMI, to create more equal treatment groups. We found that BMI is not the main predictor for sub-therapeutic plasma levels of cefazolin. We did find that during and after surgery many patients have sub-therapeutic levels of cefazolin. This could potentially lead to deep sternal wound infections, with increased morbidity and mortality (51). As discussed previously, a loading dose, or an additional dose at onset of CPB could stabilize cefazolin concentrations within the therapeutic range. Another option is continuous administration of cefazolin. Recent data has shown that continuous administered cefazolin may reduce surgical site infection and provides more stable serum levels (52, 53).

To investigate all the above mentioned changes in PK *in vivo* research in necessary. When considering drug PK and PD parameters of Vd and clearance, the cell saver and CPB cannot be seen separately from the patient. The cell saver and CPB systems are both peripheral compartments, with the patient as the central compartment. Shifts of plasma and drugs occur between those compartments, and the extent of the shifts may vary during surgery. Large randomized controlled trials are not feasible given the variation in expected alterations in PK and PD of drugs and the sparse clinical patient data, especially in children. A large prospective observational cohort study is the best approach to investigate PK and PD in children undergoing cardiac surgery. We have conducted this study, called the CPB-PHARM study and data will become available on the coming year. We have taken blood samples through an indwelling arterial catheter before, during and after surgery. We included 150 patients in the CPB-PHARM study, therefore enabling us to investigate various variables in patient, surgery and CPB characteristics. Ideally, this should result in a more individualized pharmaco-therapeutic regimen. Next to the general variables as age and weight, other potential covariables for this individualized regimen could be cyanotic or non-cyanotic cardiac defect, CPB run time, type of CPB system used; use of hypothermia yes/no and duration of surgery.

All these factors have been shown to influence Vd and clearance, but no data is yet available at individual patient level.

Key messages:

- PK parameters during surgery are altered due to use of CPB, cell saver and patient variables.
- Sequestration in CPB systems is mainly influenced by foreign body surface area and changes depending on the size of CPB system.
- The cell saver system could account for perioperative drug loss, but drug concentration in autotransfused blood is only relevant in small children.
- In adult patients undergoing cardiac surgery, a new cefazolin dosing guideline based on non-cardiac surgery data does often not obtain the target concentration, emphasizing the need for solid perioperative data in these cardiac patients.

Postoperative phase

As discussed in the preoperative and perioperative sections, children after cardiac surgery are expected to have different PK and PD parameters compared to children after non-cardiac surgery. Most of these changes will reverse to the preoperative state, however this requires time. Also SIRS is increased with longer CPB runtime, with elevated C-reactive protein and procalcitonine until the third day after surgery and may contribute to a low cardiac output syndrome (LCOS) (54-56). LCOS is a clinical condition characterized by decreased systemic perfusion due to myocardial dysfunction. LCOS may be observed within 6 to 18 hours after cardiac surgery and is associated with increased morbidity and mortality (57). Decreased systemic perfusion could lead to decreased clearance of drugs due to impaired renal and hepatic blood flow. The kidneys are particularly vulnerable to decreased perfusion. After cardiac surgery, renal impairment occurs in approximately 40% of patients (58, 59). This acute kidney injury may be due to renal vasoconstriction and hemodilution during surgery (60). Considering the percentage of children with impaired kidney function in the postoperative phase, renal clearance is expected to be decreased. With the normalization of the Vd to the preoperative state, this may lead to accumulation of drugs. We know that midazolam is cleared more slowly in critically ill children, but this could also hold true for other drugs.

Postoperative drug dosing schemes are similar in children after cardiac surgery, compared to children after non-cardiac surgery. However, for the direct postoperative period, this may not be the best approach in these children. Ideally, drugs should be titrated to effect to obtain optimal drug dosing. To assess the desired effect, validated PD assessment tools should be in place. Unfortunately, these assessment tools cannot be used for appropriate interpretation of the effects of all drugs. For analgesics and sedatives validated tools are available (61). For drugs such as antibiotics, no tools are available and outcome measures like sternal wound infections are only detected over a longer period of time. Titrating drug dosing to effect may decrease drug related adverse events due to overdosing or underdosing by finding the best dosing regimen per individual patient.

In the postoperative period, this thesis mainly focusses on analgesia in children aged 0-3 years. An international survey investigating analgesic type and dose in children after cardiac surgery showed large variability per hospital (see Chapter 7) (3). This is mainly due to lack of international guidelines on optimal analgesic treatment in this patient group. Analgesic of first choice in most hospitals is morphine, but dosing is variable. The first report of morphine as primary analgesic in children included dose finding based on respiratory adverse effects (62). After this, morphine PK and PD has been investigated, but this has not resulted in dose adjustments for children after cardiac surgery (20, 63, 64). In children after major, non-cardiac surgery, morphine could be replaced by intravenous (IV) paracetamol as primary analgesic (65). Recently, data became available on the PK of paracetamol in children after cardiac surgery indicating a decreased clearance and increased Vd compared to children after non-cardiac surgery (8, 20). We have investigated the replacement of morphine by IV paracetamol in children after cardiac surgery. Concomitant use of sedatives was also registered. Since little is known about the effects of cardiac surgery on PD postoperatively, we investigated PD with validated outcome measures. To date, the study protocol of the PACS study is published, but the PACS study has endured some difficult phases in the start-up at several participating hospitals. These delays are discussed in Chapter 9.

The delayed time-path of the PACS study is not new in pediatric drug research. Children admitted to the PICU differ from non-critically ill children. The variability in disease, treatment and patient and parental burden during PICU admission makes research in these patients

complicated. We will discuss future perspective on research in critically ill patients, with a focus on children undergoing cardiac surgery in the following paragraphs.

Key messages:

- Current guidelines and local protocols on analgesia do not account for the expected differences in PK in children after cardiac surgery compared to non-cardiac surgery patients.
- Multicenter drug research in children is challenging and delays occur both during the design and patient inclusion phases.

Future perspectives

In this thesis we have investigated changes in PK and some PD aspects in children before, during and after cardiac surgery. We have reflected on the effects these PKPD changes potentially have on drug plasma concentration and the risk of underdosing and overdosing of drugs. Most changes are patient and drug specific. Ideally, based on the results presented in this thesis and the future results of the CPB-PHARM and PACS study individualized dosing regimen can be made, thus preventing underdosing and overdosing. The data of the CPB-PHARM study extensively cover the perioperative period and extends to the postoperative period. Data from the PACS study, representing the first RCT on analgesics following pediatric cardiac surgery, can add information on the postoperative period with a special focus on PK and PD of analgesics. Patient safety and optimal treatment is intended by creating an individualized dosing regimen before surgery, based on patient characteristics.

These individualized dosing regimens are based upon population PK models, derived from data of the CPB-PHARM and PACS study. These studies have been extensive, including over 350 children undergoing cardiac surgery. Even though population PK with sparse sampling regimens has been used in these patients, the number of covariates and variation in pathology remain significant, necessitating this large number of patient enrollment in these studies. With potential future changes in drugs, patients or CPB techniques, important covariates in the PK models change. Repeating these studies in full to maintain accurate PK prediction models is not desirable. Recently, physiologically based pharmacokinetic models are being developed, based on previously investigated physiological and biochemical parameters (66). These PBPK

models are more predictive compared to the traditional population PK models. A recent study showed accurate prediction of paracetamol and it's metabolites in pregnant and non-pregnant women (67). In vivo data will be needed to validate and refine the PBPK models, but nevertheless an accurate prediction on dosing and toxicity can be made with minimal burden to patients. In children after cardiac surgery PBPK models could aid in individualized dosing regimens. These models could investigate if dosing or drugs need to be adjusted during surgery depending on changes in physiologic parameters, for instance, in case of prolonged CPB use. Also, if the use of traditional CPB would change towards mini CPB in children, PBPK models could be used to accurately predict the effects on drug concentration. Using PBPK models, a limited amount of patients need to be studied to incorporate these new techniques into predictable PK models. Another use of PBPK models is to predict shifts in drug concentration at specific moments during surgery. Immediately at the onset of CPB is such an interesting moment due to the sudden increase of Vd, but due to patient safety usually no blood sample can be taken at that time. Acute drops in drug concentration because of this hemodilution, as predicted by PBPK models, could necessitate an additional drug dose at onset of CPB to maintain the target concentration.

Ideally, not every drug needs to be studied in these critically ill children. One commonly used drug may serve as a proxy for an entire group of drugs, such as midazolam as proxy for CYP3A4 metabolized drugs. CYP3A4 is the most common subtype of the CYP P450 family and is involved in the metabolism of approximately 50% of drugs (29). The age effect of CYP3A4 is already well known, with low activity at birth increasing to adult values during infancy. One example in which this proxy drug approach has been used is the novel technique of radiolabeled drug dosing so called microdosing (68). This new technique does not interfere with general patient care and minimalizes the burden to participate in research. In radiolabeled drug dosing a radiolabeled drug is administered either concomitantly with a therapeutic dose of the same drug (microtracer) or as a sub-therapeutic dose without a concomitant therapeutic dose (microdosing). Due to the sub-therapeutic nature of the administered drugs, these doses do not interfere with clinical care. Microtracer and microdosing studies have been successfully conducted for [^{14}C]paracetamol and [^{14}C]midazolam (69, 70). Paracetamol glucuronidation and sulfation was investigated in 50 children who were administered oral [^{14}C]paracetamol microtracer dosing with concomitant

intravenous paracetamol. Both in plasma and in urine the [14C]paracetamol glucuronide/sulfate ratio increased with age, most likely reflecting a hepatic and intestinal maturation (69). A microdosing and microtracer study in [14C]midazolam showed the PK of midazolam to be comparable to therapeutic doses, thus supporting these new techniques to investigate drug PK in critically ill children (70).

Recently, it has been suggested that use of the CPB may alter Cytochrome P450 enzyme activity. Adiraju et al. describe the general influence of CPB use, with an increased Vd and decreased Cl (71). However, an overview of available literature comprised by the authors, shows that decreased clearance seems to be more pronounced in hepatic cleared drugs, compared to renal cleared drugs. The authors suggest that use of CPB increases pro-inflammatory cytokines, leading to inhibition of one or more CYPs. It has been shown that also C-reactive protein and procalcitonin levels are increased in children after cardiac surgery (55). There is no previous connection reported between CPB use and changes in CYP enzyme activity. However, influence of inflammation and infection has been known to reduce CYP substrate activity by 20-70% (72).

Another important factor in drug research is the target organ. We routinely measure drugs in plasma, however some drugs need an optimal concentration in the brain, such as analgesic drugs, or subcutaneous tissue, such as antibiotics. The CefCheck study investigates cefazolin PK in adults during and after cardiac surgery. We measured cefazolin concentration in plasma. However, the optimal cefazolin concentration is needed most in the subcutaneous tissue to prevent sternal wound infections. Subcutaneous tissue concentration of cefazolin has been investigated using microdialysis (73, 74). In morbidly obese patients tissue distribution of cefazolin was lower compared to non-obese patients (73). In children during cardiac surgery, mainly the use of deep hypothermia was essential in skeletal muscle cefazolin concentration (74). This stresses the importance of drug measurements in the target organ, but also the feasibility of these measurements. Potentially the same results could be obtained when obtaining a small tissue biopsy during wound closure to measure cefazolin tissue concentration, considering the costs and infrastructure needed for the implementation of the microdialysis technique.

Key messages:

- Proxy drugs need to be incorporated in studies, reducing the number of investigated drugs, but building the knowledge on groups of drugs.
- The influence of the CPB on CYP P450 and thus drug metabolism should be further explored and should be incorporated in future studies.
- Ideally, drug concentration should be measured in the target organ when this is easily accessible, or PBPK models could be used to accurately predict the target organ concentration guiding evidence-based dosing in the future

Conclusion

This thesis investigates the knowledge gap of drug PK and partly PD in children with cardiac defects undergoing cardiac surgery. Changes in drug PK parameters, largely induced by CPB and cell saver use, are shown to change in vitro and in vivo drug concentrations. These changes could lead to sub-therapeutic doses or toxicity and should be incorporated in individualized dosing regimens to enhance patient care of this vulnerable group of children.

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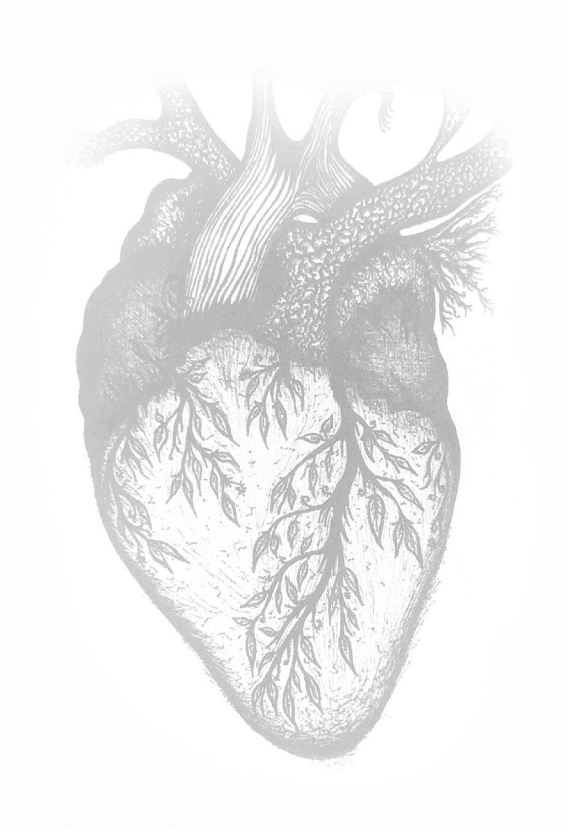

Chapter 11

Summary
Samenvatting

Summary

Use of the cardiopulmonary bypass (CPB) is often necessary during pediatric cardiac surgery to replace the function of the heart and lungs. However, CPB may influence the concentration of drugs that are administered during and after surgery. There are several causes for these changes in drug concentration, such as hemodilution and absorption of drugs in the CPB system. Hemodilution occurs at the onset of CPB, when the total circulating volume increases to sometimes double of the patients own blood volume. Absorption of drugs in the CPB system is most likely dependent on type of CPB and type of drug. Both these changes could cause a decrease in drug concentration, leading to subtherapeutic and thus ineffective drug doses. Opposite to this, unexpected accumulation of drugs could lead to toxicity. In children, influence of CPB on plasma drug concentration has not been investigated.

The first part of this thesis investigates the influence of CPB on pharmacokinetic (PK) parameters of routinely used drugs. A clearer insight into the PK of drugs during and after cardiac surgery with the use of CPB could lead to more precise dosing in pediatric patients, preferably based on patient and procedure specific factors.

PK in children during the first days after cardiac surgery may continue to be different compared to children after non-cardiac surgery. Optimal pain treatment after cardiac surgery may therefore also be different in these children. There are no clear international guidelines on optimal pain treatment in children after cardiac surgery. Because of this, considerable variation in type and dose of analgesia remains in worldwide practice. Morphine is the drug of first choice in many hospitals even though morphine has several adverse drug effects, such as hypotension and respiratory depression. Moreover, the use of validated pharmacodynamic (PD) assessment tools to assess clinical efficacy of pain treatment is often not implemented in clinical practice. Several years ago it was shown that intravenous (IV) paracetamol was equally effective to morphine as primary analgesic in children after major abdominal surgery. Also, children who received IV paracetamol had less drug related adverse events. In children after cardiac surgery, who are often hemodynamically unstable and may be more prone to morphine related drug reactions, use of IV paracetamol may be preferable.

The second part of this thesis investigates the pain treatment in children after cardiac surgery with the use of the CPB. We aim to achieve a more uniform and evidence based approach

towards pain treatment after pediatric cardiac surgery and to reduce morphine related adverse effects by decreasing the postoperative morphine consumption.

Chapter 2 gives an overview of PK in relation to pain treatment in several patient groups on the pediatric intensive care unit (PICU). General aspects of PK are described and PK changes in patients after cardiac surgery compared to patients after non-cardiac surgery are discussed.

The CPB-PHARM study and its sub studies are described in **Chapter 3** to **5**. **Chapter 3** investigates the potential drug loss and recovery due to use of the cell saver system. The cell saver system is a autotransfusion system that is used to reduce blood loss during surgery. Drugs could be lost during surgery because of removal by the cell saver system. However, drugs could also be returned to the patient through the autotransfused blood. We describe the proceedings of the cell saver system and the measurements of drugs at different stages during the procedure and in the autotransfused blood. We measured cefazolin, a hydrophilic drug, and sufentanil, propofol and midazolam, lipophilic drugs. Cefazolin was washed out during the cell saver procedure and was not measurable in the autotransfused blood. Of the three lipophilic drugs we tested, only sufentanil reached a potentially clinically relevant concentration in the autotransfused blood. The discrepancy between sufentanil and the other lipophilic drugs, propofol and midazolam, is not well understood. Propofol binds to erythrocytes and measurements of drugs in plasma may therefore not be representative of the actual total bound concentration of propofol.

The in vitro experiments of the CPB-PHARM study are discussed in the **Chapters 4** and **5**. We have divided the measured drugs in antibiotics in **Chapter 4** and anesthetic drugs and methylprednisolone in **Chapter 5**. Neonatal, infant and pediatric CPB systems, similar to the ones we use in clinical practice, were spiked with routinely used drugs. Blood samples were taken at several time points and after six hours the experiments were stopped. Propofol and sufentanil decreased substantially during the CPB runtime. Concentrations of midazolam and cefazolin remained relatively stable during the experiments. The measured concentrations of clindamycine and methylprednisolone remained stable as well, however, the first measured concentration showed great discrepancy with the theoretical starting concentration. This means that either, both drugs sequestered very rapidly in the CPB systems, or both drugs were not mixed with the priming fluid enough to reach an observed concentration similar to the

theoretical concentration. Type of CPB system was of significant influence in drug recovery, possibly due to surface to volume ratio.

Chapter 6 discusses the only adult data in this thesis. The CefCheck study was designed because of the new Dutch guideline on peri-operative antibiotic prophylaxis in adults undergoing cardiac surgery, introducing a decrease in cefazolin dosing. Evidence for this change was driven by non-cardiac surgery. However, during cardiac surgery, use of the CPB system may lead to a decrease in plasma levels of cefazolin and sub-optimal antibiotic prophylaxis. Our data show that the plasma cefazolin concentration gradually drops at onset of CPB. We also found that the protein bound fraction of cefazolin is much more variable than previously described in literature.

In **Chapter 7** the focus is shifted from routinely used drugs during the peri-operative period to pain treatment in the postoperative period. Our international survey showed a multitude of types and doses of analgesics used in children after cardiac surgery. Morphine is the drug of first choice, but dosing regimens differ substantially between hospitals. Validated assessment tools to determine and assess pharmacodynamics (PD) endpoints are often not used in the clinic. Without these tools, efficacy of pain treatment, and potential underdosing or overdosing are difficult to assess and correct.

In neonates and infants after major abdominal, non-cardiac surgery, pain treatment with IV (intravenous) paracetamol proved equally effective as morphine. Considering the hemodynamic undesirable effects of morphine, such as hypotension, IV paracetamol may also be preferable in children after cardiac surgery. We have designed the PACS (Pediatric Analgesia after Cardiac Surgery) study, a multi-center randomized controlled trial, to investigate this. **Chapter 8** describes the PACS study protocol. 208 children, aged 0-3 years, were randomized to receive IV morphine or IV paracetamol after cardiac surgery with the use of CPB. The primary endpoint was a 30% reduction in cumulative morphine consumption during the first 48 hours after surgery.

Much time and effort of conducting a randomized controlled trial is in the design, preparation and logistics. However, this is rarely presented in literature and fellow researchers cannot use previous experience to their own advantage when designing a randomized controlled drug trial. We describe our experiences from the PACS study in **Chapter 9**. The main delay in our

study timeline was due to pharmacy regulations, late withdrawal of a trial site, and negotiations with the judicial departments of the participating hospitals.

Samenvatting

Het gebruik van de hartlong machine (HLM) is vaak nodig tijdens cardiochirurgie om de functie van het hart en de longen over te nemen. Het gebruik van de HLM heeft invloed op de plasmaconcentratie van medicijnen die tijdens én na de operatie worden toegediend. Er zijn verschillende redenen voor deze beïnvloeding, zoals toename van het circulerend volume, verminderde uitscheiding van medicijnen en opname van medicatie in de HLM. Het starten van de HLM geeft een toename van het totale circulerend volume, tot soms een verdubbeling van het eigen circulerend volume van de patiënt. Opname van medicijnen in de HLM is waarschijnlijk afhankelijk van het type HLM en de eigenschappen van het medicijn. Deze veranderingen kunnen leiden tot een verminderde plasmaconcentratie van medicijnen en daarmee een subtherapeutische en ineffectieve dosering. Echter, een onverwachte opstapeling van medicijnen kan leiden tot toxiciteit. Bij kinderen zijn de effecten van de HLM op medicijn concentraties niet onderzocht.

Het eerste deel van deze thesis gaat over de invloed van de HLM op de farmacokinetiek (PK) van medicijnen die zeer vaak worden gebruikt tijdens cardiochirurgie. Een beter inzicht in de PK van medicatie tijdens en na de operatie kan leiden tot een preciezer doseringsadvies in kinderen, bij voorkeur gebaseerd op specifieke eigenschappen van de patiënt en de operatie.

Bij kinderen na cardiochirurgie blijft de PK gedurende de eerste dagen na de operatie anders dan bij kinderen na grote, niet-cardiochirurgische operaties. Optimale post-operatieve pijnbestrijding is in kinderen na cardiochirurgie dus mogelijk ook anders. Er zijn geen duidelijke wereldwijde richtlijnen voor pijnbestrijding bij kinderen na cardiochirurgie. Mede om deze reden is er wereldwijd grote variatie in het type en de dosering van de gebruikte pijnstillers. In veel ziekenhuizen is morfine de pijnstiller van eerste keus, terwijl morfine veel ongewenste bijwerkingen kan hebben zoals hypotensie en ademhalingsdepressie. Het gebruik van gevalideerde meetinstrumenten om de effectiviteit van de pijnstilling te meten worden vaak niet gebruikt in de kliniek. Enkele jaren geleden werd aangetoond dat pijnstilling met paracetamol intraveneus (i.v.) even effectief was als pijnstilling met morfine bij kinderen na grote niet-cardiochirurgische operaties. Kinderen die paracetamol i.v. kregen hadden ook minder medicatie gerelateerde bijwerkingen. Kinderen na cardiochirurgie hebben mogelijk meer last van morfine gerelateerde bijwerkingen door de hemodynamisch instabiele situatie

na de operatie. Deze kinderen hebben dus potentieel baat bij het gebruik van paracetamol i.v. als eerste keus pijnstiller.

Het tweede deel van deze thesis onderzoekt de pijnstilling in kinderen na cardiochirurgie. Het doel van het onderzoek is het vaststellen van een meer evidence-based benadering van pijnstilling in kinderen na cardiochirurgie en het verminderen van de morfine gerelateerde bijwerkingen door het verminderen van de totale morfine consumptie na de operatie.

Hoofdstuk 2 geeft een overzicht van verschillende patiënten groepen op de intensive care kinderen (ICK) in relatie tot de verschillen in PK en pijnstilling. Algemene aspecten van PK worden beschreven en de verschillen tussen patiënten na cardiochirurgie in vergelijking tot andere patiënt groepen wordt besproken.

De CPB-PHARM studie en verschillende substudies worden besproken in **Hoofdstukken 3** tot en met **5**. **Hoofdstuk 3** onderzoekt het potentiele medicatie verlies en terugwinning door gebruik van het cell saver systeem. Het cellsaver systeem is een auto-transfusie systeem wat wordt gebruikt tijdens cardiochirurgie om het peri-operatieve bloedverlies te verminderen. Tijdens de operatie kan medicatie samen met bloed worden weggezogen van het operatie gebied door het cell saver systeem. Via het autotransfusie bloed kan vervolgens medicatie aan de patiënt worden toegediend. In dit hoofdstuk wordt de werking van het cell saver systeem beschreven. Tevens zijn plasmaconcentraties van medicijnen gemeten op verschillende momenten tijdens de cell saver procedure en in het autotransfusie bloed. Tijdens de experimenten werden cefazoline, een hydrofiel medicament, en sufentanil, propofol en midazolam, lipofiele medicamenten, gemeten. Cefazoline werd uitgescheiden tijdens de cell saver procedure en werd niet teruggevonden in het autotransfusie bloed. Van de lipofiele medicamenten die zijn getest, werd alleen voor sufentanil een potentieel klinisch relevante concentratie in het autotransfusie bloed gemeten. Het verschil in plasmaconcentraties tussen de lipofiele medicamenten in het autotransfusie bloed is onduidelijk. Propofol bind aan erytrocyten en het meten van propofol in plasma geeft daarom mogelijk een foutieve weergave van de werkelijke concentratie.

De *in vitro* experimenten van de CPB-PHARM studie worden beschreven in **Hoofdstukken 4** en **5**. In drie verschillende typen HLM werd de plasmaconcentratie van verschillende medicijnen gemeten en een tijd-concentratie curve per systeem en per medicijn

samengesteld. De medicamenten werden onderverdeeld in antibiotica in **Hoofdstuk 4** en anesthetica en methylprednisolon in **Hoofdstuk 5**. Medicatie werd toegediend in neonatale, infant en pediatrische HLM. Bloedsamples werden afgenomen op verschillende tijdspunten en na zes uur werden de experimenten gestopt. Concentraties van propofol en sufentanil verminderden aanzienlijk tijdens het experiment, terwijl concentraties van midazolam en cefazoline relatief stabiel bleven. Concentraties van clindamycine en methylprednisolon bleven relatief stabiel, echter, de eerst gemeten concentratie van beide medicamenten was substantieel anders dan de theoretische start concentratie. Dit betekent dat beide medicamenten zeer snel werden opgenomen door de HLM, of beiden onvoldoende mengden met de priming vloeistof in het systeem om tot vergelijkbare geobserveerde en theoretische concentraties te komen. Het type HLM had een significante invloed op de afname van de plasmaconcentraties van medicijnen, mogelijk door een verandering in de oppervlakte/volume ratio.

In **Hoofdstuk 6** wordt de enige data van volwassen patiënten besproken. De CecfCheck studie werd opgezet vanwege de invoering van de nieuwe Nederlandse peri-operatieve richtlijn voor antibiotica profylaxe bij volwassen cardiochirurgische patienten. Gebaseerd op data van niet-cardiochirurgische patiënten, wordt in deze nieuwe richtlijn de cefazoline dosering tijdens cardiochirurgische operaties verminderd. Echter, het gebruik van de HLM zou kunnen leiden tot een lagere cefazoline concentratie en suboptimale antibiotica profylaxe. Onze data laat zien dat de cefazoline concentratie daalt na het starten van de HLM. De eiwit binding van cefazoline is veel variabeler in onze studie, vergeleken met de beschikbare literatuur.

In **Hoofdstuk 7** wordt de focus van de thesis verschoven van peri-operatieve gebruikte medicatie naar post-operatieve pijnbestrijding. Onze internationale survey toont een grote variatie in type en dosering van analgetica in kinderen na cardiochirurgie. Morfine is bijna altijd de eerste keus analgetica, maar de dosering verschilt sterk tussen ziekenhuizen. Gevalideerde methoden om de effectiviteit van de pijnstilling te meten worden vaak niet gebruikt in de kliniek. Bij gebrek aan methoden om de effectiviteit van de pijnstilling te meten, kunnen onder- of overdosering moeilijk worden opgemerkt en gecorrigeerd.

In neonaten en jonge kinderen na grote niet-cardiochirurgische operaties, werkt pijnstilling met paracetamol i.v. even goed als pijnstilling met morfine. Vanwege de negatieve

bijwerkingen van morfine op onder andere de bloeddruk, zou pijnstilling met paracetamol i.v. ook in kinderen na cardiochirurgie de voorkeur kunnen hebben. We hebben de PACS (Pediatric Analgesia after Cardiac Surgery) studie ontworpen om deze hypothese te testen. De PACS studie is een multi-center, gerandomizeerde, dubbel-blinde studie. In **Hoofdstuk 8** wordt het PACS studie protocol beschreven. In de PACS studie kregen 208 kinderen, in de leeftijd van nul tot drie jaar, gerandomiseerd paracetamol i.v. of morfine i.v. na cardiochirurgie toegediend. Het primaire doel van de studie was een 30% vermindering van de morfine consumptie in de eerste 48 uur na de operatie.

De opzet, voorbereiding en logistiek vergen een groot deel van de tijd en energie bij het uitvoeren van een multi-center, gerandomiseerde studie. Deze facetten worden bijna nooit in de literatuur besproken, waardoor collega onderzoekers geen gebruik kunnen maken van eerder opgedane ervaring. Wij beschrijven onze ervaringen met de PACS studie in **Hoofdstuk 9**. De grootste vertraging tijdens de PACS studie werd veroorzaakt door regelgeving van de trialapotheken, de late terugtrekking van een deelnemend centrum en langdurige onderhandelingen met de juridische afdelingen van deelnemende ziekenhuizen.

Appendices

About the author
List of publications
PHD portfolio
Dankwoord

About the author

Gerdien Zeilmaker was born on February 21, 1987, in Dordrecht, the Netherlands. From 2005-2012 she studied medicine at the Medical Faculty of the Erasmus MC in Rotterdam. As part of her clinical rotations she did a clinical elective at general practitioners offices in Curacao. After Medical school she worked as an pediatric resident at the Maasstad Ziekenhuis in Rotterdam. In 2013 she started her dissertation on pediatric pharmacology and analgesia at the pediatric intensive care unit and the department of cardiothoracic surgery (promotor prof. dr. D. Tibboel, promotor prof. dr. A.J.J.C. Bogers, supervisor dr. E.D. Wildschut). During this period she was the president of Promeras, the PhD association of the Erasmus MC. In January 2019 Gerdien started her pediatric residency at the Erasmus MC-Sophia Children's hospital in Rotterdam and the Amphia hospital in Breda.

Gerdien lives in Rotterdam with her husband Gert-Jan and their daughter Lizzy.

List of publications

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PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student: Gerdien Zeilmaker Erasmus MC Department: Thoracic surgery/Pediatric ICU Research School: COEUR	PhD period: 1-10-2013 / 30-09-2018 Promotor(s): prof. A.J.J.C. Bogers, prof. D. Tibboel Supervisor: dr. E.D. Wildschut

1. PhD training

	Year	Workload (Hours/ECTS)
General academic skills		
- CPO course	2014	0.3
- Biomedical English Writing and Communication	2015	4.0
- Research Integrity	2015	0.3
- BROK (Basic course Rules and Organization for Clinical researchers)	2016	1.5
Research skills		
- Nihes - Principles of Research in Medicine	2014	0.7
- Nihes - Master Class: Advances in Epidemiologic Analysis	2014	0.4
- Nihes - Methods of Clinical Research	2014	0.7
- Nihes - Fundamentals of Medical Decision Making	2014	0.7
- Nihes - Master Class: Advances in Genomic Research	2014	0.4
- Nihes - The Practice of Epidemiologic Analysis	2014	0.7
- COEUR - Arrhythmia Research Methodology	2014	1.5
- COEUR - Pathophysiology of Ischemic Heart Disease	2014	1.5
- COEUR - Intensive Care Research	2014 & 2017	2.5
- COEUR - Congenital Heart Disease	2014 & 2017	2.0
- Nihes - Biostatistical Methods 1: Basic Principles	2015	5.7

- COEUR – cardiovascular Imaging and Diagnostics	2017	0.5
Presentations		
- Pediatric Pharmacology Research Meetings: *multiple oral presentation*	2014-2017	1.5
- Sophia Scientific Research Committee *(Molenaar Award)*	2015	0.3
- Annual Leiden-Rotterdam Pharma Day: *oral presentation (3x)*	2015-2017	1.5
- Clinical lessons surgical and anesthesiologist assistants on drugs during CPB and analgesics *(n=2)*	2016	0.6
- Pediatric Cardiac Anesthesiology Meeting *(n=2)*	2016	0.6
- Annual Research Day dept. of Anesthesiology	2018	0.5
International conferences		
- 4th meeting on European Pediatric and Neonatal Cardiac Intensive Care, Montreux, Suisse: *poster presentation*	2014	0.9
- 27th Annual meeting of World Society of Cardiovascular and Thoracic Surgeons, Astana, Kazakhstan: *oral presentation*	2017	1.5
Seminars and workshops		
- Klinisch onderzoek met Kinderen	2014	0.3
- Nihes - Women's Health	2014	0.9
- COEUR debate (team dept. Cardiothoracic Surgery)	2014	0.3
- Research Integrity: Challenges, Commitments, Culture congress	2015	0.3
- COEUR PhD day	2014-2018	1.5
- Sophia Research Day	2014-2018	1.5
- ZonMw congres Goed Gebruik Geneesmiddelen	2016-2017	0.6
- Annual SICK symposium	2017	0.3
- Pulmonary Hypertension Symposium	2018	0.3

- IPOKRaTES Clinical Seminar: Evidence Based Pharmacotherapy in Newborns and Children: Implementation in Daily Clinical Practice	2018	0.9
Teaching activities		
- Supervising final year high school student's thesis	2015	0.6
- Supervising thesis clinical perfusionist	2017	1.0
Other		
- Coordinating Journal Club dept. of Cardiothoracic Surgery	2015-2017	1.0
- Promeras board	2016-2018	3.0

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