DISSERTATIONES
MEDICINAE
UNIVERSITATIS
TARTUENSIS
301

MAARJA HALLIK

Pharmacokinetics and pharmacodynamics of inotropic drugs in neonates

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

301

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
301

MAARJA HALLIK

Pharmacokinetics and pharmacodynamics of inotropic drugs in neonates

UNIVERSITY OF TARTU
Press

Department of Anesthesiology and Intensive Care, Institute of Clinical Medicine, Faculty of Medicine, University of Tartu

This dissertation is accepted for the commencement of the degree of Doctor of Philosophy in Medicine on April 15th, 2020 by the Council of the Faculty of Medicine, University of Tartu, Estonia.

Supervisor: Professor Tuuli Metsvaht, MD, PhD
Department of Pediatrics, Institute of Clinical Medicine, Faculty of Medicine, University of Tartu

Co-supervisor: Professor Joel Starkopf, MD, PhD
Department of Anesthesiology and Intensive Care, Institute of Clinical Medicine, Faculty of Medicine, University of Tartu

Reviewers: Professor Irja Lutsar, MD, PhD
Department of Microbiology, Institute of Biomedicine and Translational Medicine, University of Tartu

Professor Anti Kalda, MD, PhD
Department of Pharmacology, Institute of Biomedicine and Translational Medicine, University of Tartu

Opponent: Professor Saskia de Wildt, MD, PhD
Department of Pharmacology and Toxicology, Radboud University Medical Center, Nijmegen, The Netherlands
Intensive Care and Department of Pediatric Surgery, Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands

Commencement: June 9th, 2020, Tartu

Publication of this dissertation is granted by the University of Tartu.

ISSN 1024-395X
ISBN 978-9949-03-335-5 (print)
ISBN 978-9949-03-336-2 (pdf)

University of Tartu Press
www.tyk.ee

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

Hallik M, Tasa T, Starkopf J, Metsvaht T. Dosing of Milrinone in Preterm Neonates to Prevent Postligation Cardiac Syndrome: Simulation Study Suggests Need for Bolus Infusion. Neonatology. 2016 Aug 5;111(1):8–11.

Hallik M, Ilmoja ML, Tasa T, Standing JF, Takkis K, Veigure R, Kipper K, Jalas T, Raidmäe M, Uibo K, Starkopf J, Metsvaht T. Population Pharmacokinetics and Dosing of Milrinone After Patent Ductus Arteriosus Ligation in Preterm Infants. Pediatr Crit Care Med. 2019 Jul;20(7):621–629.

Hallik M, Ilmoja M, Standing JF, Soeorg H, Jalas T, Raidmäe M, Uibo K, Köbas K, Sõnajalg M, Takkis K, Veigure R, Kipper K, Starkopf J, Metsvaht T. Population Pharmacokinetics and Pharmacodynamics of Dobutamine in Neonates on the First Days of Life. Br J Clin Pharmacol. 2020;86(2):318–28.

Applicant's personal contribution:

In article I: participated in the study design, conducted data collection, drafted the manuscript and was responsible for the responses throughout the review process.

In article II: participated in the study design, patient recruitment and data collection, performed data analysis including PK modelling and interpretation, drafted the manuscript and was responsible for the responses throughout the review process.

In article III: participated in the study design, patient recruitment and data collection, performed data analysis including PKPD modelling and interpretation, drafted the manuscript and was responsible for the responses throughout the review process.

LIST OF PRESENTATIONS AT INTERNATIONAL CONFERENCES

Hallik M, Starkopf J, Nellis G, Varendi H, Turner M, Lutsar I, Metsvaht T on behalf of the ESNEE Consortium. Use of Cardiovascular Medications in European Neonatal Intensive Care Units: Sub-Analysis of Esnee Point Prevalence Study. Poster presentation at 7th World Congress on Pediatric Intensive and Critical Care. 2014, Istanbul, Turkey.

Hallik M, Ilmoja M-L, Starkopf J, Metsvaht T. Feasibility of Complex Monitoring of Haemodynamics in Premature Newborns During First Days of Life (Pilot Study). Poster presentation at 26th Annual Meeting of the European Society of Paediatric and Neonatal intensive Care (ESPNIC). 2015, Vilnius, Lithuania.

Hallik M, Tasa T, Starkopf J, Metsvaht T. Milrinone for post ligation cardiac syndrome (PLCS) in preterm neonates – is bolus infusion needed? Poster presentation at 4th European Conference on Paediatric and Neonatal Cardiac Intensive Care (EPNCIC). 2015, Montreux, Switzerland.

Hallik M, Ilmoja M-L, Väli M, Starkpf J, Metsvaht T. Does dobutamine improve early postnatal cardiac output? Central hemodynamic dose-response study. Poster presentation at 34th Congress of The Scandinavian Society of Anaesthesiology and Intensive Care Medicine (SSAI). 2017, Malmö, Sweden.

Hallik M, Ilmoja ML, Tasa T, Standing JF, Takkis K, Veigure R, Kipper K, Jalas T, Raidmäe M, Uibo K, Starkopf J, Metsvaht T. Population PK and Dosing of Milrinone After PDA Ligation in Preterm Infants. Poster presentation at 7th Congress of the European Academy of Paediatric Societies. 2018, Paris, France.

Hallik M, Starkopf J, Ilmoja M-L, Soeorg H, Metsvaht T. Population Pharmacokinetics and Pharmacodynamics of Dobutamine in Neonates on the First Days of Life. Oral presentation at 11th European Congress on Paediatric Anaesthesia. 2019, Rotterdam, Netherlands.

ABBREVIATIONS

AR	adrenoreceptor
BP	blood pressure
BSV	between subject variability
BW	birth weight
CBF	cerebral blood flow
cFTOE	cerebral fractional oxygen extraction
CL	clearance
CO	cardiac output
Css	steady state plasma concentration
ELBW	extremely low birth weight, < 1000 g
GA	gestational age
GFR	glomerular filtration rate
GOF	goodness-of-fit
HD	haemodynamic/haemodynamics
HR	heart rate
IVH	intraventricular haemorrhage
LLOQ	lower limit of quantification
LV	left ventricle
LVO	left ventricular output
MAP	mean arterial blood pressure
NICOM	non-invasive electrical cardiometry
NICU	neonatal intensive care unit
NIRS	near-infrared spectroscopy
OFV	objective function value
OHS	open heart surgery
OPS	orthogonal polarization spectral imaging
PD	pharmacodynamic/pharmacodynamics
PDA	patent *ductus arteriosus*
PK	pharmacokinetic/pharmacokinetics
PKPD	pharmacokinetic-pharmacodynamic
PLCS	post-ligation cardiac syndrome
PMA	postmenstrual age
PMA_{50}	postmenstrual age when the maturation of PK parameter reaches 50% of adult values
PNA	postnatal age
PPHN	persistent pulmonary hypertension of the newborn
PTA	probability of target attainment
RBC	red blood cells
$rScO_2$	cerebral regional oxygen saturation
RVO	right ventricular output
SAE	serious adverse event
SaO_2	arterial oxygen saturation

SCr	serum creatinine
SD	standard deviation
SDF	side stream dark field imaging
SE	standard error
SVCF	superior vena cava flow
SVR	systemic vascular resistance
V	volume of distribution
VLBW	very low birth weight, <1500 g
VPC	visual predictive check

1. INTRODUCTION

The neonatal period, covering only the first 28 days after birth, includes major developmental differences. As premature birth is the main reason for critical illness within this period, neonatologists are confronted with a more than ten-fold weight range as well as variable gestational age (GA), postnatal age (PNA) and dysmaturity. Furthermore, in addition to rapid maturational changes, critical illness and intensive and invasive therapies increase the physiological variability.

We use many medications in neonatal intensive care units (NICU), at least up to 65% of them off-label (Lass et al. 2011; Nir-Neuman et al. 2018). Preterm neonates – the most vulnerable paediatric age group – have the highest exposure to drugs that are insufficiently documented (Kimland et al. 2012; Lass et al. 2011). Difficulties interpreting the sparse evidence also lead to large differences in drug prescribing practices and local administration protocols between NICUs (Batton et al. 2013, 2016; Burns et al. 2016; Sehgal et al. 2012; Stranak et al. 2014; Wong et al. 2015).

The same goes for cardiovascular drug therapies in neonates. There is little evidence for which vasoactive medications to use in which patient and when, at what dose to start, how to titrate the drug, and which parameters to monitor (Barrington 2008; Evans 2006; Kluckow et al. 2008). Administration of inotropes is based on a variety of clinical assessments, including low blood pressure (BP), poor cardiac function or low cardiac output (CO), poor peripheral perfusion or decreased urine output, and lactic acidosis, all considered to be indicators of circulatory failure. Neonatal cardiovascular physiology has many important unique features; thus, the dosing and presumptive effects of therapeutic interventions cannot be extrapolated from adult studies.

Pharmacokinetic-pharmacodynamic (PKPD) modelling is an important tool in clinical pharmacology research, and the population approach is suitable for sparse data in terms of the number of samples per subject, as is often the case in neonates. The identification of predictive developmental and non-maturational covariates provides the scientific basis for rational and individualised, patient-tailored dosing schemes. Without an adequate understanding of the PKPD relationship, its variability at the inter- and intra-individual levels, and how it changes during the rapid maturation in the neonatal period, it is possible that inappropriate doses will be studied and potentially useful drugs will be discarded because of inappropriate dosing. It has recently been shown that failure to properly appreciate dose-response relationships has been a leading factor in problems with generating information useful for licensing paediatric medicinal products (Jadhav et al. 2010).

It is necessary to study the pharmacokinetic (PK) and pharmacodynamic (PD) properties of medications in the specific neonatal populations for which they are used. The present thesis concentrates on two particular situations and their respective populations: transitional circulation in critical illness within the first days of life and post-ligation cardiac syndrome (PLCS) prevention.

We started with finding the optimal dosage of milrinone for our study patients according to the best knowledge about the PK of the drug and the peculiarity of the population of neonates undergoing patent *ductus arteriosus* (PDA) ligation. The dosing scheme was carried forward to a prospective study in this population to get more precise information about the PK of milrinone in neonates of different ages. Finally, we conducted a prospective PKPD study of dobutamine to describe concentration-related effects on haemodynamics (HD) in critically ill neonates within the first days of life.

2. REVIEW OF LITERATURE

2.1 Transitional circulation of a newborn

The neonatal cardiovascular system has many special characteristics that make the HD compromise of a newborn different from that of other paediatric and adult populations. The function of the newborn heart is characterized by a relatively high resting CO with little contractile reserve due to the presence of non-contractile fibrous tissue, which results in high sensitivity to any increase in afterload. Newborn babies can increase their CO by tachycardia, but severe tachycardia may result in decreased stroke volume. Neonatal vasoregulation is characterized by a high concentration of endogenous catecholamines balanced by activated vasodilating systems such as prostaglandins, bradykinin and nitric oxide (Toth-Heyn et al. 2012). The developmentally regulated differences in vascular α- and β-adrenoreceptor (AR) expression affect the cardiac and peripheral vascular response (Felder et al. 1983; Noori et al. 2012; Vapaavouri et al. 1973).

Preterm babies tend to have a hypoperfusion reperfusion cycle over the first 24–48 hours. The hypoperfusion seems to relate to maladaptation to high vascular resistance, while the reperfusion relates to loss of vascular resistance (Evans 2006, 2009). Factors probably aggravating hypotension and its consequences on systemic and cerebral blood flow (CBF) in preterm infants include hypovolaemia, PDA, sepsis, persistent pulmonary hypertension of the newborn (PPHN), and severe respiratory distress.

2.2 Patent *ductus arteriosus* and cardiovascular response after ligation

The *ductus arteriosus* is a physiological structure shunting blood from the pulmonary artery to the descending aorta during foetal life (Gentile et al. 1981). The *ductus arteriosus* closes in the majority of term infants within the first 48 hours after birth. In about 50–70% of extremely low birth weight (ELBW) infants, it remains open and may cause various clinical problems (Clyman et al. 2012; Koch et al. 2006; Nemerofsky et al. 2008). Today, no consensus exists on the definition of hemodynamically significant PDA, and it remains difficult to identify the patients most likely to benefit from surgical duct closure. Observational studies show a 10–21% incidence of surgical closure (Boghossian et al. 2017; Hagadorn et al. 2016; Lokku et al. 2016; Weinberg et al. 2016). Surgical ligation of a PDA leads to sudden changes in cardiovascular physiology, specifically a rise in left ventricular (LV) afterload and a fall in LV preload, with a resulting sudden drop in LV output (LVO) (El-Khuffash et al. 2012; Jain et al. 2012; Noori et al. 2007). Prospective observational studies in human neonates have demonstrated declining LV performance in the immediate postoperative period, coinciding with decreased pulmonary venous return and increased SVR.

The clinical effects of this low CO state usually become apparent at 6–12 hours after ligation (McNamara et al. 2010; Teixeira et al. 2008).

Given that the effect of preload change is greatest within the first 1–2 hours after surgery, this change is unlikely the major determinant of the deterioration in clinical condition. An increase in the slope of the inverse relationship between end-systolic wall stress and the velocity of circumferential fibre shortening suggests that changes in myocardial performance are related to LV afterload. The timing of clinical deterioration (8–12 hours after surgery) coincides with the period of maximal afterload exposure (McNamara et al. 2010). Infants weighing <1000 g are at the greatest risk of HD compromise, which is biologically plausible, considering that the preterm myocardium is not conditioned to handle substantial changes in either preload or afterload. A prospective study using non-invasive electrical cardiometry (NICOM) to measure hemodynamic changes during surgical ligation of PDA in very low birth weight (VLBW) infants concludes that reduced stroke volume and elevated vascular resistance contribute to the major hemodynamic aberrations in VLBW infants receiving PDA ligation surgery (Lien et al. 2015).

The presence of prolonged left-to-right shunting across the PDA leads to altered pulmonary compliance. Improvement in lung compliance has been shown with successful medical as well as surgical PDA closure (Balsan et al. 1991; Farstad et al. 1994; Stefano et al. 1991; Szymankiewicz et al. 2004). If dynamic lung compliance improvement is coupled with an increase in tidal volume and minute ventilation, these changes may lead to lung overdistention, possibly further compromising vena cava and pulmonary venous flow, leading to impaired ventricular filling and contributing to decreased CO (El-Khuffash et al. 2013).

Changes in cardiopulmonary physiology after surgery may lead to a severe HD and respiratory deterioration – PLCS, defined as systolic BP below the 3rd percentile expected for GA requiring one or more cardiotropic agents accompanied by ventilation or oxygenation failure – in 10–40% of infants (Jain et al. 2012; Teixeira et al. 2008). PLCS is related to an increase in mortality (33% vs 11% compared with controls) (Harting et al. 2008). Risk factors reportedly associated with PLCS include earlier age at ligation, lower BW, younger GA, large PDA and the level of preoperative cardiorespiratory support (Harting et al. 2008; McNamara et al. 2010; Mertens et al. 2011; Moin et al. 2003; Noori et al. 2007, 2015; Teixeira et al. 2008; Ulrich et al. 2018). The definition cited above may be too restrictive, however, as PLCS is likely a spectrum disorder of varying severity, with some infants developing profound hypotension and others suffering exclusively from ventilation and oxygenation failure (El-Khuffash et al. 2013). PLCS typically occurs between 6–12 hours after surgery; therefore, there is a temporal window for early intervention with inotropic therapy (Jain et al. 2012; McNamara et al. 2010; Teixeira et al. 2008). Left ventricular output (LVO) <200 ml/kg/min at one hour after PDA ligation has been found to be a sensitive predictor of systemic hypotension and the need for inotropes (Jain et al. 2012).

2.3 The use of inotropes in neonates

2.3.1 Current practice

Inotropic treatment is started in response to low BP, low CO or signs of deficient tissue perfusion in critically ill neonates. The challenge for clinicians in the NICU is to identify the aetiology of the hemodynamic changes, decide if the changes are transitionally appropriate or not, and based on this understanding, tailor the treatment regimen for the patient.

Arterial hypotension during the early postnatal period is common in preterm infants and sick term neonates. Low tissue perfusion as a consequence of hypotension and failure of cerebral vascular auto-regulation are suggested as important risk factors for postnatal brain injury and neonatal morbidity of preterm and sick term neonates, including multi-organ dysfunction and acquired brain lesions (Evans 2009; Osborn et al. 2003; Volpe 2001). However, the threshold of treatment to improve short- and long-term outcomes has not been established (Laughon et al. 2007).

Studies in Europe, North America, and Australia highlight variable practices across countries and continents with respect to which patient, when and how to treat with which inotropic drugs (Batton et al. 2013, 2016; Burns et al. 2016; Sehgal et al. 2012; Stranak et al. 2014; Wong et al. 2015). An observational study in extremely preterm infants with GA less than 27 weeks reports that 55% of this population received any anti-hypotensive therapy and 28% received vasoactive drugs within the first 24 hours of life (Batton et al. 2013). A recent Norwegian population database study indicates that 2.7% of all NICU patients received inotropes at some point during their NICU stay, including 28% and 4.1% of neonates born at <28 and <36 weeks of GA, respectively, and 13% of <1,500 g infants (Burns et al. 2016). Cardiovascular drugs were the second most commonly used medication group after anti-infectives in extremely and very preterm (GA <32 weeks) neonates in Estonian neonatal units: 59% and 54% of extremely low GA (<28 weeks) neonates received dobutamine and dopamine, respectively (Lass et al. 2011). According to a study including 1507 extremely low GA (23–27 weeks) newborns, 73–93% received any treatment for hypotension and 25–52% were treated with vasopressors during the first week of life. The treatment of shock, cardiovascular instability or low systemic flow in neonates and preterm infants is mainly empirical and not based upon evidence obtained from controlled prospective clinical trials. The decision to provide treatment is associated more strongly with the centre where care was provided than with infant attributes (Laughon et al. 2007).

2.3.2 Cardiovascular drugs used to treat acute haemodynamic compromise in neonates

Clinical outcome-based data on the significance of different inotropes to prevent sequelae of hypotension/ hypoperfusion, including cerebral morbidity, are limited and controversial. The inotropes mainly used in the short-term treatment of systemic hypotension in preterm and term infants are dopamine and dobutamine (Burns et al. 2016; Dempsey et al. 2019).

Dopamine is a naturally occurring precursor of norepinephrine and has specific dopaminergic actions in addition to well-recognised α- and β-adrenergic effects (Keeley et al. 1988). At moderate doses (5–10 μg/kg/min), dopamine increases contractility and heart rate (HR) by stimulating the cardiac β1-, β 2-, and α1-AR and the dopaminergic receptors; at high doses (≥10–20 μg/kg/min), dopamine also increases systemic and probably pulmonary vascular resistance by stimulating vascular α1-AR (D'Orio et al. 1984; Goldberg 1972). Approximately 50% of the positive inotropic effect of dopamine is caused by the dopamine2-receptor stimulation-induced release of norepinephrine stored in the peripheral sympathetic nerve endings in the myocardium. Because myocardial norepinephrine stores get depleted within 8 to 12 hours, dopamine, especially in the preterm neonate with decreased myocardial norepinephrine stores, is a less effective positive inotrope in the long run compared with epinephrine (Seri 1995). Because of maturational differences in the expression of the α- and β-AR in neonates, without AR down-regulation, clinical manifestations of vascular α-AR simulation (e.g. increase in SVR) become apparent even at low-to-medium doses (Davies et al. 1984; Felder et al. 1983; Ruffolo et al. 1986; Seri 1995, 2006; Seri et al. 1984; Vapaavouri et al. 1973). Therefore, in neonates with escalating dopamine infusion, the pattern of receptor stimulation is first dopaminergic, then α-adrenergic, and finally β-adrenergic. Thus, peripheral vasoconstriction dominates over the inotropic effect when dopamine is given (Seri 1995, 2006; Seri et al. 1984).

Dobutamine is a synthetic analogue of dopamine and isoprenaline (hydroxyphenyl-isobutyl-dopamine), designed to increase contractility without significant tachycardia or vasodilation (Tuttle et al. 1975). However, unlike dopamine, its action is independent of endogenous norepinephrine release, and it does not have any dopaminergic activity. It stimulates both α- and β-AR, with a pronounced relative β1 cardio selective action and less affinity for peripheral α1- and β2- receptors. (Keeley et al. 1988)

It has been suggested that dobutamine offers the same inotropic effects as dopamine without the tendency for peripheral vasoconstriction (Gupta et al. 2014). The expected net effect is increased systemic and local blood flow without a significant change in BP (Cheung et al. 1999). Dobutamine is the drug of choice to increase CO in neonates with myocardial dysfunction. However, its effect on organ blood flow may be variable and seems to depend on the underlying patho-physiology. Thus, the hemodynamic response to dobutamine makes it suitable

for clinical situations in which poor myocardial contractility with unchanged or increased SVR is the primary cause of the cardiovascular compromise, such as perinatal asphyxia or a very preterm neonate with poor systemic blood flow and vasoconstriction (Noori et al. 2012). In contrast, in conditions with low SVR (e.g. vasodilation), such as those seen in the early stages of septic shock or in a very preterm neonate with vasodilatory shock during the transitional period, dobutamine is not appropriate (Evans 2006). However, the addition of dobutamine to dopamine in patients with cardiovascular compromise caused by impaired myocardial function and vasodilation is a reasonable approach and may be effective. An additional effect of dobutamine on increasing CO has been demonstrated in hypotensive preterm infants receiving dopamine (Lopez et al. 1997).

There is considerable controversy as to whether dopamine or dobutamine should be the first-line pharmacological agent for the treatment of neonatal hypotension. Proponents of dopamine often argue that it brings about a faster and more effective increase in BP. This is not surprising, since it is a potent vasoconstrictor. However, proponents of dobutamine argue that intense vasoconstriction by dopamine may further compromise tissue perfusion because of decreased blood flow (Gupta et al. 2014). Randomized controlled trials of dopamine versus dobutamine in preterm neonates have demonstrated that dopamine is more effective in increasing BP, while dobutamine increases CO (Osborn et al. 2002; Roze et al. 1993). In a study of preterm neonates with low systemic blood flow within 24 hours of birth, dobutamine resulted in a significantly greater increase in superior vena cava flow (SVCF) and right ventricular output (RVO), while dopamine resulted in a significantly greater increase in BP. Overall 40% of neonates failed to increase or maintain SVCF in response to either inotrope, and no significant differences in mortality or morbidity were found (Osborn et al. 2002).

Epinephrine has combined α- and β-AR agonist effects. At low doses, it is a potent inotrope, chronotrope, and systemic and pulmonary vasodilator. At higher doses, it has differential effects on the systemic and pulmonary circulation, increasing systemic pressure more than pulmonary pressure (Barrington et al. 1995). Epinephrine is frequently used in neonates for the treatment of hypotension and pulmonary hypertension, although data on the cardiovascular effects of epinephrine in neonates is limited (Zaritsky et al. 1984). A randomized controlled clinical trial of dopamine versus epinephrine demonstrated that the two medications have similar efficacy in improving BP and increasing CBF in hypotensive preterm infants (Pellicer et al. 2005). However, patients randomized to epinephrine more frequently developed increased serum lactate levels, independent of the improvement in their hemodynamic status, and hyperglycemia requiring insulin treatment. (Valverde et al. 2006) These metabolic effects are most likely explained by the drug-induced stimulation of β2-AR in the liver and skeletal muscles, resulting in decreased insulin release and increase in glycogenolysis, leading to an increase in lactate production, respectively (Noori et al. 2012).

Norepinephrine is an endogenous catecholamine that is released from adrenergic nerve endings. It has strong stimulating effects on α- and β1-AR and weaker effects on β2-AR. Norepinephrine has more potent α-mediated effects compared with epinephrine, which results in an increase in SVR and BP. While it is seldom used, it may be useful to improve BP and urine output during neonatal septic shock in preterm and term infants unresponsive to dopamine and dobutamine (Rizk et al. 2018; Tourneux et al. 2008). Norepinephrine might have a role in treating circulatory failure in severe PPHN due to a reported pulmonary vasodilatory effect (Tourneux et al. 2008). A retrospective study looking at norepinephrine use in preterm neonates over a 10 year period reported effectiveness in increasing BP with no major side effects (Rowcliff et al. 2016).

Milrinone is a selective phosphodiesterase type III inhibitor that raises intracellular cyclic adenosine monophosphate levels, which increases intracellular calcium concentrations and promotes contractile protein phosphorylation, resulting in improved systolic cardiac contractility, diastolic cardiac relaxation, and vascular relaxation. As milrinone has inotropic effects independent of β-AR stimulation, it does not rise myocardial oxygen consumption (Honerjäger et al. 1992; Yano et al. 2000).

Its usefulness in treatment of low CO states has been well demonstrated in adults. The drug has certain advantages, such as fewer tachyarrhythmias and the absence of tachyphylaxis when compared to dobutamine (Copp et al. 1992; Rettig et al. 1989). In a double-blind, randomised, placebo-controlled trial, 90 high-risk preterm infants born at <30 weeks of GA were randomised within the first 6 hours of life to receive milrinone (loading dose 0.75 μg/kg/min for 3 h, then maintenance 0.2 μg/kg/min until 18 h after birth) or a placebo to prevent low systemic blood flow. The primary outcome was maintenance of SVC flow >45 ml/kg/min through the first 24 hours of life. No difference in preventing low systemic blood flow between the two groups was found. Furthermore, milrinone-treated subjects had an increased incidence of tachycardia, low BP, and hemodynamically significant PDA (Paradisis et al. 2009). However, in another small pilot study in term infants with pulmonary hypertension, milrinone was reported to improve oxygenation and pulmonary and systemic HD in patients with suboptimal response to inhaled nitric oxide (McNamara et al. 2006). Administration of milrinone to neonates with low CO after surgical closure of PDA may lead to improved postoperative stability (Jain et al. 2012).

Arginine vasopressin is a neuropeptide hormone secreted by the posterior pituitary that regulates sodium homeostasis and serum osmolality. Vasopressin exerts a direct vasoconstrictive effect by acting on the V1 receptors that are predominantly found on vascular smooth muscle cells and the myocardium. In addition, it has vasodilatory effects in cerebral and renal arterioles by simulation of V2 receptors. Currently, vasopressin and its analogue, terlipressin, are increasingly being used as a rescue therapy for hypotension refractory to high-dose catecholamine and corticosteroids in neonates with sepsis, cardiogenic shock, necrotizing enterocolitis, non-septic shock with acute renal injury, and

systemic inflammatory response syndrome following surgery (Bidegain et al. 2010; Filippi et al. 2008; Leone et al. 2008). One study of vasopressin for refractory hypotension in 22 extremely low-birthweight infants suggests that low-dose vasopressin therapy should be considered as a rescue therapy when high-dose catecholamine therapy and/or steroid administration do not produce a sufficient increase in BP (Ikegami et al. 2009). A recent pilot study in 20 very preterm neonates compared vasopressin to dopamine during the first 24 hours of life. Both agents resulted in a similar increase in BP, but less tachycardia was seen in the vasopressin group (Rios et al. 2015).

Levosimendan is a calcium sensitizer and inodilator. It binds to cardiac troponine and enhances the sensitivity of contractile myofilaments to intracellular calcium in the cardiac muscle cell, thus improving myocardial contractility. It also activates the sarcolemmal potassium-sensitive adenosine triphosphate channels of vascular smooth muscle cells, which has a vasodilatory effect (Egan et al. 2006; Udvary et al. 1995). Levosimendan has been used in neonates, particularly in infants undergoing cardiac surgery, and initial reports are encouraging (Lechner et al. 2012). A small phase 1 study indicated that it may be superior to milrinone in this context (Pellicer et al. 2013). Improved tissue oxygenation measured with near-infrared spectroscopy (NIRS) has been reported in a cohort of neonates undergoing cardiac surgery (Ricci et al. 2012). While the drug shows promise, its use may be restricted to cases of shock with myocardial dysfunction as a major contributor, but as there are no reliable data on levosimendan in preterm neonates, such a use cannot be recommended (Bhat et al. 2015; Dempsey et al. 2019).

Despite their ongoing use, there is surprisingly very little neonatal PK and PD data available on the drugs discussed above (Dempsey et al. 2019).

2.4 Developmental pharmacology

Children differ from adults in their response to drugs. These differences may be caused by changes in the PK and/or PD between children and adults and may also vary among children of different ages. The PK of a drug includes the processes of absorption, distribution, metabolism and elimination, whereas the PD comprises the physiological and biological response to the administered drug and, therefore, may represent both efficacy and safety measures. While a child grows, elimination pathways and the function and expression of receptors and proteins mature, which can be referred to as “developmental changes” in childhood. Developmental pharmacology aims to understand the impact of growth and maturation on drug disposition and action in the neonatal and paediatric population. The maturation rates of developmental changes vary between the distribution, elimination and effect pathways and often do not correlate solely with increases in the body weight or age of the child. The question is therefore how to obtain data in children that contribute to understanding of these developmental changes, ultimately resulting in evidence-based dosing regimens for drugs in children (De Cock et al. 2011).

2.4.1 Rapid changes in neonatal physiology affecting pharmacokinetics

The rate of maturation of PK processes is most pronounced in the first 2 years of life.

Distribution is dependent on the extent of protein binding, pH, systemic and regional blood flow, permeability of natural "barriers" (e.g. blood–brain, placenta), and body composition. Clearly, these covariates will display both inter- as well as intra-patient variability, partly explained by maturational changes or disease-related differences (Batchelor et al. 2015).

Age-dependent maturational changes in body composition change the physiological spaces into which a drug will distribute. Neonates and young infants have a proportionally higher amount of body water per weight when compared to children and adults, and preterm neonates have an even higher value when compared to term neonates (Allegaert et al. 2014, 2018; Kearns et al. 2003). The total body water content is about 80% to 90% of total body weight in preterm and 70% in term neonates, with a progressive decrease to about 60% at the end of the first 1–2 years and subsequent stabilization throughout childhood (Friis-Hansen 1971). This pattern is similar for extracellular water content, starting at 40% of body weight and decreasing to about 25% to 30% at the end of infancy. In preterm neonates weighing less than 1,500 g, postnatal weight loss up to 10% of birth weight (BW) within the first 3 days of life occurs due to isotonic dehydration of the extracellular volume (Bauer et al. 1989).

Further, the presence of a PDA has been associated with an increase in the distribution volume of water-soluble compounds in preterm neonates. In an ibuprofen PK study in preterm neonates receiving the medication for PDA closure, a decrease in the central compartment volume of distribution was most pronounced in patients with a closing duct (Van Overmeire et al. 2001). The same tendency has been shown with other drugs (Gal et al. 1991; Thalji et al. 1980; Watterberg et al. 1987; Williams et al. 1997).

For the lipid compartment, the trends are more complex, with an initial increase from 10% to 15% of body weight at birth to 20% to 25% at the end of infancy, and a subsequent decrease back to 10% to 15% until adolescence (Allegaert et al. 2014, 2018; Friis-Hansen 1971).

These developmental changes in body composition have an impact on both water- and lipid-soluble medications. A decreased volume of distribution from neonates throughout childhood has been shown with aminoglycosides and acetaminophen (Allegaert and van den Anker 2017; Allegaert et al. 2011, 2015; Pacifici 2009). Increase in the volume of distribution in the first year of life has been shown with propofol (Allegaert, de Hoon et al. 2007).

Drug distribution is also influenced by protein binding. Compared to adults, infants and children have lower concentrations of the most relevant plasma binding proteins such as albumin, α-1 acid glycoprotein, or plasma globulins. Because protein concentrations reach adult values in infancy, this effect is likely to be most pronounced in neonates and young infants. In addition to the absolute

values or concentrations of proteins, competitive binding with endogenous compounds (bilirubin, free fatty acids) may further affect the binding capacity (van den Anker et al. 2018; Roberts et al. 2013; Smits et al. 2012). Clinical implications of alterations in the extent of protein binding of a drug are most relevant for those drugs that are highly protein bound and also have a narrow therapeutic index (Roberts et al. 2013). Reduced protein binding increases the free concentration and the free fraction of drugs, thereby enhancing the capacity of the active drug to diffuse more easily to other compartments. This will result in more interaction with receptors, but it also will increase the clearance rate of the drug (van den Anker et al. 2018).

Drug metabolism and excretion is a complex process involving different tissues with regional blood flow, the extraction rate, and the intrinsic drug-metabolizing enzyme specific capacity, and all these aspects may display age-related differences. Thinking of drug metabolism as being low in neonates and rising throughout infancy, early childhood, and prepuberty to reach adult levels in puberty is too simplistic (Hines 2007).

Elimination of drugs by the kidneys is dependent on glomerular filtration rate (GFR), tubular excretion, and tubular reabsorption. From a maturation standpoint, each of these processes exhibits an independent rate and pattern of development (van den Anker et al. 2018). Nephrogenesis starts in the embryo at approximately week 5, and nephrons become functional by week 8; it is complete at gestational week 36, when there are approximately 1,000,000 nephrons in each kidney (Anderson et al. 2008). The kidneys are anatomically and functionally immature at birth. The GFR is reported to double by one week of age (Chen et al. 2006). Rhodin et al. describe the maturation of the GFR with a consistent relationship with postmenstrual age (PMA) from early prematurity to adulthood (Rhodin et al. 2009). Tubular secretory and reabsorptive capacity appear to mature at much slower rates compared to GFR, and thus, they do not have a significant impact on elimination during the neonatal period (Halkin et al. 1978).

These age- and size-related developmental changes describe only a part of the inter-subject variability of PK parameters in neonatal populations. Genetics, comorbidity, and environmental issues further interact with these developmental changes (Blake et al. 2006; Leeder et al. 2010; Linakis et al. 2018).

2.4.2 Pharmacokinetic/pharmacodynamic modelling in paediatric populations

Population PKPD modelling using mixed effects models provides an opportunity to study between-individual variability in paediatric drug responses among children and neonates. Covariate analysis allows us to discover parameters that explain the predictable part of the between-individual variability. The main explanatory covariates in paediatric populations are size and age, representing growth and development, and varying in a range far greater than that seen in adults.

Size standardisation is achieved using allometric scaling, a mechanistic approach that has a strong theoretical and empirical basis (Anderson et al. 2006).

The factor for size (F_{size}) for total drug clearance may be expected to scale to weight with an allometric exponent of 3/4 (Equation 1). It is useful to report parameter values in terms of a standard subject (W_{st}), and this holds most obviously for size (Holford 1996).

$$F_{size} = \left(\frac{W}{W_{st}}\right)^{\frac{3}{4}} \quad \text{Equation 1}$$

For ease of comparison with other results, the size factor is usually standardized to a value of 70 kg (Anderson et al. 2011). When other size standards (e.g. 1 kg) have been used for children, comparison with adult estimates was not readily obvious. (Anderson et al. 2011; Knibbe et al. 2009; Su et al. 2010).

Age is used to describe maturation processes that are independent of size but are associated with the passage of time. The quantitative models (linear, exponential, first-order, variable slope sigmoidal) used to describe this maturation process vary depending on the span of the ages under investigation (Anderson et al. 2006). Maturation of clearance begins before birth, which means that post-conception age is a better predictor of the state of maturation than PNA. However, for pragmatic reasons, PMA has been recommended for studies of biologic age (Engle et al. 2004). The sigmoid hyperbolic or Hill model has been found useful for describing this maturation process (F_{mat}; Equation 2) (Anderson et al. 2011),

$$F_{mat} = \frac{PMA^{Hill}}{PMA_{50}^{Hill} + PMA^{Hill}} \quad \text{Equation 2}$$

where Hill is the sigmoidicity coefficient and PMA_{50} is the PMA when the maturation of clearance reaches 50% of adult values (Germovsek et al. 2017).

The utility of paediatric PK models can be increased by using a standardized approach to scaling: a suggested method for scaling clearance is a combination of allometric weight scaling with a sigmoid function to account for organ maturation (Germovsek et al. 2017). Extrapolations of developmental covariate models between drugs sharing an elimination pathway would also be useful for paediatric dose adjustment, but this applies only in specific cases, depending on a drug's extraction ratio, unbound fraction, type of binding plasma protein, and the fraction metabolized by the isoenzyme pathway for which clearance is scaled (Brussee et al. 2019; Calvier et al. 2018).

The nonlinear change in GFR with growth and age is also described by allometric scaling to bodyweight with coefficient of 0.75 or 0.632 and sigmoid E_{max} model describing the maturation with PMA. (Rhodin et al. 2009)

Additional to the growth and maturation taking place since conception, there may also be changes in drug clearance related to birth. Transition from the intrauterine to the extrauterine environment is associated with major changes in blood flow. There may also be an environmental trigger for the expression of

some metabolic enzyme activities (Anderson et al. 2011). PNA can be used to identify and describe effects beyond those predicted from PMA alone (Allegaert, Peeters et al. 2007). Two models are proposed that could be used to investigate this PNA effect (F_{birth}) (Anderson et al. 2011). The first one is an asymptotic exponential model (Equation 3):

$$F_{birth} = \frac{1+FB_{max}\cdot\left(1-exp\left[-PNA\cdot\frac{ln(2)}{TB_{50}}\right]\right)}{1+FB_{max}} \quad \text{Equation 3}$$

where FB_{max} is the fractional increase relative to the value at birth and TB_{50} is the half-time to achieve 50% of this relative change. F_{birth} approaches an asymptote of 1 as the baby gets older. The second is a sigmoid E_{max} model (Equation 4):

$$F_{birth} = \frac{1+FB_{max}/\left(1+\left[\frac{PNA}{TB_{50}}\right]^{-FB_{Hill}}\right)}{1+FB_{max}} \quad \text{Equation 4}$$

where additional parameter (FB_{Hill}) allows greater flexibility with the rate of change of maturation after birth. This factor for a change associated with PNA after birth (F_{birth}) can be included in the descriptors of parameter (e.g. CL) maturation (Equation 5) (Anderson et al. 2011):

$$CL = CL_{standard} \cdot F_{size} \cdot F_{mat} \cdot F_{birth} \quad \text{Equation 5}$$

This should be used a priori as a 'base' approach, allowing the effects of age and size to be delineated from other patient-specific factors, such as disease state and organ (dys)function.

Significant progress has recently been made on paediatric PK modelling; a greater emphasis now needs to be placed on PD modelling to understand age-related changes in drug effects (Germovsek et al. 2019). Measures of response are not always straightforward and can be more difficult to quantify in children. Variability in PD response may be even greater than that in PK. There is no precise boundary between PK and PD, and exploring the concentration-related effects often requires a link describing the movement of a drug from plasma to the target tissue and receptors. It is postulated that PD factors such as the number, affinity and type of receptors or the availability of natural ligands alter the drug–receptor interaction and are altered in childhood, but there are few data concerning PD differences between adults and children (Anderson et al. 2006). There is some evidence from experimental data that the PD effects of inotropes, the relationship between drug concentration and the extent of specific response vary at different ages (Akita et al. 1994; Artman et al. 1988; Felder et al. 1983; Vapaavouri et al. 1973).

2.5 Pharmacokinetics of studied inotropes in children and neonates

2.5.1 Pharmacokinetics of dobutamine in children and neonates

Studies describing the PK of dobutamine in the paediatric population date from the 1990s and have been conducted on small numbers of patients of various ages. Except for Banner et al., data from those studies indicate that the dobutamine PK in the neonatal and paediatric patient population follows first-order elimination kinetics within the dose range of 2.5 to 10 μg/kg/min for neonates and 2.5 to 17.5 μg/kg/min for infants and children. The plasma clearance rate varies widely between subjects in paediatric studies, with values ranging from 12.5 to 1319 ml/kg/min (Banner et al. 1991; Berg, Padbury et al. 1993; Berg, Donnerstein et al. 1993; Berg et al. 1997; Habib et al. 1992; Martinez et al. 1992; Schwartz et al. 1991). The only study describing the PK of dobutamine in neonates has reported a mean (standard deviation: SD) plasma clearance of 90 (38) ml/min/kg with variability independent of GA and BW. Plasma dobutamine levels correlated relatively well with infusion rates, indicating linear clearance in the dose range of 2.5–7.5 μg/kg/min (Martinez et al. 1992). The overview of PK studies of dobutamine in the paediatric population is presented in Table 1. Only one study has reported the volume of distribution with extremely high interindividual variability (Schwartz et al. 1991). Similarly, clearance appears to vary largely in study populations covering wide age ranges.

Dobutamine is metabolised through plasma catechol-O-methyltransferase, like other catecholamines, and is eliminated predominantly renally. About 80% of dobutamine administered intravenously at a steady state to a child with heart failure was detected in the urine, largely as 3-O-methyldobutamine-related derivatives (47%) and dobutamine metabolites (32%). Sulphate conjugates of both 3-O-methyl-dobutamine and dobutamine predominated, comprising 33 and 16% of the infused dobutamine (Yan et al. 2002). Sulphoconjugation and renal excretion are important determinants of the wide interpatient variability in plasma free dobutamine clearance rates (Berg et al. 1997).

Table 1. Pharmacokinetic studies of dobutamine in paediatric populations. Parameter values are presented as mean (standard error) or median[range].

		Dosing	PK parameters			
Study	Population	Infusion (µg/kg/min)	C_{ss} (ng/ml)	V (l/kg)	Clearance (ml/kg/min)	PK model
Banner et al. 1991	2 days–9 years paediatric intensive care patients (n=12)	2–15	6.4–374	not reported	[32– 625]	Non-comp. Non-Linear
Schwartz et al. 1991	0.13–16.6 years Shock and post-cardiac surgery (n=27)	1–25	105 (19) 76 [3.79–400] *	0.97 (1.71) 0.29 [0.07–5.64]	151.1 (47.5) 66 [12.5–1319]	Two-comp. Linear Non-linear
Habib et al. 1992	1 month–17 years paediatric intensive care patients (n=12)	2.5 5 7.5 10	46 >160	not reported	82 (7) [40–130]	Non-comp. Linear
Martinez et al. 1992	27–42 weeks GA neonates (n = 13)	2.5 5 7.5	not reported	not reported	90 (38)	Non-comp. Linear
Berg, Donnerstein et al. 1993	2–168 months stable, critically ill children (n=11)	0.5 2.5 5 10 20	not reported	not reported	82 (3) [53–119]	Non-comp. Linear
Berg, Padbury et al. 1993	10–22 years normal children and adolescents (n=12)	0.5 2.5 5	not reported	not reported	115 (63) [38–214]	Non-comp. Linear
Berg et al. 1997	1 d–17 years stable, critically ill neonates and children (n=38)	not reported	not reported	not reported	102 (15)	Non-comp. Linear

*PK – pharmacokinetic; Css – steady state plasma concentration; V – volume of distribution; GA – gestational age; * – infusion rate corrected to 5.0 µg/kg/min.*

2.5.2 Pharmacokinetics of milrinone in children and neonates

The PK of milrinone is well described in the perioperative period of open heart surgery (OHS); there are studies involving adults, paediatric populations, neonates with hypoplastic left heart syndrome, and children, infants and neonates with acute kidney injury (Bailey et al. 1994, 1999, 2004; Gist et al. 2015; Mizuno et al. 2019; Ramamoorthy et al. 1998; Zuppa et al. 2006). There are also PK studies in paediatric patients with septic shock, neonates with PPHN and very preterm infants on the first day of life (Lindsay et al. 1998; Mc Namara et al. 2013; Paradisis et al. 2007).

Early studies in healthy subjects confirmed that milrinone is predominantly eliminated by renal excretion, with approximately 80% excreted unchanged. The renal clearance of milrinone was calculated to be approximately 10 times higher than the expected renal clearance by filtration of the unbound drug, clearly indicating that renal secretion is a major contributor to milrinone renal excretion (Stroshane et al. 1984). The exact renal secretion pathway has not been investigated.

An overview of milrinone PK studies in paediatric populations is presented in Table 2. Different model standardisation, parameterisation and presentation of the clearance values in different units makes it difficult to compare the results of these studies.

Allometric scaling of milrinone clearance to body weight with a power coefficient of 0.75 is described in three studies and maturation as a E_{max} function of PMA with PMA_{50} estimated as 46.9 weeks in one study (Gist et al. 2015; Mizuno et al. 2019; Paradisis et al. 2007).

A physiology-based PK drug-disease model for milrinone in paediatric patients with and without low CO syndrome after OHS highlights that age, disease and surgery influence the PK of milrinone, and it proposes optimised dosing strategies to ensure safe and effective prescribing to maintain the therapeutic target range across the entire paediatric age range (Vogt 2014).

Table 2. Pharmacokinetic studies of milrinone in paediatric populations. Parameter values are presented as mean (standard error) or median[range].

		Dosing		PK parameters				
Study	Population	Bolus (µg/kg)	Infusion (µg/kg/min)	C_{ss} (ng/ml)	V (ml/kg)	$T_{1/2}$ (h)	Clearance	PK model
Lindsay et al. 1998	1.16–15 years, with septic shock (n=11)	50	0.5	81.3 (38.6)	1470 (1030)	2.88 (3.21) 1.47 [0.62–10.85]	0.0106 (0.0053) l/kg/min	Non-comp.
Ramamoorthy et al. 1998	Infants and children <13 years, after OHS (n = 19)	25+25 (+25) 50+25 (+25)	0.25+0.5 0.5+0.75	113 (39) 206 (74)	830 (400)	not reported	4.5 (1.8) ml/kg/min	Two-comp.
Bailey et al. 1999	3–22 months, after CBP (n=20)	50	not reported	not reported	190 (19)	not reported	2.5 (1.4) ·weight (kg) · [1+0.058 (0.038) · age (m)] ml/min	Three-comp.
Bailey et al. 2004 (PRIMACORP)	Neonates, infants and children <6 years, after OHS (n=157)	25 75	0.25 0.75	not reported	482 (39.3)	not reported	2.42 (0.228) · [1+age·0.0396 (0.0139)] ml/kg/min	One-comp.
Zuppa et al. 2006	Neonates with HLHS, CPB, DHCA, MUF (n=16)	100* 250*	0.5 0.5	151 (31) 358 (81)	502 (19)	not reported	0.4 ml/kg/min** 2,6 ml/kg/min***	Two-comp. ****
Paradisis at al. 2007	<29 weeks GA and <12 hours PNA (n=29)	not reported	0.25 0.5 0.75+0.2	not reported	603 (31)	10.3	35 (2) ml/h 0.64 (0.04) ml/kg/min	One-comp.

Table 2. (continued)

Study	Population	Dosing		PK parameters				
		Bolus (µg/kg)	Infusion (µg/kg/min)	C_{ss} (ng/ml)	V (ml/kg)	$T_{1/2}$ (h)	Clearance	PK model
McNamara et al. 2013	Neonates with PPHN (n=11)	50	0.33–0.99	290.9 (77,7)	560 (190)	4.1 (1.1)	0,11 (0,01) l/kg/h	Non-comp.
Gist et al. 2015	21 d–21 years, with AKI (n=11)	not reported	0.125–0.75	[44–1343]	not reported	not reported	4.72 (2.26) l /70kg/h [2.91–13.6] l /70kg/h	Two-comp.
Mizuno et al. 2019	Neonates and infants after OHS 4.8 [0.19–11.2] months (n=94)	50 (n=23)	0.25–1.2	not reported	184 (20.1) *****	not reported	7.91 (1.26) l/70 kg/h	Two-comp.
Hornik et al. 2019	Neonates, infants and children 2.9 [0.01–18] years (n=74)	not reported	0.5 [0.1–41]	not reported	0.46 (0.14)	not reported	15.9 (3.66) l/70 kg/h	One-comp.

*PK – pharmacokinetic; Css – steady state plasma concentration; V – volume of distribution; $T_{1/2}$ – elimination half-life of milrinone; GA – gestational age; PNA – postnatal age; OHS – open heart surgery; PPHN – persistent pulmonary hypertension of the newborn; HLHS – hypoplastic left heart syndrome; CPB – cardiopulmonary bypass; DHCA – deep hypothermic circulatory arrest; MUF – modified ultrafiltration (technique using ultrafiltration of the patient's intravascular volume and hemofiltration of the bypass circuit after separation from CPB to reverse hemodilution); AKI – acute kidney injury; * – bolus dose of the study drug was administered into the venous reservoir of the CPB circuit; ** – 0–12 hours after OHS; *** – on the 4th postoperative day; **** – plus MUF as a third compartment; ***** – central compartment.*

2.6 Pharmacodynamics of studied inotropes in children and neonates

2.6.1 Monitoring and targets of inotropic treatment

While aiming for population PKPD modelling of inotropic drugs, a key issue is to define proper methods for assessing systemic blood flow and organ perfusion in response to the drug under investigation.

For central HD monitoring in preterm infants, heart ultrasonography is the method of choice, although high inter- and intra-observer variability have been highlighted as potential problems (Mahoney et al. 2018). The term "functional echocardiography" was introduced by Kluckow et al. to describe the bedside use of echocardiography to longitudinally assess myocardial function, systemic and pulmonary blood flow, and intracardiac and extra-cardiac shunts (Kluckow et al. 2007). SVCF represents upper body blood flow unaffected by intra- and extra-cardiac shunts, and low SVCF has been shown to be associated with intra-ventricular haemorrhage (IVH), adverse neurodevelopmental outcomes, and death in premature infants (Hunt et al. 2004; Kluckow et al. 2000; Osborn et al. 2007, 2003). The effect of repeated neonatal echocardiography on HR, arterial oxygen saturation (SaO_2), cerebral regional oxygen saturation ($rScO_2$) and cerebral fractional tissue oxygen extraction (cFTOE) in extremely preterm infants during the first 3 postnatal days has been found to be minimal, and consequently, echocardiography is considered to be well tolerated during the postnatal transitional period (Noori et al. 2014).

More recently, NICOM has been used as a hemodynamic assessment tool in neonates. Electrical cardiometry is an impedance-based monitor that measures the electrical current produced by electrodes and changes in thoracic electrical bioimpedance, which is most closely related to thoracic aortic flow and red blood cells (RBC) alignment. When aortic flow stops, RBCs in the aorta are randomly orientated and impede conduction. Once the LV contracts, the ejection flow would compel the RBC to go parallel with the flow, which results in higher conductivity and decreased bioimpedance. The magnitude and frequency of pulsatile bioimpedance changes are used to calculate varied hemodynamic measures (Osypka et al. 2012). This method has been compared with echocardiography, demonstrating a consistent systematic bias in unstable, extremely preterm infants with PDA. NICOM may be used as a trending tool for continuous monitoring in this population, but it is not interchangeable with echocardiography (Hsu et al. 2017; Lien et al. 2015; Smits et al. 2017; Weisz et al. 2014).

Central blood flow measurements do not always correlate well with organ- and peripheral perfusion, and restoration of global HD does not always mean that adequate tissue perfusion is achieved, especially in conditions of impaired autoregulation, as may occur in premature neonates. Measurement of regional and microcirculatory blood flow can be used as an indicator of the success of the cardiovascular system to provide adequate oxygen and nutrients to the tissues (Weil et al. 2007).

NIRS was introduced by Jöbsis in 1977 as a safe and non-invasive technique to monitor cerebral oxygenation and HD in humans (Jöbsis 1977). It was first applied in neonates in 1985 by Brazy et al. (Brazy et al. 1985). The technique is based on the transparency of biological tissue to light in the near infrared part of the spectrum (700–1000 nm) and its subsequent absorption by chromophores present in oxygenated haemoglobin and deoxygenated haemoglobin in the cerebral blood vessels at different wavelengths. Current monitoring devices incorporate an absolute measure of $rScO_2$ or the cerebral tissue oxygenation index (Tisdall et al. 2009). To investigate the balance between oxygen delivery and oxygen consumption, the relative cFTOE can be formulated as a ratio: $(SaO_2\text{-}rScO_2)/SaO_2$, where SaO_2 stands for arterial oxygen saturation. An increase of cFTOE may indicate reduced oxygen delivery to the brain with constant oxygen consumption or brain oxygen consumption that exceeds oxygen delivery. A decrease of cFTOE suggests a decrease of oxygen extraction of the brain or constant oxygen consumption with increased oxygen delivery (Naulaers et al. 2007). GA-specific reference curves for $rScO_2$ and cFTOE are provided for the first 72 hours of life in preterm infants (Alderliesten et al. 2016).

Microcirculation plays a crucial role in the interaction between blood and tissue, both in physiological and pathophysiological states. It has been shown that a haemodynamically significant PDA causes major changes in the microcirculation of premature neonates (Hiedl et al. 2010), and in premature neonates with sepsis, microcirculatory alterations were already observed in the skin one day before changes in laboratory parameters were detectable (Weidlich et al. 2009). Analysis of these alterations may provide a unique perspective on disease processes at the microscopic level (Fagrell et al. 1997).

Orthogonal polarization spectral (OPS) and side stream dark field (SDF) imaging are the first imaging techniques that allow bedside visualization of microcirculation using hand-held imaging device. OPS imaging is based on the optical technique introduced by Slaaf et al., in which green polarized light is used to illuminate the tissue area of interest, which at the bedside is usually the buccal or sublingual mucosa (Slaaf et al. 1987). The green light is absorbed by haemoglobin within the RBC in the microcirculation. The reflected light is detected by an orthogonally placed analyser, which filters out surface reflections in order to produce a high-contrast reflected light image of flowing RBC within the microcirculation (Groner et al. 1999). SDF imaging is the improved successor to OPS imaging based on the dark field illumination technique introduced by Sherman et al. (Goedhart et al. 2007; Sherman et al. 1971). In this technique, the microcirculation can be observed without the need to use trans-illumination. Instead, SDF imaging uses a stroboscopic light-emitting diode ring-based imaging device, thus providing better image quality of the microcirculation (Balestra et al. 2010).

OPS and SDF imaging have been validated in several studies. Quantification of images is standardized, reproducible and validated (De Backer et al. 2007; Boerma et al. 2005). OPS and SDF imaging of the skin have been used in premature and term infants (Genzel-Boroviczény et al. 2002, 2004; Hiedl et al.

2010; Weidlich et al. 2009). These observations show that microcirculation in premature infants can be quantified and RBC velocity can be measured.

Drugs acting on the cardiovascular system display relatively fast clinical responses in the different levels of circulatory system. Complex multimodal continuous or easily repeatable bed-side monitoring is needed to get a good evaluation of the PD effects of inotropes. However, we have to be aware that parameters measured by monitoring tools reflect the sum of multiple actions within the same timeframe, and extraction of the drug-specific PD effects from these measurements is currently not yet feasible (Smits et al. 2017). Another important issue is to find reasonable relationships between these immediate HD effects and long-term outcomes.

2.6.2 Pharmacodynamics of dobutamine in children and neonates

Because dobutamine contains an asymmetric carbon atom, there are two enantiomers of the drug. The available preparation is a 1:1 racemic mixture of the (–)- and (+)-enantiomers. However, the AR affinity of the two optical isomers differs substantially. The (–)-dobutamine is a highly selective α_1-AR agonist, and the (+)-dobutamine exerts a potent and selective β_1- and β_2-AR stimulatory effect. In addition, (+)-3-O-methyldobutamine, the (+) optical isomer of the major dobutamine metabolite, is a relatively potent and highly selective α_1-AR blocker. Thus, the interaction of the two dobutamine enantiomers and (+)-3-O-methyl-dobutamine with the ARs and receptor expression/developmental pattern determine the cardiovascular response to dobutamine (Bhatt-Mehta et al. 1989).

In adults, cardiac contractility, stroke volume and CO increase with little effect on BP. In infants and young children, dobutamine has a significant chronotropic effect, which may limit the inotropic response. In general, when studied in infants and children, the improvement in CO is in excess of the impact on HR. Improvement in CO fosters improved renal function and urine output in conjunction with the resulting relaxation of sympathetic tone. Dobutamine also has modest vasodilator effects in the pulmonary vascular bed (Notterman 1988; Steinberg et al. 1994).

The response of the immature cardiovascular system of neonates to dobutamine may differ from that of older children and adults (Martinez et al. 1992). Neonates with myocardial dysfunction respond favourably to dobutamine, as evidenced by increased CO minutes after the initiation of the drug infusion, followed by increases in organ blood flow hours later (Robel-Tillig et al. 2007). The cardiovascular effects of dobutamine are dose-dependent. A dose escalation study in neonates with cardiovascular compromise showed a non-linear threshold effect. At very low doses (2.5 μg/kg/min), no significant hemodynamic effects were observed, whereas moderate doses (5–7.5 μg/kg/min) increased CO (Martinez et al. 1992). Whether these thresholds are physiologically important or related to a particular patient population is not yet clear. At doses of 5–20 μg/kg/min, dobutamine has been found to increase CO and BP even in hypotensive preterm infants (Roze et

al. 1993). Dobutamine at doses of 10 or 20 μg/kg/min was found to be more effective than the same doses of dopamine for increasing low SVC flow in preterm infants during the first postnatal day (Osborn et al. 2002).

In a foetal sheep hypoxia-induced brain injury model, dobutamine pre-treatment decreased neuroinflammation in the white matter and caudate and did not exacerbate cerebral injury or inflammation in the sham group. Early administration of dobutamine in preterm infants has been found to be relatively safe and may even be neuroprotective (Brew et al. 2018). In a study assessing long-term neuro-developmental outcomes in preterm babies with low systemic blood flow on the first day of life, dobutamine was found to be associated with greater increases in systemic blood flow and reduced rates of late severe periventricular or IVH compared with infants receiving dopamine (Osborn et al. 2007). In a recent randomised placebo-controlled study of 28 preterm neonates (<31 weeks of GA) developing circulatory impairment (SVCF <41 ml/kg/min) within the first 12 hours of life, earlier achievement of normal SVCF in patients receiving dobutamine did not reach statistical significance (Bravo et al. 2015).

2.6.3 Pharmacodynamics of milrinone in children and neonates

Experimental data suggest low activity of PDE III in extreme prematurity (Akita et al. 1994; Artman et al. 1988). However, no data are currently available on the postnatal maturation of the PDE system in humans.

Few studies in children and neonates after cardiac surgery or in septic shock have shown that milrinone also significantly improves cardiac function in this population (Bailey et al. 1999; Barton et al. 1996; Chang et al. 1995; Hoffman et al. 2003; Lindsay et al. 1998; Ramamoorthy et al. 1998). In very preterm infants, milrinone did not prevent low systemic blood flow during the first 24 hours of life (Paradisis et al. 2009).

The PD profile of milrinone has been described in 11 neonates with PPHN: milrinone led to an improvement in arterial partial pressure of oxygen and a reduction in the fraction of inspired oxygen, oxygenation index, mean airway pressure, and inhaled nitric oxide dose, transient reduction in systolic arterial pressure following the bolus, improvement in base deficit and plasma lactate, lower pulmonary artery pressure, improved RVO and LVO, and reduced bidirectional or right-left shunting (Mc Namara et al. 2013).

The concentration-related effect of milrinone has been described in 11 adult patients who had cardiac indices less than 2.5 l/min/m^2 immediately after separation from cardiopulmonary bypass. The relationship between the percentage increase in the cardiac index and milrinone plasma concentration was described with sigmoidal function, with the fastest change around a concentration of 167 ng/ml (Bailey et al. 1994). The plasma concentration of milrinone with the fastest increase in the velocity of circumferential fibre shortening corrected for HR (measurement of LV function) was around 139 ng/ml based on sigmoidal E_{max} dose-response curve (Kikura et al. 1997).

In a PK study in a population of infants and children, 50 and 25 μg/kg boluses followed by infusion of 0.75 μg/kg/min resulted in a steady state plasma concentration (Css) of 206 ± 74 μg/l (Ramamoorthy et al. 1998). In a randomized double-blind study comparing milrinone to a placebo in a similar population, a comparable milrinone dosage (bolus of 75 μg/kg followed by 0.75 μg/kg/min infusion) led to a 55% relative risk reduction for low CO syndrome (Hoffman et al. 2003).

Based on the above data, target concentration ranges of 180–300 ng/ml and 100–300 ng/ml have also been suggested in paediatric and neonatal populations (Gist et al. 2015; Mizuno et al. 2019; Paradisis et al. 2007; Vogt 2014).

EL-Khuffash et al. have demonstrated that administration of milrinone to patients with low LVO (<200 ml/kg/min) 1 hour after PDA surgical closure recovered LV performance to levels comparable with the high-LVO group within 18 hours. Milrinone was administered in 0.33 μg/kg/min intravenous infusion, and plasma concentrations were not measured (El-Khuffash et al. 2014).

Potential adverse effects of milrinone are hypotension, tachyarrhythmias and thrombocytopenia (Ramamoorthy et al. 1998; Stroshane et al. 1984). Although often reported in adult patients, they are infrequent in paediatric patients and are probably not always caused by milrinone (Hoffman et al. 2003).

2.7 Summary of the literature

Inotropic therapy in neonates is mainly based on knowledge of the pathophysiology behind the cardiovascular dysfunctional status and the PK and PD properties of the medications based on adult data. There is limited evidence about short-term effects of dobutamine and milrinone in critically ill neonates (Bravo et al. 2015; El-Khuffash et al. 2014; Martinez et al. 1992; Paradisis et al. 2009). However, the dose-concentration-response relationships remain poorly understood. The long-term neurodevelopmental effects of inotropic therapies used during critical illness of neonates has remained unestablished.

If not appropriately dosed, these medications may induce abrupt, excessive, and potentially harmful fluctuations in BP and systemic and organ blood flow (Greisen 2008; O'Leary et al. 2009; du Plessis 2009; Seri et al. 1993). Thus, in addition to cardiovascular compromise, treatment by suboptimal administration of inotropes, lusitropes, and vasopressors may contribute to short- and long-term morbidities in critically ill preterm and term neonates (Barrington 2008; O'Leary et al. 2009; du Plessis 2009).

Prompt diagnosis of neonatal cardiovascular compromise by state-of-the-art hemodynamic monitoring of BP and systemic and organ blood flow and careful titration of the vasoactive agents to the optimal hemodynamic response are thought to be of importance in decreasing mortality and morbidity associated with shock in preterm and term neonates (Goldstein et al. 1995; Martens et al. 2003; Seri 2001; Seri et al. 1993; Soleymani et al. 2010).

For milrinone, there is data about the PK properties in different specific patient populations, but pooling the information together, extrapolating between different populations and implementing conclusions into clinical practice has remained difficult. For dobutamine, existing PKPD information dates largely from the early 1990s. No studies have described dose-concentration-effect relationships through methods of non-linear mixed effects modelling.

3. AIMS OF THE RESEARCH

The general aim of the thesis was to describe the population pharmacokinetics and pharmacodynamics of two cardiovascular drugs, dobutamine and milrinone, in two clinical situations in which these medications are often used – post-ligation cardiac syndrome prevention after surgical closure of patent *ductus arteriosus* and transitional circulation of critically ill neonates.

The specific aims were:

1. To review the existing data on pharmacokinetics of milrinone in preterm infants and, by using the *in silico* probability of target attainment simulations, work out the best suitable dosing regimen for subsequent trial on neonates undergoing surgical closure of patent *ductus arteriosus*.

2. To describe the pharmacokinetics of milrinone when used for the prevention of post-ligation cardiac syndrome in neonates undergoing ligation of patent *ductus arteriosus*.

3. To develop dosing recommendations for milrinone in this patient group, based on prospectively collected pharmacokinetic data.

4. To describe the pharmacokinetics of dobutamine during the transitional circulation period in neonates, i.e. during their first three days of life

5. To describe the concentration-effect relationship of dobutamine on central and regional cerebral hemodynamic responses in neonates during the transitional circulation period.

4.PATIENTS AND METHODS

The thesis is based on one *in silico* simulation study using demographic data of a retrospective patient population and two prospective clinical trials conducted in NICUs of Tallinn Children's Hospital and Tartu University Hospital. The details of the study design are summoned in Table 3. Study-specific details of the patient population selection and data analysis will be outlined separately below for each study.

Table 3. Description of the studies

Study/Design	Timing	Population	Aim	Publication
Milrinone simulation study *In silico* simulations	Retrospective population from January 2012 – December 2014	31 neonates undergoing PDA surgical closure	To propose a dosing regimen for a prospective PK study of milrinone.	I
Milrinone PK study Prospective PK study using nonlinear mixed effects modelling	June 2015 – October 2017	10 neonates undergoing PDA surgical closure and at risk of PLCS	To describe the population PK of milrinone in premature neonates at risk of PLCS and give dosing recommendations.	II
Dobutamine PKPD study Prospective PKPD study using nonlinear mixed effects modelling	April 2016 – December 2017	28 neonates within PNA of 72 hours	To describe the PK and concentration-related effects of dobutamine in the population of critically ill neonates in the first days of life.	III

4.1 Ethical considerations

The two prospective clinical studies were approved by the Research Ethics Committee of the University of Tartu and by the State Agency of Medicines of Estonia (EU Clinical Trials Register No. 2015-000486-31 and 2015-004836-36). Written informed consent was signed by the parent(s) before inclusion in the study.

To find the best dosing of the studied medications based on already existing knowledge, an *in silico* simulation study preceded the milrinone PK study. As anonymized demographic data were available from a prior clinical practice review, for this analysis, a separate Ethics Committee approval as well as informed consent were not considered necessary.

Blood samples for PK analysis were obtained from indwelling catheters, and the overall sample volume did not exceed 3% of calculated circulating blood volume (Committee for Medicinal Products for Human Use (CHMP) et al. 2008). A sensitive method was developed and validated for detecting low levels of dobutamine and milrinone in a small amount of plasma (Takkis et al. 2019).

4.2 Study populations

4.2.1 Milrinone simulation study population (Study I)

Demographic characteristics, including GA, BW, weight, and PNA on the day of surgery, were collected from the hospital records of a retrospective population. The study population consisted of all neonates who underwent PDA ligation in the NICUs of the Tartu University Children's Clinic and Tallinn Children's Hospital between January 2012 and December 2014.

4.2.2 Milrinone PK study population (Study II)

All neonates and infants up to 90 days of age requiring PDA ligation during the study period (June 2015 to October 2017) were screened for the PK study.

Neonates were included if they fulfilled all of the following criteria: (a) were up to 90 days of age; (b) had haemodynamically significant PDA requiring surgical ligation; (c) had an arterial catheter and/or central venous catheter in place on clinical indication and (d) informed consent was signed by the parents or guardians.

Neonates with any of the following were excluded: (a) renal failure requiring renal replacement therapy (peritoneal dialysis) or serum creatinine (SCr) >100 μmol/l or oliguria <0.5 ml/kg/min within 6 hours; (b) hypersensitivity to milrinone or any other component of the study drug; (c) treating physician considered milrinone contraindicated; (d) major congenital malformation; (e) known metabolic disease; (f) informed consent from parents or guardians not obtained; (g) critically unstable state of the patient, likely to be fatal within 72 hours; or (h) already recruited in another clinical trial investigating a medical product.

In dose optimisation simulations, PMA and weight data from all PDA ligation patients treated in the study units between 2013 and 2017 were used. The population included the retrospective population of the milrinone simulation study and the prospective milrinone PK study population, including those who did not receive milrinone treatment.

4.2.3 Dobutamine PKPD study population (Study III)

All neonates hospitalized to NICU within the first 72 hours of life from April 2016 to December 2017 were screened for eligibility. We included neonates with: (a) PNA below 72 hours; (b) the need for inotropic therapy according to the decision of the treating physician (based on echocardiographic assessment, $rScO_2$, mean arterial blood pressure (MAP), acid-base balance, serum lactate and capillary refill time); (c) an arterial catheter and/or central venous catheter in place on clinical indication and (e) parents' signed informed consent. Neonates with the following were excluded: (a) a congenital defect that impacts HD response to inotropic therapy (congenital heart disease); (b) congenital hydrops; (c) other unresolved cause of low blood flow (air leak); (d) known metabolic disease; (e) informed consent not signed by parents or guardians; (f) situation where the treating physician considered a different vasoactive treatment necessary/ dobutamine contraindicated; or (g) known hypersensitivity to dobutamine or any other component of the study drug.

4.3 Study drug administration

4.3.1 Milrinone administration (Study II)

Milrinone was started in infants determined to be at risk of PLCS based on echocardiographic assessment of LVO of less than 200 ml/kg/min 1 hour after the end of PDA surgery. Milrinone (Corotrope 1mg/ml, Sanofi-Aventis, France) was diluted with 0.9% NaCl to a concentration of 15 μg/ml within 20 min before administration. A loading dose of 0.73 μg/kg/min over 3 hours was followed by a maintenance infusion of 0.16 μg/kg/min for 21 hours, based on the milrinone simulation study results, as shown in Figure 1.

To ensure adequate intravascular volume, all infants received a bolus of 9 ml/kg of normal saline over three hours with the milrinone loading dose infusion. Further hemodynamic support with fluid boluses and inotropes was provided according to the routines of the unit. The milrinone infusion rate could be changed or discontinued at the discretion of the treating physician.

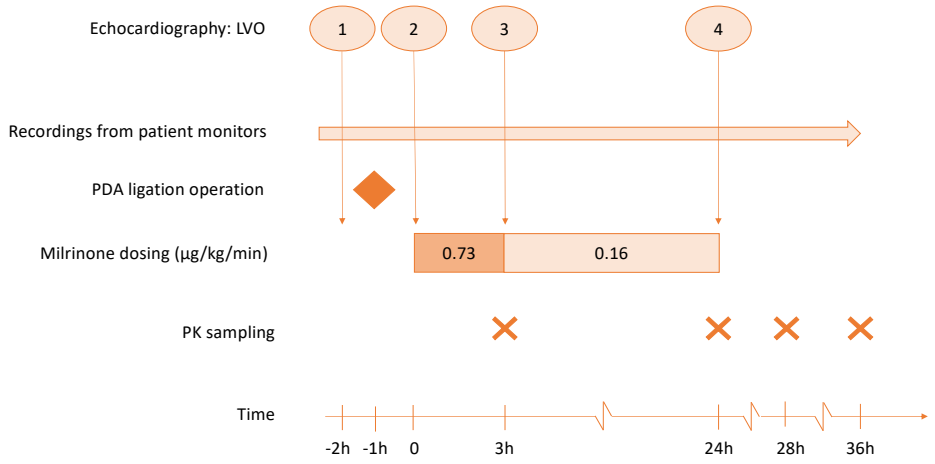

Figure 1. Milrinone PK study time-flow: LVO – left ventricular output, PDA – patent ductus arteriosus, PK – pharmacokinetic, vital signs (heart rate, blood pressure, peripheral arterial oxygen saturation, cerebral regional oxygen saturation) were continuously recorded from patient monitors.

4.3.2 Dobutamine administration (Study III)

Infusion of the studied medication was started immediately after the first echocardiographic assessment. Dobutamine in a 12.5 mg/ml concentrate for solution for infusion (Dobutamine Claris, Claris Lifesciences (UK) Limited, UK) was diluted with 0.9% NaCl to a concentration of 1.25 mg/ml within 20 min before administration.

Treatment was started at a dose of 5 µg/kg/min and raised by 5 µg/kg/min approximately every 30 min to a maximum of 20 µg/kg/min, as shown in Figure 2. Dose change times were recorded with the precision of the nearest minute.

The individual dose escalation maximum was decided based on echocardiographic and clinical findings. Dose-limiting effects of the study medication were (1) persistent tachycardia (HR >200 min^{-1} in preterm and >180 min^{-1} in term neonates) or arrhythmias, (2) no additional effect from the last dose increase or (3) sufficient effect of the last dose. To ensure adequate intravascular volume, all neonates received a bolus of 10 ml/kg of 0.9% NaCl over 10 min, unless any fluid bolus was already received within 4 hours prior to study inclusion. Concomitant treatment with volume bolus and inotropes other than the study medication was provided on the decision of the treating physician without any restrictions in the study protocol.

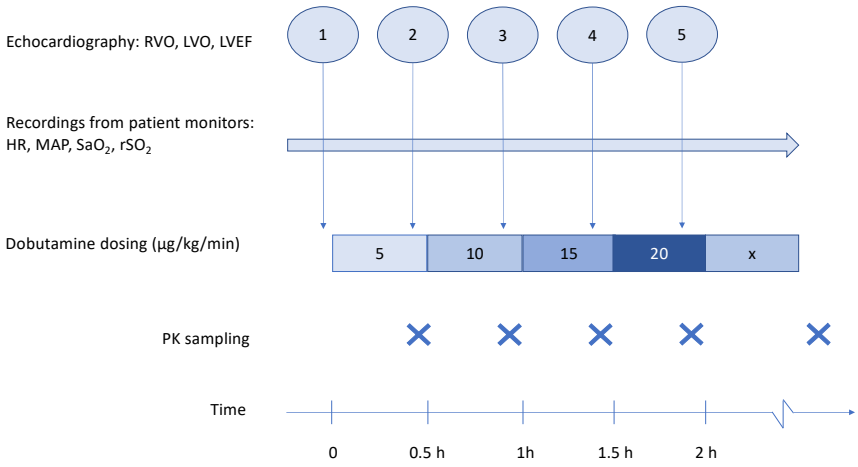

Figure 2. Dobutamine PKPD study time-flow: RVO – right ventricular output, LVO – left ventricular output, LVEF – left ventricular ejection fraction, HR – heart rate, MAP – mean arterial blood pressure, SaO_2 – peripheral arterial oxygen saturation, rSO_2 – cerebral regional oxygen saturation, PK – pharmacokinetic. PK sampling 15–30 min after every dose change and 15 min after completion of therapy. Recordings from patient monitors were made continuously.

4.4 Pharmacokinetic sampling

For the concentration measurement of the studied drugs, blood samples of 0.3 ml were collected from an indwelling arterial line into Na-heparin vials. Actual blood collection time was recorded with the precision of the nearest minute. The samples were centrifuged immediately (10 min at 3500 rpm), plasma separated and stored at –80 °C (up to 12 h storage at –20 °C prior to transfer to –80 °C was accepted) and transported on dry ice for analysis. Study-specific sampling times are outlined in Figures 1 and 2.

4.4.1 Milrinone PK study pharmacokinetic sampling (Study II)

Blood samples for milrinone plasma concentration measurements were collected at the end of loading infusion (3h), at the end of maintenance infusion (24 h) and twice after the end of treatment (28 h, 36 h), as shown in Figure 1.

4.4.2 Dobutamine PKPD study pharmacokinetic sampling (Study III)

Blood samples for dobutamine plasma concentration measurements were collected 15–30 min after every dobutamine dose change and 15 min after termination of dobutamine infusion, as shown in Figure 2. A maximum of six blood samples were drawn from one subject.

4.5 Drug concentration measurements

A method to obtain the plasma concentration measurements for the studied medications was developed and validated by Karin Kipper, Kalev Takkis and Rūta Veigure, and all the plasma samples were analysed at the Institute of Chemistry, University of Tartu.

Milrinone and dobutamine concentrations were measured by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). A sensitive method for both investigated medications with the quantification range of 1–300 μg/l and sample volume of 20 μl was developed (Takkis et al. 2019).

The problem of extreme instability of dobutamine in plasma samples was overcome by adding a stabilising agent (ascorbic acid) to the plasma during the thawing phase. Extensive stability testing was undertaken to ensure the validity of the results.

The within- and between-run accuracy (coefficient of variability) for milrinone was 97–106% (3–7%) and 97–106% (3–9%), respectively; for dobutamine 95–99% (4–7%) and 94–102% (3–5%), respectively. The lower limit of quantification (LLOQ) was determined as 0.97 ng/ml for both analytes.

All plasma concentrations detected below the LLOQ were included in the analysis with the value of 0.5 μg/l (half the LLOQ); this was the case only in dobutamine study (Study III).

4.6 Haemodynamic data collection and safety monitoring

All studied neonates and infants were treated in a Level 3 NICU and monitored according to routine clinical practice. During the treatment period with the study medications, HR, arterial BP, SaO_2 and other vital signs were continuously monitored with IntelliVue MP30, M70 or MX800 patient monitors (Philips Medical Systems, The Netherlands), cerebral regional saturation with NIRS monitor Invos Oxymeter (Medtronic, USA) and recorded with 2.5 s intervals by BedBase software (University Medical Center Utrecht, the Netherlands).

Cardiac function and central blood flows were measured using echocardiography by cardiologists. Studies were performed using the Vivid 7 Dimension or Vivid E9 cardiovascular ultrasound system (GE Healthcare, USA) with 10 or 12 MHz transducers, respectively. Evaluations included measurements of RVO and LVO, SVCF and shunting trough PDA and *foramen ovale* (when applicable), systolic function of left and right ventricle and diastolic function of the heart (Evans et al. 1996; Kluckow 2000). Study-specific timing of measurements is shown on Figure 1 and Figure 2.

The PD effect measures of RVO, LVO, SVCF and left ventricular ejection fraction (LVEF) were recorded before the dosing and once at each infusion rate of dobutamine, approximately 20–30 min after each dose escalation; HR, MAP, SaO_2 and $rScO_2$ data recorded during a 15 min period (5 min before and 10 min

after each dose change) were selected, artefacts removed manually and averaged over 1 min, and cFTOE was calculated as the ratio: $(SaO_2\text{-}rScO_2)/SaO_2$.

All neonates were monitored for adverse events for 7 days after the end of treatment with a study medication. Monitoring included clinical evaluation, cerebral ultrasonography, laboratory and vital parameters and administration of concomitant medications.

4.7 Pharmacokinetic and pharmacodynamic modelling

PKPD modelling was undertaken with nonlinear mixed-effects software NONMEM version 7.3 (ICON Development Solutions, MD, USA). The first-order conditional estimation method with interaction was used. The selection of the population model and residual error model was based on goodness-of-fit (GOF) plots and an improvement in the objective function value (OFV). For a nested model, a parameter was added if their inclusion resulted in improvement of OFV value >3.84 ($P < 0.05$ by likelihood ratio test for 1 degree of freedom).

Between subject variability (BSV) of the model parameters was described using an exponential error model (Equation 6), which assumes parameters are log-normally distributed:

$$p_i = \theta \cdot \exp(\eta_i) \qquad \text{Equation 6}$$

where p_i refers to the individual value of the respective PK or PD parameter in the i-th individual, θ is the typical parameter value, and $\exp(\eta_i)$ expresses the random difference between θ and p_i.

For describing and quantifying residual unexplained variability, an additive (Equation 7), proportional (Equation 8) and a combination of the two (Equation 9) were tested:

$$Y_{ij} = C_{ij} + \varepsilon_{ij} \qquad \text{Equation 7}$$

$$Y_{ij} = C_{ij} \cdot (1 + \varepsilon_{ij}) \qquad \text{Equation 8}$$

$$Y_{ij} = C_{ij} \cdot (1 + \varepsilon_{PROP\ ij}) + \varepsilon_{ADD\ ij} \qquad \text{Equation 9}$$

where C_{ij} is the j-th drug plasma concentration or PD effect of the i-th individual predicted by the PK or PD model, and Y_{ij} is the measured plasma concentration or effect. The residual departure of Y_{ij} from C_{ij} is represented by ε_{ij}.

To assess the GOF of the models, population and individual predicted vs. observed dobutamine C_p and PD effect measurements, absolute value of individual weighted residuals versus individual predictions, and conditional weighted residual error versus time plots were constructed with R software using package xpose4 (Jonsson et al. 1999; R Core Team 2014a).

4.7.1 Milrinone simulation study analysis (Study I)

Published milrinone population PK studies were reviewed for the characteristics of the study population and intervention.

We created 1,000 individual PK parameter value sets by simulating the chosen PK model with bootstrapped collected demographic data from our retrospective study population. Individual PK estimates were used in simulating time-concentration profiles with varied loading and maintenance doses.

First, the concentration-time profile of milrinone was simulated with a continuous infusion of 0.3 μg/kg/min, a dose proposed by a previous study of PLCS prevention (Jain et al. 2012). According to the timely course of PLCS, a 3-hour loading dose with maintenance infusion until 24 hours was chosen for dose optimisation simulations.

The concentration target was chosen based on data from previous PKPD studies of milrinone in paediatric and neonatal patients undergoing OHS (Ramamoorthy et al. 1998; Zuppa et al. 2006). In a PK study by Ramamoorthy et al., 50 and 25 μg/kg boluses followed by infusion of 0.75 μg/kg/min resulted in a steady state plasma concentration of 206 ± 74 μg /l (Ramamoorthy et al. 1998). In a randomized double-blind study in a similar population that compared milrinone to a placebo, a milrinone bolus of 75 μg/kg followed by 0.75 μg/kg/min infusion led to a 55% relative risk reduction for low cardiac output syndrome (Hoffman et al. 2003). Based on these data, a milrinone concentration of around 200 μg /l was considered effective in the prevention of low cardiac output. Bearing in mind the preventive character of intervention and the presumed lower volume of distribution in our study population compared to the PK model population of preterm neonates aged 1–2 days, a more conservative upper limit of target concentration was chosen to avoid overdosing. The two former studies addressing optimal dosing of milrinone in preterm and term neonates targeted concentrations of 180–300 and 100–300 μg /l, respectively (Paradisis et al. 2007; Vogt 2014). In our study, maximising the number of simulated subjects in the concentration range of 150–200 ng/ml for at least 80% of time in the maintenance phase was targeted.

All simulations were conducted by Tõnis Tasa (Institute of Computer Science, University of Tartu) using R software version 3.0 (R Core Team 2014b).

4.7.2 Milrinone PK study analysis (Study II)

One- (Equation 10) and two-compartment linear structural models (Equation 11) were considered to describe the PK of milrinone:

$$\frac{\Delta A}{\Delta t} = Rate - \frac{CL \cdot A}{V} \qquad \text{Equation 10}$$

where rate represents the infusion of the drug, A is the amount of the drug in the body, CL is first-order elimination clearance, and V is the volume of distribution;

$$\frac{\Delta A_c}{\Delta t} = Rate - \frac{CL \cdot A_c}{V_c} - k_{cp} \cdot A_c + k_{pc} \cdot A_p$$

$$\frac{\Delta A_p}{\Delta t} = k_{cp} \cdot A_c - k_{pc} \cdot A_p$$

Equation 11

where A_c and A_p are the amounts of drug in the central and peripheral compartments, respectively. CL is the first-order elimination clearance, and V_c is the volume of the central compartment, and k_{cp} and k_{pc} are the first order intercompartmental rate constants.

Body weight and PMA were included *a priori* in the model with allometric weight scaling and a function describing GFR maturation, respectively (Equation 1 and Equation 2) (Germovsek et al. 2017). The power coefficient of clearance and parameters of the maturation function were fixed to values from a previous study of human glomerular filtration development (Equation 12 and Equation 13) (Rhodin et al. 2009)

$$CL_i = \theta_{CL} \cdot \left(\frac{W_{D1i}}{W_{st}}\right)^{0.632} \cdot \frac{PMA_i^{3.4}}{47.7^{3.4} + PMA_i^{3.4}}$$

Equation 12

$$V_i = \theta_V \cdot \left(\frac{W_{D1i}}{W_{st}}\right)$$

Equation 13

where CL_i and V_i are the individual predicted values for clearance; V, θ_{CL} and θ_V are the typical values of clearance; V, W_{D1i} is the patient's weight on day one of the study; W_{st} is the population mean bodyweight; and PMA_i is the patient's PMA on day one of the study.

Other patient covariates (such as sex, Apgar score, albumin level, fluid intake, CO and co-administration with dopamine or dobutamine) were tested for correlation with model parameters.

A nonparametric bootstrap with 2000 replicates was undertaken to assess the robustness of parameter estimates to changes in data, and a visual predictive check (VPC) was performed to assess the simulation properties of the final model, using the software Perl speaks NONMEM and R Version 3.3.3 (Lindbom et al. 2005; R Core Team 2014a).

Probability of target attainment (PTA) simulations aimed to develop a dosing schedule that maximally attains milrinone concentration within the pre-specified target range of 150–250 µg/l by the 6th postoperative hour for the majority of patients. Compared to the milrinone simulation study, the upper limit of target concentration was raised based on the safety profile seen in the prospective milrinone PK study. Severe hypotension with additional vasopressor treatment was recorded only in two infants with milrinone plasma concentrations exceeding 300 µg/l. Patient covariate (PMA and weight) data were bootstrapped to 1,000 samples. Next, patient PK parameters were simulated using parameter equations and variability estimates from the PK model. Milrinone time-concentration curves were simulated with doses ranging from 0 to 1 µg/kg/min, with a step of 0.05 µg/kg/min. The dosing scheme consisted of a 3-hour loading dose

immediately followed by a 21-hour maintenance infusion, as explained by the time course of PLCS, also in Study I.

First, an optimal loading dose with a precision of 0.05 µg/kg/min was identified, maximising the PTA within the concentration target range of 150–250 µg/l at three hours after start of the initial infusion, and the result was used as a pre-condition for maintenance dose optimization. The optimal maintenance dose achieved a maximum of mean PTA values measured at 6, 12, 18 and 24 hours of treatment. The probability of concentrations above 300 µg/l was taken into account to minimize potential side effects from excessive concentrations. Specifically, dose optimisation of maintenance and loading dose maximised the objective function (Equation 14):

$$\arg \max_{x \in \{0,0.05,\ldots,1\}} \left(\sum_{i=1}^{n} (Target1_i(x) - w * Target2_i(x))\right)/n \qquad \text{Equation 14}$$

where $Target1_i$ is the proportion of simulated concentrations within 150–250 µg/l range at the i-th observation time and $Target2_i$ is the same for concentrations above 300 µg/l with maintenance dose x starting at the end of the loading phase (3 hours after start). Weight constant w is fixed at 1.

Additionally, to consider dose requirement changes related to PMA, we aimed to find a PMA breakpoint that divides the population into two patient subgroups with different unknown maintenance doses. The optimization found a combination of parameters that provided maximal PTA and used the following optimization function (Equation 15):

$$\arg \max_{\substack{x,y \in \{0,0.05,\ldots,1\}, \\ PMA \in \{24,25,\ldots,31\}}} \left(\sum_{i=1}^{n} (Target1_i(x,y,PMA) - w * Target2_i(x,y,PMA))\right)/n$$

Equation 15

where $Target1_i$ is the proportion of simulated concentrations within 150–250 µg/l range at the i-th observation time and $Target2_i$ is the same for concentrations above 300 µg/l with maintenance dose x (in subjects age < PMA) and y (in subjects age ≥ PMA) starting at the end of the loading phase (3 hours after start), PMA is the postmenstrual age determining the two different maintenance dose groups and w is again set as 1.

All simulations were conducted by Tõnis Tasa (Institute of Computer Science, University of Tartu) using R software Version 3.3.3 (R Core Team 2014a).

4.7.3 Dobutamine PKPD study analysis (Study III)

To describe the PK of dobutamine one-compartmental structural PK models with first-order elimination (Equation 10), Michaelis-Menten elimination (Equation 16) and elimination by parallel first-order and Michaelis-Menten processes (Equation 17) were evaluated:

$$\frac{\Delta A}{\Delta t} = Rate - \frac{V_{max} \cdot A}{KM + A}$$ Equation 16

$$\frac{\Delta A}{\Delta t} = Rate - \frac{CL \cdot A}{V} - \frac{V_{max} \cdot A}{KM + A}$$ Equation 17

where A is the amount of dobutamine in the body, CL is first-order elimination clearance, V is the volume of distribution; V_{max} the maximal rate of Michaelis-Menten elimination and KM (Michaelis Constant) is the amount of dobutamine in the body when the elimination rate by the Michaelis-Menten mechanism is half maximal.

Clearance and volume of distribution were *a priori* allometrically scaled to the population median BW, and a maturation function, describing the maturation of dobutamine clearance with PMA, was applied (Equation 18 and Equation 19):

$$CL_i = \theta_{CL} \cdot \left(\frac{BW_i}{W_{st}}\right)^{0.75} \cdot \frac{PMA_i^{Hill}}{PMA_{50}^{Hill} + PMA_i^{Hill}}$$ Equation 18

$$V_i = \theta_V \cdot \left(\frac{BW_i}{W_{st}}\right)$$ Equation 19

where CL_i and V_i are the individual predicted values for clearance and V, θ_{CL} and θ_V typical values for clearance; V, BW_i and PMA_i are the patient's individual BW and PMA; W_{st} is the population median BW; Hill is the sigmoidicity coefficient; and PMA_{50} is the PMA when the maturation of the dobutamine clearance reaches 50% of adult values (Germovsek et al. 2017). PMA_{50} and Hill's coefficient for the maturation function of dobutamine clearance were estimated from PK data.

In covariate analysis parameterization of the PK model with PNA, antenatal glucocorticoid hormone administration, coadministration of dopamine, blood haemoglobin and albumin concentration, PDA diameter, baseline LVEF and baseline RVO were tested.

Using the final linear PK structural model with fixed Hill's coefficient and PMA_{50} parameter values from the PK model fitting process, simultaneous PKPD modelling was undertaken with HD parameters as PD effect.

Considering that the HR, MAP, SaO_2 and $rScO_2$ measurements were recorded continuously around the dose change, we attempted to estimate the effect time which represents the distribution of the drug from plasma to the effect site at a steady state. An effect compartment with a first-order equilibration rate constant (k_{eo}) was added to the final PK structural model for HR, MAP and cFTOE PKPD models, assuming that the amount of drug distributing into the effect compartment does not influence the overall PK (Equation 20):

$$\frac{\Delta C_e}{\Delta t} = k_{eo} \cdot C_p - k_{eo} \cdot C_e$$ Equation 20

where C_p is the plasma concentration and C_e the effect compartment concentration, and k_{eo} is the equilibration rate constant between effect and observed (plasma) compartments.

Baseline HD parameter values were estimated. Changes in HD parameter values were explored using linear (Equation 21), E_{max} (Equation 22) and sigmoidal E_{max} (Equation 23) models:

$$E_{ij} = E_{0,i} + SL_i \cdot C_{ij} \quad \text{Equation 21}$$

$$E_{ij} = E_{0,i} + \left(E_{max,i} - E_{0,i}\right) \cdot \frac{C_{ij}}{(EC_{50,i} + C_{ij})} \quad \text{Equation 22}$$

$$E_{ij} = E_{0,i} + (E_{max,i} - E_{0,i}) \cdot \frac{C_{ij}^{\gamma}}{(EC_{50,i}^{\gamma} + C_{ij}^{\gamma})} \quad \text{Equation 23}$$

where E_{ij} is the j-th observed effect at the corresponding plasma or effect compartment concentration (C_{ij}) of the i-th individual, $E_{0,i}$ represents the estimated baseline value, SL_i is the slope of the linear relationship between E_{ij} and $C_{p,ij}$ for the i-th individual, $E_{max,i}$ is the estimated maximum HD parameter value for the i-th individual, and $EC_{50,i}$ represents the concentration at half-maximal effect for the i-th individual. The Hill coefficient (γ) describes the steepness of the concentration response relation.

BSV was estimated for most of the PK and PD model parameters using an exponential variance model (Equation 6), assuming log-normal parameter distribution. Individual η-s for clearance and the volume of distribution were highly correlated (r =1.0), so a shared η was used with an estimated scale factor applied to the shared η for the volume of distribution (Equation 24),

$$\begin{aligned} CL_i &= \theta_{CL} \cdot EXP\ \eta_i \\ V_i &= \theta_V \cdot EXP\ (\theta_n \cdot \eta_i) \end{aligned} \quad \text{Equation 24}$$

where CL_i is the individual value of CL, θ_{CL} the typical value of CL, η_i the shared interindividual variance, V_i the individual value of V, θ_V the typical value of the volume of distribution and θ_n is the estimated variance scale factor. The residual unexplained variability was explored to be described by additive (Equation 7), proportional (Equation 8), or combined additive and proportional error models (Equation 9). The residual error was estimated separately for the PK and PD observations.

For the final PK model and all PKPD models, prediction corrected VPC was constructed and a nonparametric bootstrap with 1,000 replicates was undertaken with the software Perl speaks NONMEM and R Version 3.6.0 (Lindbom et al. 2005; R Core Team 2019).

Descriptive statistics of the study populations are presented as median (range) or mean (range). Echocardiography recordings of 20% of study patients attained

by three different cardiologists were reanalysed by one observer for intra- and inter-observer variability, according to a recent recommendation (Popović et al. 2017).

Sequential measurements of central blood flow and cardiac function were analysed using a paired Wilcoxon test with R software Version 3.6.0 to assess the overall change in those parameter values (R Core Team 2019).

5. RESULTS AND DISCUSSION

5.1 Milrinone simulation study results (Study I)

5.1.1 Demographics and PK model selection

The retrospective population consisted of 31 patients who underwent a PDA closure operation during the study period. The median (range) GA was 26 (23–35) weeks, PNA 13 (3–29) days, and body weight on the day of surgery 760 (500–2,351) g.

We found five paediatric population PK models in the literature. Four of them were for populations undergoing OHS and included various age groups and one was for preterm neonates within the first 12 hours of life (Bailey et al. 1999, 2004; Paradisis et al. 2007; Ramamoorthy et al. 1998; Zuppa et al. 2006). For population and PK details of those models, see Table 2, studies 2–6.

A one-compartmental population PK model by Paradisis for preterm neonates was chosen for milrinone plasma concentration simulations as the one with the highest resemblance to the target population. Population estimates for clearance and the volume of distribution were 35 ml/h and 512 ml, respectively. Between-subject variability estimates for model parameters were 24 and 21%, respectively (Paradisis et al. 2007).

As body weight was a single covariate affecting both clearance and the volume of distribution of milrinone via allometric scaling with a power coefficient of 0.75 and 1, respectively, only the body weights of the study population were used to create the individual PK parameter value sets for dose optimization simulations.

5.1.2 Dose finding simulation results

With a dosage of 0.33 μg/kg/min, at 6 hours of therapy, the simulated plasma concentrations lie within the target range of 150–200 μg/l only in 35.9% of subjects. After 18 hours of milrinone infusion, simulated plasma concentrations remained in the therapeutic range for just 0.8% of subjects, exceeding the literature-based recommended/safe window for the rest (Figure 3).

The plasma concentration target was best achieved with a 3-hour loading infusion of 0.73 μg/kg/min followed by a maintenance infusion of 0.16 μg/kg/min (Figure 3). The proportion of subjects with simulated milrinone plasma concentrations within the target range (150–200 μg/l) was 7% after 2 hr and 32.2% after 2.5 hr of treatment. At 3 hr of treatment, at the beginning of the maintenance dose, 51.2% of subjects had simulated plasma concentrations within the target range. This percentage also remained similar after 6, 18, and 24 hr of treatment, with 56.1, 58.1, and 55.7%, respectively.

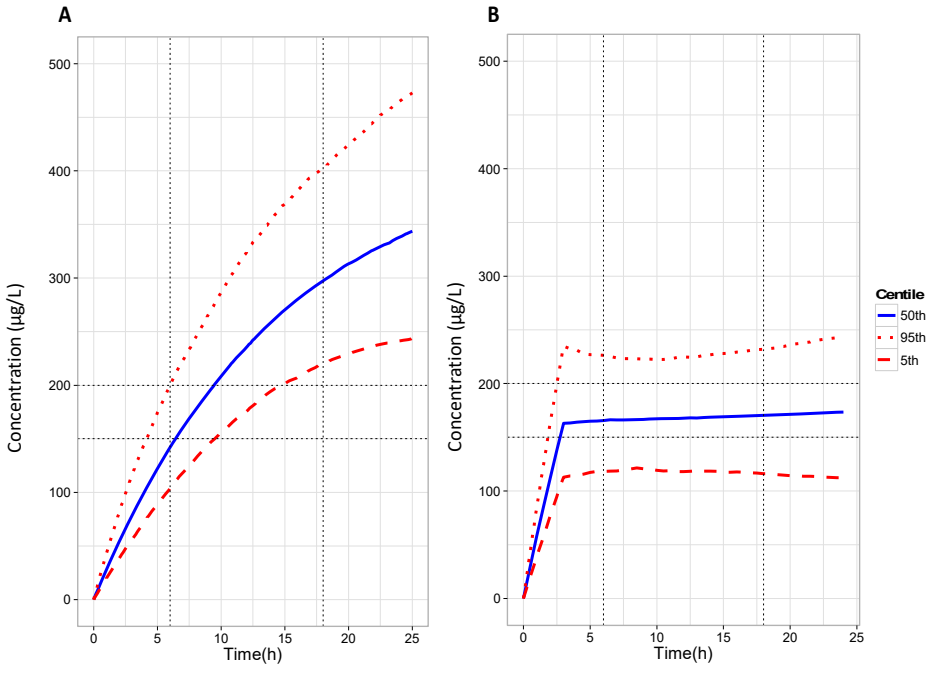

Figure 3. Milrinone concentration-time profiles of 1,000 simulated subjects: A with a continuous infusion of 0.33 µg/kg/min; B with a 3-hour loading infusion of 0.73 µg/kg/min followed by a 21-hour maintenance infusion of 0.16 µg/kg/min. Median estimates are presented with blue solid lines, red dashed lines show 90% CI, and the target concentration of 150–250 µg/l and the time period of 6–18 hr after the start of drug administration are marked with grey dotted lines.

The long half-life of milrinone in neonates, 10 hours according to Paradisis et al., suggested the need for a loading dose to achieve therapeutic concentration within a reasonable time, while low clearance refers to possible accumulation of the drug when administered continuously (Paradisis et al. 2007). The simulation study results confirmed improvement of therapeutic concentration attainment within a clinically relevant time frame with loading infusion and a lower maintenance dose compared to an unchanged continuous infusion rate.

We carried the best simulated dosing regimen forward to our next prospective milrinone PK study.

5.2 Milrinone PK study results (Study II)

5.2.1 Demographics of the milrinone PK study population

Altogether, 21 neonates underwent PDA ligation surgery within the study period. All operations were performed in NICU by one experienced surgeon and under intravenous anaesthesia with ketamine, fentanyl and atracurium. Seven neonates were excluded for the following reasons: (1) participation in another clinical study (n=3), (2) absent parental consent (n=2) and (3) renal failure with creatinine >100 μmol/l (n=2). We recruited 14 patients into the study, of which 10 received milrinone according to the study protocol (publication II, Figure 1).

Low LVO (<200 ml/kg/min) at 1 hour after PDA ligation was measured in 10 patients, and all of them were treated with milrinone according to the study protocol. The population mean (range) PMA and body weight on the day of PDA ligation were 27.4 (24.6–30.1) weeks and 857.3 (568.0–1114.0) g, respectively (Table 4).

Dose optimization simulations included PMA and weight data from 52 patients with mean (range) PMA and bodyweight of 27.6 (23.3–37.3) weeks and 875.5 (500.0–2351.0) g, respectively.

Table 4. Demographics and clinical data of the milrinone PK study patients

	Sex	GA (full wk)	BW (g)	Resp. support (FiO_2)	Inotrope/ vasopressor	Prior Ibuprofen	PNA (days)	W (g)	SCr (µmol/l)
1	M	23	540	Inv (40%)	dobutamine	no	11	568	37
2	M	23	700	Inv (35%)	–	no	12	610	48
3	F	24	602	Inv (27%)	–	no	17	795	49
4	F	24	700	Inv (45%)	dopamine	no	27	895	46
5	F	24	815	Non-inv (30%)	–	yes	11	805	73
6	F	25	892	Inv (40%)	–	no	22	1100	41
7	F	27	824	Inv (21%)	–	no	12	845	44
8	F	27	920	Non-inv (23%)	–	no	22	915	27
9	F	27	925	Inv (21%)	dopamine	no	5	926	64
10	F	27	1100	Non-inv (30%)	–	no	22	1114	41
mean	NA	25.1	801.8	NA	NA		16.1	857.3	47
median	NA	24.5	819.5	NA	NA		14.5	870	45

GA – gestational age at birth; wk – weeks; BW – birth weight; Resp. support – preoperative respiratory support; inv – invasive conventional mechanical ventilation; non-inv – non-invasive pressure control ventilation, nasal CPAP or high flow nasal cannula; Inotrope/vasopressor – perioperative inotropic and vasopressor treatment; Prior ibuprofen – administered within 7 days before duct ligation; NA – not applicable; PNA – postnatal age at the day of PDA ligation; W – weight on the day of PDA surgery; SCr – serum creatinine on the day of study recruitment.

5.2.2 Pharmacokinetics of milrinone

Forty time-concentration data points, four from each patient, were available for PK analysis. The median (range) milrinone plasma concentrations at 3, 24, 28 and 36 hr were 279.7 (213.6–366.2), 186.7 (84.3–361.0), 105.1 (27.1–227.3) and 45.4 (3.0–113.3) µg/l, respectively. Individual time-concentration profiles of the study patients are presented in Figure4.

A one-compartmental linear structural model with a proportional residual error model adequately described the population PK data. Adding a second compartment to the PK structural model did not lower the OFV (292.8 vs. 291.9). Adding allometric scaling to body weight and maturation function of GFR of the model parameters to the structural model (Equation 12 and Equation13) reduced the OFV from 291.9 to 273.1.

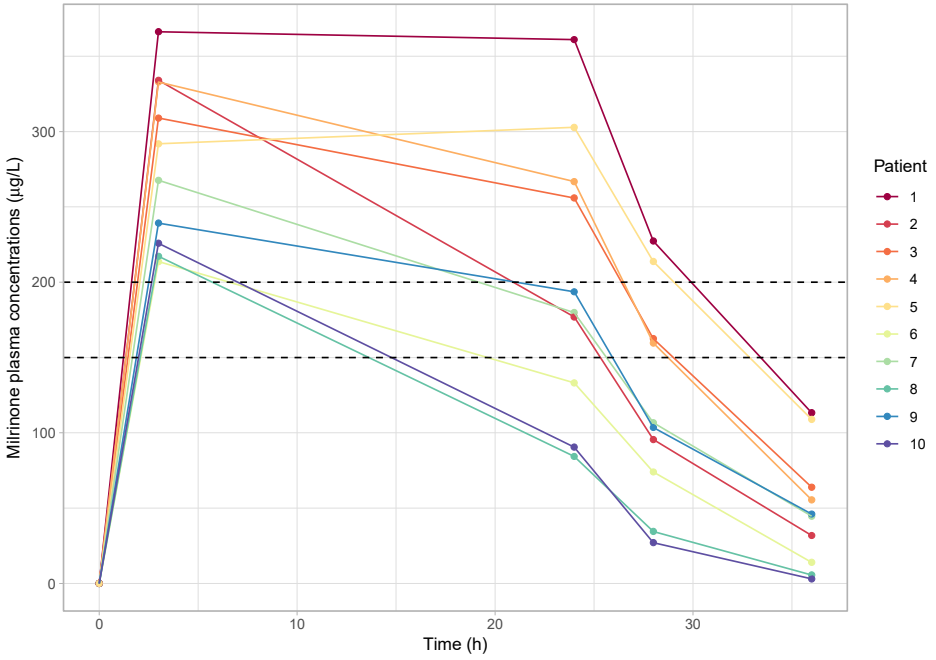

Figure 4. Measured milrinone plasma concentrations. Each colour refers to a patient as presented in Table 4. Dashed lines refer to the target concentration range of Study I simulations. The patient numbers are in concordance with Table 4.

The final population mean (coefficient of variation) parameter estimates for clearance and volume of distribution were 0.350 l/h/857.3g (11.6%) and 0.329 l/857.3g (32.6%), respectively. Similar PK parameter estimates from the final model NONMEM run and bootstrap analysis demonstrate the robustness of the model (Table 5).

Table 5. Final parameter estimates with uncertainty from the NONMEM output file and from bootstrap analysis results.

	Values from the final model		Values from bootstrap analysis		
Parameter	Mean	SE	Median	2.5th percentile	97.5th percentile
TVCL (l/h/857.3g)	0.350	0.036	0.347	0.285	0.430
TVV (l/857.3g)	0.329	0.014	0.329	0.305	0.357
BSV on CL (CV%)	32.6	15.8	31.1	19.2	38.2
BSV on V(CV%)	11.6	7.1	11.0	5.5	14.5
Proportional residual error	0.07	0.05	0.07	0.04	0.10
CL-V covariance	0.521	0.220	0.513	–0.230	0.686

TV – population typical parameter value; BSV – between subject variability; CL – clearance; V – volume of distribution; SE – standard error; CV% – presented as coefficient of variation, calculated as: (square root of ω2)·100%.

GOF plots of the final PK model showing the correlation between the model-predicted and observed plasma concentrations and time- and concentration-independence of residual variability are presented in Figure 5, and a VPC showing all the observed concentration within the confidence intervals of model predictions is presented in Figure 6.

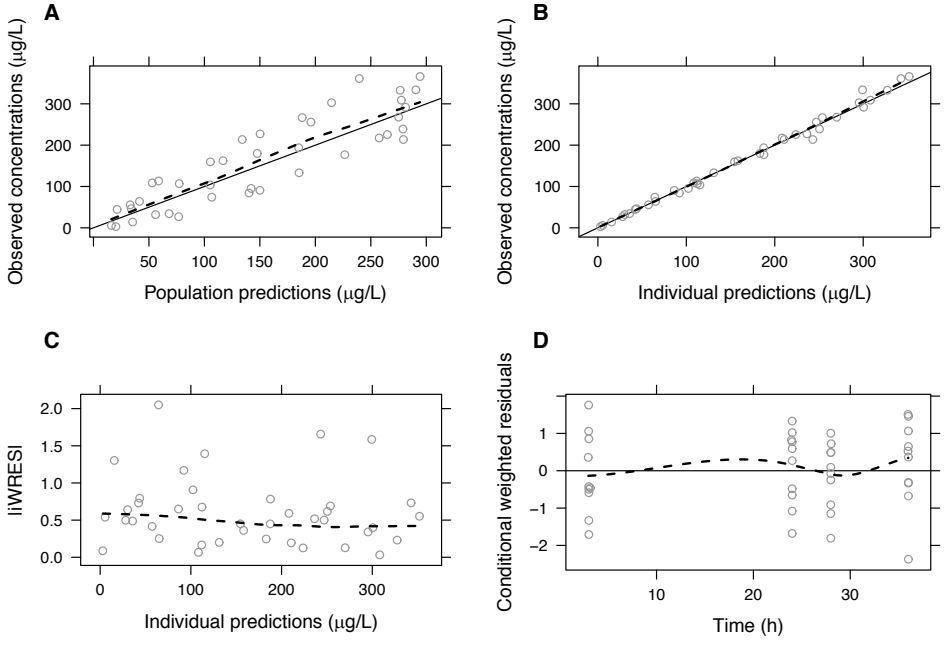

Figure 5. Basic goodness-of-fit plots of the final PK model: A observed versus population predicted milrinone concentrations, B observed versus individual predicted milrinone concentrations, C absolute value of individual weighted residuals (|iWRES|) versus individual predictions, D conditional weighted residuals over time.

All measured plasma concentrations at 3 hr exceeded the target concentration range of 150–200 µg/l from the milrinone simulation study, and in 9 of 10 patients, the plasma concentration decreased over the maintenance phase. The discordance can be explained by a significant reduction in total body water (accompanied by proportional weight loss) within the first 3–4 days of life, resulting in a lower volume of distribution for water-soluble agents and rapid changes in GFR and renal secretion within the first weeks of life (Bauer et al. 1989; Chen et al. 2006; Pacifici 2009). Consequently, the dosing of milrinone, based on the milrinone simulation study using the milrinone population PK model developed in preterm neonates within the first days of life (Paradisis et al. 2007), resulted in higher than expected loading and lower maintenance concentrations in the population with a PNA of 5–27 days. The lower population mean volume of distribution (0.329 vs 0.512 l) and higher clearance (0.350 vs 0.035 l/h) found in our study are in concordance with this explanation.

The differences in milrinone PK parameter estimates between two populations of preterm neonates studied at different ages underline the importance of collecting data in neonates of different GA and PNA to adequately describe the maturation of PK properties. A standardised GFR maturation function can be applied to the description of the PK of drugs mainly cleared by the kidneys.

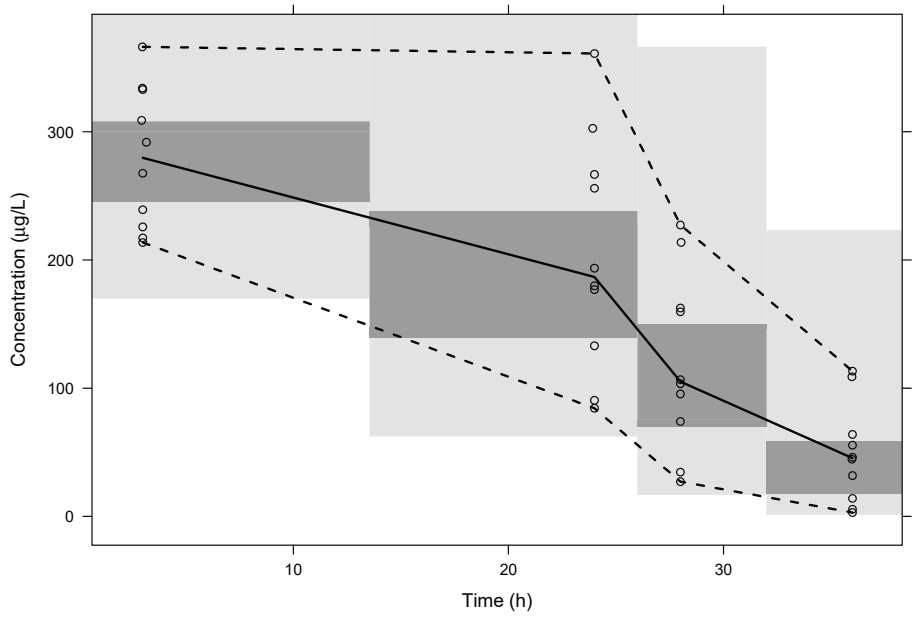

Figure 6. Visual predictive check (VPC) of 1,000 simulated milrinone concentration-time data sets from the final model. Open circles represent the observations, a solid line the 50th percentile, dashed lines the 2.5th and 97.5th percentiles, and shaded areas the 95% confidence intervals of the corresponding predicted milrinone concentrations.

5.2.3 Simulation results for milrinone dose optimisation

The best probability of concentration target (150–250 µg/l) attainment was achieved with a 3-hour loading dose of 0.50 µg/kg/min followed by a maintenance infusion rate of 0.15 µg/kg/min. Differentiation of maintenance doses by PMA improved PTA from 0.561 to 0.589, with a PMA threshold of 27 weeks and maintenance doses of 0.15 and 0.20 µg/kg/min, in the PMA<27 and PMA≥27 groups, respectively. Figure 7 shows the simulated milrinone plasma concentrations of 1,000 subjects with uniform and PMA-based maintenance doses. Application of a higher maintenance dose (0.20 instead of 0.15 µg/kg/min) for neonates with a PMA greater than or equal to 27 weeks reduced the proportion of patients with simulated plasma concentrations below 100 µg/l, at the cost of higher proportions exceeding 300 µg/l.

As the present milrinone PK model was developed based on data from a small population of 10 neonates, external validation of the model and suggested dosing regimen would be necessary to improve the reliability.

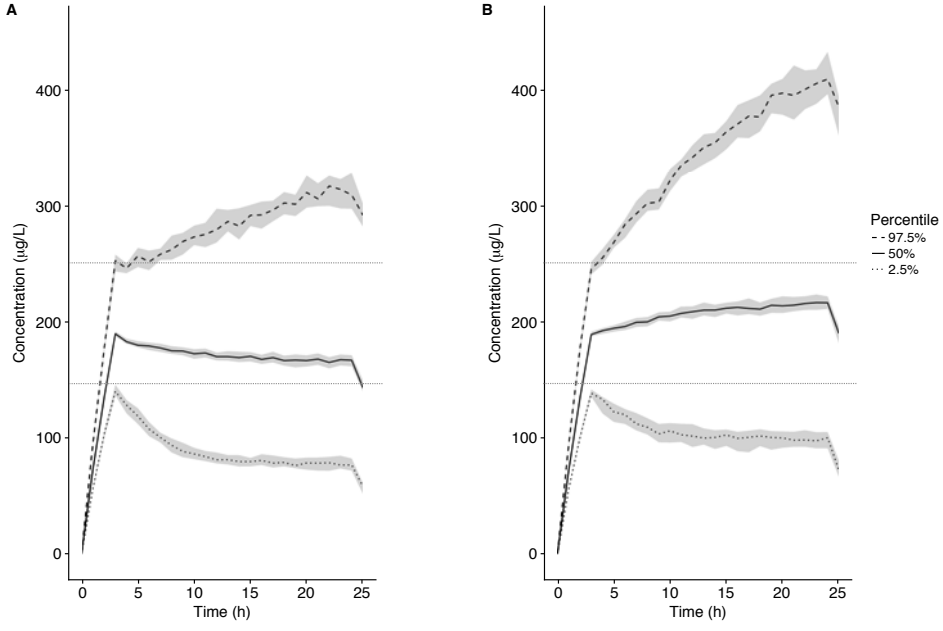

Figure 7. Simulated milrinone concentration-time curves: A 3-hour 0.5 µg/kg/min loading dose followed by a 21-hour maintenance dose of 0.15 µg/kg/min; B 3-hour 0.5µg/kg/min loading dose followed by a 21-hour maintenance dose of 0.15 µg/kg/min in subjects under 27 weeks of PMA and 0.2 µg/kg/min in subjects with a PMA≥27 weeks. The lines show 2.5%, 50% and 97.5% percentiles of the simulated concentrations, shaded areas show bootstrapped 95% confidence intervals, and dotted grey lines mark the target concentration of 150–250 µg/l.

5.2.4 Clinical outcome and safety of the milrinone PK study

None of the study patients developed PLCS, defined as low systolic BP requiring one or more cardiotropic agents accompanied by ventilation or oxygenation failure. There was no significant overall LVO improvement observed at 3 hr of milrinone infusion, but at 24 hr of treatment, LVO improved in all but two patients by an average (SD) of 54 (64) ml/kg/min (p=0.037). Intra- and inter-observer variability (mean ±SD) of LVO measurements were 10±5 ml/kg/min (6±4 %) and 37 ± 43 ml/kg/min (15±16%), respectively.

Tachycardia (HR >200 min^{-1}), registered in 3 patients during milrinone therapy with cumulative time over the threshold of 6, 3 and 37 min, respectively, triggered no change in treatment. Arterial hypotension (MAP < 30 mmHg) was registered in 6 patients with cumulative time under the threshold of 21, 327, 183, 13, 101 and 30 min, respectively. Vasoactive treatment was started in two cases, and one patient was already on dopamine with no increase in dose during milrinone treatment. In both cases of additional vasoactive treatment, milrinone concentrations at 3 hours of therapy exceeded 300 μg/l, and the neonates had a PMA of less than 27 weeks. One patient with thrombocytopenia (platelet count $< 100 \times 10^9$ /l) present prior to PDA ligation was treated with platelet transfusion.

5.3 Dobutamine PKPD study results (Study III)

5.3.1 Demographics of the dobutamine PKPD study population

We recruited 31 neonates, and data from 28 were included in the final analysis. The reason for exclusion from the final analysis was therapeutic hypothermia in two cases, considered to possibly affect the PK of dobutamine, and withdrawal of parental consent in one case. Demographic and clinical data of the study patients at recruitment are presented in Table 6. The main underlying diagnoses were respiratory distress syndrome (n=14), early onset neonatal sepsis (n=8), perinatal asphyxia (n=2), meconium aspiration (n=2) and feto-fetal transfusion syndrome (n=2).

Table 6. Demographic and clinical data of the study population. Data are presented as median (range) or count (percent of the population), if not stated differently.

Patient characteristics (n=28)	Values
Demographic data	
GA at birth, weeks	30.4 (22.7–41.0)
GA <28 weeks	7 (25%)
GA 28–32 weeks	9 (32%)
GA 32–37 weeks	7 (25%)
GA >37 weeks	5 (18%)
Gender, male	18 (64%)
BW, g	1618 (465–4380)
Age at recruitment, hours	6 (2 – 28)
Birth history	
Multiple birth	6 (21%)
Small for GA	2 (7%)
Born from Caesarean section	22 (79%)
SNAPPE II score	16 (3–89)
Apgar score at 5’	7 (1–8)
Antenatal glucocorticoids (% of neonates born <34 weeks of GA)	15 (71%)
Laboratory data at recruitment	
Haemoglobin, g/l	163 (117–203)
Albumin, g/l	27.7 (21.6–41.3)
pH	7.327 (7.137–7.536)
HCO_3, mmol/l	19.1 (15.2–24.9)
Concomitant medications	
Dopamine	5 (18%)
Caffeine	5 (18%)
Opiates	6 (21%)
Sedatives	5 (18%)
Nitric Oxide inhalation	1 (4%)
Ventilation support at recruitment	
Invasive ventilation/FiO2	23 (82%)/0.30 (0.21–1)
Non-invasive ventilation or nasal CPAP/FiO2	5 (18%)/0.25 (0.21–0.6)
Circulatory status at recruitment	
RVO, ml/kg/min	136 (75–306)
LVO, ml/kg/min	128 (71–338)
LVEF, %	64 (51–79)

n-number; GA – gestational age; BW – birth weight; SNAPPE II – Score for Neonatal Acute Physiology Perinatal Extension; RVO-right ventricular output; LVO – left ventricular output; LVEF – left ventricular ejection fraction

5.3.2 Pharmacokinetics of dobutamine

The maximum dobutamine dose was 10, 15 and 20 µg/kg/min in one, 17 and 10 patients, respectively, with a median (range) infusion duration of 3.5 (1.4–17.7) days. A total of 119 dobutamine plasma concentrations were collected. In two samples, dobutamine was not detected, and in nine samples, the dobutamine concentration remained below LLOQ. The maximum measured concentration was 330 µg/l. The dose-concentration graph is shown inFigure 8.

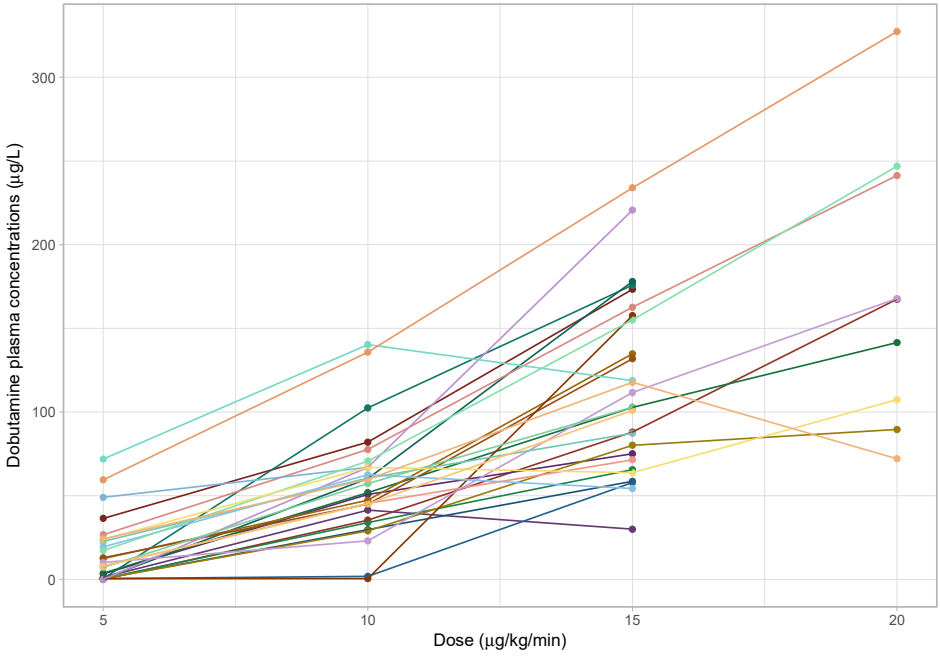

Figure 8. Measured dobutamine plasma concentrations at different continuous infusion rates; each colour refers to a patient.

The PK data was well described by one-compartmental linear structural model with a proportional error model for residual variability. Michaelis-Menten elimination did not improve fit to the observed data. Combining the Michaelis-Menten process parallel to linear elimination in the PK model resulted only in a slightly better fit (ΔOFV=10) with estimated mean (SE) values for V_{max} and KM of 436(351) µg/h and 192(131) µg/l, respectively. Therefore, to avoid over-parameterisation, a linear PK model was carried forward to PKPD modelling.

Adding allometric weight scaling and the clearance maturation function significantly improved model fit (ΔOFV=26 and 15, respectively) and lowered BSV by 62%, without further improvement by other covariates.

The final linear PK model parameter estimates from NONMEM and bootstrap analysis are presented in Table 7. GOF plots show population and individual predicted vs. observed dobutamine concentrations, as well as residuals errors depending neither on individual predicted concentrations, nor time (Figure 9). Prediction corrected VPC is presented in Figure 10

Table 7. Final dobutamine pharmacokinetic model parameter estimates with uncertainty from NONMEM output file and from bootstrap analysis results.

	Values from the final model		Values from bootstrap analysis		
Model parameters	Mean (SE)	Shrinkage	Median	2.5th percentile	97.5th percentile
TVCL (l/h/1618 g)	41.2 (44.5)	–	27.4	15.8	55.1
TVV (l/1618 g)	5.29 (0.821)	–	5.1	3.5	6.8
BSV of CL and V (CV%)	29% (17.2%)	17%	27	14	38
Shared BSV scale factor	1.34 (0.373)	–	1.3	0.5	3.1
Hill	2.67 (1.90)	–	4.3	1.9	33.0
PMA_{50} (weeks)	37.4 (30.6)	–	28.2	22.7	44.1
Residual error (proportional)	0.581 (0.229)	5%	0.316	0.231	0.433

TV – population typical parameter value; CL – clearance ; V – volume of distribution; BSV – between subject variability; CV% – presented as coefficient of variation, calculated as: (square root of ω2)·100%; Hill – the sigmoidicity coefficient of maturation of CL; PMA_{50} – the PMA when the maturation of the CL reaches 50% of adult values.

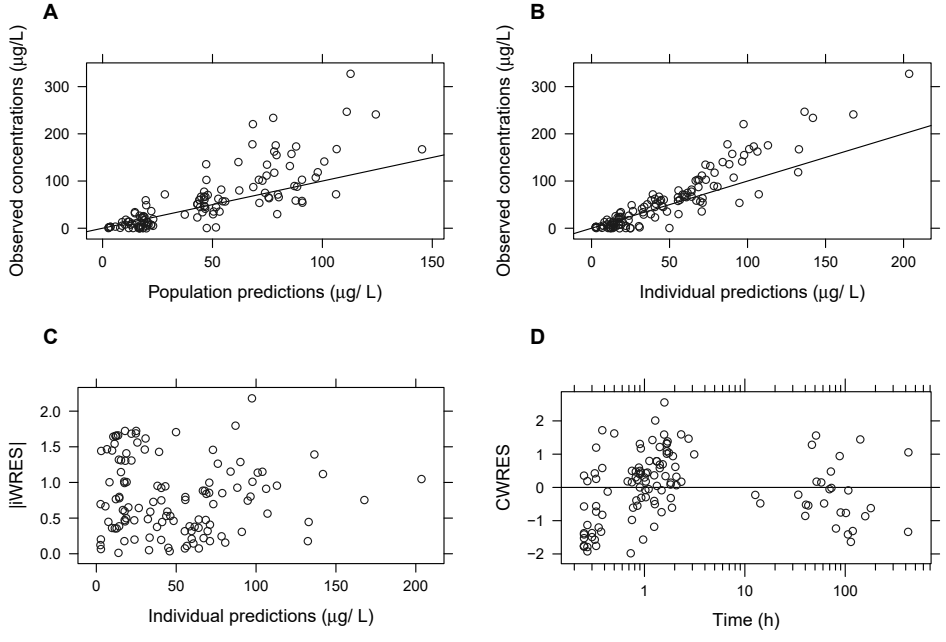

Figure 9. Basic goodness-of-fit plots of the final dobutamine linear PK model: A observed versus population predicted dobutamine plasma concentrations, B observed versus individual predicted dobutamine plasma concentrations, C absolute value of individual weighted residuals (|iWRES|) versus individual predictions, D conditional weighted residuals (CWRES) over time (log-scale).

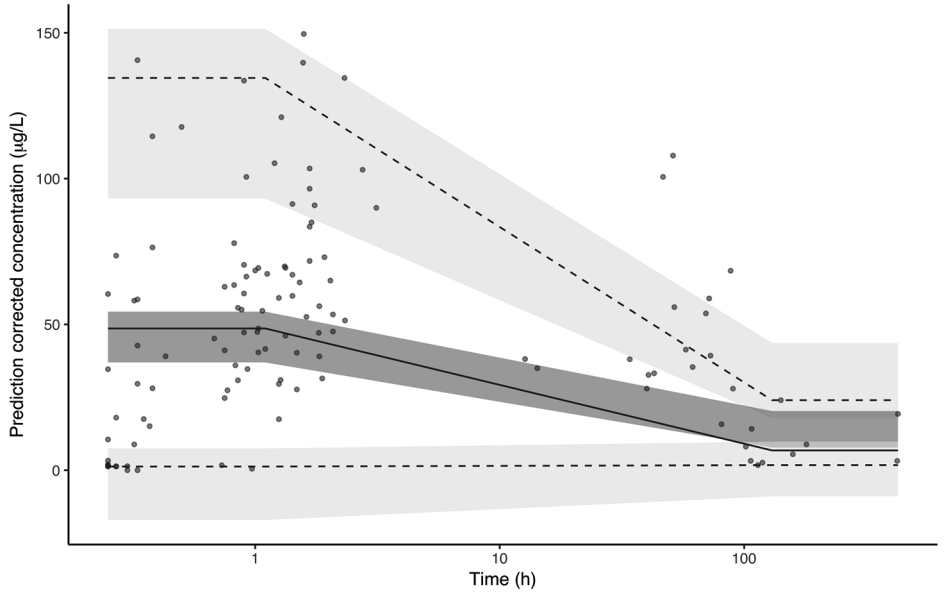

Figure 10. Prediction corrected visual predictive check (VPC) of 1,000 simulated dobutamine concentration-time datasets from the final linear PK model. Open circles represent the observations, solid line the 50th percentile, dashed lines the 2.5th and 97.5th percentiles, and shaded areas the 95% confidence intervals of the corresponding predicted dobutamine concentrations.

This is the first study to show how dobutamine's clearance is related to body size and PMA; that could be expected based on the neonatal PK data of other medications but had remained undetectable in early dobutamine PK studies (Martinez et al. 1992).

In previous paediatric studies, an extremely wide BSV in dobutamine plasma clearance has been noticed and explained by variation in sulfoconjugation and renal function (Berg, Padbury et al. 1993; Berg et al. 1997; Habib et al. 1992).

We have chosen an approach in which the whole elimination process maturation is described by the Hill model, with estimated mean parameter values of 37.4 weeks for PMA_{50} and 2.67 for the Hill coefficient, similar to those identified for clearance maturation in other drugs in neonates (Germovsek et al. 2017).

Adding PNA did not improve the PK model fit with our data, but we would suggest addressing this again in future studies. The reason why we could not show a significant effect may be the relatively small study population within a narrow range of PNAs.

In previous paediatric studies, an extremely wide BSV in dobutamine plasma clearance has been noticed and explained by variation in sulfoconjugation and renal function (Berg, Padbury et al. 1993; Berg et al. 1997; Habib et al. 1992).

We have chosen an approach in which the whole elimination process maturation is described by the Hill model, with estimated mean parameter values

of 37.4 weeks for PMA_{50} and 2.67 for the Hill coefficient, similar to those identified for clearance maturation in other drugs in neonates (Germovsek et al. 2017).

Adding PNA did not improve the PK model fit with our data, but we would suggest addressing this again in future studies. The reason why we could not show a significant effect may be the relatively small study population within a narrow range of PNAs.

There is one study that reports a highly variable volume of distribution of dobutamine with a mean (range) of 1.14 (0.09 to 5.65) l/kg in paediatric patients aged 1 month to 16 years of age (Schwartz et al. 1991). The mean value is higher than reported in adults (0.202 l/kg) (Kates et al. 1978). The typical value for the volume of distribution of 5.29 l/1618 g or 3.27 l/kg in the present study population is even higher than that reported in the paediatric study (Schwartz et al. 1991). A higher volume of distribution of water-soluble agents in neonates in their first days of life can be explained by significantly higher total body water content, which undergoes major reduction (accompanied by proportional weight loss) within the first 3 to 4 days of life (Bauer et al. 1989; Pacifici 2009), as also described for milrinone in the present thesis. In our study, the continuous administration of medication and small available cumulative PK sample volume limited the yield of informative data for the individual volume of distribution estimation. The PK model development process showed high correlation between BSV of clearance and volume of distribution; consequently, the common BSV estimate was given for clearance and the volume of distribution of dobutamine. Our approach yielded a shared BSV coefficient of 29% for clearance and volume of distribution, which is notably smaller than what was described earlier for dobutamine clearance or the volume of distribution.

5.3.3 Pharmacodynamics of dobutamine

Echocardiography measurements (RVO, LVO and LVEF) were obtained for 27 of the 28 study patients at all timepoints; one patient only had baseline LVEF measured. The maximum values of RVO, LVO and LVEF achieved during dobutamine infusion significantly exceeded pre-treatment values: RVO increased from 136 (75–306) to 182 (100–442) ml/kg/min, LVO from 128 (71–338) to 162 (94–350) ml/kg/min, and LVEF from 63.5 (51–79) to 69.5 (59–92) % (p-value < 0.01 in all cases). Intra- and inter-observer variability for echocardiography measurements are presented in Table 8. The variation of individual trends of RVO and LVO are shown in Figure 11.

Table 8. Intra- and inter-observer variability of echocardiography measurements, presented as absolute and relative variability.

	Intra-observer variability				Inter-observer variability			
Measurement	Mean	SD	Mean%	SD%	Mean	SD	Mean%	SD%
RVO (ml/kg/min)	7	4	4	2	16	14	11	10
LVO (ml/kg/min)	5	5	4	3	18	14	12	10
LVEF (%)	4	3	5	5	2	1	3	2

RVO – right ventricular output; LVO – left ventricular output; LVEF – left ventricular ejection fraction; Mean – mean absolute difference (in original units ml/kg/min); SD – standard deviation of absolute difference; Mean% – mean relative difference (difference between the two measurements as a percentage of the mean of the two measurements); SD% – standard deviation of relative difference.

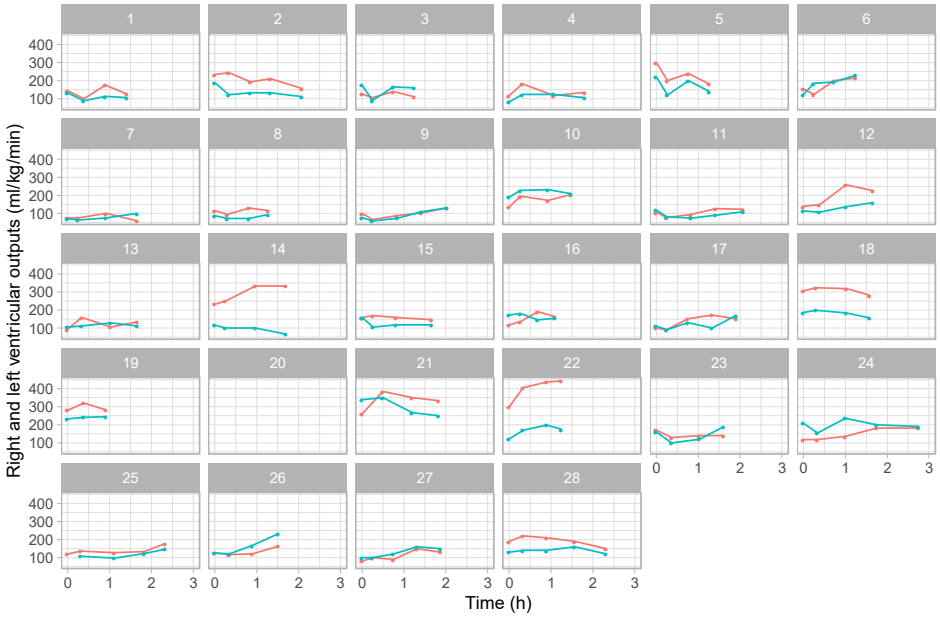

Figure 11. Individual right (blue) and left (red) ventricular cardiac output (RVO, LVO) measures during dobutamine dose escalation. Every numbered plot refers to a study patient, and every point refers to a measurement at doses 0, 5, 10, 15 and 20 µg/kg/min, if administered. Patient nr. 20 did not have any RVO or LVO measurements done.

We described the concentration-related effect of dobutamine on RVO, LVO, LVEF, HR, MAP and cFTOE. OFVs of all the tested PKPD models with different PD model structures are presented in Table 9. Residual variability for both concentration and effect were best described with a proportional error model, remaining >50% in PK observations and between 5–20% in PD observations.

Table 9. Objective function values of tested pharmacokinetic-pharmacodynamic models.

PD model structure	RVO	LVO	LVEF	HR	HR+ K_{EO}	MAP	MAP+ K_{EO}	cFTOE	cFTOE +K_{EO}
linear	1849	1806	1451	7655	7598	5418	5418	–5080	–5108
E_{max}	1847	1801	1451	7654	7513	5240	5145	–5288	–5139
sigmoidal E_{max}	1847	1797	1451	7457	7411	5076	5101	–5567	–5152

RVO – right ventricular output; LVO – left ventricular output; LVEF – left ventricular ejection fraction; HR – heart rate; K_{EO} – equilibration rate constant between effect and observed (plasma) compartment, indicating that effect compartment was added to the model; MAP – mean arterial blood pressure; cFTOE – cerebral fractional oxygen extraction; the underlined objective function values indicate the final models.

Changes in RVO and LVEF were best described with linear PD models and in LVO with a sigmoidal E_{max} model with an estimated mean (SD) EC_{50} of 117 (37.0) µg/l. The linear concentration-effect relationship within the studied concentration range for RVO, LVEF and the relatively high mean EC_{50} estimate for LVO suggest improvement of cardiac function and central blood flow throughout the studied dose range.

Final RVO, LVO and LVEF PKPD model parameter estimates together with values from bootstrap analysis are presented in Table 10. GOF plots of concentration and effect predictions are presented in the Appendix (Figures A1–A3) and predictions corrected VPC plots in Figure 12.

Table 10. Right ventricular output (RVO), left ventricular output (LVO) and left ventricular ejection fraction (LVEF) pharmacokinetic-pharmacodynamic model final parameter estimates with uncertainty from the NONMEM output file and from the bootstrap analysis.

	Values from the final model		Values from bootstrap analysis		
Model parameters	Mean (SE)	Shrinkage	Median	2.5th percentile	97.5th percentile
RVO					
TVCL (l/h/1618 g)	41.0 (3.15)	–	41.0	35.2	48.2
TVV (l/1618 g)	5.31 (0.753)	–	5.1	3.5	7.0
BSV of CL and V (CV%)	27% (18.7%)	18%	25	13	37
Shared BSV scale factor	1.50 (0.479)	–	1.5	0.6	3.7
TVE_0 (ml/kg/min)	151 (12.8)	–	151	127	180
BSV of E_0 (CV%)	41% (22.2%)	3%	41	29	50
TVSL	0.214 (0.067)	–	0.214	0.061	0.386
PK residual error (proportional)	0.583 (0.052)	8%	0.575	0.484	0.664
PD residual error (proportional)	0.184 (0.014)	8%	0.182	0.163	0.200

Table 10. (continued)

	Values from the final model		Values from bootstrap analysis		
Model parameters	Mean (SE)	Shrinkage	Median	2.5th percentile	97.5th percentile
LVO					
TVCL (l/h/1618 g)	40.7 (3.03)	–	41.0	34.9	47.6
TVV (l/1618 g)	5.14 (0.726)	–	5.0	3.4	6.6
BSV of CL and V (CV%)	25% (17.5%)	21%	24	11	34
Shared BSV scale factor	1.33 (0.490)	–	1.3	0.4	3.6
TVE_0 (ml/kg/min)	131 (9.64)	–	131	114	151
BSV of E_0 (CV%)	36% (19.8%)	3%	35	25	45
TVγ	2.82 (1.25)	–	3.3	1.0	7.3
$TVEC_{50}$ (µg/l)	117 (37.0)	–	110.4	80.6	217.3
TVE_{max} (ml/kg/min)	157 (21.8)	–	155	119	200
BSV of E_{max} (CV%)	44% (39.7%)	29%	38	11	79
PK residual error (proportional)	0.589 (0.053)	11%	0.576	0.493	0.670
PD residual error (proportional)	0.167 (0.015)	11%	0.164	0.138	0.191
LVEF					
TVCL (l/h/1618 g)	41.2 (3.22)	–	41.0	35.1	47.5
TVV (l/1618 g)	5.26 (0.753)	–	5.0	3.4	6.9
BSV of CL and V (CV%)	28% (19.3%)	17%	26	15	37
Shared BSV scale factor	1.38 (0.434)	–	1.4	0.6	3.4
TVE_0 (%)	63.5 (1.46)	–	63.6	60.6	66.3
BSV of E_0 (CV%)	9% (5.6%)	10%	9	7	11
TVSL	0.029 (0.015)	–	0.029	0.008	0.053
PK residual error (proportional)	0.580 (0.051)	7%	0.571	0.486	0.660
PD residual error (proportional)	0.098 (0.007)	7%	0.096	0.063	0.141

TV – population typical parameter value; BSV – between subject variability; CV% – presented as coefficient of variation, calculated as: (square root of ω2)·100%; CL – clearance; V – volume of distribution; E_0 – estimated baseline value of PD parameter; SL – slope of the linear relationship between effect and concentration; E_0 – estimated baseline value of PD parameter; γ – steepness of concentration-effect relationship; EC_{50} – concentration at half-maximal effect; E_{max} – the estimated maximum value of the PD parameter.

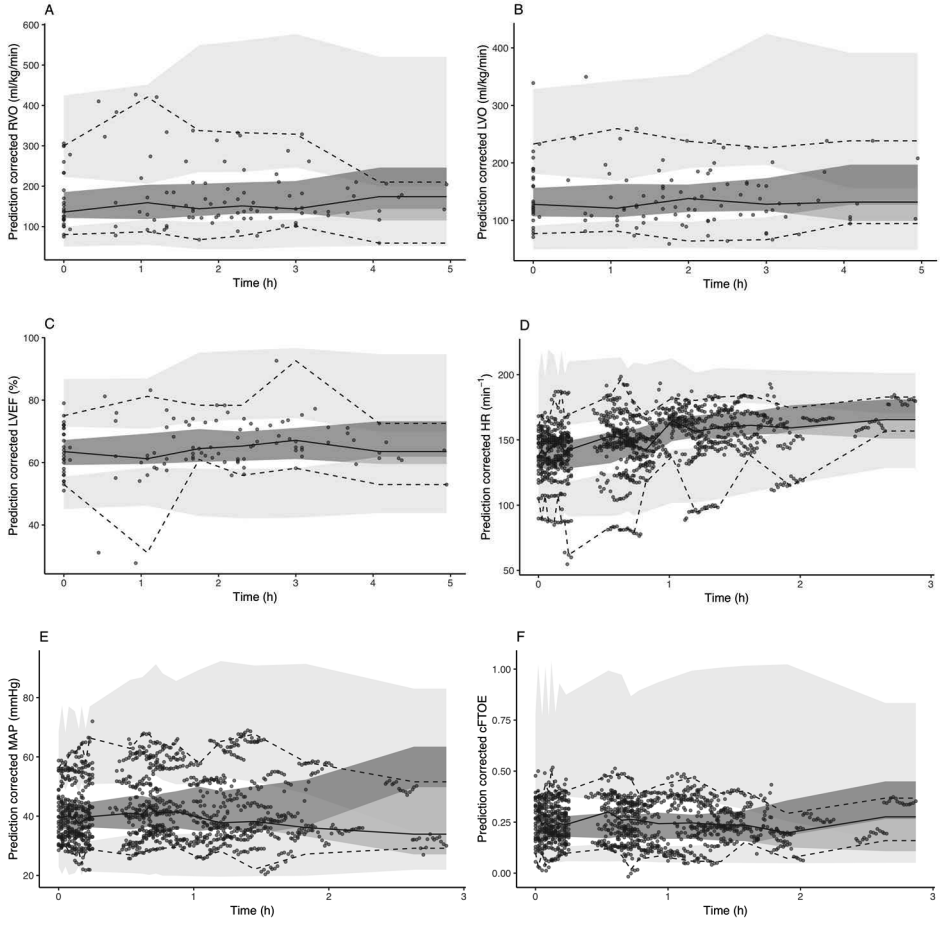

Figure 12. Prediction corrected visual predictive checks (VPC) of 1,000 simulated concentration-time and effect-time datasets from the dobutamine final pharmacokinetic-pharmacodynamic models of: A right ventricular output (RVO), B left ventricular output (LVO), C left ventricular ejection fraction (LVEF), D heart rate (HR), E mean arterial blood pressure (MAP), F cerebral fractional tissue oxygen extraction fraction (cFTOE). Circles represent the observations, solid line the 50th percentile, dashed lines the 2.5th and 97.5th percentiles, shaded areas the 95% CIs of the corresponding model predicted values.

Dobutamine's effect on HR was best described by a model with an effect compartment equilibration rate constant with mean effect time of 9 min ($k_{eo}^{-1} \cdot 60$ min) and sigmoidal E_{max} PD model with an estimated mean (SD) EC_{50} of 39.2 (5.56) µg/l. The change in MAP during dobutamine treatment was described by a sigmoidal E_{max} model without an effect compartment equilibrium rate constant with an estimated mean (SD) EC_{50} of 25.4 (2.00) µg/l. The decrease in cFTOE was described by a sigmoidal E_{max} model without an effect compartment equilibrium rate constant with an estimated mean (SD) EC_{50} of 52.9 (7.26) µg/l. The observed lower mean EC_{50} estimates (25–53 µg/l) and steep concentration-effect

relationship (ϒ of 3.4–13.5) for HR, MAP and cFTOE indicate that these changes take place at lower dobutamine concentrations and are limited.

Parameter estimates of HR, MAP and cFTOE final PKPD models from NONMEM and bootstrap analysis are presented in Table 11, GOF plots of concentration and effect predictions in the Appendix (Figures A4–A6) and prediction corrected VPC plots in Figure 12.

The BSV coefficient estimates of clearance and the volume of distribution were the lowest in the PKPD models of LVO and MAP effect, 25 and 24%, respectively (Table 10 and Table 11). These results point towards the possible impact of CO and BP on the elimination rate of dobutamine.

Table 11. Heart rate (HR), mean arterial blood pressure (MAP) and cerebral fractional tissue oxygen extraction (cFTOE) pharmacokinetic-pharmacodynamic model final parameter estimates with uncertainty from the NONMEM output file and from the bootstrap analysis.

	Values from the final model		Values from bootstrap analysis		
Model parameters	Mean (SE)	Shrinkage	Median	2.5th percentile	97.5th percentile
HR					
TVCL (l/h/1618 g)	42.3 (3.22)	–	40.6	34.9	47.5
TVV (l/1618 g)	5.42 (0.766)	–	5.2	3.4	6.9
BSV of CL and V (CV%)	27% (18.0%)	15%	25	14	36
Shared BSV scale factor	1.72 (0.421)	–	1.8	0.7	4.7
TVE_0 (min^{-1})	138 (4.2)	–	138	130	146
BSV of E_0 (CV%)	15% (8.1%)	4%	15	9	21
TVK_{E0} (h^{-1})	6.59 (2.18)	–	6.2	3.8	8.7
γ	3.36 (0.326)	–	3.4	2.7	8.0
$TVEC_{50}$ (µg/l)	39.2 (5.56)	–	38.5	29.5	48.9
BSV of EC_{50} (CV%)	50% (32.2%)	21%	47	22	66
TVE_{max} (min^{-1})	172 (2.5)	–	171	158	177
BSV of E_{max} (CV%)	5% (3.1%)	28%	5	2	11
PK residual error (proportional)	0.590 (0.052)	3%	0.582	0.498	0.682
PD residual error (proportional)	0.051 (0.001)	3%	0.050	0.033	0.070

Table 11. (continued)

Model parameters	Values from the final model		Values from bootstrap analysis		
	Mean (SE)	Shrinkage	Median	2.5th percentile	97.5th percentile
MAP					
TVCL (l/h/1618 g)	37.2 (2.88)	–	37.4	31.9	45.0
TVV (l/1618 g)	4.88 (0.958)	–	4.8	3.3	6.5
BSV of CL and V (CV%)	24% (16.6%)	4%	25	10	40
Shared BSV scale factor	3.62 (0.760)	–	3.5	1.9	8.9
TVE_0 (mmHg)	39.7 (1.75)	–	39	36	43
BSV of E_0 (CV%)	22% (11.8%)	2%	22	17	26
TVγ	13.5 (2.94)	–	13.5	7.1	21.7
$TVEC_{50}$ (µg/l)	25.4 (2.00)	–	25.5	17.4	34.9
TVE_{max} (mmHg)	41.9 (2.19)	–	41	37	47
BSV of E_{max} (CV%)	26% (13.9%)	4%	26	19	31
PK residual error (proportional)	0.675 (0.062)	2%	0.666	0.551	0.827
PD residual error (proportional)	0.065 (0.001)	2%	0.064	0.049	0.081
cFTOE					
TVCL (l/h/1618 g)	37.2 (3.61)	–	37.4	31.0	47.0
TVV (l/1618 g)	4.80 (0.993)	–	4.5	3.2	6.0
BSV of CL and V (CV%)	35% (21.5%)	9%	32	10	46
Shared BSV scale factor	2.42 (0.333)	–	2.3	0.7	6.5
TVE_0 (mmHg)	0.227 (0.023)	–	0.224	0.182	0.271
BSV of E_0 (CV%)	50% (26.8%)	2%	50	30	74
TVγ	3.65 (0.573)	–	3.9	1.2	23.1
$TVEC_{50}$ (µg/l)	52.9 (7.26)	–	50.8	18.9	93.2
TVE_{max} (mmHg)	0.206 (0.027)	–	0.208	0.147	0.260
BSV of E_{max} (CV%)	60% (36.2%)	9%	55	38	88
PK residual error (proportional)	0.653 (0.061)	2%	0.633	0.528	0.774
PD residual error (proportional)	0.181 (0.004)	2%	0.179	0.139	0.217

TV – population typical parameter value; BSV – between subject variability; CV% – presented as coefficient of variation, calculated as: (square root of ω2)·100%; CL – clearance; V – volume of distribution; E_0 – estimated baseline value of PD parameter; γ – steepness of concentration-effect relationship; K_{E0} – the equilibration rate constant between effect and observed (plasma) compartments; EC_{50} – concentration at half-maximal effect; E_{max} – the estimated maximum value of the PD parameter

The effect of dobutamine on CO was found to increase throughout the studied dose range at least up to a concentration of 200 µg/l. The improvement of CO beyond the maximum HR effect implicates the role of improved myocardial function and/or decreased SVR.

Unfortunately, there are no studies to compare our results with that report the dobutamine concentration-related increase in HR in neonates. However, according to the review by Mahoney et al., previous studies also report only a moderate average increase (up to 11,6%) in heart rate with dobutamine treatment, even with a dose of 20 µg/kg/min (Devictor et al. 1988; Klarr et al. 1994; Mahoney et al. 2016; Osborn et al. 2002; Robel-Tillig et al. 2007; Roze et al. 1993; Stopfkuchen et al. 1987, 1990). Hence tachycardia is unlikely to limit the dobutamine's effect on CO, and increasing dose up to 20 µg/kg/min appears to be feasible.

The change in BP during dobutamine treatment has also shown to be moderate in previous studies, and the maximal increase tends to be reached with doses of 5–10 µg/kg/min (Devictor et al. 1988; Klarr et al. 1994; Mahoney et al. 2016; Martinez et al. 1992; Osborn et al. 2002; Robel-Tillig et al. 2007; Roze et al. 1993; Ruelas-Orozco et al. 2000). Martinez et al. showed in one patient that the dobutamine plasma concentration threshold for systolic BP increase is much lower than for CO, although the measured plasma concentrations were much lower than in our study, 8 and 35 µg/l, respectively (Martinez et al. 1992).

The increase rather than decrease of MAP in response to dobutamine is potentially explained by developmental differences in vascular α- and β-AR expression. During early development, cardiovascular α-AR expression is upregulated while maturation of β-AR lags behind, and preterm neonates are likely to respond to dobutamine with attenuated decreases in systemic vascular resistance and thus with a more pronounced increase in BP (Felder et al. 1983; Noori et al. 2012).

In previous studies, dobutamine has also been shown to increase superior vena cava flow, which is considered to reflect cerebral blood flow (Bravo et al. 2015; Osborn et al. 2002). The increase of blood flow velocities and decrease of the pulsatility index of the anterior cerebral artery is shown by Robel-Tilling et al. in 20 neonates with myocardial dysfunction (Robel-Tillig et al. 2007). The present study describes dobutamine's effect on cFTOE as a surrogate for cerebral blood flow. The modest decrease in cFTOE may refer to an increase in cerebral blood flow already at a relatively low plasma concentration.

Neonatal circulatory management is aimed at preventing cerebral hypoperfusion, particularly in preterm neonates in whom cerebral autoregulation may be impaired or absent. Although the role of inotropes in the prevention of prematurity-related brain injury has been difficult to establish, there is some evidence that early administration of dobutamine may be relatively safe (Brew et al. 2018). In a study in preterm neonates with short- and long-term follow ups, dobutamine was found to be associated with a greater increase in systemic blood flow, reduced rates of severe IVH and fewer disabilities at 3 years of age, but similar combined rates of death or disability were found in infants treated with dopamine (Osborn et al. 2007, 2002).

A concentration-dependent increase in CO has been previously described by Martinez et al. in 13 critically ill neonates. They showed an average (standard error, SE) increase of 25% in CO, from 205 (13) to 256 (12) ml/kg/min. Dobutamine was administered in doses up to 7.5 μg/kg/min, and most of the measured plasma concentrations remained below 100 μg/l (Martinez et al. 1992). In our study, the average baseline CO was lower, 151(12.8) and 131(9.64) ml/kg/min for RVO and LVO, respectively, and the average CO remained below 200 ml/kg/min even with dobutamine plasma concentrations up to 200 μg/l. Nevertheless, the proportional change in CO was found to be similar: 20–30%.

High BSV of the PD response suggests the need for individual dose titration rather than targeting a specific dosing regimen in neonates receiving dobutamine for stabilisation of transitional circulation.

5.3.4 Clinical outcome and safety of the dobutamine PKPD study

14 of the 28 study patients had 21 serious adverse events (SAE) during the study follow-up period. Four study patients died, and the fatal SAEs were gastric perforation, necrotising enterocolitis with intestinal perforation, pulmonary interstitial emphysema and pulmonary interstitial emphysema combined with haemolysis and pulmonary hypertension. Deterioration of respiratory status requiring intubation with invasive ventilation occurred in two and intestinal perforation in two patients, and one patient had ileus as a complication of surgery for anal atresia. None of the aforementioned SAEs were considered to be related to dobutamine.

Twenty-seven patients had cerebral ultrasonography performed within the follow up period, IVH grade III–IV occurred in two, grade I–II in four, periventricular leukomalacia in three and other intracranial pathologies in three patients. The incidence is comparable to that of previous studies (Alderliesten et al. 2013; Osborn et al. 2003).

The high incidence of SAEs and mortality reflects that we have conducted the study in critically ill neonates in the true dobutamine use clinical scenario.

6. GENERAL DISCUSSION

Cardiovascular PKPD data in neonates is sparse, and extrapolating information between different maturational stages PMA- or PNA-wise, as well as between different clinical situations, could lead to a significant offset in clinical results (Allegaert, Simons et al. 2017; van den Anker et al. 2018). Therefore, the PK and PD properties of medications should be properly understood in the particular populations and clinical situations in which they are used.

6.1 Challenges in neonatal pharmacokinetic-pharmacodynamic research

Planning and conducting neonatal pharmacological studies involve many challenges. Working with a vulnerable population with an urgent need to start treatment makes the study recruitment process and obtaining informed consent difficult and demanding for the medical staff (Harter et al. 2016). In a situation in which treatment needs to be started immediately, informing the parents in an emotionally difficult situation is not an easy task for the researchers and puts additional pressure on the parents.

The fact that the medications are studied in situations in which they are prescribed on clinical indication likely makes it easier for parents to accept their baby's participation in the study (Baker et al. 2005; Tooher et al. 2008). That was the case in the present studies in which we investigated the PK and PD of the studied medications in situations when the treating physician believed them to be the best treatment choice for the patient. The parents were informed about the need to collect more detailed information about the studied medication, perform some additional monitoring and haemodynamic measurements and to collect some extra blood for concentration measurements. The need to collect and analyse data with the intent to have more precise treatment abilities in the future was well understood by the parents.

However, future developments should also carefully account for the potential benefits of deferred consent or other alternative approaches to "unload" parents, especially in situations already holding huge emotional stress for them (McCarthy et al. 2019; Megone et al. 2016; Rich et al. 2019). Increasing patient-parent involvement in planning clinical research may help to reach good consensus.

Very limited acceptable cumulative sampling volume in ELBW infants requires analytical methods for a minimal sample volume and excellent knowledge and analytical skills to optimise the sampling times to get most of the sparse data (Committee for Medicinal Products for Human Use (CHMP) et al. 2008).

Lack of standardised acute period outcome parameters and a small signal-to-noise ratio makes acquiring HD data for PKPD modelling a complex and challenging process. The need for simultaneous application of different monitoring techniques further adds to complexity. The feasibility of simultaneous recording

from different monitoring devices, ultrasound and PK sampling and the reliability of the data acquirement systems may need to be tested in pilot studies. To test the applicability of the HD monitoring, data acquisition methods and repeated echocardiography procedures, we performed a pilot study with 10 participants (Hallik et al. 2015).

While the technical challenges are likely to be alleviated by fast development of technology, ensuring maximum information gain through data repositories and sharing is only just taking off. Furthermore, if such demanding studies are performed and PK models developed and fitted, they often lack external validation. Validating the PK models in different populations would increase reliability and usefulness and would help us to standardise the covariate model structures for different drugs.

6.2 Pharmacokinetic studies of cardiovascular medications in neonates

If we look at the populations of paediatric PK studies of inotropes, they also include some (usually a few) neonates, and in most cases, the neonatal data is pooled together with data from older children (Bailey et al. 2004; Banner et al. 1991; Berg et al. 1997; Gist et al. 2015; Hornik et al. 2019; Mizuno et al. 2019; Oualha, Tréluyer et al. 2014; Oualha, Urien et al. 2014). In populations with a wide age range, the maturation and development of PK parameters and covariates can be described more generally for the whole population age range, but the important rapid maturation processes within the first days and weeks of life remain marginal, undetectable and undescribed. The inappropriateness of extrapolating these findings and dosing regimens to patients at the extremes of the weight-for-age range or ELBW infants without dedicated studies has been pointed out in a recent review (Allegaert, Simons et al. 2017).

Even extrapolation of the PK data from one neonatal population to another may not yield appropriate and clinically applicable results. The results of our research (the milrinone simulation study and milrinone PK study) showed that using a PK model for a preterm neonatal population for dose optimisation in a slightly different age group of preterm neonates (difference in PNA within 4 weeks), causes significant offset between simulated and measured concentrations (Figure 4). In the end, we recommended a 33% lower loading dose of milrinone for the PDA surgical closure population than was estimated based on the PK model from preterm neonates within the first days of life.

Furthermore, with cardiovascular drugs, the PD effect can also affect PK processes, and PD effects can be different from those seen in older populations. Thus, extrapolating PK data on cardiovascular medications from adult, paediatric and even different neonatal studies to neonates may cause unpredictable biases (Allegaert, Simons et al. 2017).

There are a few PK studies of cardiovascular drugs in neonates describing milrinone PK in neonates with PPHN, after OHS, and in preterm neonates within the first days of life, as well as dobutamine PK in neonates (Martinez et al. 1992; Mc Namara et al. 2013; Paradisis et al. 2007; Zuppa et al. 2006). Most of these studies, including ours, are limited to specific clinical indications. We believe that only population PK studies focusing on particular patient populations and clinical situations can give us accurate information about BSV, important covariates and the extent of their effect on PK parameters within a specific disease state. However, future developments in physiology-based PK modelling will likely lead to merging these fragmented data into a more complex, yet detailed, understanding of the drug-body interactions.

6.3 Pharmacodynamic studies of cardiovascular medications in neonates

Simultaneous population PKPD modelling is a tool that can be used to improve our knowledge about the effects of inotropes in neonatal populations. Particularly in the present situation in which randomised controlled trials in many cases have proven unfeasible and the results of prospective observational studies describing the effects of antihypotensive therapy on clinical outcomes are contradictory (Batton et al. 2016; Bravo et al. 2015; Dempsey et al. 2014; Durrmeyer et al. 2017; Pellicer et al. 2009).

Better understanding the drug-response relationship may shed some light on these discrepancies. For example, the hemodynamic effects and the clinical response to vasopressors and inotropes is altered by downregulation of adrenergic receptors caused by critical illness-associated prolonged endogenous and treatment-associated exogenous receptor stimulation (Ruffolo et al. 1986).

Furthermore, because cardiovascular adrenergic receptor expression is regulated by corticosteroids, the documented higher incidence of relative adrenal insufficiency also contributes to the observed attenuated hemodynamic response and the development of vasopressor-dependence in preterm and term neonates (Davies et al. 1984; Fernandez et al. 2009). These effects, especially seen in patients with prolonged exposure to dopamine and other vasopressors and inotropes, likely have implications for long-term outcomes (Seri et al. 2001).

Collecting and analysing PK and PD data together allows us to consider the clinical effectiveness of vasopressors and inotropes in a situation in which certain exposure to medication does not guarantee the expected PD effect.

There are many methodological issues to be considered when planning neonatal PD studies of inotropes. First, the PD effect parameters are to be chosen. When treating haemodynamically compromised neonates, the immediate treatment targets can be BP or central blood flow thresholds or prevention of regional tissue hypoxia. As long as we do not have evidence-based consensus on which

measurable HD parameters are related to short- and long-term mortality and morbidity, it is difficult to find ideal treatment targets.

With more precise knowledge about how drugs act in particular situations, in terms of change in the HD parameter values, it will be increasingly possible to identify the advantages and disadvantages of these medications in terms of short- and long-term clinical outcomes for critically ill neonates.

6.4 Limitations of the studies

These studies have several limitations. First, the small number of patients in the milrinone PK study precluded the exploration of the impact of additional patient characteristics potentially affecting the PK of milrinone, including prior ibuprofen therapy and the status of renal function. Considering the homogenous population, we could give dosing recommendations only in preterm neonates without severe renal function impairment.

We could not provide PD analysis, and thus the effect on blood flow and effectiveness of preventing PLCS of the recommended milrinone dosing scheme will be the objective of future studies.

The limited number of PK samples per patient in the dobutamine PKPD study did not yield informative data on the volume of distribution (hence BSV could not be separately estimated for clearance and V). Although we tested adding Michaelis-Menten processes in the dobutamine PK model, having only a few PK samples >200 μg/l in the dataset minimised the significance of saturable elimination pathways in model fitting. The saturation of elimination pathways of dobutamine may become important at higher plasma concentrations, but we may not have been able to capture the higher end of concentrations sufficiently.

If we could collect PKPD data from larger populations, more diverse and infrequent covariates such as co-medications and co-morbidities could be explored and their effects to PK and PD described. Although adding postnatal age, concomitant medications and severity of illness in covariate analysis did not improve the PK model fit in our study, a larger and more variable study sample may be needed to describe these covariate effects.

In neonatal PK studies, an important limitation/source of residual error is drug administration accuracy. Dilution of the formula, low infusion rates and large dead space may lead to a discrepancy between the prescribed and actual infusion rates, resulting in a PK observation residual error of over 50% in the dobutamine PKPD study.

Despite all the aforementioned limitations, we need to recognise that these studies were conducted in critically ill neonates in real-life conditions, with all the related challenges.

6.5 Clinical implementation and future considerations

The main clinical importance of this research is to improve pharmacological treatment of haemodynamically compromised neonates based on PK and PKPD knowledge.

As a result of the research, we have suggested a precise dosing regimen for milrinone in preterm neonates after PDA ligation.

The results of the PKPD study of dobutamine inform us about expected HD responses during dobutamine administration to neonates within the transition period. Dobutamine can be used to increase CO in a dose up to 20 μg/kg/min without excessive effect to BP or HR. It can also be considered to be a proof of concept study, as PKPD studies of cardiovascular drugs relying on rich PD datasets, as in our study, are scarce in neonates.

Future studies will have to define methods for individual dose/effect optimisation of cardiovascular drugs in neonates. However, today we do not know what immediate HD parameter effects to target for better long-term outcomes. Collecting and analysing the complex and multimodal HD data to predict short- and long-term mortality and morbidity is a research area that needs to develop together with the understanding of the PKPD of vasopressors and inotropes. Integrating continuous monitoring and analysis of multimodal HD data with PKPD methods may provide tools to individualise vasopressor and inotrope therapy in critically ill neonates.

It is important to arrive at an agreement as to how the PK parameter maturation is described in the models for preterm neonates. For example, should it be based on PMA, which includes intrauterine and postnatal development, to which PNA-based changes can be added, or should it incorporate GA- and PNA-based changes separately? Standardising model parameterisation will benefit the paediatric PK community by facilitating parameter value interpretation and model sharing across studies (Germovsek et al. 2017).

Through implementing PK and PD knowledge into maturational physiology, precise drug dosing regimens can be developed for critically ill neonates to optimise the efficacy and minimise the negative effects of pharmacotherapies. Translating and implementing the results of PKPD studies into clinical practice is an important task that needs to involve specialists from different fields of neonatal medicine, including clinical practice, research and pharmacometrics.

7. CONCLUSIONS

1. Simulations suggest that the plasma time-concentration profile of milrinone in neonates after patent *ductus arteriosus* ligation could be optimized with a slow loading dose followed by maintenance infusion. The objective target of most simulations with maintenance phase plasma concentrations in the range of 150–200 μg/l for at least 80% of time was maximized with a 3-hour infusion of 0.73 μg/kg/min, followed by a 0.16 μg/kg/min maintenance dose.

2. The pharmacokinetics of milrinone in premature neonates was best described by a one-compartmental linear model with parameters allometrically scaled to body weight and the age maturation function of the glomerular filtration rate taken into account. Rapid physiological changes during the first weeks of life have a significant impact on the drug metabolism – modelling the milrinone pharmacokinetics in the patent *ductus arteriosus* ligation population resulted in a smaller distribution volume and higher clearance when compared to preterm neonates in their very first days after birth.

3. Our modelling and simulation results allow us to recommend two PMA-dependent dosing regimens of milrinone for the prevention of post-ligation cardiac syndrome. For neonates younger than 27 weeks of PMA, a regimen of 0.5 μg/kg/min 3-hr loading followed by a maintenance infusion of 0.15 μg/kg/min can be recommended, while infants at least 27 weeks of PMA should receive a maintenance dose of 0.2 μg/kg/min after an identical loading dose. The effectiveness of this dosing scheme in preventing post-ligation cardiac syndrome needs to be elucidated in future studies.

4. The pharmacokinetics of dobutamine in critically ill neonates in the first days of life fits with a one-compartmental linear model in which clearance and the volume of distribution are allometrically scaled to body weight and the model includes the maturation function of elimination mechanisms with post-menstrual age.

5. The effects of dobutamine on cardiac function, blood pressure and cerebral blood flow during transitional circulation occur at different plasma concentrations. The effect on the heart rate and arterial blood pressure is limited and saturable at lower plasma concentrations, contrary to the effect on cardiac output, which continues to increase with higher plasma concentrations throughout the currently used dose range.

8. REFERENCES

Akita T, Joyner RW, Lu C, Kumar R, Hartzell HC. 1994. Developmental Changes in Modulation of Calcium Currents of Rabbit Ventricular Cells by Phosphodiesterase Inhibitors. *Circulation* 90(1):469–78.

Alderliesten T, Dix L, Baerts W, Caicedo A, Van Huffel S, Naulaers G, Groenendaal F, Van Bel F, Lemmers P. 2016. Reference Values of Regional Cerebral Oxygen Saturation during the First 3 Days of Life in Preterm Neonates. *Pediatric Research* 79(1):55–64.

Alderliesten T, Lemmers PMA, Smarius JJM, Van De Vosse RE, Baerts W, Van Bel F. 2013. Cerebral Oxygenation, Extraction, and Autoregulation in Very Preterm Infants Who Develop Peri-Intraventricular Hemorrhage. *Journal of Pediatrics* 162(4):698–704.e2.

Allegaert K, van den Anker J. 2011. Pharmacokinetics and Pharmacodynamics of Intravenous Acetaminophen in Neonates. *Expert Review of Clinical Pharmacology* 4(6):713–18.

Allegaert K, van den Anker JN. 2014. Clinical Pharmacology in Neonates: Small Size, Huge Variability. *Neonatology* 105(4):344–49.

Allegaert K, van den Anker JN. 2017. Perinatal and Neonatal Use of Paracetamol for Pain Relief. *Seminars in Fetal & Neonatal Medicine* 22(5):308–13.

Allegaert K, Cossey V, van den Anker JN. 2015. Dosing Guidelines of Aminoglycosides in Neonates: A Balance Between Physiology and Feasibility. *Current Pharmaceutical Design* 21(39):5699–5704.

Allegaert K, de Hoon J, Verbesselt R, Naulaers G, Murat I. 2007. Maturational Pharmacokinetics of Single Intravenous Bolus of Propofol. *Paediatric Anaesthesia* 17(11):1028–34.

Allegaert K, Mian P, N. van den Anker J. 2018. Developmental Pharmacokinetics in Neonates: Maturational Changes and Beyond. *Current Pharmaceutical Design* 23(38):5769–78.

Allegaert K, Peeters MY, Verbesselt R, Tibboel D, Naulaers G, De Hoon JN, Knibbe CA. 2007. Inter-Individual Variability in Propofol Pharmacokinetics in Preterm and Term Neonates. *British Journal of Anaesthesia* 99(6):864–70.

Allegaert K, Simons SHP, Tibboel D, Krekels EH, Knibbe CA, van den Anker JN. 2017. Non-Maturational Covariates for Dynamic Systems Pharmacology Models in Neonates, Infants, and Children: Filling the Gaps beyond Developmental Pharmacology. *European Journal of Pharmaceutical Sciences* 109(May):S27–31.

Anderson BJ, Allegaert K, Holford NHG. 2006. Population Clinical Pharmacology of Children: General Principles. *European Journal of Pediatrics* 165(11):741–46.

Anderson BJ, Holford NHG. 2008. Mechanism-Based Concepts of Size and Maturity in Pharmacokinetics. *Annual Review of Pharmacology and Toxicology* 48(1):303–32.

Anderson BJ, Holford NHG. 2011. Tips and Traps Analyzing Pediatric PK Data. *Paediatric Anaesthesia* 21(3):222–37.

van den Anker J, Reed MD, Allegaert K, Kearns GL. 2018. Developmental Changes in Pharmacokinetics and Pharmacodynamics. *Journal of Clinical Pharmacology* 58(June):S10–25.

Artman M, Kithas PA, Wike JS, Strada SJ. 1988. Inotropic Responses Change during Postnatal Maturation in Rabbit. *The American Journal of Physiology* 255(2 Pt 2):H335-42.

De Backer D, Hollenberg S, Boerma C, Goedhart P, Büchele G, Ospina-Tascon G, Dobbe I, Ince C. 2007. How to Evaluate the Microcirculation: Report of a Round Table Conference. *Critical Care* 11(5):R101.

Bailey JM, Hoffman TM, Wessel DL, Nelson DP, Atz AM, Chang AC, Kulik TJ, Spray TL, Akbary A, P.Miller R, Wernovsky G. 2004. A Population Pharmacokinetic Analysis of Milrinone in Pediatric Patients after Cardiac Surgery. *Journal of Pharmacokinetics and Pharmacodynamics* 31(1):43–59.

Bailey JM, Levy JH, Kikura M, Szlam F, Hug CC. 1994. Pharmacokinetics of Intravenous Milrinone in Patients Undergoing Cardiac Surgery. *Anesthesiology* 81(3):616–22.

Bailey JM, Miller BE, Lu W, Tosone SR, Kanter KR, Tam VK. 1999. The Pharmacokinetics of Milrinone in Pediatric Patients after Cardiac Surgery. *Anesthesiology* 90(4):1012–18.

Baker L, Lavender T, Tincello D. 2005. Factors That Influence Women's Decisions about Whether to Participate in Research: An Exploratory Study. *Birth (Berkeley, Calif.)* 32(1):60–66.

Balestra GM, Bezemer R, Boerma EC, Yong Z-Y, Sjauw KD, Engstrom AE, Koopmans M, Ince C. 2010. Improvement of Sidestream Dark Field Imaging with an Image Acquisition Stabilizer. *BMC Medical Imaging* 10(1):15.

Balsan MJ, Jones JG, Guthrie RD. 1991. Effects of a Clinically Detectable PDA on Pulmonary Mechanics Measures in VLBW Infants with RDS. *Pediatric Pulmonology* 11(2):161–65.

Banner W, Vernon DD, Minton SD, Dean JM. 1991. Nonlinear Dobutamine Pharmacokinetics in a Pediatric Population. *Critical Care Medicine* 19(7):871–73.

Barrington KJ. 2008. Hypotension and Shock in the Preterm Infant. *Seminars in Fetal & Neonatal Medicine* 13(1):16–23.

Barrington KJ, Finer NN, Chan WK. 1995. A Blind, Randomized Comparison of the Circulatory Effects of Dopamine and Epinephrine Infusions in the Newborn Piglet during Normoxia and Hypoxia. *Critical Care Medicine* 23(4):740–48.

Barton P, Garcia J, Kouatli A, Kitchen L, Zorka A, Lindsay C, Lawless S, Giroir B. 1996. Hemodynamic Effects of i.v. Milrinone Lactate in Pediatric Patients with Septic Shock. A Prospective, Double-Blinded, Randomized, Placebo-Controlled, Interventional Study. *Chest* 109(5):1302–12.

Batchelor HK, Marriott JF. 2015. Paediatric Pharmacokinetics: Key Considerations. *British Journal of Clinical Pharmacology* 79(3):395–404.

Batton B, Li L, Newman NS, Das A, Watterberg KL, Yoder BA, Faix RG, Laughon MM, Stoll BJ, Higgins RD, Walsh MC, Eunice Kennedy Shriver National Institute of Child Health & Human Development Neonatal Research Network. 2016. Early Blood Pressure, Antihypotensive Therapy and Outcomes at 18–22 Months' Corrected Age in Extremely Preterm Infants. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 101(3):F201-6.

Batton B, Li L, Newman NS, Das A, Watterberg KL, Yoder BA, Faix RG, Laughon MM, Stoll BJ, Van Meurs KP, Carlo WA, Poindexter BB, Bell EF, Sánchez PJ, Ehrenkranz RA, Goldberg RN, Laptook AR, Kennedy KA, Frantz ID, Shankaran S, Schibler K, Higgins RD, Walsh MC, Eunice Kennedy Shriver National Institute of Child Health & Human Development Neonatal Research Network MC, Network for the EKSNI of CH& HDNR. 2013. Use of Antihypotensive Therapies in Extremely Preterm Infants. *Pediatrics* 131(6):e1865-73.

Bauer K, Versmold H. 1989. Postnatal Weight Loss in Preterm Neonates Less than 1,500 g Is Due to Isotonic Dehydration of the Extracellular Volume. *Acta Paediatrica Scandinavica. Supplement* 360:37–42.

Berg RA, Donnerstein RiL, Padbury JF. 1993. Dobutamine Infusions in Stable, Critically Ill Children. *Critical Care Medicine* 21(5):678–86.

Berg RA, Padbury JF. 1997. Sulfoconjugation and Renal Excretion Contribute to the Interpatient Variation of Exogenous Catecholamine Clearance in Critically Ill Children. *Critical Care Medicine* 25(7):1247–51.

Berg RA, Padbury JF, Donnerstein RL, Klewer SE, Hutter JJJ. 1993. Dobutamine Pharmacokinetics and Pharmacodynamics in Normal Children and Adolescents. *The Journal of Pharmacology and Experimental Therapeutics* 265(3):1232–38.

Bhat BV, Plakkal N. 2015. Management of Shock in Neonates. *Indian Journal of Pediatrics* 82(10):923–29.

Bhatt-Mehta V, Nahata MC. 1989. Dopamine and Dobutamine in Pediatric Therapy. *Pharmacotherapy* 9(5):303–14.

Bidegain M, Greenberg R, Simmons C, Dang C, Cotten CM, Smith PB. 2010. Vasopressin for Refractory Hypotension in Extremely Low Birth Weight Infants. *The Journal of Pediatrics* 157(3):502–4.

Blake MJ, Abdel-Rahman SM, Pearce RE, Leeder JS, Kearns GL. 2006. Effect of Diet on the Development of Drug Metabolism by Cytochrome P-450 Enzymes in Healthy Infants. *Pediatric Research* 60(6):717–23.

Boerma EC, Mathura KR, van der Voort PHJ, Spronk PE, Ince C. 2005. Quantifying Bedside-Derived Imaging of Microcirculatory Abnormalities in Septic Patients: A Prospective Validation Study. *Critical Care* 9(6):R601-6.

Boghossian NS, Do BT, Bell EF, Dagle JM, Brumbaugh JE, Stoll BJ, Vohr BR, Das A, Shankaran S, Sanchez PJ, Wyckoff MH, Bethany Ball M, Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. 2017. Efficacy of Pharmacologic Closure of Patent Ductus Arteriosus in Small-for-Gestational-Age Extremely Preterm Infants. *Early Human Development* 113:10–17.

Bravo MC, López-Ortego P, Sánchez L, Riera J, Madero R, Cabañas F, Pellicer A. 2015. Randomized, Placebo-Controlled Trial of Dobutamine for Low Superior Vena Cava Flow in Infants. *Journal of Pediatrics* 167(3):572-578.e2.

Brazy JE, Lewis D V, Mitnick MH, Jöbsis vander Vliet FF. 1985. Noninvasive Monitoring of Cerebral Oxygenation in Preterm Infants: Preliminary Observations. *Pediatrics* 75(2):217–25.

Brew N, Nakamura S, Hale N, Azhan A, Davies GI, Nitsos I, Miller SL, Walker DW, Wong FY. 2018. Dobutamine Treatment Reduces Inflammation in the Preterm Fetal Sheep Brain Exposed to Acute Hypoxia. *Pediatric Research* 84(3):442–50.

Brussee JM, Krekels EHJ, Calvier EAM, Palić S, Rostami-Hodjegan A, Danhof M, Barrett JS, de Wildt SN, Knibbe CAJ. 2019. A Pediatric Covariate Function for CYP3A-Mediated Midazolam Clearance Can Scale Clearance of Selected CYP3A Substrates in Children. *The AAPS Journal* 21(5):81.

Burns ML, Stensvold HJ, Risnes K, Guthe HJ, Astrup H, Nordhov SM, Selberg TR, Rønnestad A, Lang AM, Norwegian Neonatal Network. 2016. Inotropic Therapy in Newborns, A Population-Based National Registry Study. *Pediatric Critical Care Medicine* 17(10):948–56.

Calvier EAM, Krekels EHJ, Yu H, Välitalo PAJ, Johnson TN, Rostami-Hodjegan A, Tibboel D, van der Graaf PH, Danhof M, Knibbe CAJ. 2018. Drugs Being Eliminated

via the Same Pathway Will Not Always Require Similar Pediatric Dose Adjustments. *CPT: Pharmacometrics & Systems Pharmacology* 7(3):175–85.
Chang AC, Atz AM, Wernovsky G, Burke RP, Wessel DL. 1995. Milrinone: Systemic and Pulmonary Hemodynamic Effects in Neonates after Cardiac Surgery. *Critical Care Medicine* 23(11):1907–14.
Chen N, Aleksa K, Woodland C, Rieder M, Koren G. 2006. Ontogeny of Drug Elimination by the Human Kidney. *Pediatric Nephrology (Berlin, Germany)* 21(2):160–68.
Cheung PY, Barrington KJ, Bigam D. 1999. The Hemodynamic Effects of Dobutamine Infusion in the Chronically Instrumented Newborn Piglet. *Critical Care Medicine* 27(3):558–64.
Clyman RI, Couto J, Murphy GM. 2012. Patent Ductus Arteriosus: Are Current Neonatal Treatment Options Better or Worse Than No Treatment at All? *Seminars in Perinatology* 36(2):123–29.
De Cock RFW, Piana C, Krekels EHJ, Danhof M, Allegaert K, Knibbe CAJ. 2011. The Role of Population PK-PD Modelling in Paediatric Clinical Research. *European Journal of Clinical Pharmacology* 67(SUPPL. 1):5–16.
Committee for Medicinal Products for Human Use (CHMP), Paediatric Committee (PDCO). 2008. *Guideline on the Investigation of Medicinal Products in the Term and Preterm Neonate*. London.
Copp M V, Hill AJ, Feneck RO. 1992. Overview of the Effects of Intravenous Milrinone in Acute Heart Failure Following Surgery. *European Journal of Anaesthesiology. Supplement* 5:35–41.
D'Orio V, el Allaf D, Juchmès J, Marcelle R. 1984. The Use of Low Doses of Dopamine in Intensive Care Medicine. *Archives Internationales de Physiologie et de Biochimie* 92(4):S11–20.
Davies AO, Lefkowitz RJ. 1984. Regulation of β-Adrenergic Receptors by Steroid Hormones. *Annual Review of Physiology* 46(1):119–30.
Dempsey E, Rabe H. 2019. The Use of Cardiotonic Drugs in Neonates. *Clinics in Perinatology* 46(2):273–90.
Dempsey EM, Barrington KJ, Marlow N, O'Donnell CP, Miletin J, Naulaers G, Cheung PY, Corcoran D, Pons G, Stranak Z, Van Laere D. 2014. Management of Hypotension in Preterm Infants (The HIP Trial): A Randomised Controlled Trial of Hypotension Management in Extremely Low Gestational Age Newborns. *Neonatology* 105(4): 275–81.
Devictor D, Verlhac S, Pariente D, Huault G. 1988. Hemodynamic Effects of Dobutamine in Asphyxiated Newborn Infants. *Archives Françaises de Pédiatrie* 45(7):467–70.
Durrmeyer X, Marchand-Martin L, Porcher R, Gascoin G, Roze J-C, Storme L, Favrais G, Ancel P-Y, Cambonie G. 2017. Abstention or Intervention for Isolated Hypotension in the First 3 Days of Life in Extremely Preterm Infants: Association with Short-Term Outcomes in the EPIPAGE 2 Cohort Study. *Archives of Disease in Childhood – Fetal and Neonatal Edition* 102(6):490–96.
Egan JR, Clarke AJB, Williams S, Cole AD, Ayer J, Jacobe S, Chard RB, Winlaw DS. 2006. Levosimendan for Low Cardiac Output: A Pediatric Experience. *Journal of Intensive Care Medicine* 21(3):183–87.
El-Khuffash AF, Jain A, Dragulescu A, Mcnamara PJ, Mertens L. 2012. Acute Changes in Myocardial Systolic Function in Preterm Infants Undergoing Patent Ductus Arteriosus Ligation: A Tissue Doppler and Myocardial Deformation Study. *Journal of the American Society of Echocardiography* 25(10):1058–67.

El-Khuffash AF, Jain A, McNamara PJ. 2013. Ligation of the Patent Ductus Arteriosus in Preterm Infants: Understanding the Physiology. *Journal of Pediatrics* 162(6): 1100–1106.

El-Khuffash AF, Jain A, Weisz D, Mertens L, McNamara PJ. 2014. Assessment and Treatment of Post Patent Ductus Arteriosus Ligation Syndrome. *Journal of Pediatrics* 165(1):1–8.

Engle WA, American Academy of Pediatrics Committee on Fetus and Newborn HJ. 2004. *Age Terminology during the Perinatal Period.* Vol. 114. American Academy of Pediatrics.

Evans N. 2006. Which Inotrope for Which Baby? *Archives of Disease in Childhood. Fetal and Neonatal Edition* 91(3):F213–20.

Evans N. 2009. Support of the Preterm Circulation: Keynote Address to the Fifth Evidence vs Experience Conference, Chicago, June 2008. *Journal of Perinatology : Official Journal of the California Perinatal Association* 29 Suppl 2(S2):S50–7.

Evans N, Kluckow M. 1996. Early Determinants of Right and Left Ventricular Output in Ventilated Preterm Infants. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 74(2):F88–94.

Fagrell B, Intaglietta M. 1997. Microcirculation: Its Significance in Clinical and Molecular Medicine. *Journal of Internal Medicine* 241(5):349–62.

Farstad T, Bratlid D. 1994. Pulmonary Effects of Closure of Patent Ductus Arteriosus in Premature Infants with Severe Respiratory Distress Syndrome. *European Journal of Pediatrics* 153(12):903–5.

Felder RA, Pelayo JC, Calcagno PL, Eisner GM, Jose PA. 1983. Alpha-Adrenoceptors in the Developing Kidney. *Pediatric Research* 17(2):177–80.

Fernandez EF, Watterberg KL. 2009. Relative Adrenal Insufficiency in the Preterm and Term Infant. *Journal of Perinatology : Official Journal of the California Perinatal Association* 29 Suppl 2(S2):S44–9.

Filippi L, Poggi C, Serafini L, Fiorini P. 2008. Terlipressin as Rescue Treatment of Refractory Shock in a Neonate. *Acta Paediatrica (Oslo, Norway : 1992)* 97(4):500–502.

Friis-Hansen B. 1971. Body Composition during Growth. In Vivo Measurements and Biochemical Data Correlated to Differential Anatomical Growth. *Pediatrics* 47(1): 264+.

Gal P, Ransom JL, Weaver RL, Schall S, Wyble LE, Carlos RQ, Brown Y. 1991. Indomethacin Pharmacokinetics in Neonates: The Value of Volume of Distribution as a Marker of Permanent Patent Ductus Arteriosus Closure. *Therapeutic Drug Monitoring* 13(1):42–45.

Gentile R, Stevenson G, Dooley T, Franklin D, Kawabori I, Pearlman A. 1981. Pulsed Doppler Echocardiographic Determination of Time of Ductal Closure in Normal Newborn Infants. *The Journal of Pediatrics* 98(3):443–48.

Genzel-Boroviczény O, Christ F, Glas V. 2004. Blood Transfusion Increases Functional Capillary Density in the Skin of Anemic Preterm Infants. *Pediatric Research* 56(5): 751–55.

Genzel-Boroviczény O, Strötgen J, Harris AG, Messmer K, Christ F. 2002. Orthogonal Polarization Spectral Imaging (OPS): A Novel Method to Measure the Microcirculation in Term and Preterm Infants Transcutaneously. *Pediatric Research* 51(3):386–91.

Germovsek E, Barker CIS, Sharland M, Standing JF. 2017. Scaling Clearance in Paediatric Pharmacokinetics: All Models Are Wrong, Which Are Useful? *British Journal of Clinical Pharmacology* 83(4):777–90.

Germovsek E, Barker CIS, Sharland M, Standing JF. 2019. Pharmacokinetic–Pharmacodynamic Modeling in Pediatric Drug Development, and the Importance of Standardized Scaling of Clearance. *Clinical Pharmacokinetics* 58(1):39–52.

Gist KM, Mizuno T, Goldstein SL, Vinks A. 2015. Retrospective Evaluation of Milrinone Pharmacokinetics in Children with Kidney Injury. *Therapeutic Drug Monitoring* 37(6):792–96.

Goedhart PT, Khalilzada M, Bezemer R, Merza J, Ince C. 2007. Sidestream Dark Field (SDF) Imaging: A Novel Stroboscopic LED Ring-Based Imaging Modality for Clinical Assessment of the Microcirculation. *Optics Express* 15(23):15101.

Goldberg LI. 1972. Cardiovascular and Renal Actions of Dopamine: Potential Clinical Applications. *Pharmacological Reviews* 24(1):1–29.

Goldstein RF, Thompson RJ, Oehler JM, Brazy JE. 1995. Influence of Acidosis, Hypoxemia, and Hypotension on Neurodevelopmental Outcome in Very Low Birth Weight Infants. *Pediatrics* 95(2):238–43.

Greisen G. 2008. Autoregulation of Vital and Nonvital Organ Blood Flow in the Preterm and Term Neonate. Pp. 19–38 in *Hemodynamics and cardiology. Neonatal questions and controversies.*, edited by C. Kleinman and I. Seri. Philadelphia: Saunders Elsevier.

Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, Nadeau RG. 1999. Orthogonal Polarization Spectral Imaging: A New Method for Study of the Microcirculation. *Nature Medicine* 5(10):1209–12.

Gupta S, Donn SM. 2014. Neonatal Hypotension: Dopamine or Dobutamine? *Seminars in Fetal and Neonatal Medicine* 19(1):54–59.

Habib DM, Padbury JF, Anas NG, Perkin RM, Minegar C. 1992. Dobutamine Pharmacokinetics and Pharmacodynamics in Pediatric Intensive Care Patients. *Critical Care Medicine* 20(5):601–8.

Hagadorn JI, Brownell EA, Trzaski JM, Johnson KR, Lainwala S, Campbell BT, Herbst KW. 2016. Trends and Variation in Management and Outcomes of Very Low-Birth-Weight Infants with Patent Ductus Arteriosus. *Pediatric Research* 80(6):785–92.

Halkin H, Radomsky M, Millman P, Almog S, Blieden L, Boichis H. 1978. Steady State Serum Concentrations and Renal Clearance of Digoxin in Neonates, Infants and Children. *European Journal of Clinical Pharmacology* 13(2):113–17.

Hallik M, Ilmoja M-L, Starkopf J, Metsvaht T. 2015. Feasibility of Complex Monitoring of Haemodynamics in Premature Newborns During First Days of Life (Pilot Study). in *Poster presentation at 26th Annual Meeting of the European Society of Paediatric and Neonatal intensive Care (ESPNIC).* Vilnius.

Harter G, Darden J, McMenemy N, McElvy T, Hendrich A. 2016. Consent and Enrollment Process: Achieving High Enrollment Rates for Obstetric Research. *Applied Nursing Research* 29:101–6.

Harting MT, Blakely ML, Cox CS, Lantin-Hermoso R, Andrassy RJ, Lally KP. 2008. Acute Hemodynamic Decompensation Following Patent Ductus Arteriosus Ligation in Premature Infants. *Journal of Investigative Surgery : The Official Journal of the Academy of Surgical Research* 21(3):133–38.

Hiedl S, Schwepcke A, Weber F, Genzel-Boroviczeny O. 2010. Microcirculation in Preterm Infants: Profound Effects of Patent Ductus Arteriosus. *Journal of Pediatrics* 156(2):191–96.

Hines RN. 2007. Ontogeny of Human Hepatic Cytochromes P450. *Journal of Biochemical and Molecular Toxicology* 21(4):169–75.

Hoffman TM, Wemovsky G, Atz AM, Kulik TJ, Nelson DP, Chang AC, Bailey JM, Akbary A, Kocsis JF, Kaczmarek R, Spray TL, Wessel DL. 2003. Efficacy and Safety

of Milrinone in Preventing Low Cardiac Output Syndrome in Infants and Children after Corrective Surgery for Congenital Heart Disease. *Circulation* 107(7):996–1002.
Holford NHG. 1996. A Size Standard for Pharmacokinetics. *Clinical Pharmacokinetics* 30(5):329–32.
Honerjäger P, Nawrath H. 1992. Pharmacology of Bipyridine Phosphodiesterase III Inhibitors. *European Journal of Anaesthesiology. Supplement* 5:7–14.
Hornik CP, Yogev R, Mourani PM, Watt KM, Sullivan JE, Atz AM, Speicher D, Al-Uzri A, Adu-Darko M, Payne EH, Gelber CE, Lin S, Harper B, Melloni C, Cohen-Wolkowiez M, Gonzalez D, Best Pharmaceuticals for Children Act-Pediatric Trials Network Steering Committee. 2019. Population Pharmacokinetics of Milrinone in Infants, Children, and Adolescents. *The Journal of Clinical Pharmacology* 59(12): 1606–19.
Hsu K-H, Wu T-W, Wu I-H, Lai M-Y, Hsu S-Y, Huang H-W, Mok T-Y, Lien R. 2017. Electrical Cardiometry to Monitor Cardiac Output in Preterm Infants with Patent Ductus Arteriosus: A Comparison with Echocardiography. *Neonatology* 112(3):231–37.
Hunt RW, Evans N, Rieger I, Kluckow M. 2004. Low Superior Vena Cava Flow and Neurodevelopment at 3 Years in Very Preterm Infants. *Journal of Pediatrics* 145(5):588–92.
Ikegami H, Funato M, Tamai H, Wada H, Nabetani M, Nishihara M. 2009. Low-Dose Vasopressin Infusion Therapy for Refractory Hypotension in ELBW Infants. *Pediatrics International* 52(3):368–73.
Jadhav PR, Kern SE. 2010. The Need for Modeling and Simulation to Design Clinical Investigations in Children. *Journal of Clinical Pharmacology* 50(9 Suppl):121S–129S.
Jain A, Sahni M, El-Khuffash A, Khadawardi E, Sehgal A, McNamara PJ. 2012. Use of Targeted Neonatal Echocardiography to Prevent Postoperative Cardiorespiratory Instability after Patent Ductus Arteriosus Ligation. *Journal of Pediatrics* 160(4):584–589.e1.
Jöbsis FF. 1977. Noninvasive, Infrared Monitoring of Cerebral and Myocardial Oxygen Sufficiency and Circulatory Parameters. *Science* 198(4323):1264–67.
Jonsson EN, Karlsson MO. 1999. Xpose--an S-PLUS Based Population Pharmacokinetic/ Pharmacodynamic Model Building Aid for NONMEM. *Computer Methods and Programs in Biomedicine* 58(1):51–64.
Kates RE, Leier C V. 1978. Dobutamine Pharmacokinetics in Severe Heart Failure. *Clinical Pharmacology & Therapeutics* 24(5):537–41.
Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. 2003. Developmental Pharmacology – Drug Disposition, Action, and Therapy in Infants and Children edited by A. J. J. Wood. *New England Journal of Medicine* 349(12):1157–67.
Keeley SR, Bohn DJ. 1988. The Use of Inotropic and Afterload-Reducing Agents in Neonates. *Clinics in Perinatology* 15(3):467–89.
Kikura M, Levy JH, Michelsen LG, Shanewise JS, Bailey JM, Sadel SM, Szlam F. 1997. The Effect of Milrinone on Hemodynamics and Left Ventricular Function after Emergence from Cardiopulmonary Bypass. *Anesthesia and Analgesia* 85(1):16–22.
Kimland E, Odlind V. 2012. Off-Label Drug Use in Pediatric Patients. *Clinical Pharmacology & Therapeutics* 91(5):796–801.

Klarr JM, Faix RG, Pryce CJE, Bhatt-Mehta V. 1994. Randomized, Blind Trial of Dopamine versus Dobutamine for Treatment of Hypotension in Preterm Infants with Respiratory Distress Syndrome. *Journal of Pediatrics* 125(1):117–22.

Kluckow M. 2000. Superior Vena Cava Flow in Newborn Infants: A Novel Marker of Systemic Blood Flow. *Archives of Disease in Childhood – Fetal and Neonatal Edition* 82(3):182F – 187.

Kluckow M, Evans N. 2000. Low Superior Vena Cava Flow and Intraventricular Haemorrhage in Preterm Infants. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 82(3):F188–94.

Kluckow M, Seri I, Evans N. 2007. Functional Echocardiography: An Emerging Clinical Tool for the Neonatologist. *The Journal of Pediatrics* 150(2):125–30.

Kluckow M, Seri Istvan. 2008. Clinical Presentations of Neonatal Shock: The VLBW Infant during the First Postnatal Day. Pp. 147–177 in *Hemodynamics and cardiology. Neonatal questions and controversies.*, edited by C. Kleinman and I Seri. Philadelphia: Saunders Elsevier.

Knibbe CAJ, Krekels EHJ, van den Anker JN, DeJongh J, Santen GWE, van Dijk M, Simons SHP, van Lingen RA, Jacqz-Aigrain EM, Danhof M, Tibboel D. 2009. Morphine Glucuronidation in Preterm Neonates, Infants and Children Younger than 3 Years. *Clinical Pharmacokinetics* 48(6):371–85.

Koch J, Hensley G, Roy L, Brown S, Ramaciotti C, Rosenfeld CR. 2006. Prevalence of Spontaneous Closure of the Ductus Arteriosus in Neonates at a Birth Weight of 1000 Grams or Less. *Pediatrics* 117(4):1113–21.

Lass J, Käär R, Jõgi K, Varendi H, Metsvaht T, Lutsar I. 2011. Drug Utilisation Pattern and Off-Label Use of Medicines in Estonian Neonatal Units. *European Journal of Clinical Pharmacology* 67(12):1263–71.

Laughon M, Bose C, Allred E, O'Shea TM, Marter LJ V., Bednarek F, Leviton A. 2007. Factors Associated With Treatment for Hypotension in Extremely Low Gestational Age Newborns During the First Postnatal Week. *Pediatrics* 119(2):273–80.

Lechner E, Hofer A, Leitner-Peneder G, Freynschlag R, Mair R, Weinzettel R, Rehak P, Gombotz H. 2012. Levosimendan versus Milrinone in Neonates and Infants after Corrective Open-Heart Surgery: A Pilot Study. *Pediatric Critical Care Medicine : A Journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies* 13(5):542–48.

Leeder JS, Kearns GL, Spielberg SP, van den Anker J. 2010. Understanding the Relative Roles of Pharmacogenetics and Ontogeny in Pediatric Drug Development and Regulatory Science. *Journal of Clinical Pharmacology* 50(12):1377–87.

Leone M, Martin C. 2008. Role of Terlipressin in the Treatment of Infants and Neonates with Catecholamine-Resistant Septic Shock. *Best Practice & Research. Clinical Anaesthesiology* 22(2):323–33.

Lien R, Hsu K-H, Chu J-J, Chang Y-S. 2015. Hemodynamic Alterations Recorded by Electrical Cardiometry during Ligation of Ductus Arteriosus in Preterm Infants. *European Journal of Pediatrics* 174(4):543–50.

Linakis MW, Cook SF, Kumar SS, Liu X, Wilkins DG, Gaedigk R, Gaedigk A, Sherwin CMT, van den Anker JN. 2018. Polymorphic Expression of UGT1A9 Is Associated with Variable Acetaminophen Glucuronidation in Neonates: A Population Pharmacokinetic and Pharmacogenetic Study. *Clinical Pharmacokinetics* 57(10):1325–36.

Lindbom L, Pihlgren P, Jonsson N, Jonsson N. 2005. PsN-Toolkit – A Collection of Computer Intensive Statistical Methods for Non-Linear Mixed Effect Modeling Using NONMEM. *Computer Methods and Programs in Biomedicine* 79(3):241–57.

Lindsay CA, Barton P, Lawless S, Kitchen L, Zorka A, Garcia J, Kouatli A, Giroir B. 1998. Pharmacokinetics and Pharmacodynamics of Milrinone Lactate in Pediatric Patients with Septic Shock. *The Journal of Pediatrics* 132(2):329–34.

Lokku A, Mirea L, Lee S, Shah P. 2016. Trends and Outcomes of Patent Ductus Arteriosus Treatment in Very Preterm Infants in Canada. *American Journal of Perinatology* 34(05):441–50.

Lopez SL, Leighton JO, Walther FJ. 1997. Supranormal Cardiac Output in the Dopamine- and Dobutamine-Dependent Preterm Infant. *Pediatric Cardiology* 18(4):292–96.

Mahoney L, Fernandez-Alvarez JR, Rojas-Anaya H, Aiton N, Wertheim D, Seddon P, Rabe H. 2018. Intra- and Inter-Rater Agreement of Superior Vena Cava Flow and Right Ventricular Outflow Measurements in Late Preterm and Term Neonates. *Journal of Ultrasound in Medicine* 37(9):2181–90.

Mahoney L, Shah G, Crook D, Rojas-Anaya H, Rabe H. 2016. A Literature Review of the Pharmacokinetics and Pharmacodynamics of Dobutamine in Neonates. *Pediatric Cardiology* 37(1):14–23.

Martens SE, Rijken M, Stoelhorst GMSJ, van Zwieten PHT, Zwinderman AH, Wit JM, Hadders-Algra M, Veen S. 2003. Is Hypotension a Major Risk Factor for Neurological Morbidity at Term Age in Very Preterm Infants? *Early Human Development* 75(1–2):79–89.

Martinez AM, Padbury JF, Thio S. 1992. Dobutamine Pharmacokinetics and Cardiovascular Responses in Critically Ill Neonates. *Pediatrics* 89(1):47–51.

Mc Namara PJ, Shivananda SP, Sahni M, Freeman D, Taddio A. 2013. Pharmacology of Milrinone in Neonates with Persistent Pulmonary Hypertension of the Newborn and Suboptimal Response to Inhaled Nitric Oxide*. *Pediatric Critical Care Medicine* 14(1):74–84.

McCarthy KN, Ryan NC, O'Shea DT, Doran K, Greene R, Livingstone V, Ryan CA, Boylan GB, Dempsey EM. 2019. Parental Opinion of Consent in Neonatal Research. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 104(4):F409–14.

McNamara PJ, Laique F, Muang-In S, Whyte HE. 2006. Milrinone Improves Oxygenation in Neonates with Severe Persistent Pulmonary Hypertension of the Newborn. *Journal of Critical Care* 21(2):217–22.

McNamara PJ, Stewart L, Shivananda SP, Stephens D, Sehgal A. 2010. Patent Ductus Arteriosus Ligation Is Associated with Impaired Left Ventricular Systolic Performance in Premature Infants Weighing Less than 1000 G. *The Journal of Thoracic and Cardiovascular Surgery* 140(1):150–57.

Megone C, Wilman E, Oliver S, Duley L, Gyte G, Wright J. 2016. The Ethical Issues Regarding Consent to Clinical Trials with Pre-Term or Sick Neonates: A Systematic Review (Framework Synthesis) of the Analytical (Theoretical/Philosophical) Research. *Trials* 17(1):443.

Mertens L, Seri I, Marek J, Arlettaz R, Barker P, McNamara P, Moon-Grady AJ, Coon PD, Noori S, Simpson J, Lai WW. 2011. Targeted Neonatal Echocardiography in the Neonatal Intensive Care Unit: Practice Guidelines and Recommendations for Training. *European Journal of Echocardiography* 12(10):715–36.

Mizuno T, Gist KM, Gao Z, Wempe MF, Alten J, Cooper DS, Goldstein SL, Vinks AA. 2019. Developmental Pharmacokinetics and Age-Appropriate Dosing Design of Milrinone in Neonates and Infants with Acute Kidney Injury Following Cardiac Surgery. *Clinical Pharmacokinetics* 58(6):793–803.

Moin F, Kennedy KA, Moya FR. 2003. Risk Factors Predicting Vasopressor Use after Patent Ductus Arteriosus Ligation. *American Journal of Perinatology* 20(6):313–20.

Naulaers G, Meyns B, Miserez M, Leunens V, Van Huffel S, Casaer P, Weindling M, Devlieger H. 2007. Use of Tissue Oxygenation Index and Fractional Tissue Oxygen Extraction as Non-Invasive Parameters for Cerebral Oxygenation. *Neonatology* 92(2):120–26.

Nemerofsky SL, Parravicini E, Bateman D, Kleinman C, Polin RA, Lorenz JM. 2008. The Ductus Arteriosus Rarely Requires Treatment in Infants > 1000 Grams. *American Journal of Perinatology* 25(10):661–66.

Nir-Neuman H, Abu-Kishk I, Toledano M, Heyman E, Ziv-Baran T, Berkovitch M. 2018. Unlicensed and Off-Label Medication Use in Pediatric and Neonatal Intensive Care Units: No Change Over a Decade. *Advances in Therapy* 35(7):1122–32.

Noori S, Friedlich P, Seri I, Wong P. 2007. Changes in Myocardial Function and Hemodynamics after Ligation of the Ductus Arteriosus in Preterm Infants. *Journal of Pediatrics* 150(6):597–602.

Noori S, McNamara P, Jain A, Lavoie PM, Wickremasinghe A, Merritt TA, Solomon T, Sekar K, Attridge JT, Swanson JR, Gillam-Krakauer M, Reese J, Poindexter BB, Brook M, Auchus RJ, Clyman RI, PDA Ligation/Hypotension Trial Investigators PLT. 2015. Catecholamine-Resistant Hypotension and Myocardial Performance Following Patent Ductus Arteriosus Ligation. *Journal of Perinatology : Official Journal of the California Perinatal Association* 35(2):123–27.

Noori S, Seri I. 2012. Neonatal Blood Pressure Support: The Use of Inotropes, Lusitropes, and Other Vasopressor Agents. *Clinics in Perinatology* 39(1):221–38.

Noori S, Seri I. 2014. Does Targeted Neonatal Echocardiography Affect Hemodynamics and Cerebral Oxygenation in Extremely Preterm Infants? *Journal of Perinatology* 34(11):847–49.

Notterman DA. 1988. Pharmacology of the Cardiovascular System. Pp. 329–46 in *Pediatric Critical Care*, edited by T. Fuhrman, BP; Zimmerman. Mosby.

O'Leary H, Gregas MC, Limperopoulos C, Zaretskaya I, Bassan H, Soul JS, Di Salvo DN, du Plessis AJ. 2009. Elevated Cerebral Pressure Passivity Is Associated with Prematurity-Related Intracranial Hemorrhage. *Pediatrics* 124(1):302–9.

Osborn D, Evans N, Kluckow M. 2002. Randomized Trial of Dobutamine versus Dopamine in Preterm Infants with Low Systemic Blood Flow. *Journal of Pediatrics* 140(2):183–91.

Osborn DA, Evans N, Kluckow M. 2003. Hemodynamic and Antecedent Risk Factors of Early and Late Periventricular/Intraventricular Hemorrhage in Premature Infants. *Pediatrics* 112(1 Pt 1):33–39.

Osborn DA, Evans N, Kluckow M, Bowen JR, Rieger I. 2007. Low Superior Vena Cava Flow and Effect of Inotropes on Neurodevelopment to 3 Years in Preterm Infants. *Pediatrics* 120(2):372–80.

Osypka M, Soleymani S, Seri I, Noori S. 2012. Assessment of Cardiac Output in Neonates: Techniques Using the Fick Principle, Pulse Wave Form Analysis, and Electrical Impedance. in *Hemodynamics and cardiology: neonatology questions and controversies*, edited by C. Kleinman and I. Seri. Philadelphia: Saunders.

Oualha M, Tréluyer JM, Lesage F, de Saint Blanquat L, Dupic L, Hubert P, Spreux-Varoquaux O, Urien S. 2014. Population Pharmacokinetics and Haemodynamic Effects of Norepinephrine in Hypotensive Critically Ill Children. *British Journal of Clinical Pharmacology* 78(4):886–97.

Oualha M, Urien S, Spreux-Varoquaux O, Bordessoule A, D'Agostino I, Pouard P, Tréluyer JM. 2014. Pharmacokinetics, Hemodynamic and Metabolic Effects of

Epinephrine to Prevent Post-Operative Low Cardiac Output Syndrome in Children. *Critical Care* 18(1):1–14.
Van Overmeire B, Touw D, Schepens PJ, Kearns GL, van den Anker JN. 2001. Ibuprofen Pharmacokinetics in Preterm Infants with Patent Ductus Arteriosus. *Clinical Pharmacology and Therapeutics* 70(4):336–43.
Pacifici GM. 2009. Clinical Pharmacokinetics of Aminoglycosides in the Neonate: A Review. *European Journal of Clinical Pharmacology* 65(4):419–27.
Paradisis M, Evans N, Kluckow M, Osborn D. 2009. Randomized Trial of Milrinone Versus Placebo for Prevention of Low Systemic Blood Flow in Very Preterm Infants. *Journal of Pediatrics* 154(2):189–95.
Paradisis M, Jiang X, McLachlan AJ, Evans N, Kluckow M, Osborn D. 2007. Population Pharmacokinetics and Dosing Regimen Design of Milrinone in Preterm Infants. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 92(3):F204–9.
Pellicer A, del Carmen Bravo M, Madero R, Salas S, Quero J, Cabanas F. 2009. Early Systemic Hypotension and Vasopressor Support in Low Birth Weight Infants: Impact on Neurodevelopment. *Pediatrics* 123(5):1369–76.
Pellicer A, Riera J, Lopez-Ortego P, Bravo MC, Madero R, Perez-Rodriguez J, Labrandero C, Quero J, Buño A, Castro L, Lubomirov R, Cabañas F. 2013. Phase 1 Study of Two Inodilators in Neonates Undergoing Cardiovascular Surgery. *Pediatric Research* 73(1):95–103.
Pellicer A, Valverde E, Elorza MD, Madero R, Gayá F, Quero J, Cabañas F. 2005. Cardiovascular Support for Low Birth Weight Infants and Cerebral Hemodynamics: A Randomized, Blinded, Clinical Trial. *Pediatrics* 115(6):1501–12.
du Plessis AJ. 2009. The Role of Systemic Hemodynamic Disturbances in Prematurity-Related Brain Injury. *Journal of Child Neurology* 24(9):1127–40.
Popović ZB, Thomas JD. 2017. Assessing Observer Variability: A User's Guide. *Cardiovascular Diagnosis and Therapy* 7(3):317–24.
R Core Team. 2014a. R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing. Retrieved March 24, 2017 (http://www.r-project.org/).
R Core Team. 2014b. R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing. Retrieved March 28, 2014 (http://www.r-project.org/).
R Core Team. 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved May 28, 2019 (https://www.r-project.org/).
Ramamoorthy C, Anderson GD, Williams GD, Lynn AM. 1998. Pharmacokinetics and Side Effects of Milrinone in Infants and Children after Open Heart Surgery. *Anesthesia and Analgesia* 86(2):283–89.
Rettig GF, Schieffer HJ. 1989. Acute Effects of Intravenous Milrinone in Heart Failure. *European Heart Journal* 10 Suppl C:39–43.
Rhodin MM, Anderson BJ, Peters AM, Coulthard MG, Wilkins B, Cole M, Chatelut E, Grubb A, Veal GJ, Keir MJ, Holford NHG. 2009. Human Renal Function Maturation: A Quantitative Description Using Weight and Postmenstrual Age. *Pediatric Nephrology* 24(1):67–76.
Ricci Z, Garisto C, Favia I, Vitale V, Di Chiara L, Cogo PE. 2012. Levosimendan Infusion in Newborns after Corrective Surgery for Congenital Heart Disease: Randomized Controlled Trial. *Intensive Care Medicine* 38(7):1198–1204.

Rich WD, Katheria AC. 2019. Waived Consent in Perinatal/Neonatal Research-When Is It Appropriate? *Frontiers in Pediatrics* 7:493.
Rios DR, Kaiser JR. 2015. Vasopressin versus Dopamine for Treatment of Hypotension in Extremely Low Birth Weight Infants: A Randomized, Blinded Pilot Study. *The Journal of Pediatrics* 166(4):850–55.
Rizk M, Lapointe A, Lefebvre F, Barrington K. 2018. Norepinephrine Infusion Improves Haemodynamics in the Preterm Infants during Septic Shock. *Acta Paediatrica* 107(3):408–13.
Robel-Tillig E, Knüpfer M, Pulzer F, Vogtmann C. 2007. Cardiovascular Impact of Dobutamine in Neonates with Myocardial Dysfunction. *Early Human Development* 83(5):307–12.
Roberts JA, Pea F, Lipman J. 2013. The Clinical Relevance of Plasma Protein Binding Changes. *Clinical Pharmacokinetics* 52(1):1–8.
Rowcliff K, de Waal K, Mohamed A-L, Chaudhari T. 2016. Noradrenaline in Preterm Infants with Cardiovascular Compromise. *European Journal of Pediatrics* 175(12): 1967–73.
Roze JC, Tohier C, Maingueneau C, Lefevre M, Mouzard A. 1993. Response to Dobutamine and Dopamine in the Hypotensive Very Preterm Infant. *Archives of Disease in Childhood* 69(1 Spec No):59–63.
Ruelas-Orozco G, Vargas-Origel A. 2000. Assessment of Therapy for Arterial Hypotension in Critically Ill Preterm Infants. *American Journal of Perinatology* 17(2):95–99.
Ruffolo RR, Kopia GA. 1986. Importance of Receptor Regulation in the Pathophysiology and Therapy of Congestive Heart Failure. *The American Journal of Medicine* 80(2B): 67–72.
Schwartz PH, Eldadah MK, Newth CJL. 1991. The Pharmacokinetics of Dobutamine in Pediatric Intensive Care Unit Patients. *Drug Metabolism and Disposition* 19(3):614–19.
Sehgal A, Osborn D, McNamara PJ. 2012. Cardiovascular Support in Preterm Infants: A Survey of Practices in Australia and New Zealand. *Journal of Paediatrics and Child Health* 48(4):317–23.
Seri I. 1995. Cardiovascular, Renal, and Endocrine Actions of Dopamine in Neonates and Children. *The Journal of Pediatrics* 126(3):333–44.
Seri I. 2001. Circulatory Support of the Sick Preterm Infant. *Seminars in Neonatology* 6(1):85–95.
Seri I. 2006. Management of Hypotension and Low Systemic Blood Flow in the Very Low Birth Weight Neonate during the First Postnatal Week. *Journal of Perinatology* 26 Suppl 1(S1):S8–13; discussion S22–3.
Seri I, Rudas G, Bors Z, Kanyicska B, Tulassay T. 1993. Effects of Low-Dose Dopamine Infusion on Cardiovascular and Renal Functions, Cerebral Blood Flow, and Plasma Catecholamine Levels in Sick Preterm Neonates. *Pediatric Research* 34(6):742–49.
Seri I, Tan R, Evans J, Elorza D, Quero J, Cabañas F. 2001. Cardiovascular Effects of Hydrocortisone in Preterm Infants With Pressor-Resistant Hypotension. *Pediatrics* 107(5):1070–74.
Seri I, Tulassay T, Kiszel J, Machay T, Csömör S. 1984. Cardiovascular Response to Dopamine in Hypotensive Preterm Neonates with Severe Hyaline Membrane Disease. *European Journal of Pediatrics* 142(1):3–9.
Sherman H, Klausner S, Cook WA. 1971. Incident Dark-Field Illumination: A New Method for Microcirculatory Study. *Angiology* 22(5):295–303.

Slaaf DW, Tangelder GJ, Reneman RS, Jäger K, Bollinger A. 1987. A Versatile Incident Illuminator for Intravital Microscopy. *International Journal of Microcirculation, Clinical and Experimental* 6(4):391–97.

Smits A, Kulo A, Verbesselt R, Naulaers G, Hoon J, Vermeersch P, Allegaert K. 2012. Cefazolin Plasma Protein Binding and Its Covariates in Neonates. *European Journal of Clinical Microbiology & Infectious Diseases* 31(12):3359–65.

Smits A, Thewissen L, Dereymaeker A, Dempsey E, Caicedo A, Naulaers G. 2017. The Use of Hemodynamic and Cerebral Monitoring to Study Pharmacodynamics in Neonates. *Current Pharmaceutical Design* 23(38):5955–63.

Soleymani S, Borzage M, Seri I. 2010. Hemodynamic Monitoring in Neonates: Advances and Challenges. *Journal of Perinatology* 30 Suppl(S1):S38–45.

Stefano JL, Abbasi S, Pearlman SA, Spear ML, Esterly KL, Bhutani VK. 1991. Closure of the Ductus Arteriosus with Indomethacin in Ventilated Neonates with Respiratory Distress Syndrome. Effects of Pulmonary Compliance and Ventilation. *The American Review of Respiratory Disease* 143(2):236–39.

Steinberg C, Notterman DA. 1994. Pharmacokinetics of Cardiovascular Drugs in Children. Inotropes and Vasopressors. *Clinical Pharmacokinetics* 27(5):345–67.

Stopfkuchen H, Queisser-Luft A, Vogel K. 1990. Cadiovascular Responses to Dobutamine Determined by Systolic Time Intervals in Preterm Infants. *Critical Care Medicine* 18(7):722–24.

Stopfkuchen H, Schranz D, Huth R, Jüngst BK. 1987. Effects of Dobutamine on Left Ventricular Performance in Newborns as Determined by Systolic Time Intervals. *European Journal of Pediatrics* 146(2):135–39.

Stranak Z, Semberova J, Barrington K, O'Donnell C, Marlow N, Naulaers G, Dempsey E, HIP consortium. 2014. International Survey on Diagnosis and Management of Hypotension in Extremely Preterm Babies. *European Journal of Pediatrics* 173(6):793–98.

Stroshane RM, Koss RF, Biddlecome CE, Luczkowec C, Edelson J. 1984. Oral and Intravenous Pharmacokinetics of Milrinone in Human Volunteers. *Journal of Pharmaceutical Sciences* 73(10):1438–41.

Su F, Nicolson SC, Gastonguay MR, Barrett JS, Adamson PC, Kang DS, Godinez RI, Zuppa AF. 2010. Population Pharmacokinetics of Dexmedetomidine in Infants after Open Heart Surgery. *Anesthesia and Analgesia* 110(5):1383–92.

Szymankiewicz M, Hodgman JE, Siassi B, Gadzinowski J. 2004. Mechanics of Breathing after Surgical Ligation of Patent Ductus Arteriosus in Newborns with Respiratory Distress Syndrome. *Biology of the Neonate* 85(1):32–36.

Takkis K, Veigure R, Metsvaht T, Hallik M, Ilmoja M-L, Starkopf J, Kipper K. 2019. A Sensitive Method for the Simultaneous UHPLC-MS/MS Analysis of Milrinone and Dobutamine in Blood Plasma Using NH4F as the Eluent Additive and Ascorbic Acid as a Stabilizer. *Clinical Mass Spectrometry* 12:23–29.

Teixeira LS, Shivananda SP, Stephens D, Van Arsdell G, McNamara PJ. 2008. Postoperative Cardiorespiratory Instability Following Ligation of the Preterm Ductus Arteriosus Is Related to Early Need for Intervention. *Journal of Perinatology* 28(12):803–10.

Thalji AA, Carr I, Yeh TF, Raval D, Luken JA, Pildes RS. 1980. Pharmacokinetics of Intravenously Administered Indomethacin in Premature Infants. *The Journal of Pediatrics* 97(6):995–1000.

Tisdall MM, Taylor C, Tachtsidis I, Leung TS, Elwell CE, Smith M. 2009. The Effect on Cerebral Tissue Oxygenation Index of Changes in the Concentrations of Inspired

Oxygen and End-Tidal Carbon Dioxide in Healthy Adult Volunteers. *Anesthesia and Analgesia* 109(3):906–13.
Tooher RL, Middleton PF, Crowther CA. 2008. A Thematic Analysis of Factors Influencing Recruitment to Maternal and Perinatal Trials. *BMC Pregnancy and Childbirth* 8:36.
Toth-Heyn P, Cataldi L. 2012. Vasoactive Compounds in the Neonatal Period. *Current Medicinal Chemistry* 19(27):4633–39.
Tourneux P, Rakza T, Abazine A, Krim G, Storme L. 2008. Noradrenaline for Management of Septic Shock Refractory to Fluid Loading and Dopamine or Dobutamine in Full-Term Newborn Infants. *Acta Paediatrica* 97(2):177–80.
Tuttle RR, Mills J. 1975. Dobutamine: Development of a New Catecholamine to Selectively Increase Cardiac Contractility. *Circulation Research* 36(1):185–96.
Udvary E, Papp JG, Végh A. 1995. Cardiovascular Effects of the Calcium Sensitizer, Levosimendan, in Heart Failure Induced by Rapid Pacing in the Presence of Aortic Constriction. *British Journal of Pharmacology* 114(3):656–61.
Ulrich TJB, Hansen TP, Reid KJ, Bingler MA, Olsen SL. 2018. Post-Ligation Cardiac Syndrome Is Associated with Increased Morbidity in Preterm Infants. *Journal of Perinatology* 38(5):537–42.
Valverde E, Pellicer A, Madero R, Elorza D, Quero J, Cabañas F. 2006. Dopamine versus Epinephrine for Cardiovascular Support in Low Birth Weight Infants: Analysis of Systemic Effects and Neonatal Clinical Outcomes. *Pediatrics* 117(6):e1213–22.
Vapaavouri EK, Shinebourne EA, Williams RL, Heymann MA, Rudolph AM. 1973. Development of Cardiovascular Responses to Autonomic Blockade in Intact Fetal and Neonatal Lambs. *Biology of the Neonate* 22(3):177–88.
Vogt W. 2014. Evaluation and Optimisation of Current Milrinone Prescribing for the Treatment and Prevention of Low Cardiac Output Syndrome in Paediatric Patients after Open Heart Surgery Using a Physiology-Based Pharmacokinetic Drug-Disease Model. *Clinical Pharmacokinetics* 53(1):51–72.
Volpe JJ. 2001. Perinatal Brain Injury: From Pathogenesis to Neuroprotection. *Mental Retardation and Developmental Disabilities Research Reviews* 7(1):56–64.
Watterberg KL, Kelly HW, Johnson JD, Aldrich M, Angelus P. 1987. Effect of Patent Ductus Arteriosus on Gentamicin Pharmacokinetics in Very Low Birth Weight (<1,500 g) Babies. *Developmental Pharmacology and Therapeutics* 10(2):107–17.
Weidlich K, Kroth J, Nussbaum C, Hiedl S, Bauer A, Christ F, Genzel-Boroviczeny O. 2009. Changes in Microcirculation as Early Markers for Infection in Preterm Infants-an Observational Prospective Study. *Pediatric Research* 66(4):461–65.
Weil MH, Tang W. 2007. Welcoming a New Era of Hemodynamic Monitoring: Expanding from the Macro to the Microcirculation. *Critical Care Medicine* 35(4):1204–5.
Weinberg JG, Evans FJ, Burns KM, Pearson GD, Kaltman JR. 2016. Surgical Ligation of Patent Ductus Arteriosus in Premature Infants: Trends and Practice Variation. *Cardiology in the Young* 26(6):1107–14.
Weisz DE, Jain A, Ting J, McNamara PJ, EL-Khuffash A. 2014. Non-Invasive Cardiac Output Monitoring in Preterm Infants Undergoing Patent Ductus Arteriosus Ligation: A Comparison with Echocardiography. *Neonatology* 106(4):330–36.
Williams BS, Ransom JL, Gal P, Carlos RQ, Smith M, Schall SA. 1997. Gentamicin Pharmacokinetics in Neonates with Patent Ductus Arteriosus. *Critical Care Medicine* 25(2):273–75.

Wong J, Shah PS, Yoon EW, Yee W, Lee S, Dow K. 2015. Inotrope Use among Extremely Preterm Infants in Canadian Neonatal Intensive Care Units: Variation and Outcomes. *American Journal of Perinatology* 32(1):9–14.

Yan M, Webster LT, Blumer JL. 2002. 3-O-Methyldobutamine, a Major Metabolite of Dobutamine in Humans. *Drug Metabolism and Disposition* 30(5):519–24.

Yano M, Kohno M, Ohkusa T, Mochizuki M, Yamada J, Kohno M, Hisaoka T, Ono K, Tanigawa T, Kobayashi S, Matsuzaki M. 2000. Effect of Milrinone on Left Ventricular Relaxation and Ca(2+) Uptake Function of Cardiac Sarcoplasmic Reticulum. *American Journal of Physiology. Heart and Circulatory Physiology* 279(4):H1898–905.

Zaritsky A, Chernow B. 1984. Use of Catecholamines in Pediatrics. *The Journal of Pediatrics* 105(3):341–50.

Zuppa AF, Nicolson SC, Adamson PC, Wernovsky G, Mondick JT, Burnham N, Hoffman TM, Gaynor JW, Davis LA, Greeley WJ, Spray TL, Barrett JS. 2006. Population Pharmacokinetics of Milrinone in Neonates with Hypoplastic Left Heart Syndrome Undergoing Stage I Reconstruction. *Anesthesia and Analgesia* 102(4):1062–69.

9. SUMMARY IN ESTONIAN

Inotroopsete ravimite farmakokineetika ja farmakodünaamika vastsündinutel

Vastsündinueaks loetakse inimese esimesed 28 elupäeva. See lühike ajaperiood hõlmab väga olulisi arengulisi muutusi. Kuna vastsündinute raske haigestumise peamiseks riskifaktoriks on enneaegne sünd, tuleb neonatoloogidel tegeleda patsientidega, kes on sündinud raseduse erinevatel etappidel, kellel esineb lisaks ebaküpsusele ka arenguhäireid ning kelle kehakaal varieerub rohkem kui kümnekordselt. Lisaks kiiretele loomuliku küpsemise protsessidele kujundavad vastsündinute populatsiooni heterogeensust veel kriitiline haigusseisund ning intensiivne ravitegevus.

Kriitlises seisundis vastsündinutel kasutatakse palju erinevaid ravimeid. Enamike ravimite kasutamist sellel populatsioonil ei ole piisavalt uuritud. Nii on näidatud, et kuni 65% vastsündinute intensiivravi osakondades kasutatavatest ravimitest on nn. *off-label* ehk mitte litsentseeritud kasutamiseks sel vanusegrupil. (Nir-Neuman et al. 2018) Seejuures on just enneaegsed vastsündinud enim eksponeeritud ravimitele, mille näidustused, toimed ja kõrvaltoimed ei ole piisavalt uuritud. (Kimland et al. 2012) Seetõttu on ravimite kasutuspraktika vastsündinutel väga varieeruv ja osakonniti erinev.

See kehtib ka südame- ja veresoonkonna ravimite kohta. Tõenduspõhisus, millist ravimit millises olukorras vastsündinul kasutada, millises doosis ravi alustada, kuidas doosi tiitrida ning millised parameetreid ravi efektiivsuse hindamiseks monitoorida, on äärmiselt tagasihoidlik. (Barrington 2008; Evans 2006; Kluckow et al. 2008) Manustamisel orienteerutakse enamasti laste ja täiskasvanute meditsiinis kasutusel olevate kliiniliste sümptomite järgi: madal vererõhk, südamefunktsiooni langus, madal südame minutimaht, halb perifeerne verevarustus, vähenenud uriinieritus, laktatsidoos – kõik vereringe puudulikkuse tunnused. Vastsündinute südame-veresoonkonna füsioloogia ja patofüsioloogia on aga eripärane, mistõttu ei saa täiskasvanutel või lastel läbiviidud uuringuid ekstrapoleerida sellele populatsioonile.

Matemaatiline modelleerimine on paljulubav metoodika vastsündinute farmakokineetika ja farmakodünaamika alasteks ravimuuringuteks. Populatsioonipõhine lähenemine sobib hästi andmete vähesuse ja hõredusega, mis tuleneb vastsündinute haavatavusest nii vereanalüüside hulga kui ka paljude invasiivsete monitooringumeetodite suhtes. Matemaatiline modelleerimine annab võimaluse kirjeldada arenguliste ja mittearenguliste kovariaatide rolli patsientidevaheliste erinevuste kujunemises ning selle põhjal võimaldab jõuda täpsemate patsiendipõhiste doseerimisskeemideni. Kui me ei tea ravimite farmakokineetika ja farmakodünaamika vahelisi seoseid, nende varieeruvust indiviidide vahel või ühe indiviidi piires ja seoste muutust tulenevalt kiirest arengust vastsündinueas, võib juhtuda, et efektiivsusuuringutes kasutatakse valesid doose ning potentsiaalselt vajalikud ravimid tunnistatakse ebaefektiivseks. (Jadhav et al. 2010)

Oluline on uurida ravimite farmakokineetikat ja farmakodünaamikat just nendes vastsündinute populatsioonides ja kliinilistes situatsioonides, kus neid enim kasutatakse. Käesolev doktoritöö keskendub kahele kliinilisele situatsioonile ja vastavatele patsiendipopulatsioonidele: vahetus sünnijärgses perioodis kriitilises seisundis vastsündinud, kellel uurisime dobutamiini farmakokineetikat ja kontsentratsioonist sõltuvat toimet südamele ja vereringele, ning arterioosjuha kirurgilise sulgemise järgselt pulmo-kardiaalse seisundi olulise halvenemise riskiga enneaegsed vastsündinud, kellel uurisime milrinooni farmakokineetikat.

Uurimistöö eesmärgid

Töö üldine eesmärk oli kirjeldada dobutamiini ja milrinooni populatsiooni farmakokineetikat ja farmakodünaamikat kahes kliinilises olukorras, kus neid ravimeid vastsündinutel sageli kasutatakse.

Töö täpsemad eesmärgid olid järgmised:

- Analüüsida seniseid teaduskirjanduses avaldatud andmeid milrinooni farmakokineetikast lastel ja vastsündinutel. Nende põhjal töötada välja optimaalne doseerimisskeem arterioosjuha kirurgilise sulgemise järgseks perioodiks, kasutades arvutipõhist eesmärkkontsentratsiooni saavutamise tõenäosuste modelleerimist. Saadud doseerimisskeemi rakendada järgnevas prospektiivses uuringus.
- Kirjeldada milrinooni farmakokineetikat enneaegsetel vastsündinutel arterioosjuha kirurgilise sulgemise järgses perioodis.
- Prospektiivses uuringus kogutud andmete põhjal anda soovitused milrinooni optimaalseks doseerimiseks selles situatsioonis.
- Kirjeldada dobutamiini farmakokineetikat kriitilises seisundis vastsündinutel vahetus sünnijärgses perioodis.
- Kirjeldada dobutamiini kontsentratsioonist sõltuvaid toimeid tsentraalsele ja regionaalsele hemodünaamikale selles situatsioonis.

Uuritavad ja metoodika

Käesoleva doktoritöö raames viidi läbi kolm uuringut:

- Retrospektiivses uuringus koguti demograafilised andmed patsientide kohta, kellel tehti arterioosjuha kirurgiline sulgemine Tartu Ülikooli Kliinikumis või Tallinna Lastehaiglas ajavahemikul jaanuar 2012 kuni detsember 2014. Kasutades kirjanduses leitavaid populatsiooni farmakokineetika mudeleid, simuleeriti sellele populatsioonile milrinooni aja-kontsentratsiooni kõverad ning leiti nende põhjal doseerimisskeem, mis kõige suurema tõenäosusega võiks anda ravimi plasmakontsentratsiooni 150–200 µg/l 6–24 tundi peale arterioosjuha ligeerimist.
- Prospektiivne uuring milrinooni farmakokineetikast patsientidel, kellel tehti arterioosjuha sulgemise operatsioon Tartu Ülikooli Kliinikumis või Tallinna

Lastehaiglas ajavahemikul juuni 2015 kuni oktoober 2017. Milrinooniga raviti neid patsiente, kellel üks tund peale arterioosjuha ligeerimise operatsiooni oli südame vasaku vatsakese väljutusmaht mõõdetuna ehhokardiograafial alla 200 ml/kg/min. Milrinooni manustati vastavalt eelmises uuringus saadud doseerimisskeemile. Kindlaksmääratud ajahetkedel võeti vereanalüüsid ravimi plasmakontsentratsioonimääramiseks, saadud andmeid analüüsiti mittelineaarsete sega-mõjudega farmakokineetika mudelite abil. Mõõdetud ravimikontsentratsioonidega kõige paremini sobiva farmakokineetika mudelit kasutati seejärel aja-kontsentratsioonikõverate simuleerimiseks ja leiti optimaalseim doseerimisskeem eesmärkkontsentratsiooni 150–250 µg/l saavutamiseks.

- Prospektiivne uuring dobutamiini farmakokineetikast ja farmakodünaamikast kriitlises seisundis vastsündinutel. Uuritavateks olid vastsündinud, kes hospitaliseeriti Tartu Ülikooli Kliinikumi või Tallinna Lastehaigla laste- ja vastsündinute intensiivraviosakonda nende esimese 72 elutunni jooksul ajavahemikul aprill 2016 kuni detsember 2017. Ravi dobutamiiniga alustati kliinilisel näidustusel raviarsti otsusel. Ravimit manustati püsiinfusioonina 5–20 µg/kg/min, doosi tõsteti iga 20–30 minuti järel. Koguti vereanalüüsid dobutamiini plasmakontsentratsioonimääramiseks ja salvestati hemodünaamika parameetrid patsiendimonitoridest ja ehhokardiograafial. Saadud andmeid analüüsiti segamõjudega mitteleneaarsete mudelite abil, kasutades samaaegset farmakokineetika ja farmakodünaamika modelleerimist.

Peamised tulemused ja arutelu

Esimese uuringu populatsioon koosnes 31 vastsündinust, keskmise (vahemik) gestatsioonivanusega 26 (23–35) nädalat, sünnijärgse vanusega 13 (3–29) päeva ja operatsioonipäeva kehakaaluga 760 (500–2,351) g. Leidsime kirjandusest viis milrinooni laste populatsiooni farmakokineetika mudelit, millest neli olid kirjeldatud kardiokirurgia ja kunstliku vereringe patsientide populatsioonil. Meie uuringupopulatsioonile kõige lähem oli esimese 12 elutunni enneaegsete vastsündinute populatsioonil kirjeldatud ühekambriline mudel. (Paradisis et al. 2007) Mudeli ainukeseks vastsündinu suurust ja küpsust kirjeldavaks kovariaadiks oli kehakaal, mille suhtes totaalne kliirens ja suhteline jaotusruumala olid kohandatud. Eesmärgiks seatud kontsentratsioonivahemik saavutatakse selle mudeli põhjal kõige paremini 0.73 µg/kg/min kolmetunnise küllastusinfusiooni ja sellele järgneva 0.16 µg/kg/min säilitusdoosiga. Simulatsiooniuuringuga näitasime, et arterioosjuha sulgemise järgses kliinilises situatsioonis on terapeutilise kontsentratsiooni saavutamiseks sobivam aeglane e küllastusdoos ja selle järgnev säilitusinfusioon kui pideva ühesugune infusioonikiirus kohe manustamise algusest. Leitud doseerimisskeemi kasutasime oma prospektiivses milrinooni farmakokineetika uuringus.

Teise uuringu perioodil tehti kahes uuringukeskuses kokku 21 arterioosjuha sulgemise operatsiooni. Nendest 14 patsienti arvati uuringusse, ning omakorda 10 patsienti said ravi milrinooniga vastavalt protokollile. Nende vastsündinute keskmine (vahemik) postmenstruaalne vanus ja kehakaal operatsioonipäeval olid

27.4 (24.6–30.1) nädalat ja 857.3 (568.0–1114.0) g. Parima doseerimisskeemi leidmiseks tegime järgnevalt simulatsioonid kõikide esimese ja teise uuringuperioodi jooksul opereeritud patsientidega, kokku 52 vastsündinuga. Nende keskmine (vahemik) postmenstruaalne vanus ja kehakaal olid vastavalt 27.6 (23.3–37.3) nädalat ja 875.5 (500.0–2351.0) g. Kõige paremini kirjeldas uuringus kogutud farmakokineetika andmeid ühekambriline lineaarne mudel, mille parameetrid olid allomeetriliselt skaleeritud kehakaalule ning totaalse kliirensi arvutamisel arvestatud glomerulaarfiltratsiooni küpsemist vastavalt postmenstruaalvanusele. Populatsiooni keskmine (inter-individuaalne varieeruvus) hinnang milrinooni totaalsele kliirensile ja suhtelisele jaotusruumalale oli vastavalt 0.350 l/h/857.3g (11.6%) ja 0.329 l/857.3g (32.6%). Simulatsioonide tulemusena saame öelda, et parim eesmärkkontsentratsiooni tabamine on saavutatav kolmetunnise küllastusinfusiooniga 0.50 µg/kg/min, millele järgneb säilitusdoos sõltuvalt postmenstruaalvanusest. Vastsündinutel postmenstruaalses vanuses alla 27 nädala on sobivaks säilitusdoosiks 0.15 µg/kg/min ja alates 27 nädalast 0.20 µg/kg/min. Võrreldes esimese elupäeva vastsündinutega (kirjanduse analüüs esimeses uuringus) on neil patsientidel väiksem milrinooni suhteline jaotusruumala ja suurem eliminatsioonikiirus, mis on seletatav keha veesisalduse languse ja glomerulaarfiltratsiooni kiire tõusuga esimesel elunädalal. Sellest tulenevalt on meie mudeli põhjal soovitatud küllastusdoos väiksem ja säilitusannus osadel vastsündinutel suurem.

Dobutamiini farmakokineetika ja farmakodünaamika uuringusse võtsime 31 vastsündinut, kellest 28 vastsündinu andmeid analüüsisime. Uuringupopulatsiooni mediaan (vahemik) gestatsioonivanus oli 30.4 (22.7–41.0) nädalat, sünnijärgne vanus uuringusse arvamisel 6 (2 – 28) tundi ja sünnikaal 1618 (465–4380) g. Dobutamiini farmakokineetikat kirjeldasime ühekambrilise lineaarse mudeliga, milles parameetrid olid allomeetriliselt skaleeritud kehakaalule ja totaalse kliirensi arvutamisel arvestati eliminatsioonimehhanismide küpsemist vastavalt postmenstruaalvanusele. Populatsiooni keskmine (inter-individuaalne varieeruvus) hinnang dobutamiini totaalsele kliirensile ja suhtelisele jaotusruumalale oli vastavalt 41.2 l/h/1618g (29%) ja 5.29 l/1618g (29%). Kirjeldasime dobutamiini kontsentratsioonist sõltuvat toimet südame minutimahule, väljutusfraktsioonile, südamesagedusele, keskmisele arteriaalsele vererõhule ja ajukoe fraktsionaalne hapnikuekstraktsioonile. Näitasime, et südamesagedus ja vererõhk tõusevad dobutamiini toimel vähe, see muutus ilmneb madalatel kontsentratsioonidel ja on küllastuv. Ajukoe fraktsionaalne hapnikuekstraktsioon dobutamiini toimel langeb. Sellest võime järeldada, et aju verevool paraneb juba suhteliselt madalate dobutamiini kontsentratsioonide juures. Dobutamiini südamefunktsiooni ja -minutimahtu suurendav toime jätkub ka kõrgematel, kuni 200 µg/l plasmakontsentratsioonidel.

Järeldused

- Simulatsiooniuuring näitas, et enneaegsetel vastsündinutel on milrinooni terapeutilise plasmakontsentratsiooni saavutamiseks arterioosjuha sulgemise järgselt sobivaim aeglane küllastusdoos järgneva säilitusinfusiooniga. Et tabada eesmärkkontsentratsiooni 150– 200 µg/l vähemalt 80% ajast 3–24 tundi peale ravi alustamist võimalikult paljudel patsientidel, peaksime kasutama kolmetunnist küllastusinfusiooni 0.73 µg/kg/min ja järgnevat säilitusdoosi 0.16 µg/kg/min.
- Milrinooni farmakokineetikat vastsündinutel kirjeldab ühekambriline lineaarne mudel, mille parameetrid on allomeetriliselt skaleeritud kehakaalule ning kliirensi arvutamisel arvestatakse glomerulaarfiltratsiooni vanuselist küpsemist. Kiired füsioloogilised muutused esimese elunädala jooksul keha koostises ja neerufunktsioonis mõjutavad milrinooni farmakokineetikat, mistõttu on arterioosjuha ligeerimise järgselt ravimi totaalne kliirens suurem ja suhteline jaotusruumala väiksem kui enneaegsetel vastsündinutel esimesel elupäeval.
- Modelleerimise ja simulatsioonide tulemusel soovitame arterioosjuha sulgemise järgse kardiopulmonaalse seisundi halvenemise vältimiseks doseerimisskeemi, mis sõltub postmenstruaalvanusest. Alla 27 nädala vanustel vastsündinutel järgneb kolmetunnisele küllastusinfusioonile 0.50 µg/kg/min säilitusdoos 0.15 µg/kg/min ja alates 27 nädala vanustest samale küllastusdoosile säilitusdoos 0.20 µg/kg/min.
- Dobutamiini farmakokineetikat vastsündinutel kirjeldab ühekambriline lineaarne mudel, milles parameetrid on allomeetriliselt skaleeritud kehakaalule ja totaalse kliirensi arvutamisel arvestatakse eliminatsioonimehhanismide küpsemist vastavalt postmenstruaalvanusele.
- Dobutamiini toimed südame funktsioonile, vererõhule ja aju verevoolule avalduvad erinevate plasmakontsentratsioonide juures. Südamesagedus ja vererõhk tõusevad dobutamiini toimel vähe, see toime ilmneb plasmakontsentratsioonidel alla 100 µg/l ja on küllastuv. Südame funktsioon ja minutimaht tõusevad veel ka dobutamiini kõrgemate, kuni 200 µg/l plasmakontsentratsioonide juures.

10. ACKNOWLEDGEMENTS

The studies for this thesis were conducted in the Department of Anaesthesiology and Intensive Care of the Institute of Clinical Medicine, University of Tartu; Department of Anaesthesiology and Intensive Care, Tallinn Children's Hospital and Department of Paediatric and Neonatal Intensive Care, Clinic of Anaesthesiology and Intensive Care, Tartu University Hospital, Estonia.

The work was funded from Estonian Research Council grant PUT1197 and Archimedes Foundation Project no. 3.2.1001-0032, and it was additionally supported by the University of Tartu Foundation: Professor Lembit Allikmets' and Heino Kruse's foundations.

The studies presented here are based on broad and successful collaboration and the contributions of many people. I wish to express my sincere gratitude and acknowledge the following persons:

My supervisors, Professor Tuuli Metsvaht and Professor Joel Starkopf, for the opportunity to start a novel and unpredictable project, for their trust in me throughout the process, and for their limit-pushing ideas and the encouragement to make them come true.

Joseph F Standing for being the key person to introduce me to the field of pharmacometrics, for his contributions to the publications and for his hospitality in London.

All my colleagues in Tallinn Children's Hospital, especially Mari-Liis Ilmoja for her exceptional support, and intensive care nurses Birgit Kiilaspää and Marika Zuihhina for their kind collaboration throughout the clinical studies.

All doctors and nurses in the Department of Paediatric and Neonatal Intensive Care, Tartu University Hospital, for their kind collaboration in clinical studies.

All the parents of critically ill neonates who decided to participate in the studies and give their contribution.

Paediatric cardiologists Tiiu Jalas, Maila Raidmäe, Karin Uibo, Margit Sõnajalg and Kristel Köbas for all their competence in neonatal echocardiography and for conducting the numerous studies.

Dr. Natan-Toomas Aro for kind collaboration during the PDA closure operations, patience and extraordinary humour.

Karin Kipper, Kalev Takkis and Rūta Veigure for developing and validating the method for quantifying milrinone and dobutamine in plasma samples of very limited volume.

Petra Lemmers, Thomas Alderliesten and René van de Vosse from University Medical Centre Utrecht for their knowledge and support in data recording.

Tõnis Tasa for the kind mathematical support and simulations and Hiie Soeorg for the interesting discussion about the results of the dobutamine study.

Professor Irja Lutsar and Professor Anti Kalda for reviewing the manuscript and for all the detailed comments that helped a lot to make things clearer for readers.

All my friends and family for being there for me for all these years and not asking very often when it will be finished.

My best friend, my love, my husband Jaanus, for the greatest support imaginable, including R-code and the best cups of coffee.

Our kids, Anette and Lennart, for their patience when waiting for me to come home and while seeing me work all these days.

APPENDIX

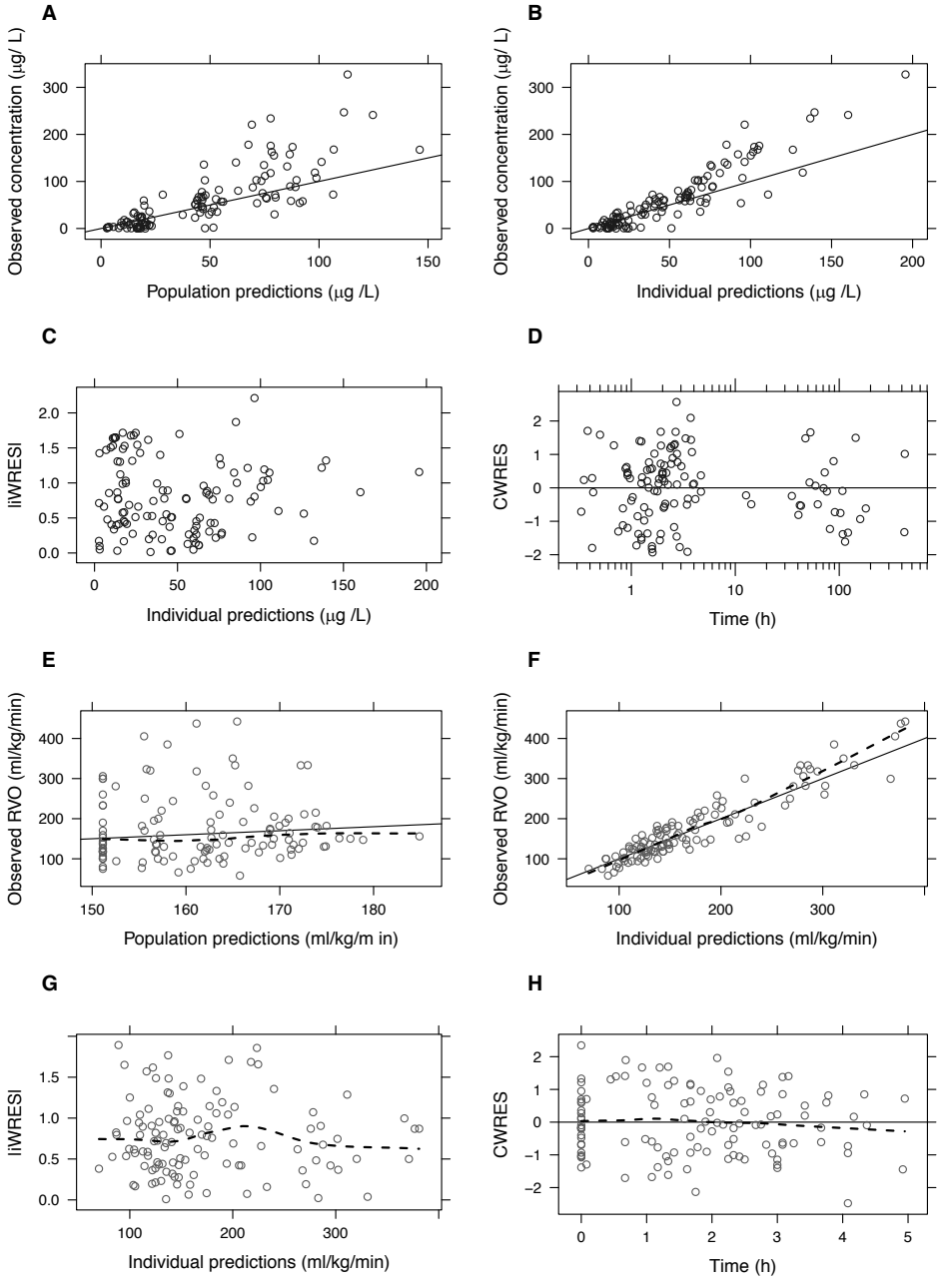

Figure A1. Basic goodness-of-fit plots of the final dobutamine-right ventricular output (RVO) pharmacokinetic-pharmacodynamic model: A observed versus population predicted dobutamine plasma concentrations, B observed versus individual predicted dobutamine plasma concentrations, C absolute value of individual weighted residuals (|iWRES|) versus individual concentration predictions, D conditional weighted residuals (CWRES) of concentrations over time (log-scale), E observed versus population predicted RVO, F observed versus individual predicted RVO, G absolute value of individual weighted residuals (|iWRES|) versus individual RVO predictions, H conditional weighted residuals of RVO over time.

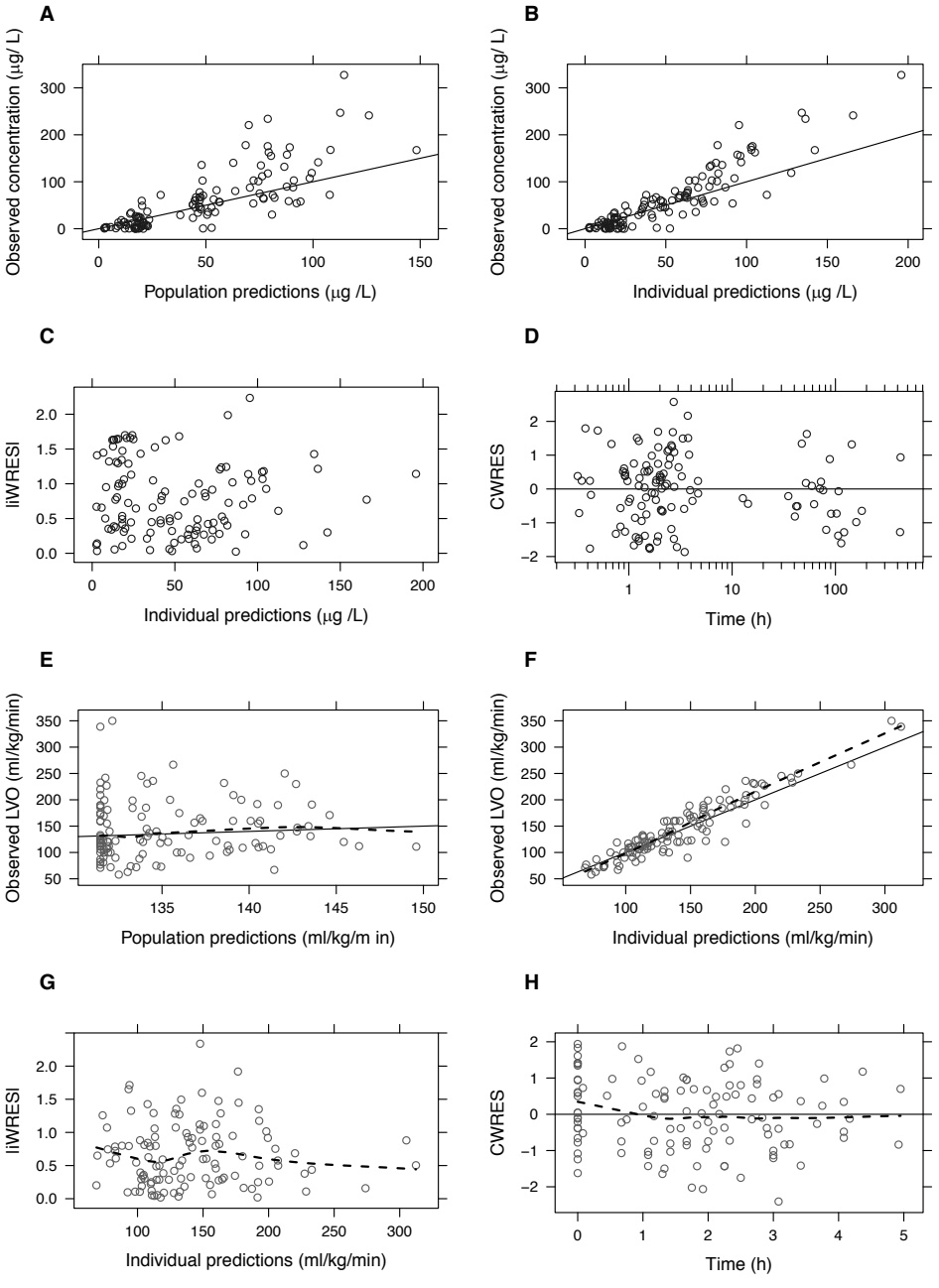

Figure A2. Basic goodness-of-fit plots of the final dobutamine-left ventricular output (LVO) pharmacokinetic-pharmacodynamic model: A observed versus population predicted dobutamine plasma concentrations, B observed versus individual predicted dobutamine plasma concentrations, C absolute value of individual weighted residuals (|iWRES|) versus individual concentration predictions, D conditional weighted residuals (CWRES) of concentrations over time (log-scale), E observed versus population predicted LVO, F observed versus individual predicted LVO, G absolute value of individual weighted residuals (|iWRES|) versus individual LVO predictions, H conditional weighted residuals of LVO over time.

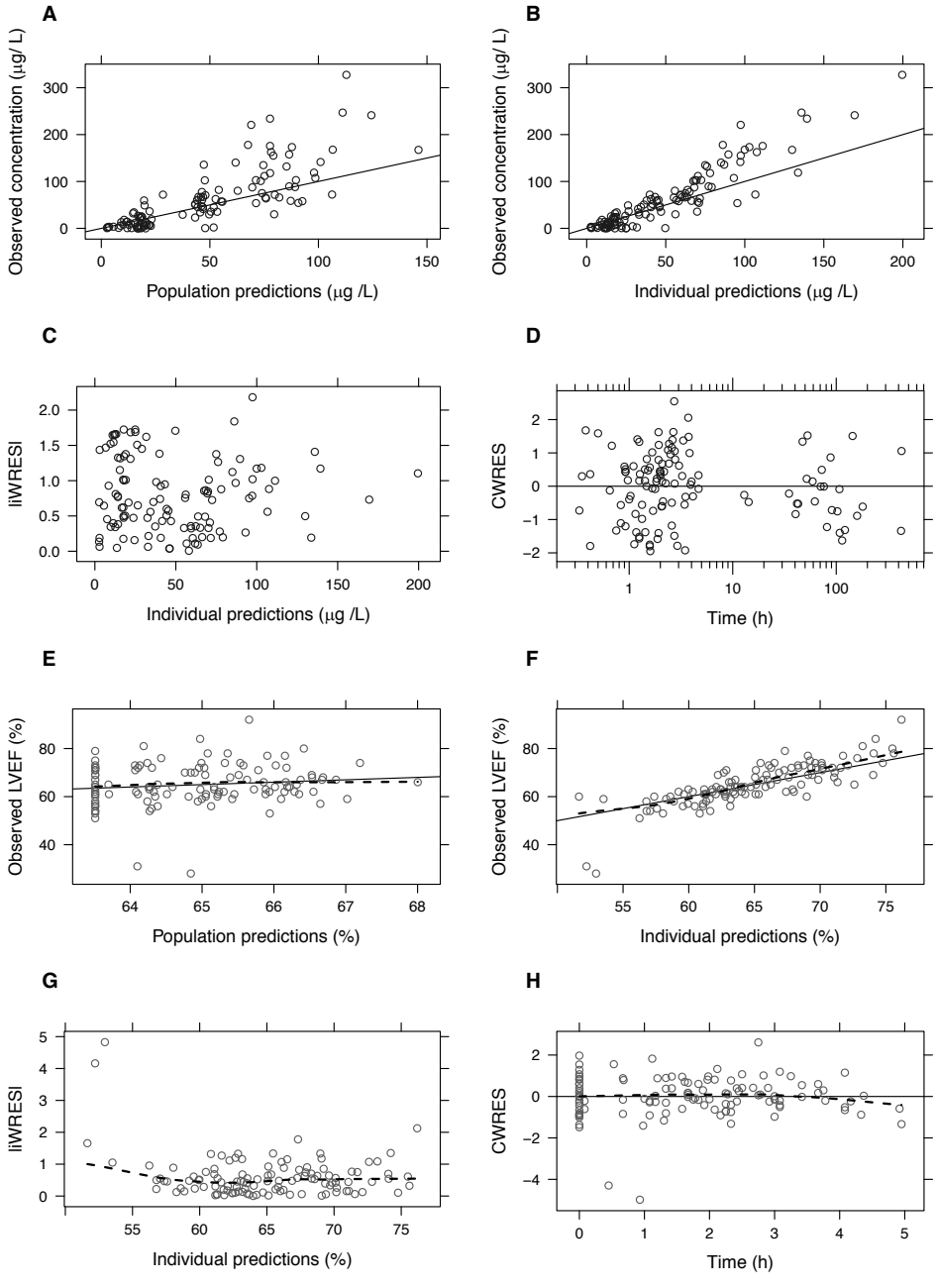

Figure A3. Basic goodness-of-fit plots of the final dobutamine-left ventricular ejection fraction (LVEF) pharmacokinetic-pharmacodynamic model: A observed versus population predicted dobutamine plasma concentrations, B observed versus individual predicted dobutamine plasma concentrations, C absolute value of individual weighted residuals (|iWRES|) versus individual concentration predictions, D conditional weighted residuals (CWRES) of concentrations over time (log-scale), E observed versus population predicted LVEF, F observed versus individual predicted LVEF, G absolute value of individual weighted residuals (|iWRES|) versus individual LVEF predictions, H conditional weighted residuals of LVEF over time.

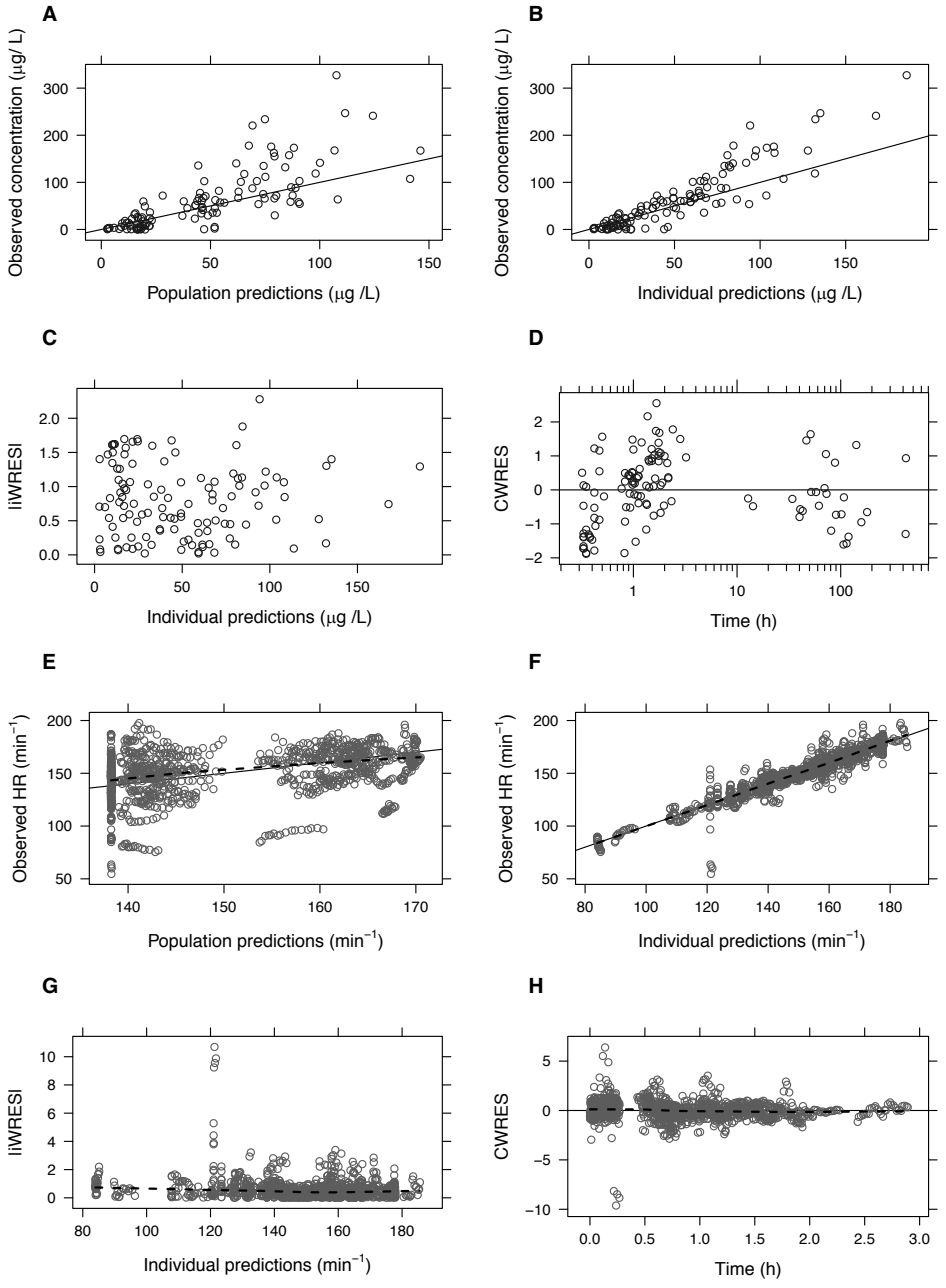

Figure A4. Basic goodness-of-fit plots of the final dobutamine-heart rate (HR) pharmacokinetic-pharmacodynamic model: A observed versus population predicted dobutamine plasma concentrations, B observed versus individual predicted dobutamine plasma concentrations, C absolute value of individual weighted residuals (|iWRES|) versus individual concentration predictions, D conditional weighted residuals (CWRES) of concentrations over time (log-scale), E observed versus population predicted HR, F observed versus individual predicted HR, G absolute value of individual weighted residuals (|iWRES|) versus individual HR predictions, H conditional weighted residuals of HR over time.

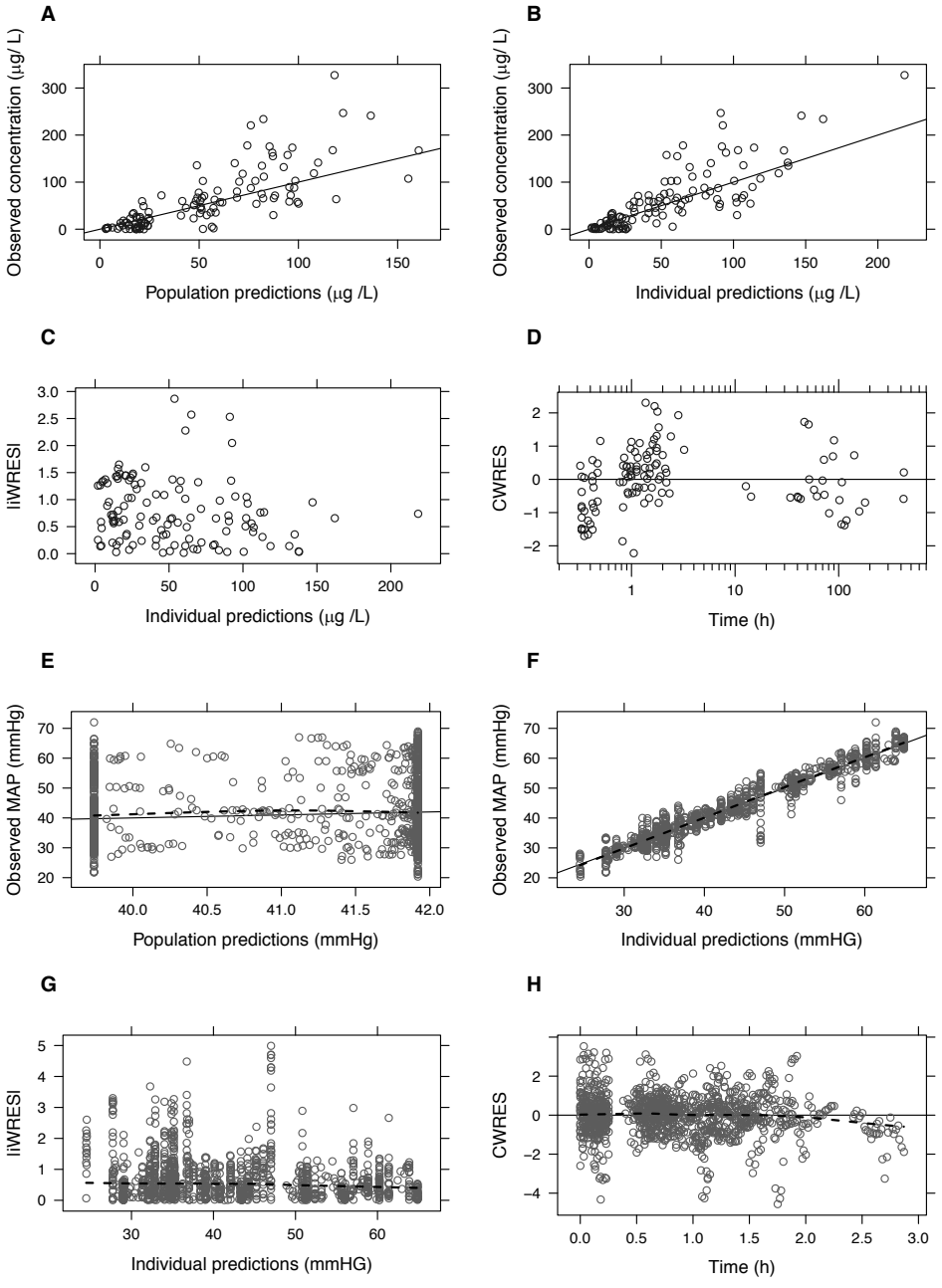

Figure A5. Basic goodness-of-fit plots of the final dobutamine-mean arterial blood pressure (MAP) pharmacokinetic-pharmacodynamic model: A observed versus population predicted dobutamine plasma concentrations, B observed versus individual predicted dobutamine plasma concentrations, C absolute value of individual weighted residuals (|iWRES|) versus individual concentration predictions, D conditional weighted residuals (CWRES) of concentrations over time (log-scale), E observed versus population predicted MAP, F observed versus individual predicted MAP, G absolute value of individual weighted residuals (|iWRES|) versus individual MAP predictions, H conditional weighted residuals of MAP over time.

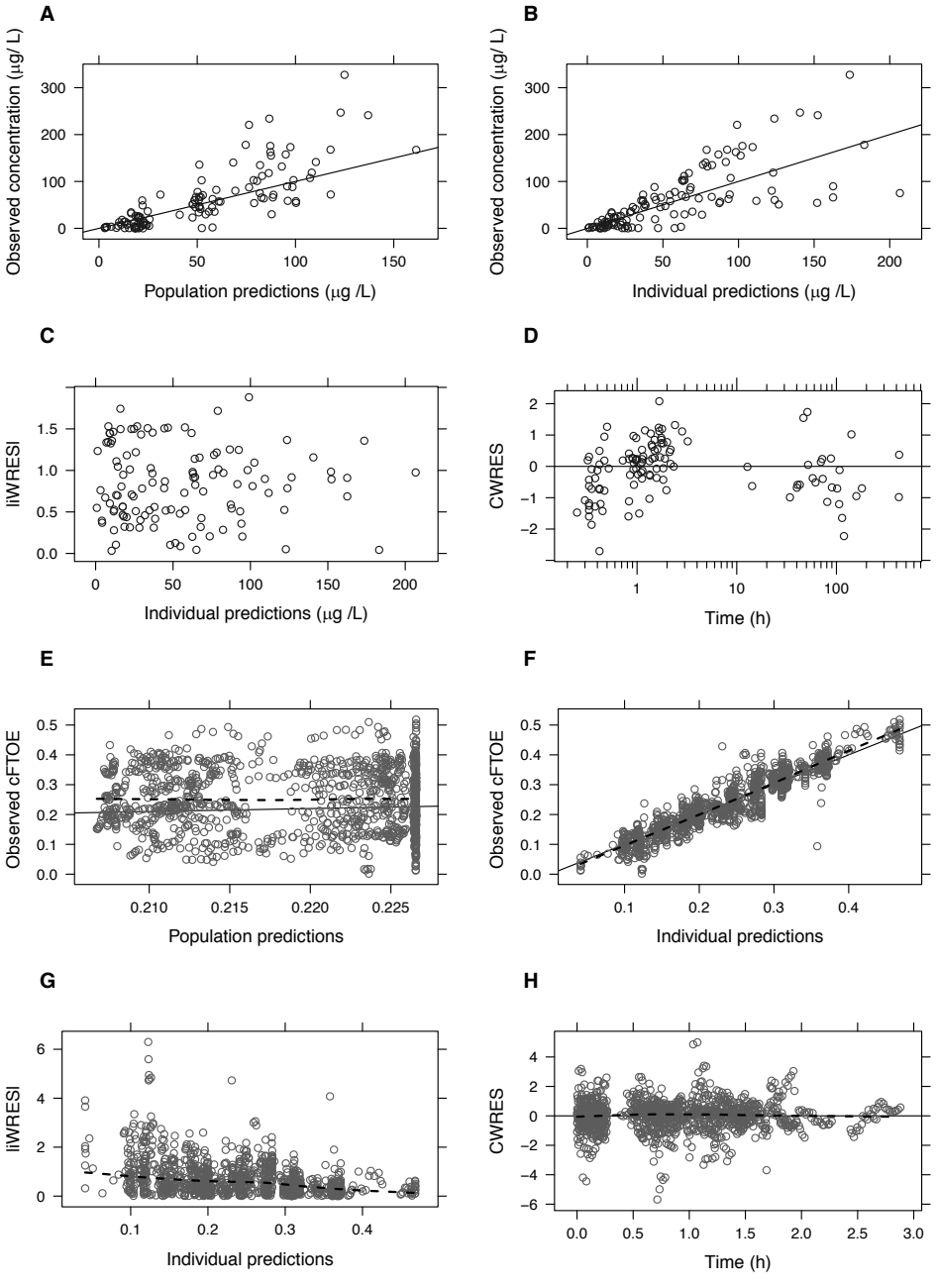

Figure A6. Basic goodness-of-fit plots of the final dobutamine-cerebral fractional tissue oxygen extraction (cFTOE) pharmacokinetic-pharmacodynamic model: A observed versus population predicted dobutamine plasma concentrations, B observed versus individual predicted dobutamine plasma concentrations, C absolute value of individual weighted residuals (|iWRES|) versus individual concentration predictions, D conditional weighted residuals (CWRES) of concentrations over time (log-scale), E observed versus population predicted cFTOE, F observed versus individual predicted cFTOE, G absolute value of individual weighted residuals (|iWRES|) versus individual cFTOE predictions, H conditional weighted residuals of cFTOE over time.

PUBLICATIONS

CURRICULUM VITAE

Name:	Maarja Hallik
Date of birth:	March 4,1982
Citizenship:	Estonia
E-mail:	maarja.hallik@gmail.com

Education

2013–	University of Tartu, PhD studies in clinical medicine
2007–2012	University of Tartu, Post-graduate training in anaesthesiology and intensive care
2000–2007	University of Tartu, Medicine

Work experience

2019–	East-Tallinn Central Hospital, Department of Anaesthesiology and Intensive Care; Department of Neonatology
2019	University of Tartu, Department of Anaesthesiology and Intensive Care, Specialist in Pharmacokinetic and -dynamic Analysis
2017–2019	University of Tartu, Department of Anaesthesiology and Intensive Care, Junior Research Fellow
2012–2018	Tallinn Children's Hospital, Department of Anaesthesiology and Intensive Care

Research interests

Pharmacokinetics and pharmacodynamics in neonatal populations. Non-liner mixed effects modelling. Haemodynamic monitoring and cardiovascular drug therapy in neonatal intensive care.

Publications

1. Hallik M, Tamme K, Väli T, Starkopf J. Successful liver transplantation after 21 days of hepatic coma. ASAIO J. 2011;57(6):545–546. https://doi.org/10.1097/MAT.0b013e318230632e
2. Hallik M, Annilo T, Ilmoja ML. Different course of lung disease in two siblings with novel ABCA3 mutations. Eur J Pediatr. 2014;173(12):1553–1556. https://doi.org/10.1007/s00431-013-2087-3
3. Hallik M, Tasa T, Starkopf J, Metsvaht T. Dosing of Milrinone in Preterm Neonates to Prevent Postligation Cardiac Syndrome: Simulation Study Suggests Need for Bolus Infusion. Neonatology. 2017;111(1):8–11. https://doi.org/10.1159/000447049

4. Takkis K, Veigure R, Metsvaht T, Hallik M, Ilmoja M-L, Starkopf J, Kipper K. A sensitive method for the simultaneous UHPLC-MS/MS analysis of milrinone and dobutamine in blood plasma using NH4F as the eluent additive and ascorbic acid as a stabilizer. Clinical Mass Spectrometry. 2019;12:23–29. https://doi.org/10.1016/J.CLINMS.2019.03.003
5. Hallik M, Ilmoja ML, Tasa T, Standing JF, Takkis K, Veigure R, Kipper K, Jalas T, Raidmäe M, Uibo K, Starkopf J, Metsvaht T. Population Pharmacokinetics and Dosing of Milrinone After Patent Ductus Arteriosus Ligation in Preterm Infants. Pediatr Crit Care Med. 2019;20(7):621–629. https://doi.org/10.1097/PCC.0000000000001879
6. Hallik M, Ilmoja M, Standing JF, Soeorg H, Jalas T, Raidmäe M, Uibo K, Köbas K, Sõnajalg M, Takkis K, Veigure R, Kipper K, Starkopf J, Metsvaht T. Population Pharmacokinetics and Pharmacodynamics of Dobutamine in Neonates on the First Days of Life. Br J Clin Pharmacol. 2020;86(2):318–28. https://doi.org/10.1111/bcp.14146
7. Lutsar I, Chazallon C, Trafojer U, de Cabre VM, Auriti C, Bertaina C, Calo Carducci FI, Canpolat FE, Esposito S, Fournier I, Hallik M, Heath PT, Ilmoja M-L, Iosifidis E, Kuznetsova J, Meyer L, Metsvaht T, Mitsiakos G, Pana ZD, Mosca F, Pugni L, Roilides E, Rossi P, Sarafidis K, Sanchez L, Sharland M, Usonis V, Warris A, Aboulker J-P, Giaquinto C, Consortium on behalf of N. Meropenem vs Standard of Care for Treatment of Neonatal Late Onset Sepsis (NeoMero1): A Randomised Controlled Trial edited by A. Scherag. PLOS ONE 2020;15(3):e0229380.

Professional training

2012 – Paediatric intensive care, Helsinki University Hospital, Finland
2012 – CITI Program's GCP course
2013 – Neonatal intensive care, Michigan University Hospital, USA
2013 – American Austrian Foundation OMI Salzburg Medical Seminar "Pediatric Anaesthesia and Intensive Care"
2014 – IPOKRaTES Seminar "Neonatal Hemodynamics"
2016 – Population Modelling and Dose Optimization with Pmetrics and BestDose: Antimicrobial Applications, ESCMID Postgraduate Technical Workshop
2017 – UCL Pharmacometrics group nonlinear mixed effects modelling course
2017 – UCL Individual training in non-linear mixed effects modelling in pharmacokinetics and pharmacodynamics with NONMEM
2018 – Syneos Health GCP course
2019 – HealthyR: quick-start course

Professional organisations

Member of Estonian Society of Anaesthesiologists, Estonian Medical Association and European Society of Paediatric Anaesthesiology.

ELULOOKIRJELDUS

Nimi: Maarja Hallik
Sünniaeg: 4. märts. 1982
Kodakondsus: Eesti
E-post: maarja.hallik@gmail.com

Haridus

2013–	Tartu Ülikool, doktoriõpe
2007–2012	Tartu Ülikool, anestesioloogia ja intensiivravi residentuur
2000–2007	Tartu Ülikool, arstiõpe
1997–2000	Gustav Adolfi Gümnaasium
1988–1997	Viimsi Keskkool

Töökäik

2019–	AS Ida-Tallinna Keskhaigla, Anestesioloogia ja intensiivravi keskus ja Neonatoloogiaosakond, anestesioloog
2019	Tartu Ülikool, Anestesioloogia ja intensiivravi kliinik, farmakokineetika ja -dünaamika analüüsi spetsialist
2017–2019	Tartu Ülikool, Anestesioloogia ja intensiivravi kliinik, nooremteadur
2012–2018	Tallinna Lastehaigla, Anestesioloogia-intensiivraviosakond, anestesioloog

Teaduslik ja arendustegevus

Peamised uurimisvaldkonnad: inotroopsete ravimite kasutamine ja nende mõju tsentraalsele ja regionaalsele verevoolule enneaegsetel vastsündinutel, populatsiooni farmakokineetika ja -dünaamika mittelineaarne segamõjudega modelleerimine.

Osalesin kaasuurijana meropeneemi efektiivsust, ohutust ja farmakokineetikat vastsündinutel käsitlevas rahvusvahelistes kliinilistes uuringutes NEOMERO1 ja NEOMERO 2.

Publikatsioonid

1. Hallik M, Tamme K, Väli T, Starkopf J. Successful liver transplantation after 21 days of hepatic coma. ASAIO J. 2011;57(6):545–546. https://doi.org/10.1097/MAT.0b013e318230632e
2. Hallik M, Annilo T, Ilmoja ML. Different course of lung disease in two siblings with novel ABCA3 mutations. Eur J Pediatr. 2014;173(12):1553–1556. https://doi.org/10.1007/s00431-013-2087-3
3. Hallik M, Tasa T, Starkopf J, Metsvaht T. Dosing of Milrinone in Preterm Neonates to Prevent Postligation Cardiac Syndrome: Simulation Study Suggests Need for Bolus Infusion. Neonatology. 2017;111(1):8–11. https://doi.org/10.1159/000447049

4. Takkis K, Veigure R, Metsvaht T, Hallik M, Ilmoja M-L, Starkopf J, Kipper K. A sensitive method for the simultaneous UHPLC-MS/MS analysis of milrinone and dobutamine in blood plasma using NH4F as the eluent additive and ascorbic acid as a stabilizer. Clinical Mass Spectrometry. 2019;12:23–29. https://doi.org/10.1016/J.CLINMS.2019.03.003
5. Hallik M, Ilmoja ML, Tasa T, Standing JF, Takkis K, Veigure R, Kipper K, Jalas T, Raidmäe M, Uibo K, Starkopf J, Metsvaht T. Population Pharmacokinetics and Dosing of Milrinone After Patent Ductus Arteriosus Ligation in Preterm Infants. Pediatr Crit Care Med. 2019;20(7):621–629. https://doi.org/10.1097/PCC.0000000000001879
6. Hallik M, Ilmoja M, Standing JF, Soeorg H, Jalas T, Raidmäe M, Uibo K, Köbas K, Sõnajalg M, Takkis K, Veigure R, Kipper K, Starkopf J, Metsvaht T. Population Pharmacokinetics and Pharmacodynamics of Dobutamine in Neonates on the First Days of Life. Br J Clin Pharmacol. 2020;86(2):318–28. https://doi.org/10.1111/bcp.14146
7. Lutsar I, Chazallon C, Trafojer U, de Cabre VM, Auriti C, Bertaina C, Calo Carducci FI, Canpolat FE, Esposito S, Fournier I, Hallik M, Heath PT, Ilmoja M-L, Iosifidis E, Kuznetsova J, Meyer L, Metsvaht T, Mitsiakos G, Pana ZD, Mosca F, Pugni L, Roilides E, Rossi P, Sarafidis K, Sanchez L, Sharland M, Usonis V, Warris A, Aboulker J-P, Giaquinto C, Consortium on behalf of N. Meropenem vs Standard of Care for Treatment of Neonatal Late Onset Sepsis (NeoMero1): A Randomised Controlled Trial edited by A. Scherag. PLOS ONE 2020;15(3):e0229380

Saadud uurimistoetused ja stipendiumid:

Eesti Anestesioloogide Seltsi koolitustoetus 2013
TÜ sihtasutuse Heino Kruse stipendium 2015
TÜ sihtasutuse professor Lembit Allikmetsa stipendium 2017

Erialane enesetäiendus:

2012 – Helsingi Ülikoolihaigla Laste intensiivraviosakond (2 nädalat)
2012 – CITI Program's GCP koolitus
2013 – Michigani Ülikoolihaigla vastsündinute intensiivraviosakond (2 nädalat)
2013 – Ameerika Austria Fondi Salzburgi seminar "Laste anesteesia ja – intensiivravi" 2014 – IPOKRaTES Seminar "Neonatal Hemodynamics"
2016 – Population Modelling and Dose Optimization with Pmetrics and BestDose: Antimicrobial Applications, ESCMID Postgraduate Technical Workshop
2017 – UCL Pharmacometrics group nonlinear mixed effects modeling course
2017 – UCL Individuaalõpe: nonlinera mixed effects modeling in pharmacokintics NONMEM tarkvaraga
2018 – Syneos Health GCP kursus
2019 – HealthyR: quick-start course

Muu teaduslik organisatsiooniline ja erialane tegevus:

Eesti Anestesioloogide Seltsi liige, European Society for Paediatric Anaesthesiology liige, Eesti Arstide Liidu liige.

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos**. The natural course of gastric ulcer in connection with chronic gastritis and *Helicobacter pylori*. Tartu, 1991.
2. **Mihkel Zilmer**. Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar**. Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik**. Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation). Tartu, 1992.
5. **Ants Peetsalu**. Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and *Helicobacter pylori*. Tartu, 1992.
6. **Marika Mikelsaar**. Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus**. Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar**. Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm**. On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross**. Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo**. Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik**. The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa**. Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk**. Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar**. Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi**. The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar**. Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang**. The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak**. Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.

20. **Tõnis Karki**. Quantitative composition of the human lactoflora and method for its examination. Tartu, 1996.
21. **Reet Mändar**. Vaginal microflora during pregnancy and its transmission to newborn. Tartu, 1996.
22. **Triin Remmel**. Primary biliary cirrhosis in Estonia: epidemiology, clinical characterization and prognostication of the course of the disease. Tartu, 1996.
23. **Toomas Kivastik**. Mechanisms of drug addiction: focus on positive reinforcing properties of morphine. Tartu, 1996.
24. **Paavo Pokk**. Stress due to sleep deprivation: focus on $GABA_A$ receptor-chloride ionophore complex. Tartu, 1996.
25. **Kristina Allikmets**. Renin system activity in essential hypertension. Associations with atherothrombogenic cardiovascular risk factors and with the efficacy of calcium antagonist treatment. Tartu, 1996.
26. **Triin Parik**. Oxidative stress in essential hypertension: Associations with metabolic disturbances and the effects of calcium antagonist treatment. Tartu, 1996.
27. **Svetlana Päi**. Factors promoting heterogeneity of the course of rheumatoid arthritis. Tartu, 1997.
28. **Maarike Sallo**. Studies on habitual physical activity and aerobic fitness in 4 to 10 years old children. Tartu, 1997.
29. **Paul Naaber**. *Clostridium difficile* infection and intestinal microbial ecology. Tartu, 1997.
30. **Rein Pähkla**. Studies in pinoline pharmacology. Tartu, 1997.
31. **Andrus Juhan Voitk**. Outpatient laparoscopic cholecystectomy. Tartu, 1997.
32. **Joel Starkopf**. Oxidative stress and ischaemia-reperfusion of the heart. Tartu, 1997.
33. **Janika Kõrv**. Incidence, case-fatality and outcome of stroke. Tartu, 1998.
34. **Ülla Linnamägi**. Changes in local cerebral blood flow and lipid peroxidation following lead exposure in experiment. Tartu, 1998.
35. **Ave Minajeva**. Sarcoplasmic reticulum function: comparison of atrial and ventricular myocardium. Tartu, 1998.
36. **Oleg Milenin**. Reconstruction of cervical part of esophagus by revascularised ileal autografts in dogs. A new complex multistage method. Tartu, 1998.
37. **Sergei Pakriev**. Prevalence of depression, harmful use of alcohol and alcohol dependence among rural population in Udmurtia. Tartu, 1998.
38. **Allen Kaasik**. Thyroid hormone control over β-adrenergic signalling system in rat atria. Tartu, 1998.
39. **Vallo Matto**. Pharmacological studies on anxiogenic and antiaggressive properties of antidepressants. Tartu, 1998.
40. **Maire Vasar**. Allergic diseases and bronchial hyperreactivity in Estonian children in relation to environmental influences. Tartu, 1998.
41. **Kaja Julge**. Humoral immune responses to allergens in early childhood. Tartu, 1998.

42. **Heli Grünberg**. The cardiovascular risk of Estonian schoolchildren. A cross-sectional study of 9-, 12- and 15-year-old children. Tartu, 1998.
43. **Epp Sepp**. Formation of intestinal microbial ecosystem in children. Tartu, 1998.
44. **Mai Ots**. Characteristics of the progression of human and experimental glomerulopathies. Tartu, 1998.
45. **Tiina Ristimäe**. Heart rate variability in patients with coronary artery disease. Tartu, 1998.
46. **Leho Kõiv**. Reaction of the sympatho-adrenal and hypothalamo-pituitary-adrenocortical system in the acute stage of head injury. Tartu, 1998.
47. **Bela Adojaan**. Immune and genetic factors of childhood onset IDDM in Estonia. An epidemiological study. Tartu, 1999.
48. **Jakov Shlik**. Psychophysiological effects of cholecystokinin in humans. Tartu, 1999.
49. **Kai Kisand**. Autoantibodies against dehydrogenases of α-ketoacids. Tartu, 1999.
50. **Toomas Marandi**. Drug treatment of depression in Estonia. Tartu, 1999.
51. **Ants Kask**. Behavioural studies on neuropeptide Y. Tartu, 1999.
52. **Ello-Rahel Karelson**. Modulation of adenylate cyclase activity in the rat hippocampus by neuropeptide galanin and its chimeric analogs. Tartu, 1999.
53. **Tanel Laisaar**. Treatment of pleural empyema — special reference to intrapleural therapy with streptokinase and surgical treatment modalities. Tartu, 1999.
54. **Eve Pihl**. Cardiovascular risk factors in middle-aged former athletes. Tartu, 1999.
55. **Katrin Õunap**. Phenylketonuria in Estonia: incidence, newborn screening, diagnosis, clinical characterization and genotype/phenotype correlation. Tartu, 1999.
56. **Siiri Kõljalg**. *Acinetobacter* – an important nosocomial pathogen. Tartu, 1999.
57. **Helle Karro**. Reproductive health and pregnancy outcome in Estonia: association with different factors. Tartu, 1999.
58. **Heili Varendi**. Behavioral effects observed in human newborns during exposure to naturally occurring odors. Tartu, 1999.
59. **Anneli Beilmann**. Epidemiology of epilepsy in children and adolescents in Estonia. Prevalence, incidence, and clinical characteristics. Tartu, 1999.
60. **Vallo Volke**. Pharmacological and biochemical studies on nitric oxide in the regulation of behaviour. Tartu, 1999.
61. **Pilvi Ilves**. Hypoxic-ischaemic encephalopathy in asphyxiated term infants. A prospective clinical, biochemical, ultrasonographical study. Tartu, 1999.
62. **Anti Kalda**. Oxygen-glucose deprivation-induced neuronal death and its pharmacological prevention in cerebellar granule cells. Tartu, 1999.
63. **Eve-Irene Lepist**. Oral peptide prodrugs – studies on stability and absorption. Tartu, 2000.

64. **Jana Kivastik**. Lung function in Estonian schoolchildren: relationship with anthropometric indices and respiratory symptomas, reference values for dynamic spirometry. Tartu, 2000.
65. **Karin Kull**. Inflammatory bowel disease: an immunogenetic study. Tartu, 2000.
66. **Kaire Innos**. Epidemiological resources in Estonia: data sources, their quality and feasibility of cohort studies. Tartu, 2000.
67. **Tamara Vorobjova**. Immune response to *Helicobacter pylori* and its association with dynamics of chronic gastritis and epithelial cell turnover in antrum and corpus. Tartu, 2001.
68. **Ruth Kalda**. Structure and outcome of family practice quality in the changing health care system of Estonia. Tartu, 2001.
69. **Annika Krüüner**. *Mycobacterium tuberculosis* – spread and drug resistance in Estonia. Tartu, 2001.
70. **Marlit Veldi**. Obstructive Sleep Apnoea: Computerized Endopharyngeal Myotonometry of the Soft Palate and Lingual Musculature. Tartu, 2001.
71. **Anneli Uusküla**. Epidemiology of sexually transmitted diseases in Estonia in 1990–2000. Tartu, 2001.
72. **Ade Kallas**. Characterization of antibodies to coagulation factor VIII. Tartu, 2002.
73. **Heidi Annuk**. Selection of medicinal plants and intestinal lactobacilli as antimicrobil components for functional foods. Tartu, 2002.
74. **Aet Lukmann**. Early rehabilitation of patients with ischaemic heart disease after surgical revascularization of the myocardium: assessment of health-related quality of life, cardiopulmonary reserve and oxidative stress. A clinical study. Tartu, 2002.
75. **Maigi Eisen**. Pathogenesis of Contact Dermatitis: participation of Oxidative Stress. A clinical – biochemical study. Tartu, 2002.
76. **Piret Hussar**. Histology of the post-traumatic bone repair in rats. Elaboration and use of a new standardized experimental model – bicortical perforation of tibia compared to internal fracture and resection osteotomy. Tartu, 2002.
77. **Tõnu Rätsep**. Aneurysmal subarachnoid haemorrhage: Noninvasive monitoring of cerebral haemodynamics. Tartu, 2002.
78. **Marju Herodes**. Quality of life of people with epilepsy in Estonia. Tartu, 2003.
79. **Katre Maasalu**. Changes in bone quality due to age and genetic disorders and their clinical expressions in Estonia. Tartu, 2003.
80. **Toomas Sillakivi**. Perforated peptic ulcer in Estonia: epidemiology, risk factors and relations with *Helicobacter pylori*. Tartu, 2003.
81. **Leena Puksa**. Late responses in motor nerve conduction studies. F and A waves in normal subjects and patients with neuropathies. Tartu, 2003.
82. **Krista Lõivukene**. *Helicobacter pylori* in gastric microbial ecology and its antimicrobial susceptibility pattern. Tartu, 2003.

83. **Helgi Kolk**. Dyspepsia and *Helicobacter pylori* infection: the diagnostic value of symptoms, treatment and follow-up of patients referred for upper gastrointestinal endoscopy by family physicians. Tartu, 2003.
84. **Helena Soomer**. Validation of identification and age estimation methods in forensic odontology. Tartu, 2003.
85. **Kersti Oselin**. Studies on the human MDR1, MRP1, and MRP2 ABC transporters: functional relevance of the genetic polymorphisms in the *MDR1* and *MRP1* gene. Tartu, 2003.
86. **Jaan Soplepmann**. Peptic ulcer haemorrhage in Estonia: epidemiology, prognostic factors, treatment and outcome. Tartu, 2003.
87. **Margot Peetsalu**. Long-term follow-up after vagotomy in duodenal ulcer disease: recurrent ulcer, changes in the function, morphology and *Helicobacter pylori* colonisation of the gastric mucosa. Tartu, 2003.
88. **Kersti Klaamas**. Humoral immune response to *Helicobacter pylori* a study of host-dependent and microbial factors. Tartu, 2003.
89. **Pille Taba**. Epidemiology of Parkinson's disease in Tartu, Estonia. Prevalence, incidence, clinical characteristics, and pharmacoepidemiology. Tartu, 2003.
90. **Alar Veraksitš**. Characterization of behavioural and biochemical phenotype of cholecystokinin-2 receptor deficient mice: changes in the function of the dopamine and endopioidergic system. Tartu, 2003.
91. **Ingrid Kalev**. CC-chemokine receptor 5 (CCR5) gene polymorphism in Estonians and in patients with Type I and Type II diabetes mellitus. Tartu, 2003.
92. **Lumme Kadaja**. Molecular approach to the regulation of mitochondrial function in oxidative muscle cells. Tartu, 2003.
93. **Aive Liigant**. Epidemiology of primary central nervous system tumours in Estonia from 1986 to 1996. Clinical characteristics, incidence, survival and prognostic factors. Tartu, 2004.
94. **Andres, Kulla**. Molecular characteristics of mesenchymal stroma in human astrocytic gliomas. Tartu, 2004.
95. **Mari Järvelaid**. Health damaging risk behaviours in adolescence. Tartu, 2004.
96. **Ülle Pechter**. Progression prevention strategies in chronic renal failure and hypertension. An experimental and clinical study. Tartu, 2004.
97. **Gunnar Tasa**. Polymorphic glutathione S-transferases – biology and role in modifying genetic susceptibility to senile cataract and primary open angle glaucoma. Tartu, 2004.
98. **Tuuli Käämbre**. Intracellular energetic unit: structural and functional aspects. Tartu, 2004.
99. **Vitali Vassiljev**. Influence of nitric oxide syntase inhibitors on the effects of ethanol after acute and chronic ethanol administration and withdrawal. Tartu, 2004.

100. **Aune Rehema**. Assessment of nonhaem ferrous iron and glutathione redox ratio as markers of pathogeneticity of oxidative stress in different clinical groups. Tartu, 2004.
101. **Evelin Seppet**. Interaction of mitochondria and ATPases in oxidative muscle cells in normal and pathological conditions. Tartu, 2004.
102. **Eduard Maron**. Serotonin function in panic disorder: from clinical experiments to brain imaging and genetics. Tartu, 2004.
103. **Marje Oona**. *Helicobacter pylori* infection in children: epidemiological and therapeutic aspects. Tartu, 2004.
104. **Kersti Kokk**. Regulation of active and passive molecular transport in the testis. Tartu, 2005.
105. **Vladimir Järv**. Cross-sectional imaging for pretreatment evaluation and follow-up of pelvic malignant tumours. Tartu, 2005.
106. **Andre Õun**. Epidemiology of adult epilepsy in Tartu, Estonia. Incidence, prevalence and medical treatment. Tartu, 2005.
107. **Piibe Muda**. Homocysteine and hypertension: associations between homocysteine and essential hypertension in treated and untreated hypertensive patients with and without coronary artery disease. Tartu, 2005.
108. **Külli Kingo**. The interleukin-10 family cytokines gene polymorphisms in plaque psoriasis. Tartu, 2005.
109. **Mati Merila**. Anatomy and clinical relevance of the glenohumeral joint capsule and ligaments. Tartu, 2005.
110. **Epp Songisepp**. Evaluation of technological and functional properties of the new probiotic *Lactobacillus fermentum* ME-3. Tartu, 2005.
111. **Tiia Ainla**. Acute myocardial infarction in Estonia: clinical characteristics, management and outcome. Tartu, 2005.
112. **Andres Sell**. Determining the minimum local anaesthetic requirements for hip replacement surgery under spinal anaesthesia – a study employing a spinal catheter. Tartu, 2005.
113. **Tiia Tamme**. Epidemiology of odontogenic tumours in Estonia. Pathogenesis and clinical behaviour of ameloblastoma. Tartu, 2005.
114. **Triine Annus**. Allergy in Estonian schoolchildren: time trends and characteristics. Tartu, 2005.
115. **Tiia Voor**. Microorganisms in infancy and development of allergy: comparison of Estonian and Swedish children. Tartu, 2005.
116. **Priit Kasenõmm**. Indicators for tonsillectomy in adults with recurrent tonsillitis – clinical, microbiological and pathomorphological investigations. Tartu, 2005.
117. **Eva Zusinaite**. Hepatitis C virus: genotype identification and interactions between viral proteases. Tartu, 2005.
118. **Piret Kõll**. Oral lactoflora in chronic periodontitis and periodontal health. Tartu, 2006.
119. **Tiina Stelmach**. Epidemiology of cerebral palsy and unfavourable neurodevelopmental outcome in child population of Tartu city and county, Estonia Prevalence, clinical features and risk factors. Tartu, 2006.

120. **Katrin Pudersell**. Tropane alkaloid production and riboflavine excretion in the field and tissue cultures of henbane (*Hyoscyamus niger* L.). Tartu, 2006.
121. **Külli Jaako**. Studies on the role of neurogenesis in brain plasticity. Tartu, 2006.
122. **Aare Märtson**. Lower limb lengthening: experimental studies of bone regeneration and long-term clinical results. Tartu, 2006.
123. **Heli Tähepõld**. Patient consultation in family medicine. Tartu, 2006.
124. **Stanislav Liskmann**. Peri-implant disease: pathogenesis, diagnosis and treatment in view of both inflammation and oxidative stress profiling. Tartu, 2006.
125. **Ruth Rudissaar**. Neuropharmacology of atypical antipsychotics and an animal model of psychosis. Tartu, 2006.
126. **Helena Andreson**. Diversity of *Helicobacter pylori* genotypes in Estonian patients with chronic inflammatory gastric diseases. Tartu, 2006.
127. **Katrin Pruus**. Mechanism of action of antidepressants: aspects of serotoninergic system and its interaction with glutamate. Tartu, 2006.
128. **Priit Põder**. Clinical and experimental investigation: relationship of ischaemia/reperfusion injury with oxidative stress in abdominal aortic aneurysm repair and in extracranial brain artery endarterectomy and possibilities of protection against ischaemia using a glutathione analogue in a rat model of global brain ischaemia. Tartu, 2006.
129. **Marika Tammaru**. Patient-reported outcome measurement in rheumatoid arthritis. Tartu, 2006.
130. **Tiia Reimand**. Down syndrome in Estonia. Tartu, 2006.
131. **Diva Eensoo**. Risk-taking in traffic and Markers of Risk-Taking Behaviour in Schoolchildren and Car Drivers. Tartu, 2007.
132. **Riina Vibo**. The third stroke registry in Tartu, Estonia from 2001 to 2003: incidence, case-fatality, risk factors and long-term outcome. Tartu, 2007.
133. **Chris Pruunsild**. Juvenile idiopathic arthritis in children in Estonia. Tartu, 2007.
134. **Eve Õiglane-Šlik**. Angelman and Prader-Willi syndromes in Estonia. Tartu, 2007.
135. **Kadri Haller**. Antibodies to follicle stimulating hormone. Significance in female infertility. Tartu, 2007.
136. **Pille Ööpik**. Management of depression in family medicine. Tartu, 2007.
137. **Jaak Kals**. Endothelial function and arterial stiffness in patients with atherosclerosis and in healthy subjects. Tartu, 2007.
138. **Priit Kampus**. Impact of inflammation, oxidative stress and age on arterial stiffness and carotid artery intima-media thickness. Tartu, 2007.
139. **Margus Punab**. Male fertility and its risk factors in Estonia. Tartu, 2007.
140. **Alar Toom**. Heterotopic ossification after total hip arthroplasty: clinical and pathogenetic investigation. Tartu, 2007.

141. **Lea Pehme**. Epidemiology of tuberculosis in Estonia 1991–2003 with special regard to extrapulmonary tuberculosis and delay in diagnosis of pulmonary tuberculosis. Tartu, 2007.
142. **Juri Karjagin**. The pharmacokinetics of metronidazole and meropenem in septic shock. Tartu, 2007.
143. **Inga Talvik**. Inflicted traumatic brain injury shaken baby syndrome in Estonia – epidemiology and outcome. Tartu, 2007.
144. **Tarvo Rajasalu**. Autoimmune diabetes: an immunological study of type 1 diabetes in humans and in a model of experimental diabetes (in RIP-B7.1 mice). Tartu, 2007.
145. **Inga Karu**. Ischaemia-reperfusion injury of the heart during coronary surgery: a clinical study investigating the effect of hyperoxia. Tartu, 2007.
146. **Peeter Padrik**. Renal cell carcinoma: Changes in natural history and treatment of metastatic disease. Tartu, 2007.
147. **Neve Vendt**. Iron deficiency and iron deficiency anaemia in infants aged 9 to 12 months in Estonia. Tartu, 2008.
148. **Lenne-Triin Heidmets**. The effects of neurotoxins on brain plasticity: focus on neural Cell Adhesion Molecule. Tartu, 2008.
149. **Paul Korrovits**. Asymptomatic inflammatory prostatitis: prevalence, etiological factors, diagnostic tools. Tartu, 2008.
150. **Annika Reintam**. Gastrointestinal failure in intensive care patients. Tartu, 2008.
151. **Kristiina Roots**. Cationic regulation of Na-pump in the normal, Alzheimer's and CCK_2 receptor-deficient brain. Tartu, 2008.
152. **Helen Puusepp**. The genetic causes of mental retardation in Estonia: fragile X syndrome and creatine transporter defect. Tartu, 2009.
153. **Kristiina Rull**. Human chorionic gonadotropin beta genes and recurrent miscarriage: expression and variation study. Tartu, 2009.
154. **Margus Eimre**. Organization of energy transfer and feedback regulation in oxidative muscle cells. Tartu, 2009.
155. **Maire Link**. Transcription factors FoxP3 and AIRE: autoantibody associations. Tartu, 2009.
156. **Kai Haldre**. Sexual health and behaviour of young women in Estonia. Tartu, 2009.
157. **Kaur Liivak**. Classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Estonia: incidence, genotype and phenotype with special attention to short-term growth and 24-hour blood pressure. Tartu, 2009.
158. **Kersti Ehrlich**. Antioxidative glutathione analogues (UPF peptides) – molecular design, structure-activity relationships and testing the protective properties. Tartu, 2009.
159. **Anneli Rätsep**. Type 2 diabetes care in family medicine. Tartu, 2009.
160. **Silver Türk**. Etiopathogenetic aspects of chronic prostatitis: role of mycoplasmas, coryneform bacteria and oxidative stress. Tartu, 2009.

161. **Kaire Heilman**. Risk markers for cardiovascular disease and low bone mineral density in children with type 1 diabetes. Tartu, 2009.
162. **Kristi Rüütel**. HIV-epidemic in Estonia: injecting drug use and quality of life of people living with HIV. Tartu, 2009.
163. **Triin Eller**. Immune markers in major depression and in antidepressive treatment. Tartu, 2009.
164. **Siim Suutre**. The role of TGF-β isoforms and osteoprogenitor cells in the pathogenesis of heterotopic ossification. An experimental and clinical study of hip arthroplasty. Tartu, 2010.
165. **Kai Kliiman**. Highly drug-resistant tuberculosis in Estonia: Risk factors and predictors of poor treatment outcome. Tartu, 2010.
166. **Inga Villa**. Cardiovascular health-related nutrition, physical activity and fitness in Estonia. Tartu, 2010.
167. **Tõnis Org**. Molecular function of the first PHD finger domain of Autoimmune Regulator protein. Tartu, 2010.
168. **Tuuli Metsvaht**. Optimal antibacterial therapy of neonates at risk of early onset sepsis. Tartu, 2010.
169. **Jaanus Kahu**. Kidney transplantation: Studies on donor risk factors and mycophenolate mofetil. Tartu, 2010.
170. **Koit Reimand**. Autoimmunity in reproductive failure: A study on associated autoantibodies and autoantigens. Tartu, 2010.
171. **Mart Kull**. Impact of vitamin D and hypolactasia on bone mineral density: a population based study in Estonia. Tartu, 2010.
172. **Rael Laugesaar**. Stroke in children – epidemiology and risk factors. Tartu, 2010.
173. **Mark Braschinsky**. Epidemiology and quality of life issues of hereditary spastic paraplegia in Estonia and implemention of genetic analysis in everyday neurologic practice. Tartu, 2010.
174. **Kadri Suija**. Major depression in family medicine: associated factors, recurrence and possible intervention. Tartu, 2010.
175. **Jarno Habicht**. Health care utilisation in Estonia: socioeconomic determinants and financial burden of out-of-pocket payments. Tartu, 2010.
176. **Kristi Abram**. The prevalence and risk factors of rosacea. Subjective disease perception of rosacea patients. Tartu, 2010.
177. **Malle Kuum**. Mitochondrial and endoplasmic reticulum cation fluxes: Novel roles in cellular physiology. Tartu, 2010.
178. **Rita Teek**. The genetic causes of early onset hearing loss in Estonian children. Tartu, 2010.
179. **Daisy Volmer**. The development of community pharmacy services in Estonia – public and professional perceptions 1993–2006. Tartu, 2010.
180. **Jelena Lissitsina**. Cytogenetic causes in male infertility. Tartu, 2011.
181. **Delia Lepik**. Comparison of gunshot injuries caused from Tokarev, Makarov and Glock 19 pistols at different firing distances. Tartu, 2011.
182. **Ene-Renate Pähkla**. Factors related to the efficiency of treatment of advanced periodontitis. Tartu, 2011.

183. **Maarja Krass**. L-Arginine pathways and antidepressant action. Tartu, 2011.
184. **Taavi Lai**. Population health measures to support evidence-based health policy in Estonia. Tartu, 2011.
185. **Tiit Salum**. Similarity and difference of temperature-dependence of the brain sodium pump in normal, different neuropathological, and aberrant conditions and its possible reasons. Tartu, 2011.
186. **Tõnu Vooder**. Molecular differences and similarities between histological subtypes of non-small cell lung cancer. Tartu, 2011.
187. **Jelena Štšepetova**. The characterisation of intestinal lactic acid bacteria using bacteriological, biochemical and molecular approaches. Tartu, 2011.
188. **Radko Avi**. Natural polymorphisms and transmitted drug resistance in Estonian HIV-1 CRF06_cpx and its recombinant viruses. Tartu, 2011, 116 p.
189. **Edward Laane**. Multiparameter flow cytometry in haematological malignancies. Tartu, 2011, 152 p.
190. **Triin Jagomägi**. A study of the genetic etiology of nonsyndromic cleft lip and palate. Tartu, 2011, 158 p.
191. **Ivo Laidmäe**. Fibrin glue of fish (*Salmo salar*) origin: immunological study and development of new pharmaceutical preparation. Tartu, 2012, 150 p.
192. **Ülle Parm**. Early mucosal colonisation and its role in prediction of invasive infection in neonates at risk of early onset sepsis. Tartu, 2012, 168 p.
193. **Kaupo Teesalu**. Autoantibodies against desmin and transglutaminase 2 in celiac disease: diagnostic and functional significance. Tartu, 2012, 142 p.
194. **Maksim Zagura**. Biochemical, functional and structural profiling of arterial damage in atherosclerosis. Tartu, 2012, 162 p.
195. **Vivian Kont**. Autoimmune regulator: characterization of thymic gene regulation and promoter methylation. Tartu, 2012, 134 p.
196. **Pirje Hütt**. Functional properties, persistence, safety and efficacy of potential probiotic lactobacilli. Tartu, 2012, 246 p.
197. **Innar Tõru**. Serotonergic modulation of CCK-4- induced panic. Tartu, 2012, 132 p.
198. **Sigrid Vorobjov**. Drug use, related risk behaviour and harm reduction interventions utilization among injecting drug users in Estonia: implications for drug policy. Tartu, 2012, 120 p.
199. **Martin Serg**. Therapeutic aspects of central haemodynamics, arterial stiffness and oxidative stress in hypertension. Tartu, 2012, 156 p.
200. **Jaanika Kumm**. Molecular markers of articular tissues in early knee osteoarthritis: a population-based longitudinal study in middle-aged subjects. Tartu, 2012, 159 p.
201. **Kertu Rünkorg**. Functional changes of dopamine, endopioid and endocannabinoid systems in CCK2 receptor deficient mice. Tartu, 2012, 125 p.
202. **Mai Blöndal**. Changes in the baseline characteristics, management and outcomes of acute myocardial infarction in Estonia. Tartu, 2012, 127 p.

203. **Jana Lass**. Epidemiological and clinical aspects of medicines use in children in Estonia. Tartu, 2012, 170 p.
204. **Kai Truusalu**. Probiotic lactobacilli in experimental persistent *Salmonella* infection. Tartu, 2013, 139 p.
205. **Oksana Jagur**. Temporomandibular joint diagnostic imaging in relation to pain and bone characteristics. Long-term results of arthroscopic treatment. Tartu, 2013, 126 p.
206. **Katrin Sikk**. Manganese-ephedrone intoxication – pathogenesis of neurological damage and clinical symptomatology. Tartu, 2013, 125 p.
207. **Kai Blöndal**. Tuberculosis in Estonia with special emphasis on drug-resistant tuberculosis: Notification rate, disease recurrence and mortality. Tartu, 2013, 151 p.
208. **Marju Puurand**. Oxidative phosphorylation in different diseases of gastric mucosa. Tartu, 2013, 123 p.
209. **Aili Tagoma**. Immune activation in female infertility: Significance of autoantibodies and inflammatory mediators. Tartu, 2013, 135 p.
210. **Liis Sabre**. Epidemiology of traumatic spinal cord injury in Estonia. Brain activation in the acute phase of traumatic spinal cord injury. Tartu, 2013, 135 p.
211. **Merit Lamp**. Genetic susceptibility factors in endometriosis. Tartu, 2013, 125 p.
212. **Erik Salum**. Beneficial effects of vitamin D and angiotensin II receptor blocker on arterial damage. Tartu, 2013, 167 p.
213. **Maire Karelson**. Vitiligo: clinical aspects, quality of life and the role of melanocortin system in pathogenesis. Tartu, 2013, 153 p.
214. **Kuldar Kaljurand**. Prevalence of exfoliation syndrome in Estonia and its clinical significance. Tartu, 2013, 113 p.
215. **Raido Paasma**. Clinical study of methanol poisoning: handling large outbreaks, treatment with antidotes, and long-term outcomes. Tartu, 2013, 96 p.
216. **Anne Kleinberg**. Major depression in Estonia: prevalence, associated factors, and use of health services. Tartu, 2013, 129 p.
217. **Triin Eglit**. Obesity, impaired glucose regulation, metabolic syndrome and their associations with high-molecular-weight adiponectin levels. Tartu, 2014, 115 p.
218. **Kristo Ausmees**. Reproductive function in middle-aged males: Associations with prostate, lifestyle and couple infertility status. Tartu, 2014, 125 p.
219. **Kristi Huik**. The influence of host genetic factors on the susceptibility to HIV and HCV infections among intravenous drug users. Tartu, 2014, 144 p.
220. **Liina Tserel**. Epigenetic profiles of monocytes, monocyte-derived macrophages and dendritic cells. Tartu, 2014, 143 p.
221. **Irina Kerna**. The contribution of *ADAM12* and *CILP* genes to the development of knee osteoarthritis. Tartu, 2014, 152 p.

222. **Ingrid Liiv**. Autoimmune regulator protein interaction with DNA-dependent protein kinase and its role in apoptosis. Tartu, 2014, 143 p.
223. **Liivi Maddison**. Tissue perfusion and metabolism during intra-abdominal hypertension. Tartu, 2014, 103 p.
224. **Krista Ress**. Childhood coeliac disease in Estonia, prevalence in atopic dermatitis and immunological characterisation of coexistence. Tartu, 2014, 124 p.
225. **Kai Muru**. Prenatal screening strategies, long-term outcome of children with marked changes in maternal screening tests and the most common syndromic heart anomalies in Estonia. Tartu, 2014, 189 p.
226. **Kaja Rahu**. Morbidity and mortality among Baltic Chernobyl cleanup workers: a register-based cohort study. Tartu, 2014, 155 p.
227. **Klari Noormets**. The development of diabetes mellitus, fertility and energy metabolism disturbances in a Wfs1-deficient mouse model of Wolfram syndrome. Tartu, 2014, 132 p.
228. **Liis Toome**. Very low gestational age infants in Estonia. Tartu, 2014, 183 p.
229. **Ceith Nikkolo**. Impact of different mesh parameters on chronic pain and foreign body feeling after open inguinal hernia repair. Tartu, 2014, 132 p.
230. **Vadim Brjalin**. Chronic hepatitis C: predictors of treatment response in Estonian patients. Tartu, 2014, 122 p.
231. **Vahur Metsna**. Anterior knee pain in patients following total knee arthroplasty: the prevalence, correlation with patellar cartilage impairment and aspects of patellofemoral congruence. Tartu, 2014, 130 p.
232. **Marju Kase**. Glioblastoma multiforme: possibilities to improve treatment efficacy. Tartu, 2015, 137 p.
233. **Riina Runnel**. Oral health among elementary school children and the effects of polyol candies on the prevention of dental caries. Tartu, 2015, 112 p.
234. **Made Laanpere**. Factors influencing women's sexual health and reproductive choices in Estonia. Tartu, 2015, 176 p.
235. **Andres Lust**. Water mediated solid state transformations of a polymorphic drug – effect on pharmaceutical product performance. Tartu, 2015, 134 p.
236. **Anna Klugman**. Functionality related characterization of pretreated wood lignin, cellulose and polyvinylpyrrolidone for pharmaceutical applications. Tartu, 2015, 156 p.
237. **Triin Laisk-Podar**. Genetic variation as a modulator of susceptibility to female infertility and a source for potential biomarkers. Tartu, 2015, 155 p.
238. **Mailis Tõnisson**. Clinical picture and biochemical changes in blood in children with acute alcohol intoxication. Tartu, 2015, 100 p.
239. **Kadri Tamme**. High volume haemodiafiltration in treatment of severe sepsis – impact on pharmacokinetics of antibiotics and inflammatory response. Tartu, 2015, 133 p.

240. **Kai Part**. Sexual health of young people in Estonia in a social context: the role of school-based sexuality education and youth-friendly counseling services. Tartu, 2015, 203 p.
241. **Urve Paaver**. New perspectives for the amorphization and physical stabilization of poorly water-soluble drugs and understanding their dissolution behavior. Tartu, 2015, 139 p.
242. **Aleksandr Peet**. Intrauterine and postnatal growth in children with HLA-conferred susceptibility to type 1 diabetes. Tartu. 2015, 146 p.
243. **Piret Mitt**. Healthcare-associated infections in Estonia – epidemiology and surveillance of bloodstream and surgical site infections. Tartu, 2015, 145 p.
244. **Merli Saare**. Molecular Profiling of Endometriotic Lesions and Endometria of Endometriosis Patients. Tartu, 2016, 129 p.
245. **Kaja-Triin Laisaar**. People living with HIV in Estonia: Engagement in medical care and methods of increasing adherence to antiretroviral therapy and safe sexual behavior. Tartu, 2016, 132 p.
246. **Eero Merilind**. Primary health care performance: impact of payment and practice-based characteristics. Tartu, 2016, 120 p.
247. **Jaanika Kärner**. Cytokine-specific autoantibodies in AIRE deficiency. Tartu, 2016, 182 p.
248. **Kaido Paapstel**. Metabolomic profile of arterial stiffness and early biomarkers of renal damage in atherosclerosis. Tartu, 2016, 173 p.
249. **Liidia Kiisk**. Long-term nutritional study: anthropometrical and clinico-laboratory assessments in renal replacement therapy patients after intensive nutritional counselling. Tartu, 2016, 207 p.
250. **Georgi Nellis**. The use of excipients in medicines administered to neonates in Europe. Tartu, 2017, 159 p.
251. **Aleksei Rakitin**. Metabolic effects of acute and chronic treatment with valproic acid in people with epilepsy. Tartu, 2017, 125 p.
252. **Eveli Kallas**. The influence of immunological markers to susceptibility to HIV, HBV, and HCV infections among persons who inject drugs. Tartu, 2017, 138 p.
253. **Tiina Freimann**. Musculoskeletal pain among nurses: prevalence, risk factors, and intervention. Tartu, 2017, 125 p.
254. **Evelyn Aaviksoo**. Sickness absence in Estonia: determinants and influence of the sick-pay cut reform. Tartu, 2017, 121 p.
255. **Kalev Nõupuu**. Autosomal-recessive Stargardt disease: phenotypic heterogeneity and genotype-phenotype associations. Tartu, 2017, 131 p.
256. **Ho Duy Binh**. Osteogenesis imperfecta in Vietnam. Tartu, 2017, 125 p.
257. **Uku Haljasorg**. Transcriptional mechanisms in thymic central tolerance. Tartu, 2017, 147 p.
258. **Živile Riispere**. IgA Nephropathy study according to the Oxford Classification: IgA Nephropathy clinical-morphological correlations, disease progression and the effect of renoprotective therapy. Tartu, 2017, 129 p.

259. **Hiie Soeorg**. Coagulase-negative staphylococci in gut of preterm neonates and in breast milk of their mothers. Tartu, 2017, 216 p.
260. **Anne-Mari Anton Willmore**. Silver nanoparticles for cancer research. Tartu, 2017, 132 p.
261. **Ott Laius**. Utilization of osteoporosis medicines, medication adherence and the trend in osteoporosis related hip fractures in Estonia. Tartu, 2017, 134 p.
262. **Alar Aab**. Insights into molecular mechanisms of asthma and atopic dermatitis. Tartu, 2017, 164 p.
263. **Sander Pajusalu**. Genome-wide diagnostics of Mendelian disorders: from chromosomal microarrays to next-generation sequencing. Tartu, 2017, 146 p.
264. **Mikk Jürisson**. Health and economic impact of hip fracture in Estonia. Tartu, 2017, 164 p.
265. **Kaspar Tootsi**. Cardiovascular and metabolomic profiling of osteoarthritis. Tartu, 2017, 150 p.
266. **Mario Saare**. The influence of AIRE on gene expression – studies of transcriptional regulatory mechanisms in cell culture systems. Tartu, 2017, 172 p.
267. **Piia Jõgi**. Epidemiological and clinical characteristics of pertussis in Estonia. Tartu, 2018, 168 p.
268. **Elle Põldoja**. Structure and blood supply of the superior part of the shoulder joint capsule. Tartu, 2018, 116 p.
269. **Minh Son Nguyen**. Oral health status and prevalence of temporomandibular disorders in 65–74-year-olds in Vietnam. Tartu, 2018, 182 p.
270. **Kristian Semjonov**. Development of pharmaceutical quench-cooled molten and melt-electrospun solid dispersions for poorly water-soluble indomethacin. Tartu, 2018, 125 p**.**
271. **Janne Tiigimäe-Saar**. Botulinum neurotoxin type A treatment for sialorrhea in central nervous system diseases. Tartu, 2018, 109 p.
272. **Veiko Vengerfeldt.** Apical periodontitis: prevalence and etiopathogenetic aspects. Tartu, 2018, 150 p.
273. **Rudolf Bichele.** TNF superfamily and AIRE at the crossroads of thymic differentiation and host protection against *Candida albicans* infection. Tartu, 2018, 153 p.
274. **Olga Tšuiko**. Unravelling Chromosomal Instability in Mammalian Preimplantation Embryos Using Single-Cell Genomics. Tartu, 2018, 169 p.
275. **Kärt Kriisa.** Profile of acylcarnitines, inflammation and oxidative stress in first-episode psychosis before and after antipsychotic treatment. Tartu, 2018, 145 p.
276. **Xuan Dung Ho.** Characterization of the genomic profile of osteosarcoma. Tartu, 2018, 144 p.
277. **Karit Reinson.** New Diagnostic Methods for Early Detection of Inborn Errors of Metabolism in Estonia. Tartu, 2018, 201 p.

278. **Mari-Anne Vals.** Congenital N-glycosylation Disorders in Estonia. Tartu, 2019, 148 p.
279. **Liis Kadastik-Eerme**. Parkinson's disease in Estonia: epidemiology, quality of life, clinical characteristics and pharmacotherapy. Tartu, 2019, 202 p.
280. **Hedi Hunt**. Precision targeting of intraperitoneal tumors with peptide-guided nanocarriers. Tartu, 2019, 179 p.
281. **Rando Porosk**. The role of oxidative stress in Wolfram syndrome 1 and hypothermia. Tartu, 2019, 123 p.
282. **Ene-Ly Jõgeda**. The influence of coinfections and host genetic factor on the susceptibility to HIV infection among people who inject drugs. Tartu, 2019, 126 p.
283. **Kristel Ehala-Aleksejev**. The associations between body composition, obesity and obesity-related health and lifestyle conditions with male reproductive function. Tartu, 2019, 138 p.
284. **Aigar Ottas**. The metabolomic profiling of psoriasis, atopic dermatitis and atherosclerosis. Tartu, 2019, 136 p.
285. **Elmira Gurbanova.** Specific characteristics of tuberculosis in low default, but high multidrug–resistance prison setting. Tartu, 2019, 129 p.
286. **Van Thai Nguyeni.** The first study of the treatment outcomes of patients with cleft lip and palate in Central Vietnam. Tartu, 2019, 144 p.
287. **Maria Yakoreva**. Imprinting Disorders in Estonia. Tartu, 2019, 187 p.
288. **Kadri Rekker**. The putative role of microRNAs in endometriosis pathogenesis and potential in diagnostics. Tartu, 2019, 140 p.
289. **Ülle Võhma**. Association between personality traits, clinical characteristics and pharmacological treatment response in panic disorder. Tartu, 2019, 121 p.
290. **Aet Saar**. Acute myocardial infarction in Estonia 2001–2014: towards risk-based prevention and management. Tartu, 2019, 124 p.
291. **Toomas Toomsoo**. Transcranial brain sonography in the Estonian cohort of Parkinson's disease. Tartu, 2019, 114 p.
292. **Lidiia Zhytnik**. Inter- and intrafamilial diversity based on genotype and phenotype correlations of Osteogenesis Imperfecta. Tartu, 2019, 224 p.
293. **Pilleriin Soodla.** Newly HIV-infected people in Estonia: estimation of incidence and transmitted drug resistance. Tartu, 2019, 194 p.
294. **Kristiina Ojamaa.** Epidemiology of gynecological cancer in Estonia. Tartu, 2020, 133 p.
295. **Marianne Saard.** Modern Cognitive and Social Intervention Techniques in Paediatric Neurorehabilitation for Children with Acquired Brain Injury. Tartu, 2020, 168 p.
296. **Julia Maslovskaja.** The importance of DNA binding and DNA breaks for AIRE-mediated transcriptional activation. Tartu, 2020, 162 p.
297. **Natalia Lobanovskaya.** The role of PSA-NCAM in the survival of retinal ganglion cells. Tartu, 2020, 105 p.

298. **Madis Rahu.** Structure and blood supply of the postero-superior part of the shoulder joint capsule with implementation of surgical treatment after anterior traumatic dislocation. Tartu, 2020, 104 p.
299. **Helen Zirnask**. Luteinizing hormone (LH) receptor expression in the penis and its possible role in pathogenesis of erectile disturbances. Tartu, 2020, 87 p.
300. **Kadri Toome.** Homing peptides for targeting of brain diseases. Tartu, 2020, 152 p.