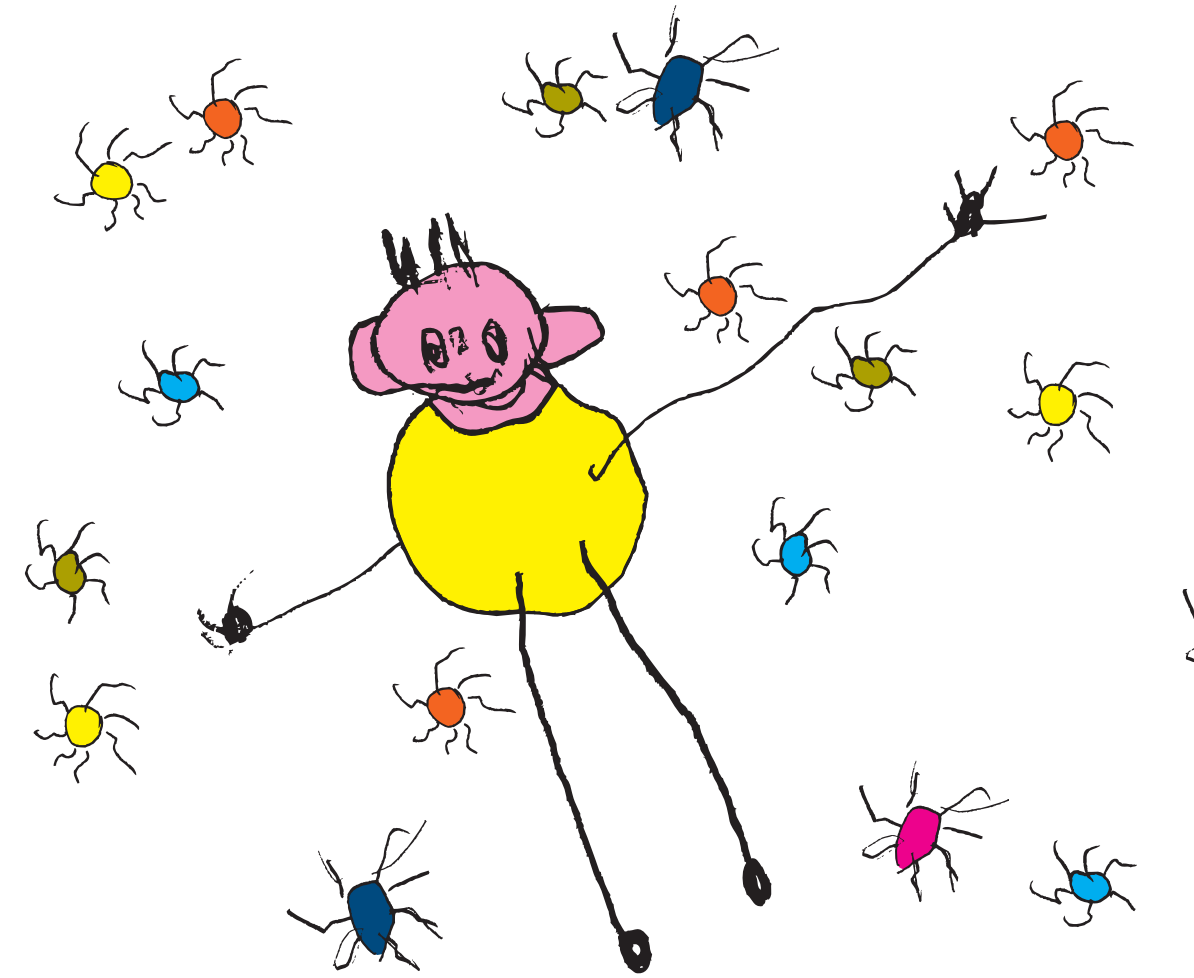
Impact of critical illness and cardiopulmonary bypass on antibiotic disposition in children



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Impact of critical illness and cardiopulmonary bypass on antibiotic disposition in children

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Take each man's censure, but reserve thy judgment
William Shakespeare

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LIST OF ABBREVIATIONS

A

ACE	Angiotensin converting enzyme
ADME	Absorption, distribution, metabolism, excretion
ADR	Alternative dosing regimen
AIC	Akaike's information criterion
AKI	Acute kidney injury
ARC	Augmented renal clearance
AUC	Area under the concentration-time curve

B

BLI	β-lactamase inhibitor
BNFc	British National Formulary for Children
BOV	Between-occasion variability
BQL	Below quantification limit
BSV	Between-subject variability

C

CD	Continuous dosing
CHMP	Committee for Medicinal Products for Human Use
CI	Continuous infusion
CKD	Chronic kidney disease
CL_i	Individual clearance
CL_{pop}	Population clearance
CLSI	Clinical and Laboratory Standards Institute
C_{max}	Peak concentration
CPB	Cardiopulmonary bypass
CrCL	Creatinine clearance
CRP	C-Reactive protein
CV	Coefficient of variation
CWRES	Conditional weighted residuals
CYP	Cytochrome P-450
CysC	Cystatin C

D

DDI	drug-drug interaction
DME	drug metabolising enzym

E

EC	Ethics committee
ECMO	Extracorporeal membrane oxygenation
eGFR	Estimated GFR
EMA	European Medicines Agency
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EU	European Union
EudraCT	European clinical trials database

F

F	Bioavailability
*f*AUC	*Free (or unbound*) AUC
FO	First-order
FOCE-I	First-order conditional estimation method with the interaction option
$fT_{>MIC}$	time during which the unbound concentration is above the MIC of the pathogen
f_u	Free or unbound fraction

G

GFR	Glomerular filtration rate

I

ICH	International Council for Harmonisation
ICU	Intensive care unit
ID	Intermittent dosing
IOV	Interoccasion variability
IQR	Interquartile range

K

K_a	Acid dissociation constant
K_D	Dissociation constant

L

LD	Loading dose
LLOQ	Lower limit of quantification
LOQ	Limit of quantification

M

MIC	Minimal inhibitory concentration

MM	Michaelis-Menten
MRSA	Methicillin-resistent *Staphylococcus aureus*

N

NPDE	Normalised prediction distribution error
NICU	Neonatal intensive care unit

O

OFV	Objective function value

P

PBPK	Physiologically-based pharmacokinetics
pcVPC	Prediction-corrected visual predictive check
PD	Pharmacodynamic(s)
PDCO	Paediatric Committee
PELOD	Pediatric logistic organ dysfunction
PICU	Paediatric intensive care unit
PIP	Paediatric investigation plan
PK	Pharmacokinetic(s)
pK_a	Negative logarithm of acid dissociation constant
PMA	Postmenstrual age
pRIFLE	Paediatric risk, injury, failure, loss, end stage renal disease
PRISM	Paediatric risk of mortality
PTA	Probability of target attainment
PUMA	Paediatric-use marketing authorisation

Q

Q	Intercompartmental clearance

R

RCT	Randomised controlled trial
RRT	Renal replacement therapy
RSE	Relative standard error

S

SCM	Stepwise covariate model
Scr	Serum creatinine
SD	Standard deviation
SDR	Study dosing regimen

SSI	Surgical site infection

T

TDM	Therapeutic drug monitoring
TM_{50}	Maturation half-life

U

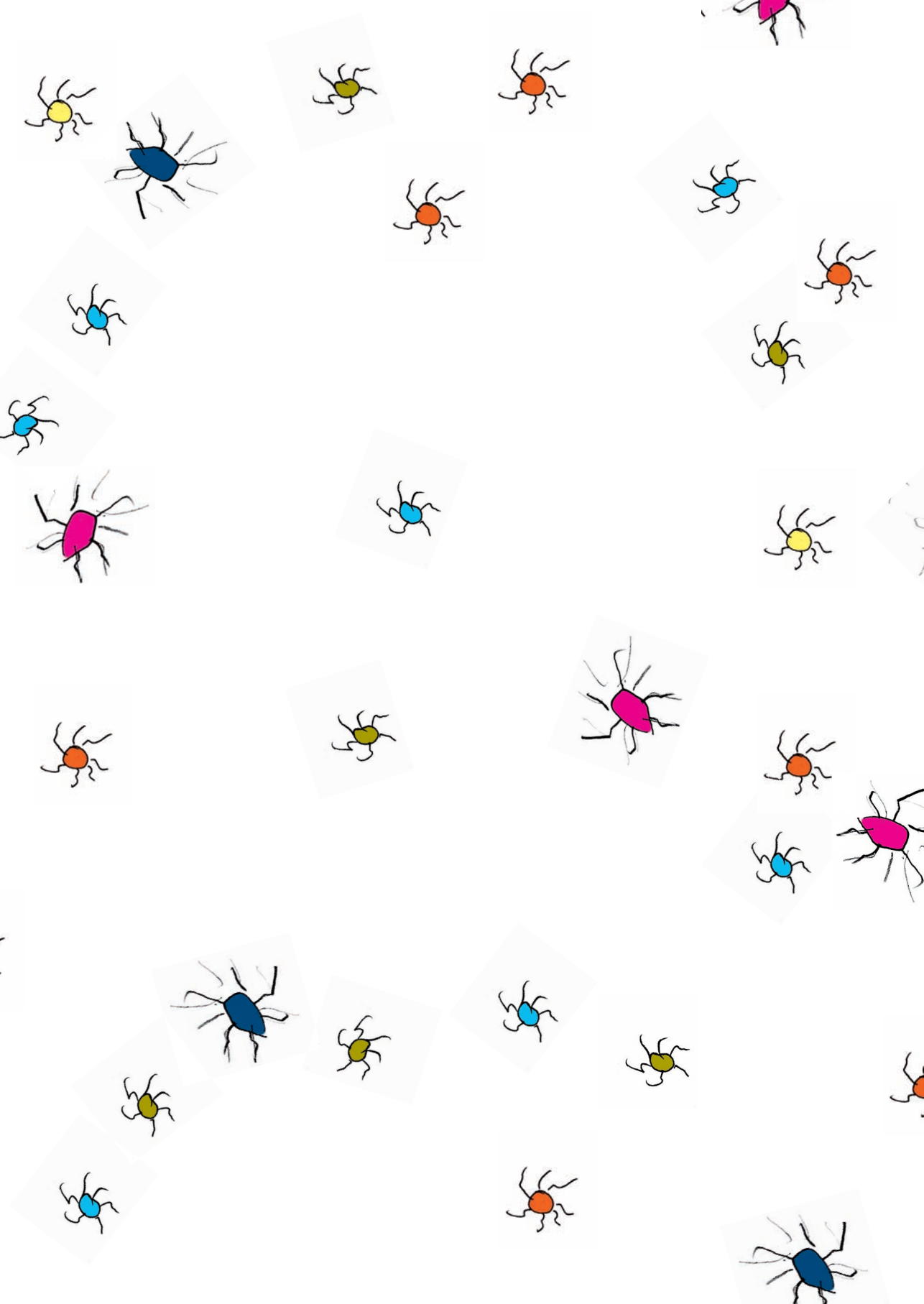
Ucr	Urine creatinine
UGT	Uridine-5′-diphospho-glucuronosyltransferase
UPLC	Ultrahigh-pressure liquid chromatography

V

VPC	Visual predictive check
Vd	Volume of distribution

W

WT	Weight
WTmed	Median weight



CHAPTER 1

GENERAL INTRODUCTION

1. BACKGROUND

In Belgium, the annual admission number to the intensive care unit (ICU) is around 5000 newborns and 4000 paediatric patients (infants, toddlers, children and adolescents till 15 years of age)*.[1,2] Infections affect 30% of those children, with a 25% mortality rate in the presence of severe sepsis and septic shock.[3] This high number is not surprising since seriously ill children are predisposed to develop infections due to their risk factor profile with invasive devices, a prolonged hospital stay, frequent surgical procedures, an immature immune response, immunoparesis due to drugs or procedures, and prior antibiotic therapy.[4,5] Consequently, antimicrobial agents are amongst the most commonly prescribed drugs on the neonatal and paediatric ICU.[6,7]

In this introductory chapter, we first discuss the impact of maturation and pathophysiological changes on the pharmacokinetics (PK) of antibiotics in children*. Second, we focus on PK changes in children using extracorporeal techniques. This is followed by a section considering the distinct pharmacodynamic (PD) properties of antibiotics. Fourth, we discuss the problem of off-label and unlicensed use in a neonatal and paediatric intensive care environment. Next, a concise overview of the European initiative to improve paediatric drug development is given. In the final part of the chapter, we consider modelling and simulation strategies to optimize antibiotic dosing in critically ill children.

2. ALTERED DISPOSITION IN THE CRITICALLY ILL PAEDIATRIC PATIENT

PK describes the drug concentration over time (*'what the body does to the drug'*) at a specific site (e.g. blood, cerebrospinal fluid) and is described by its **a**bsorption, **d**istribution and elimination through **m**etabolism or **e**xcretion (ADME) .

In healthy children, drug disposition is driven by physiological processes of growth and development. From birth up to adulthood, every child is prone to maturational changes in body composition, drug metabolising enzymes, cardiac output, blood flow and function of drug eliminating organs (liver and kidneys) **(Figure 1)**.[9] Furthermore, in children admitted to the ICU, pathophysiological and treatment induced changes can also lead to alterations in PK **(Figure 2)**.[10]

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* paediatric age groups according to the classification of the International Council for Harmonisation (ICH) : preterm newborns (less than 36 weeks of gestation), term newborns (0 to 27 days), infants and toddlers (28 days to 23 months), children (2 to 11 years), and adolescents (12-16/18 years).[8]

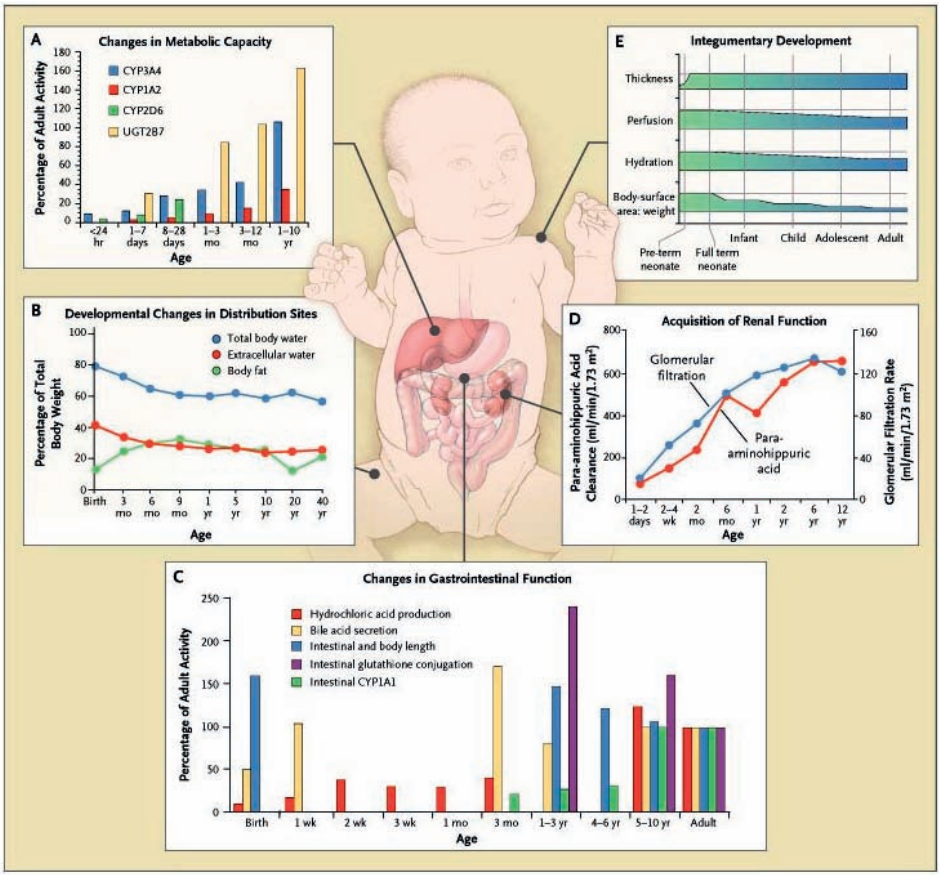

Figure 1. Developmental changes in physiological factors that influence drug disposition in infants, children, and adolescents. Physiological changes in multiple organs and organ systems during development are responsible for age-related differences in drug disposition. CYP, cytochrome P-450; UGT, glucuronosyltransferase (UGT); wk, week; mo, month; yr, year. (Reproduced with permission from Kearns G. *et al.*[9], Copyright Massachusetts Medical Society)

The ADME properties and the extent to which they vary between ICU and non-ICU patients receiving antibiotics are briefly discussed here.

2.1. Absorption

Absorption is the process of drug transport from the site of administration to the systemic circulation. The extent of absorption is described by bioavailability (F), the fraction of the administered dose of unchanged drug reaching the systemic circulation. If a drug is administered intravenously, F is 100%, for other routes, this is between 0 and 100%. Drug and patient specific factors are responsible for the rate and magnitude of absorption. Main drug specific factors include: particle size, solubility and dissolution rate, lipophilicity, ionisation state, acid dissociation constant of the drug. [11]

The main patient determinants for the rate and extent of enteral absorption are gastric emptying time and intestinal motility, gastrointestinal pH, splanchnic blood flow, intestinal secretion, absorption surface area and drug metabolism at the intestinal epithelium, of which maturational and pathophysiological changes are sequentially discussed below.

Gastric emptying matures over a period of 6-8 months to an adult level.[11] Furthermore, antral contractions and intestinal motor activity increase during the first weeks of life with possible consequences on enteral absorption. Delayed gastric emptying is estimated to be present in 50% of critically ill children, especially in neonates and infants where a developmental pattern further strengthens this phenomenon as described above.[12,13] Gastroparesis may occur as a side effect of opioids, while the use of naso-duodenal gavage feeding will bypass gastric effects.[14] Similarly, chronic kidney disease can also delay gastric emptying time through visceral neuropathy.[15] No studies have specifically evaluated the effect of developmental and disease related changes in gastric emptying and intestinal motility on absorption of antibiotics in children. Although, delayed and incomplete absorption in neonates and small infants have been suggested for amoxicillin, rifampicin and chloramphenicol.[16] In general, one could hypothesise that delayed gastric emptying leads to a delayed and more blunted peak concentration compared to a patient without delayed emptying. The clinical relevance of this depends on the concentration-effect profile of the compound. If a minimum effective concentration has to be reached, the effect could be delayed.[11]

Although there are no significant differences between neonates (a few hours after delivery), infants, toddlers, children, adolescents and adults in baseline gastric pH, one should consider that pH will rise postprandially as milk and feedings in general have a buffering effect. As a consequence, during the day, neonates and children on continuous enteral feeding tend to more often have a basic gastric environment.[17] These changes in gastric pH are important for acid-labile drugs like penicillin G which can be absorbed more efficiently in a higher gastric pH environment. Huang *et al*. showed that neonates tend to have a higher bioavailability of penicillin G as compared to older children.[18] Similar effects may occur in the case of stress ulcer prophylaxis with pH modulating agents, which are commonly prescribed on the ICU. Little is known about the age-related changes in intestinal pH.

An age-related increase in splanchnic blood flow has been observed over the first three weeks of life which may influence absorption rates.[9] Circulatory dysfunction in cardiac failure (e.g. during paediatric sepsis and septic shock) leads to shunting of blood flow towards the vital organs like brain and heart, at the expense of other peripheral tis-

sues (e.g. muscles, skin and splanchnic organs, kidney, liver). This cardiovascular failure results in a decreased forward flow (reduced tissue perfusion) and an increased back pressure (congestion) (e.g. in the gut circulation). Vasopressors and inotropes are often used in haemodynamically unstable children and are known to alter splanchnic perfusion. Although several animal and clinical studies have assessed the gut-specific effects of these vasoactive drugs, it is not really known whether these effects are beneficial or detrimental in terms of gut perfusion and at what specific dose they occur.[19–29] King *et al*. evaluated in a retrospective manner the tolerance of enteral feeding in patients admitted to the paediatric ICU receiving cardiovascular medication. Dopamine was the most commonly used vasopressor. Twenty-nine per cent of patients had feedings withheld for a perceived gastrointestinal intolerance.[30] In another study epinephrine at a dose more than 0.3 µg/kg/min was identified to be a significant factor for gastrointestinal complications in critically ill children receiving transpyloric enteral nutrition.[13] One of the explanations for this gastrointestinal intolerance could be a body's failure to meet the higher splanchnic metabolic demands when the gut is hypoperfused.[12] To the best of our knowledge, there are no studies available investigating the impact of impaired splanchnic perfusion on drug absorption in children, although it is hypothesised that drug absorption from these sites can be erratic.

Lipophilic antibiotics given enterally need biliary salts to be absorbed. One could speculate that due to maturation of conjugation and transport of bile salts up to the age of 4, absorption of these antibiotics increases with age **(Figure 1)**. Patients in the NICU/PICU are at risk for cholestasis and acute cholecystitis due to the presence of sepsis, surgery and total parenteral nutrition.[31,32] Also due to polypharmacy, NICU/PICU patients are susceptible for drug-induced cholestasis.[33] Evidently, these factors may lead to a reduced absorption of lipophilic antibiotics.

Villous formation normally ends at 20 weeks of gestation, rendering absorptive surface after birth unlikely to alter drug absorption.[17,34] ICU patients are often withheld from enteral feeding due to intolerance. It is known that starvation and diarrhoea result in intestinal atrophy and it is therefore likely that cellular dysfunction in the gut potentially leads to altered intestinal drug absorption.[36] The knowledge of ontogeny on intestinal membrane transporters with regard to drug absorption is scarce. *Ex vivo*, pharmacokinetic and pharmacogenetic studies suggest transporter-specific changes from human fetus to the adult. To date, no clear maturation patterns have been identified.[35] Diarrhoea may also compromise intestinal transporter activity.[35]

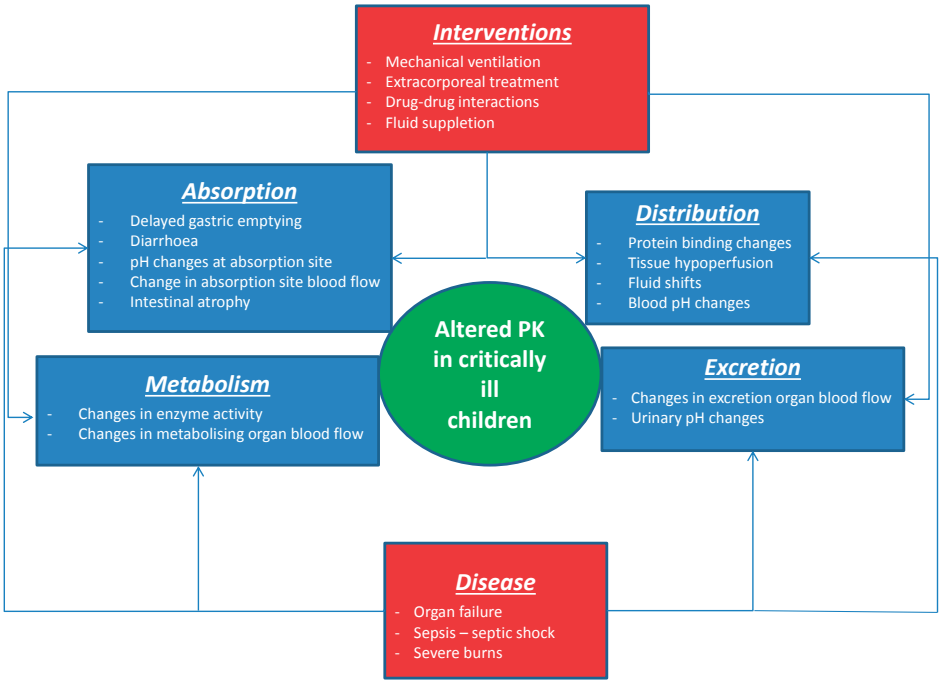

Figure 2. Major pathophysiological and treatment factors related to PK changes in critically ill children

Due to this expected high interpatient variability in absorption processes, intravenous drug administration is the preferred route in critically ill children in order to avoid compromised drug bio-availability.[14]

2.2. Distribution

The apparent volume of distribution is a theoretical measure of the extent to which a drug will migrate into extravascular tissues.[37] It is affected by developmental and pathophysiological changes in body composition and tissue permeability, protein binding, regional blood flow and membrane transporters .[9,38]

The younger the child the higher the extracellular and total body water content **(Figure 1)**, resulting in higher volumes of distribution (Vd) and lower (peak) concentrations of water soluble drugs (e.g. aminoglycosides, vancomycin, β-lactam antibiotics) when administered on a mg/kg basis. Nielsen *et al.* observed that preterm neonates had a significantly larger central Vd per kg bodyweight than term neonates.[39] Increased capillary permeability, increased hydrostatic pressure, or decreased plasma oncotic pressure due to hypoproteinemia are commonly encountered in the neonatal and paediatric intensive care unit (NICU/PICU) and may further increase the distribution volume (e.g. oedema). Lingvall *et al*. documented that the Vd of gentamicin was significantly higher

in blood culture confirmed septic neonates compared to non-septic cases.[40] Also, in children with severe burns, increased capillary permeability usually leads to an increased Vd, as shown for vancomycin and amikacin.[41,42]

Similarly, maturational changes in the overall plasma binding protein pool will have an impact on the unbound fraction of the drug and, therefore, the ability of drug to migrate into tissues. The most important plasma proteins for drug binding are albumin and the acute phase reactant α-1 acid glycoprotein. Albumin preferentially binds acidic molecules whereas α-1 acid glycoprotein tends to bind compounds with basic moieties. Plasma albumin concentrations and binding capacity will reach adult levels around the end of infancy (~2 years of age).[9] Smits *et al.* recently evaluated protein binding of the highly protein-bound antibiotic cefazolin in postoperative neonates. As expected, the median unbound cefazolin fraction was higher than in adults.[43] In states of severe illness, hypoproteinemia (<61 g/L) and hypoalbuminemia (<33 g/L) are frequently observed in children and are the result of a number of mechanisms such as increased protein catabolism, capillary permeability and decreased production due to liver failure. In contrast, α-1 acid glycoprotein levels often increase during periods of critical illness.[44,45] Besides protein concentration, the binding affinity of antibiotic to plasma proteins also depends on conformational changes of the protein molecule. These changes can be induced by changes in pH and urea concentration, phenomena likely to occur in critical illness.[46] Likewise, changes in the ionised fraction of the drug (depending on the pKa) due to pH changes may alter plasma protein binding, tissue distribution, and thus Vd.

Competitive binding of co-administered drugs or endogenous substances (e.g. bilirubin, free fatty acids) may also have an impact on the degree of drug-protein binding. In preterm neonates, competitive binding of antibiotics (e.g. ceftriaxone, cefazolin) and bilirubin to albumin has been described.[47,48] As a clinical consequence, the highly albumin-bound antibiotic ceftriaxone is currently contraindicated in neonates until a postmenstrual age of 41 weeks because of displacement of unconjugated bilirubin which could potentially result in kernicterus.[48,49] Likewise, in the case of liver failure due to critical illness, hyperbilirubinemia may induce similar competitive binding interactions.

Cardiac output undergoes a developmental decrease until adolescence.[9] Redistribution of blood during cardiac failure, as described in the absorption section, results in a reduced Vd with decreased delivery of hydrophilic drugs to the capillary system and poor peripheral tissue penetration. In a study by Joukhadar *et al.*, this resulted in a five to tenfold decrease of piperacillin distribution into fat and muscle tissues in adults. [50]

Of special note, the blood-brain barrier matures until the age of 6 months and is more permeable in the presence of inflammation. Both factors have a potential impact on antibiotic disposition when treating central nervous system infections.[51] Also, the effect of membrane transporters in the developing BBB and blood-cerebrospinal barrier (e.g. P-glycoproteine P) may play an important role in the distribution of antibiotics.[52,53]

2.3. Metabolism

Drug metabolism is the process by which a drug undergoes biotransformation to a moiety that is more readily eliminated from the body. Typically, drug metabolites are more polar, water-soluble molecules than the parent drug molecule, and often they are biologically inactive.[54] However, some molecules are prodrugs and need to be active metabolites to exert their effect, other drugs are metabolised to molecules with a higher potency than the mother molecule. The liver and intestine are the main organs where drug metabolism occurs but also kidney and lungs may be involved.[54]

Drug metabolism reactions are classified as phase I and phase II reactions and generally occur sequentially. Phase I reactions are typically modification reactions (oxidation, reduction, hydrolysis) rendering the compound more polar. Phase II reactions are conjugation reactions with hydrophilic molecules (e.g. glucuronidation, acetylation, sulphation). Most important group of enzymes involved in phase I reactions are the cytochrome P450 iso-enzymes; alcohol hydrogenase and flavine mono-oxygenase enzymes are other phase I reactions involved in drug metabolism.[54]

Maturational trends in drug metabolising enzyme (DME) activity have been classified into three distinct groups: (i) a group of enzymes that are higly active before birth but declining shortly thereafter; (ii) a group of enzymes of which the activity rises during the first 2 years after birth; (iii) a group of enzymes of which the activity remains constant throughout life (**Figure 3**).[54] Age-related changes may affect drug effect and toxicity and need to be considered when dosing antibiotics which undergo drug metabolism (e.g. chloramphenicol toxicity in neonates due to diminished glucuronidation capacity) (**Figure 3**).[55]

With regard to the impact of critical illness, Carcillo *et al*. observed a twofold reduced CYP450 mediated metabolism in children with sepsis.[56] Likewise, in the case of liver impairment of any origin, liver metabolism may be reduced as was illustrated by Acocella *et al.* for rifampicin in adults.[57]

Due to polypharmacy in the NICU/PICU, pharmacokinetic drug-drug interactions (DDI) at the level of DME (e.g. enzyme induction, inhibition) are common (e.g. clarithromycin:

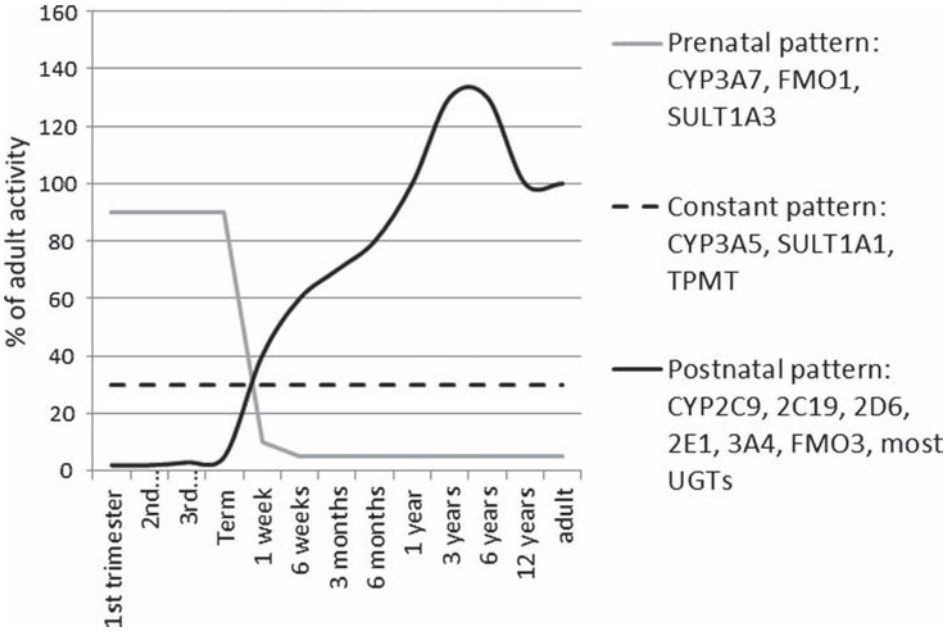

Figure 3. Ontogeny of drug metabolizing enzymes. CYp, cytochrome P450; TPMT, thiopurine S-methyltransferase; UGT, UDP-glucuronosyltransferases; FMO, flavin-containing mono-oxygenase. (Reproduced with permission from de Wildt *et al.*[54], Copyright BMJ Publishing Group Ltd.)

CYP3A4 inhibitor) and may lead to altered pharmacokinetics.[54] Since concomitant developmental changes in drug metabolism occur, children's vulnerability to DDI may differ from adults. To date, prospective studies investigating the occurrence and severity of drug-drug interactions in children are lacking.[58]

Finally, in patients with cardiac failure or ventilated patients, a decreased cardiac output may reduce the hepatic clearance of drugs with a high extraction ratio through a decreased liver blood flow (e.g. ciprofloxacin).[59]

2.4. Excretion

Drug excretion is the process by which parent drug and/or its metabolite(s) are removed from the body. This is mainly accomplished by the kidneys (glomerular filtration and proximal renal tubular secretion) and via the hepato-biliary route. Both processes undergo maturational changes and can also be affected by critical illness.

Many of the commonly used antibiotics in critically ill children are cleared by renal elimination. Glomerular integrity, physicochemical properties of the drug, and extent of protein binding determine the total amount to be filtered. Since only unbound drug can be filtered, the unbound fraction drives elimination of antibiotics excreted by glo-

merular filtration. In addition to glomerular filtration, drugs can be eliminated by active secretion in the proximal renal tubules, where transporters of cationic and anionic drugs are highly expressed. Weak acids and bases (i.e. most drugs) can be reabsorbed in non-ionised forms in the distal tubule. [37]

The glomerular filtration rate (GFR) matures starting from fetal organogenesis into late infancy. At birth, newborns experience profound hemodynamic changes. Among these changes, increased renal blood flow and decreased renal vascular resistance cause a rapid rise in GFR over the first weeks of life, with adult GFR typically attained by 2 years of age **(Figure 1)**.[9] Among preterm neonates, increments in GFR are much slower due to lower kidney perfusion, incomplete nephrogenesis and reduced nephron numbers.[60] The maturation of the active tubular secretion and reabsorption process through membrane transporters is less well known but is assumed to reach adult capacity in early childhood.[9,61] Evidently, all these maturation processes are likely to have a major impact on the dosing of renally cleared antibiotics in children below 2 years of age.

Besides maturation, disease characteristics also affect renal elimination capacity **(Figure 2)**. Acidosis and alkalosis are common in critically ill children (due to organ failure or alveolar hyperventilation) and may lead to changes in urinary pH. As only the unionised fraction is subject to tubular reabsorption, this may lead to altered drug reabsorption.[37]

In patients with cardiac failure, a decreased cardiac output may induce a lower renal blood flow and reduced renal clearance of antibiotics.[62] Also mechanical ventilation may alter renal drug clearance through a similar mechanism.[59] Acute kidney injury (AKI) and chronic kidney disease (CKD) (e.g. patients with heart failure) are commonly encountered in the NICU/PICU and may directly lead to impaired renal drug clearance.[63–65] Polypharmacy may also lead to alterations in renal drug clearance. In critically ill neonates, co-administration of the nephrotoxic non-steroidal anti-inflammatory agent ibuprofen led to decreased clearance of amikacin, vancomycin and gentamicin.[66]

Augmented renal clearance (ARC) of antibiotics is frequently observed in critically ill adults. The exact pathophysiological mechanism remains unknown but an increased renal blood flow (due to vasodilation and increased cardiac output) leading to significant alterations in glomerular filtration, tubular secretion and reabsorption are apparent.[67,68] Although the commonly used definition for ARC in adults (estimated GFR>130 ml/min) cannot be applied throughout the time span of renal maturation, there is some evidence that the concept of ARC also applies to children. Yu *et al.* and Gomez *et al.* observed significantly increased clearances of vancomycin and amikacin in children with burn wound sepsis when compared to children without burn wounds.[41,42]

3. PK ALTERATIONS IN CHILDREN USING EXTRACORPOREAL TECHNIQUES

Some treatment modalities require special attention with regard to PK changes and are discussed below **(Figure 2)**.

3.1. Renal replacement therapy

During dialysis, a countercurrent dialysate flow allows for diffusion of solutes (including the unbound fraction of drugs) according to the concentration gradient. Ultrafiltration is often used, in combination with dialysis, for management of fluid overload. In this case, plasma water removal occurs through convection over the filter membrane using a hydrostatic pressure. Solutes (including the unbound fraction of drugs) follow the direction of removed water.[37]

Drug and procedure related characteristics drive drug clearance during renal replacement therapy (RRT) **(Figure 4)**.

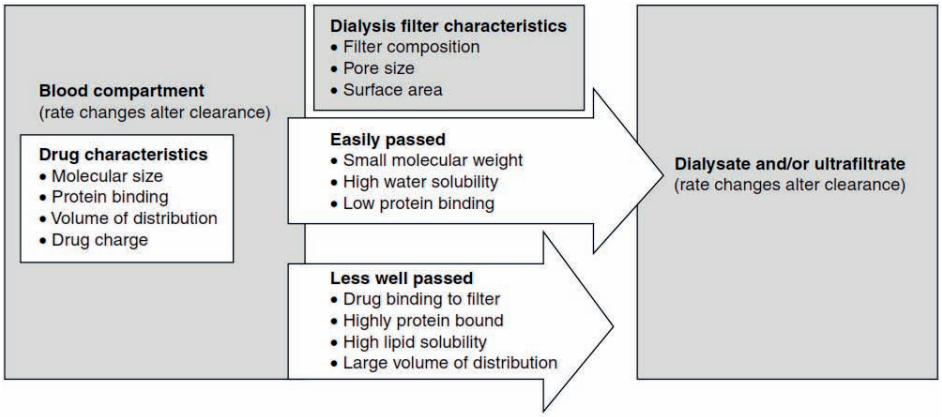

Figure 4. Determinants of drug removal during renal replacement therapies. (Reproduced with permission from Veltri *et al.*[69], Copyright Springer)

Drugs which are predominantly cleared by the kidneys are most affected by use of renal replacement therapy. Typically, low-molecular weight (<500 dalton), hydrophilic (low Vd) and low protein-bound compounds have a high extraction ratio during dialysis and continuous renal replacement therapy. In the case of antibiotics, passage across the filter is usually large due to their small molecular weight.[69] Main determinants for drug clearance related to the equipment and RRT technique, are the RRT mode and duration, blood, dialysis and ultrafiltrate flow. The more rapid the blood and dialysate flow rates the better the clearance by diffusion. Finally, dialysis membranes differ in surface area and pore size and may be subject to drug adsorption, which also may have an effect on antibiotic clearance.[70]

Depending on haemodynamic status, fluid status and catheter access, physicians will choose between intermittent haemodialysis (IDH) and continuous renal replacement therapies (CRRT) like continuous arteriovenous/venovenous haemofiltration (CAVH/CVVH), continuous arteriovenous/venovenous haemodialysis (CAVHD/CVVHD) and continuous arteriovenous/venovenous haemodiafiltration (CAVHDF/CVVHDF).

Due to a lack of paediatric-specific studies, dosing recommendations are often extrapolated from available adult literature. As general guideline for dosing renally cleared antimicrobials in patients with intermittent hemodialysis (IHD), it is important to estimate the residual renal function and start with an appropriate paediatric dosing regimen according to this estimated renal clearance. If drug properties allow for drug removal during IHD, a post IHD dose is currently suggested.[69] In NICU/PICU patients, continuous renal replacement therapies are most commonly used. Operating conditions are crucial to predict antimicrobial concentrations since over- and underdosing need to be considered.[71]

3.2. Extracorporeal membrane oxygenation

Extracorporeal membrane oxygenation (ECMO) is a cardiopulmonary bypass technique providing temporary respiratory and cardiac support. During ECMO, drugs tend to have higher volumes of distribution, mainly through haemodilution on initiation of ECMO, decreased plasma protein binding, capillary leakage and oedema. These phenomena have the greatest effect on drugs whose distribution is limited to the plasma compartment.[72]

Additionally, adsorption of drugs to ECMO component material may also occur and mainly depends on the lipophilicity of the drug, type of pump, oxygenator and circuit material.[72] Generally speaking, the higher the lipophilicity of the antibiotic, the more adsorption is to be expected. In an *in vitro* study by Wildschut *et al*., hydrophilic antibiotics vancomycin, meropenem and cefazolin showed only modest sequestration to currently used ECMO circuits.[73] Due to the expected increase in Vd of hydrophilic antibiotics, higher vancomycin and gentamycin loading doses were suggested in children on ECMO.[74,75]

Renal dysfunction is also common in the ECMO setting, and is usually related to the underlying indication. The loss in blood flow pulsatility and the inflammatory state, both induced by ECMO, may also affect renal function.[76] Whenever renal function becomes erratic, ECMO is used in combination with renal replacement therapy. Hence, renal clearance of antibiotics is usually impaired as was observed for cefotaxime, gentamycin and vancomycin and lower maintenance doses may be indicated.[75]

3.3. Cardiopulmonary bypass

The cardiopulmonary bypass (CPB) equipment and its impact on PK are similar to what has been described for ECMO.[77] Consequently, general dosing principles of high loading doses and reduced maintenance doses for hydrophilic antibiotics may also apply during CPB, as was suggested for cefazolin by Haessler *et al*.[78] Adsorption of liphophilic antibiotics (e.g. fluorochinolones, macrolides) to the CPB material may also necessitate dose increments.

Additionally, during CPB, therapeutic hypothermia is commonly used for organ protection. Due to a decreased cardiac output and reduced blood flow to tissues, this lower body temperature tends to reduce the Vd. This was illustrated by Kilbaugh *et al*. who observed decreased cefazolin tissue disposition into skeletal muscle during CPB with deep hypothermic circulatory arrest when compared to children with mild to moderate hypothermia.[79] Indirectly, a temperature drop also leads to an increase in blood pH and consequent changes in the ionised fraction of the drug, another determinant of the Vd. Finally, during hypothermia a decreased clearance by elimination organs is expected due to the reduced organ blood flow and diminished enzyme activity.[76]

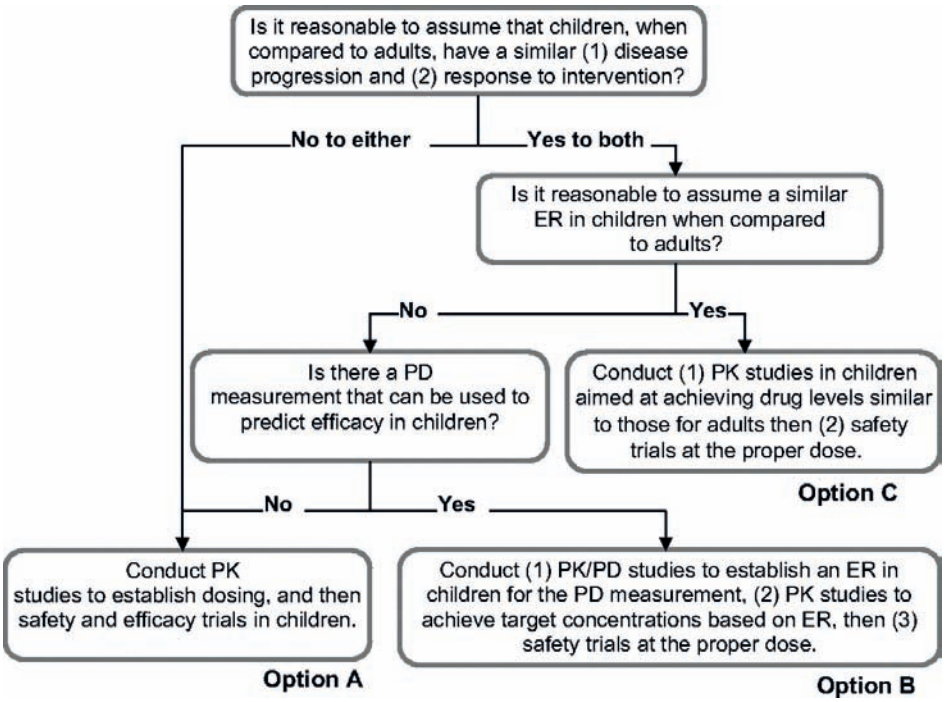

Figure 5. Paediatric study decision tree from the Food and Drug Administration (Reproduced with permission from Dunne *et al*.[80], Copyright American Academy of Pediatrics) ER, exposure-response curve

4. PHARMACODYNAMICS OF ANTIBIOTICS

PD estimates the relationship between a drug concentration and (side-) effect over time (*'what the drug does to the body')*. Whether PD of drugs needs to be studied in children, in addition to the investigation in adults, depends on the information regarding: (*i*) how similar disease progression is between adults and children, (*ii*) how similar the response to intervention is between these populations, and (*iii*) which valid and relevant PD measurements (biomarkers, outcome variables) are available **(Figure 5)**.[80]

When applying this decision tree to antibiotics **(Figure 5)**, the Food and Drug Administration (FDA) and European Medicines Agency (EMA) currently consider it to be reasonable to postulate similarities in antimicrobial PD between patient populations

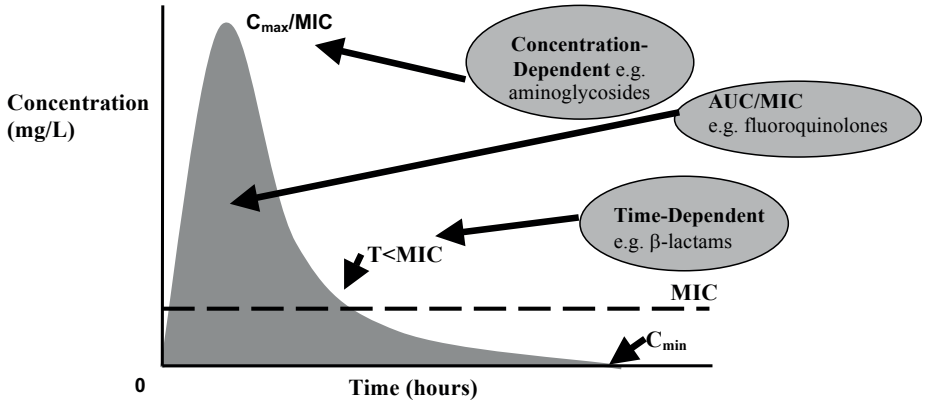

Figure 6. PK/PD parameters of antibiotics (Reproduced with permission from Roberts J. *et al.*[82], Copyright Wolters Kluwer Health, Inc.) C_{max}, peak concentration; AUC, area under the concentration time curve; T>MIC, time interval during which the unbound concentration remains above the minimal inhibitory concentration of the infecting organism

Table 1. PK/PD targets for antibiotics commonly used in critically ill children.

Antibacterial	PD index	PD target
Amikacin-tobramycin-gentamicin-netilmicin	C_{max}/MIC	8-12
Tobramycin	AUC/MIC	30
Ciprofloxacin-levofloxacin	AUC/MIC	>125
β-lactam antibiotics	%*f*T above MIC	40-100 (%)
Vancomycin	AUC/MIC	400
Teicoplanin	AUC/MIC	75-95

C_{max}, peak concentration; T, time; AUC, area under the concentration time curve; MIC, minimal inhibitory concentration of the infecting organism. Based on Moore *et al.*, Burgess D. *et al.*, Scaglioni F. *et al.*, Kanasawa N. *et al.* and Matsumoto K. *et al.*[83–87]

(concentration-response), because the treatment is aimed at the infectious organism (which is considered to behave the same between patient populations) and not the host per se. Consequently, differences in PK and safety aspects are the primary focus for optimizing antibiotic utilization across populations.[81] Three main PK/PD targets, irrespective of the patient population, have been defined for maximum killing of the infecting pathogen depending on the properties of the antibiotic: ratio of the peak plasma concentration over minimal inhibitory concentration (MIC) of the infecting organism, ratio of the area under the concentration time curve over MIC, or time during which the unbound concentration remains above the MIC **(Figure 6).** PD targets have been established in *in vitro* and animal studies and were in most cases confirmed in clinical studies in adults **(Table 1)**.

5. OFF-LABEL DRUG USE IN THE NEONATAL AND PAEDIATRIC ICU

Patients admitted to the NICU/PICU are exposed to a large number of drugs and total numbers increase with duration of ICU therapies and length of ICU stay.[88–90] Despite the importance of evidence-based drug treatment in this vulnerable population, PK, PD and efficacy data are scarce and dosing regimens remain often empirically derived from adults, relatively 'healthy' and/or older children. Consequently, most drugs used in the ICU are prescribed outside the terms of product license (off-label) or even without market authorization (unlicensed use). Frequencies of off-label and unlicensed use in the NICU/PICU between 50-85% have been reported, of which anti-infectives were one of the most commonly involved drug classes.[88,90,91] Risk factors for receiving an off-label drug included young age (<5 years), chronic health conditions, acute organ failures, mechanical ventilation, having arterial or venous catheters, dialysis treatment and receiving blood products.[91,92] Most commonly reported types of off-label use were the prescription of drugs in another dose or frequency, in a different formulation, or in an another age group.[93]

Previous research has also shown that these practices undoubtedly contribute to an extensive variability in dosing regimens.[94] Moreover, unlicensed/off-label drug prescribing has been associated with medication errors and unpredictable responses, related to either toxicity or therapeutic failure.[95,96] An illustrative historical example of antibiotic toxicity is the 'gray baby syndrome', due to chloramphenicol overdosing when given without regard for the infants' diminished capacities for glucuronidation metabolism and renal excretion. These babies presented with hypothermia, vomiting, acidosis, cyanosis and a characteristic grey skin colour.[55,97] Finally, in the case of antibiotics, adequate dosing is also warranted to reduce the emerging spread of resistant pathogens.[98] Since

2011, the European Commission (EC) and the EMA have been considering this global threat as a high priority. To tackle the problem, several areas were identified where measures have to be taken among which the appropriate use of antibiotics (including prescribing the appropriate dose) and development of new antibiotics.[99]

6. EUROPEAN PAEDIATRIC REGULATION

6.1. Introduction

To counterbalance the lack of information on the use of medicines in children, the European Paediatric Regulation came into force in 2007 to ensure that "the development of medicinal products that are potentially to be used for the paediatric population becomes an integral part of the development of medicinal products, integrated into the development programme for adults".[100] Hereby, the pharmaceutical industry is obliged to initiate a paediatric drug development program for every new drug compound. Any application for drug approval by the EMA (including new indication, pharmaceutical form or route of administration) must be accompanied by a paediatric investigation plan (PIP). This plan should include essential information on how and when the pharmaceutical company will study the pharmacokinetics and (side-)effects of the investigational compound in every subpopulation of children affected by the disease, including information on the drug formulation. This plan has to be approved *a priori* by the experts from the Paediatric Committee (PDCO) of the EMA. The PDCO is composed of 5 members of the Committee for Medicinal Products for Human Use (CHMP), 1 member appointed by each European Union member state that is not represented by the members appointed by the CHMP, 3 members representing healthcare professionals and three members representing patient associations.

A (partial) waiver for paediatric drug development can be granted by the PDCO if (i) the specific drug or drug class is likely to be ineffective or unsafe in part or all of the paediatric population, (ii) the disease or condition for which the drug or drug class is intended occurs only in specific paediatric age categories or adults, or (iii) the drug does not represent a significant therapeutic benefit over existing treatments in children. After the PIP has been completed and (positive or negative) results are submitted to the regulatory authorities, pharmaceutical companies receive a six months extension of their patent protection as a financial reward.

Off-patent drugs that are developed specifically for children are eligible for a paediatric-use marketing authorisation (PUMA). In this case, the incentive is a 10 year duration of market protection. To align scientific research for off-patent drugs, EMA has published

a priority list in 2009, which has continuously been updated in recent years.[101] The following were always considered as high-priority areas: (i) the development of age-appropriate formulations and strengths (even if not explicity stated), (ii) data in neonates for all conditions (except oncology) and, (iii) data in infants for oncological conditions and for refractory paediatric epilepsy syndromes. For orphan drugs, the incentive is an additional two years of market exclusivity.

Nine years after implementation of the Paediatric Regulation, the effect on drug development is substantial.[102] First, it had a direct positive impact on paediatric drug development since paediatric considerations have become an integral part of pharmaceutical development. Since the date of commencement by end 2015, 49 new drugs were authorised for paediatric use, 64 new paediatric indications were authorised and 13 new pharmaceutical forms, all linked to the requirements of the regulation.[102] The number has been increasing every year due to the drug development time. To date, 100 PIPs have been completed and more than 700 are ongoing.[102] Second, also 140 updates on product information and 16 new paediatric indications were approved using data from studies already completed before 2007.[102] This is a result of the mandatory submission by the marketing authorisation holder to the regulatory agency, as stipulated in article 45 of the Regulation. Other achievements of the regulation include: (i) the rising quality of trials, in particular through the possibility to receive (free-of-charge) scientific advice by the EMA for pharmaceutical companies, academia and other parties developing medicines and, (ii) the implementation of the public availability of information on PIP and protocol-related information via the European clinical trials database (EudraCT) to omit duplication, limit the patient's burden and prevent selective reporting.

A substantial amount of off-patent drugs are used in children. However, in this group, over the last 10 years, only 2 products were granted with a PUMA, 22 PIPs have been agreed and 24 applications are still ongoing or were withdrawn.[102]

6.2. Inclusion of intensive care patients

The PIP is defined in the regulation as a development programme "aimed at ensuring that the necessary data are generated determining the conditions in which a medicinal product may be authorized to treat in the paediatric population".[100] A condition is vagely defined as "any deviation(s) from the normal structure or function of the body, as manifested by a characteristic set of signs and symptoms (typically a recognised distinct disease or a syndrome)". The approach used by the PDCO to identify the scope of PIP with regard to conditions to be investigated was clarified in an EMA policy document in 2012 and is mainly based on the system organ class classification.[103] Traditionally, all

subsets of age groups between birth and 18 years that may be concerned should be specified in the PIP assessment.[8]

However, despite the ICH E11 recommendation to prioritize drug development in serious or life-threatening diseases, the paediatric regulation (nor accompanying policy documents and inventory/priority lists) does not explicitly stipulate the need to study the impact of (profound) pathophysiological changes (e.g. sepsis/septic shock), treatment characteristics (e.g. common drug-drug interactions, body cooling, extracorporeal membrane oxygenation) and/or co-morbidity (e.g. obesity) on drug PK/PD.[8]

After enforcement of the regulation, a lot of attention has been paid to the gap-of-knowledge in the neonatal subpopulation. The number of neonates to be included in trials has been increased by more than 25 times.[102] From these numbers one can assume that at least, also a proportion of seriously ill neonates should be recruited. Nevertheless, based on safety considerations, until now, neonatal studies are often deferred until experience is available in older age groups **(Figure 7)**.[102]

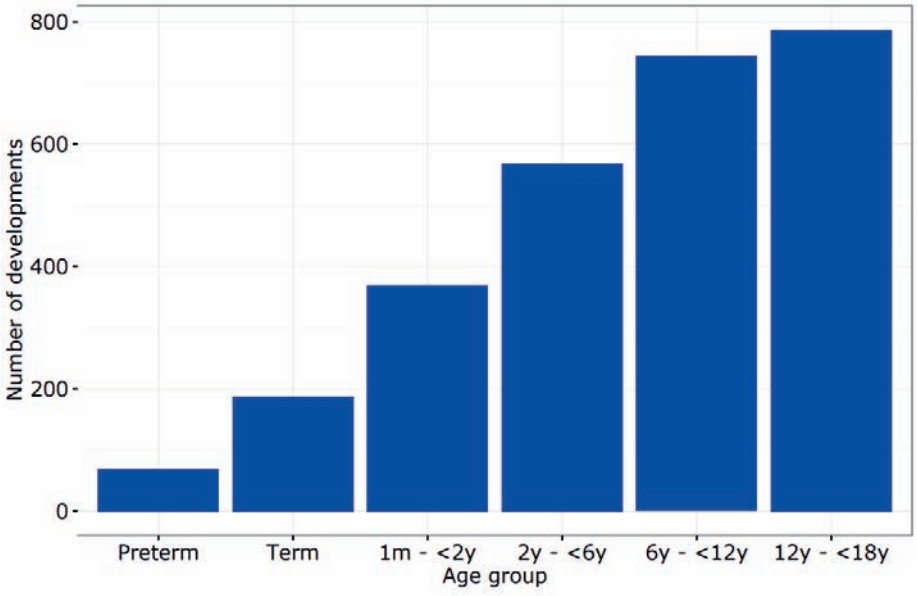

Figure 7. Involvement of children per age group within medicine developments, according to the 10-year report of the paediatric regulation[102]

This holds the risk that after marketing authorisation for adults is granted, the planned studies are delayed or never initiated and drugs remain to be prescribed off-label. There are two reasons for this: once the drug is authorised, the EMA has no means to enforce

the legislation and second, once the drug is available off-label, extra patient recruitment problems may occur. It is an issue that is currently discussed to support earlier conduct of neonatal and infant studies, considering the limited relevance of *a priori* evidence in older age groups. Alongside the classification for PIPs and waivers based on therapeutic areas (e.g. cardiovascular diseases, dermatology, haematology, infectious diseases), a class of 'neonatology and paediatric intensive care' is currently used. This classification is determined within the PIP submission by the applicant. Between 2007 and end 2015 the number of agreed PIPs within the class of neonatology/paediatric intensive care only represented 2 % of agreed PIPs.[102] Besides the chance that a compound - that mainly will be tested in a critical care setting- is classified only under its therapeutic area and not specifically under the class of 'neonatology/paediatric intensive care', this low number emphasises the room for improvement of the regulation to ensure drug development in this vulnerable subgroup of patients.

Moreover, the most recently revised priority list for off-patent products only specifies in the area of intensive care/anaesthesiology the need to study propofol for short-term procedural sedation in neonates, although a referral is made to the therapeutic areas like cardiology, haematology, infectiology, neonatology and pain.[101] In these therapeutic domains, only requirements are defined on age (neonates and infants), indication (e.g. immunocompromised patients), and type of study (e.g. PK, efficacy, safety). Similarly as for new drugs under development, one can assume that some of those patients will require intensive care depending on the patient's individual status. Besides, some indications will almost exclusively be treated on the ICU (e.g. septic shock). However, again, the risk to exclude the ICU population from clinical trials poses a real threat, since PUMAs can be obtained without a specific obligation to include them.

As such, under the current regulation, neonatologists and paediatric intensivists still face the paradox that many of those paediatric clinical trials do not meet the specific needs in daily practice and children admitted to the critical care unit remain deprived of evidence-based drug use.

6.3. Research on antibiotics

In 2010, PDCO published a survey of all paediatric uses of medicines in Europe.[104] One of the main findings of this survey was that antibiotics were extensively used off-label in young children. Based on the results of this study, the PDCO published in 2013 an inventory list of paediatric needs in the different therapeutic areas for both off-patent and new medicinal products. In the list of antimicrobial classes penicillins, cephalosporins, macrolides, carbapenems, sulphonamides, aminoglycosides, antituberculostatics, ciprofloxacin, colistin and vancomycin were listed.[105]

Between 2007-2015, 5 PIPs for antibiotics (vancomycin, meropenem, ceftriaxone/sulbactam, ciprofloxacin, azithromycin) have been agreed to study in neonates and were mostly funded through the European Union (EU) Framework Programme 7.[102] In general, only a minority of antibiotic PIPs were agreed (vancomycin and meropenem classified within the therapeutic area of neonatology/intensive care; 15 compounds within the therapeutic area of infectiology). This is presumably related to the fact that the development pipeline with antibiotics is rather small and the financial incentive to study off-patent compounds (PUMA) is not encouraging enough for pharmaceutical companies.[102,106]

7. ETHICS AND BARRIERS IN CLINICAL RESEARCH IN THE NICU/PICU

As it is our collective responsibility to obtain sufficient information to develop medicines for children, the conduct of clinical research in neonatal and paediatric intensive care is necessary but poses some unique ethical and practical challenges.

After the Second World War, the Nuremberg code stated that the voluntary consent of the human subject in clinical research is absolutely essential.[107] Later, this statement was amended by the Declaration of Helsinki and allowed the parents of minor children (or their legal representatives) to consent, as they were thought to act according to the presumed will of their child.[108] Several steps are distinguishable in the informed consent process of which objective transmission of the information by a competent physician, a good understanding by the parents of this information and sufficient time to come to a decision (without coercion) are the most important. However, the emotionally strained circumstances of intensive care carry an enormous potential to compromise this process.[109] Especially in emergency and life-threatening conditions, parents are overwhelmed by the disease severity of their beloved child in an extraordinary environment, leading to an impaired ability to understand the proposed research and take a decision.[109,110] Previous studies reported that parents regularly experienced recall-bias on the consent procedure, had difficulties understanding the proposed research and identified the timing and ways in which they received the information needed to be improved. [111–114]

In randomised controlled trials (RCT) on the ICU, the concept of 'therapeutic misconception' may also occur, with parents believing that giving consent for conducting a study is an *a priori* for getting better, while underestimating potential risks.[115] Also under these circumstances, one cannot speak of a rationally given consent.

The EU clinical trial regulation requires that in addition to the informed consent by the parents, a capable minor, should assent himself or herself to participate in a clinical trial.[116] This capability is not solely depending on the child's age but also on experience (e.g. chronically ill children). Of course, in most circumstances on the ICU, the child is often too sick to participate in the assenting process prior to inclusion.

In Western Europe and Canada, the treating physician and person asking for informed consent are in most recruitment centres the same.[117] Herein lies another ethical challenge with the physician balancing between the moral duty to treat his individual seriously ill patient according to evidence-based guidelines and his intention to generate new insights and hope on a better care for future patients. This ethical conflict was nicely illustrated in a study in which 1050 paediatric intensivists were asked about their opinion on conducting randomized, controlled trials in critically ill children.[118] In this study, 96% of respondents indicated that they believed that RCTs of potentially life-sustaining therapies must be performed, although only 10% indicated that they did not experience ethical conflict with this type of study. Eighty-four per cent indicated that earlier published data would have the potential to bias them towards the investigational product.

Enrolment of children in clinical trials should not only be considered if scientifically needed but also the potential benefit of an optimised drug treatment should outweigh the risks and burden for the individual child.[119] Both benefit, risks and burden should be considered in relation to the severity of the disease e.g. when studying life-saving therapies a higher risk level and/or burden is permittable. In vulnerable ICU children, however, it is known that side-effects can be more frequent and severe and are not always easily predictable from other (paediatric) populations.[120] Trial related burden should also be minimised as most ICU patients already undergo many invasive and painful procedures.[119]

Recruitment problems were reported as the major difficulty for conducting and completing PIP studies in the 10-year report on the paediatric regulation.[102] In the NICU/PICU environment, patient recruitment here is even more challenging due to a high population heterogeneity and a relatively low number of admissions per single centre. Duffett *et al*. previously reported that one third of initiated PICU RCTs was prematurily stopped, mainly due to recruitment problems.[121] Interestingly, main barriers for recruitment were reported as the lack of availability of parents, language barriers between physician and parents, and parents being overwhelmed when asked for consent as discussed above.[114,121] Nevertheless, more than 80% of RCTs are reported as single-centre studies, hence compromising the likelihood of producing generalisable study results.[121]

Since intensive care requires also frequent blood sampling for routine monitoring, very limited blood volume is available for PK/PD related purposes.[110] Different guidelines are available which are only specified in detail for the age category preterm and term neonates.[122–124] In particular for PK studies, the availability of a separate IV access for drug infusion and blood sampling often poses a challenge.

Finally, protocol deviations in this environment are common as reported in a survey by Morris *et al*. In this observational study, 65% of paediatric intensivists indicated that they did not adhere to the research protocol when the patient deteriorated or the parents asked for the study drug.[118] Evidently, this practice may compromise the validity of study results.

8. MODELLING STRATEGIES TO OPTIMISE ANTIBIOTIC DOSING

Biological models are defined as simplified representations of a biological system to provide knowledge and understanding of this system.[125] In the field of pharmacometrics, mathematical models are developed to describe and predict the drug's PK (and/or PD) behaviour in animals and humans. Since many years, its role has been substantially increasing in the process of drug development.

Traditional PK studies include the standard two-stage approach. In this approach, the individual parameters are first calculated from the individual concentration-time profiles. As a second step, the average of parameters and the between-subject variability are calculated. This approach has the major drawback that the inter- and intrapatient variability are not distinguishable from each other and, as such, interpatient variability is usually overestimated. Moreover, these studies require dense blood sampling in each patient which renders them difficult to conduct in a NICU/PICU setting **(Figure 8)**.[126]

In a population PK modelling approach, typically, all data from all individuals are analysed simultaneously. The mixed effects modelling technique has been commonly used for this purpose. Herein, the term 'mixed' refers to a combination of fixed effect parameters and random effects parameters that are estimated. Fixed effects are the average PK parameters, also known as the population PK parameters (or 'typical values'), and the parameters describing the relationship between the identified covariates and PK parameters. Random effects quantify variability associated with fixed effects parameters. Several sources of random effects are estimated: typically this includes between-subject and residual variability, in some cases between-occasion variability is of relevance as well.

Important advantage of a population PK analysis is that sparse and unbalanced data can be analysed, which are common in neonatal and paediatric intensive care patients, where the blood sampling volume is limited and protocol deviations are common **(Figure 8)**.

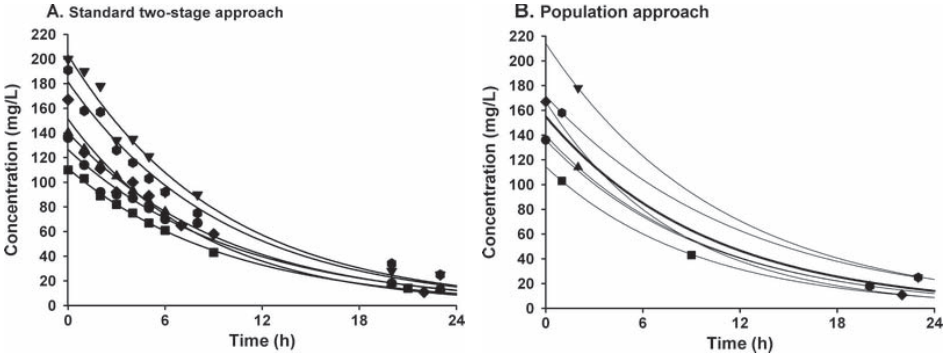

Figure 8: Concentration–time profiles after IV bolus administration of the same hypothetical study drug using (A) the standard two-stage approach applied to a rich dataset. (B) population PK modelling approach applied to the same dataset using only two data points for each individual (sparse dataset). In (A) in each of the six individuals 10 samples are available. The different symbols correspond to different individuals. Each *black line* corresponds to a separate fit to the 10 data points of each individual. In (B) two samples of the 10 per subject in (A) are used. The different symbols correspond to the six different individuals. The *black line* illustrates the concentration–time plot based on the population mean values of the PK parameters. The grey *lines* show the plots of the individual patients, which are based on the population mean values together with the measured concentrations of the specific individual. Adapted from De Cock R. *et al.* with permission[126]

A population model consists of a structural model, a statistical model and a covariate model. The structural model describes the mean trend of the data. Typically, structural models consist of compartmental models to describe the rates of change of drugs and/or biomarkers. The statistical submodel includes random effects parameters (e.g. between-subject, residual variability and between-occasion variability) **(Figure 9)**.

Next, covariates are evaluated to predict and thereby reduce between-subject variability associated with the structural model parameters. The identification and quantification of these covariate effects provides the basis for rational dose individualisation.

Finally, for dose evaluation, stochastic simulations are performed using the developed population PK model. In combination with plausible sets of patient-specific covariates included in the model, individual concentration-time profiles can be generated (e.g. simulation of 1000 concentration-time profiles. In the case of antibiotics, optimized dosing regimens are selected through probability of target attainment (PTA) evaluation, with a target PTA of minimum 90%.[127]

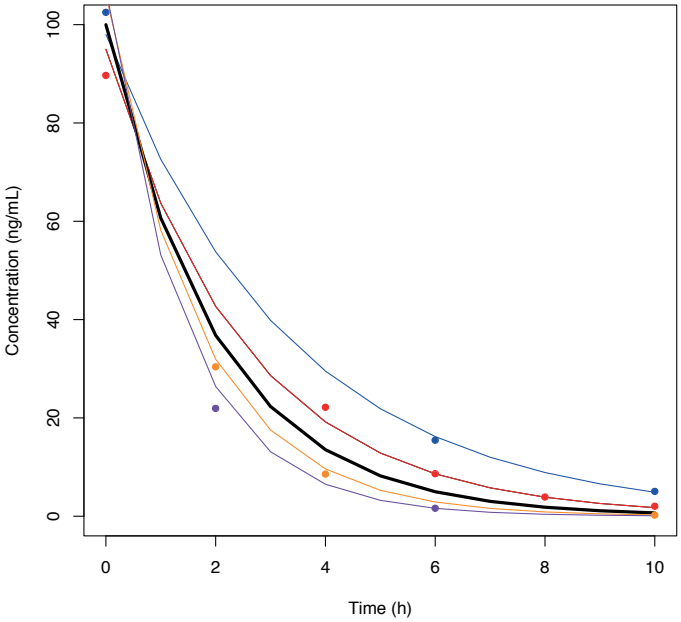

Figure 9 : In panel (A) the inter-individual variability of four individuals (coloured lines) from the mean or typical patient (black line) is shown, after receiving the same IV dose of a hypothetical drug. Panel (B) illustrates the unexplained residual variability in one individual, after receiving an IV bolus dose of a hypothetical drug. The red line represents the individually predicted concentration-time curve and the red dots the measured concentration data points.

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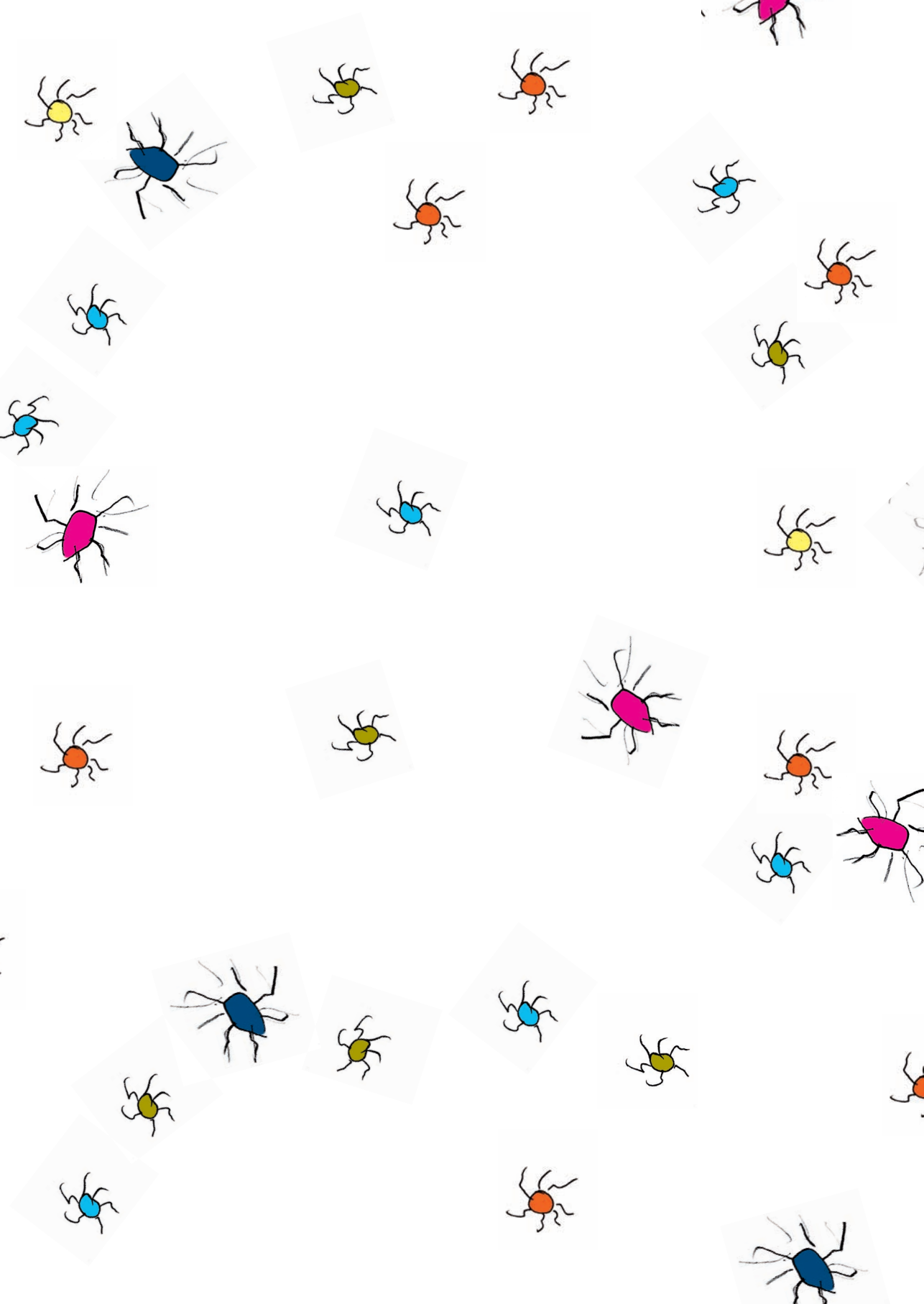


CHAPTER 2

SCOPE AND RESEARCH OBJECTIVES

As extensively illustrated in the introductory chapter, limited data on the impact of maturational and critical illness related changes on antibiotic disposition and effect are available in children. Despite global efforts to improve paediatric drug research in Europe, NICU/PICU patients remain "therapeutic orphans". This knowledge resulted in the origin of the proposed research in this doctoral dissertation.

1. GENERAL RESEARCH OBJECTIVE

The general aim of this doctoral dissertation was to optimise drug dosing in critically ill children through investigation of developmental, disease and treatment related changes on drug pharmacokinetics, using a model-based approach.

Based on their frequent use, off-knowledge, off-patent and (consequently) off-label status, the following compounds were studied: amoxicillin in combination with clavulanic acid, piperacillin in combination with tazobactam, cefazolin and vancomycin.

2. DRUG-SPECIFIC RESEARCH OBJECTIVES

Amoxicillin is a β-lactam antibiotic, used in combination with the β-lactamase inhibitor clavulanic acid to extend its spectrum. Both compounds are minimally protein bound to plasma proteins (~18-25%) and renally excreted. Typical indications within the PICU include community-acquired pneumonia, skin, soft tissue and abdominal infections. Limited data are available to guide its dosing in critically ill children.

Piperacillin is a broad-spectrum β-lactam antibiotic that is used in combination with the β-lactamase inhibitor tazobactam to extend its spectrum. Both compounds are only minimally bound to plasma proteins (~30%) and renally excreted. Typical indications within the PICU include ventilator-associated pneumonia, intra-abdominal infections and sepsis of unknown origin. Limited data are available to guide its dosing in critically children.

Cefazolin is a highly plasma protein-bound (~80%) β-lactam antibiotic that is mainly used as surgical prophylactic agent. CPB and renal function changes were previously shown to have a major impact on its disposition. To date, only sparse data on the PK of unbound cefazolin are available in children during CPB, and no data are available after paediatric cardiac surgery.

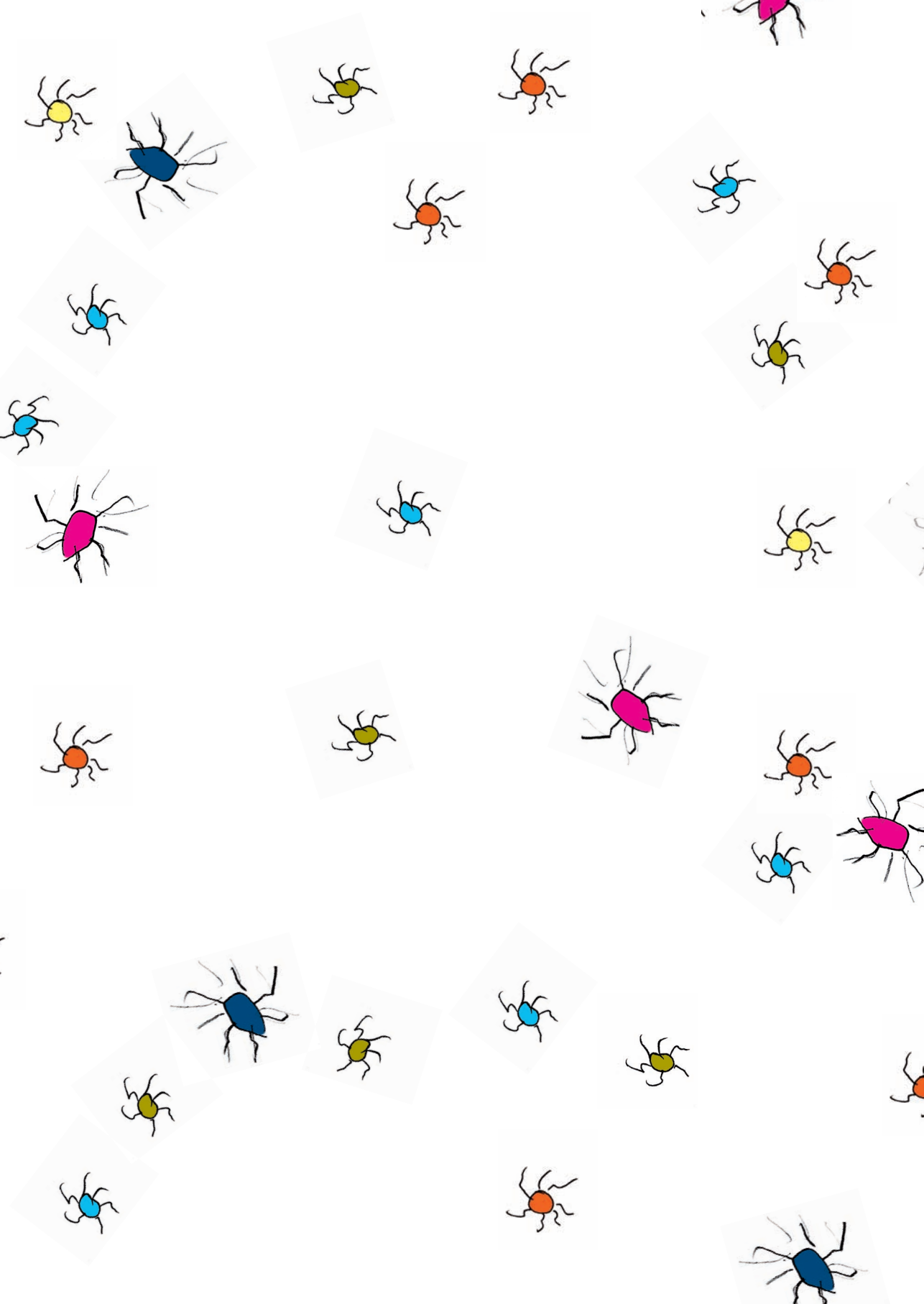
Drug-specific objectives investigated for these compounds in **Chapter 3**, **4** and **5** are:

- to quantitatively characterise the intravenous PK, including predictors of inter-individual variability
- to evaluate the PTA for the current standard dose regimen
- to develop improved evidence-based practical dosing regimens

Vancomycin is a glycopeptide antibiotic that is commonly used to treat serious Gram-positive infections in the NICU/PICU. Plasma protein binding is ranging from 50-55% in healthy volunteers and non-critically ill adults. No data on protein binding are available in critically ill children. As only the unbound drug is pharmacologically active, alterations in protein binding may have an impact on target attainment rates.

Drug-specific objectives investigated in **Chapter 6** are:

- to quantitatively characterise plasma protein binding, including predictors of inter-individual variability
- to compare attainment rates of three currently used targets: total (trough) concentration, AUC/MIC and *f*AUC/MIC
- to develop a prediction tool for the unbound vancomycin concentration



CHAPTER 3

AUGMENTED RENAL CLEARANCE IMPLIES A NEED FOR INCREASED AMOXICILLIN/ CLAVULANIC ACID DOSING IN CRITICALLY ILL CHILDREN

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ABSTRACT

Background and Objectives: There is little data available to guide amoxicillin/clavulanic acid dosing in critically ill children. The primary objective of this study was to investigate the pharmacokinetics of both compounds in this paediatric subpopulation.

Patients and Methods: Patients admitted to the paediatric ICU in whom intravenous amoxicillin/clavulanic acid was indicated (25-35 mg/kg of body weight every 6 h) were enrolled. Population pharmacokinetic analysis was conducted and the clinical outcome was documented. Clinicaltrials.gov: NCT02456974.

Results: A total of 325 and 151 blood samples were collected from 50 patients (median age, 2.58 years; age range = 1 month to 15 years) treated with amoxicillin and clavulanic acid, respectively. A three-compartment model for amoxicillin and a two-compartment model for clavulanic acid best described the data, in which allometric weight scaling and maturation functions were added *a priori* to scale for size and age. In addition, plasma cystatin C and concomitant treatment with vasopressors were identified to have a significant influence on amoxicillin clearance. The typical population values of clearance for amoxicillin and clavulanic acid were 17.97 L/h/70kg and 12.20 L/h/70kg, respectively. In 32% of the treated patients, amoxicillin/clavulanic acid therapy was stopped prematurely due to clinical failure and the patient was switched to broader-spectrum antibiotic treatment. Monte Carlo simulations demonstrated that four-hourly dosing of 25 mg/kg was required to achieve the therapeutic target for both amoxicillin and clavulanic acid. For patients with augmented renal function, a 1-h infusion was preferable to bolus dosing.

Conclusions: Current published dosing regimens result in subtherapeutic concentrations in the early period of sepsis due to augmented renal clearance, which risks clinical failure in critically ill children, and therefore need to be updated.

INTRODUCTION

Appropriate antibiotic treatment is a cornerstone in the pharmacological treatment of critically ill children. Paediatric sepsis and septic shock reportedly affect 30% of children admitted to paediatric intensive care units (ICUs), with a 25% mortality rate.[1] Due to their broad antimicrobial spectrum and relatively low toxicity, β-lactam antibiotics such as amoxicillin/clavulanic acid are commonly used in paediatric critical care for treating community-acquired infections.[2] Typical indications include community-acquired pneumonia, skin, soft tissue and abdominal infections.

During childhood many developmental changes occur, which influence both drug exposure and drug response.[3] Moreover, pathophysiological changes during critical illness frequently affect pharmacokinetics (PK) and pharmacodynamics (PD).[4–6] To date, only one report on the pharmacokinetics of amoxicillin/clavulanic acid in a limited number of critically ill children older than 2 years (n = 15 patients) is available.[7]

Broader-spectrum and newer antibiotics are now being studied more extensively in this patient population, which may predispose clinicians towards the use of such agents.[8] Therefore, research for more-targeted and well-established therapies like amoxicillin/clavulanic acid is highly relevant.

The primary aims of this study were: (i) to investigate the pharmacokinetics of intravenous amoxicillin/clavulanic acid in critically ill infants and children and (ii) to evaluate the efficiency of current and alternative dosing regimens in this population.

PATIENTS AND METHODS

Study design

A prospective, open-label, pharmacokinetic study was conducted at the paediatric ICU of the Ghent University Hospital, Ghent, Belgium between May 2012 and December 2013. Patients between 1 month and 15 years of age admitted to the paediatric ICU were included in whom treatment with intravenous amoxicillin/clavulanic acid was the standard of care. Patients were excluded if they required an extracorporeal circuit or did not have arterial or intravenous access other than the drug infusion line available for blood sampling. The research was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the institutional Ethics Committee (EC/2012/172). Written informed consent was obtained from the parents or legal representatives and also from the patients if they older than 12 years.

Drug dosing and administration

Amoxicillin/clavulanic acid (Augmentin P 500/50 mg and Augmentin 1000/200 mg, GlaxoSmithKline, Genval, Belgium; Amoxiclav Sandoz 1000/200 mg, Sandoz NV, Vilvoorde, Belgium) was prescribed in a dose range of 25 to 35 mg amoxicillin per kilogram body weight (maximum 1000 mg) every 6 h and administered intravenously over 5 to 30 min using a calibrated syringe driver, according to current dosing guidelines.[9] According to a standardized procedure, infusion lines were flushed with normal saline immediately after drug administration with a minimum of twice the dead space volume.

Blood sampling

Serial blood samples were obtained from the first and/or assumed steady-state doses from an indwelling catheter other than the drug infusion line (median of four blood samples per dose). The total number of samples collected (per patient) was limited by the predefined total maximum blood volume permitted for PK sampling per individual patient, defined as 2.4 mL/kg bodyweight.[10] A full sampling scheme per dose typically included a sample just before dosing (t = 0), a sample immediately after dosing and flush, a distribution sample between 5 and 70 min after the start of the drug infusion, a mid-dose-interval sample 3 h after the drug infusion start time and a trough sample just prior to the next dose. All samples were immediately transferred on ice to the chemistry laboratory and centrifuged (8 min, 1885g) after which the resulting plasma was frozen at -80°C for a maximum of 3 months before assay.

Drug and biochemical assays

Total plasma amoxicillin and clavulanic acid concentrations were quantified simultaneously using a validated ultraperformance ultrahigh-pressure liquid chromatography (UPLC)-tandem mass spectrometry method.[11] The lower limit of quantification (LLOQ) was 0.5 mg/L for both compounds and the imprecision was < 15% at all levels. For the first 24 patients only the amoxicillin compound was quantified. Creatinine was measured in serum (Scr) and urine (Ucr) using the rate-blanked compensated Jaffe technique (Modular P and Cobas 6000, Roche Diagnostics GmbH, Mannheim, Germany). Twenty-four-hour creatinine clearance (CrCL) was calculated using the following formula: CrCL = urine volume x Ucr/(1440 x Scr) (whenever a urinary catheter and 24-h urine collection were available). Plasma Cystatin C (CysC) was measured using the N Latex cystatin C assay on the Behring nephelometer II (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) (intra-assay coefficient of variation [CV]: 1.4%; inter-assay CV: 5.4%) and was standardized according to the ERM-DA471/IFCC reference material.[12]

Pharmacokinetic analysis

Amoxicillin/clavulanic acid pharmacokinetics were evaluated using the non-linear mixed-effects modeling software NONMEM version 7.3 (ICON PLC; Ellicott City, Maryland). The first-order conditional estimation method with the interaction option (FOCE-I) was used to estimate PK parameters and variability. R (version 3.0.2) and PsN (version 5.18.2) tools were used for pre- and postprocessing. One-, two-, and three-compartmental linear models were tested to fit plasma concentrations of both compounds independently using the NONMEM library ADVAN subroutines.[13] Following this, a simultaneous fit of concentrations of both compounds was evaluated; when both analyte measurements were obtained from the same sample, correlations in residual error were handled using the L2 method in NONMEM.[13] The L2 data item is used to group observations within an individual to indicate there may be a degree of correlation in the residual variability (usually residual variability assumes all observations are independent). All clearance parameters were scaled *a priori* using an allometric weight (WT) approach with a fixed exponent of 0.75 in combination with a Hill model using postmenstrual age (PMA) to describe the maturation process on clearance[14]:

$$CL_i = CL_{pop} \times \left(\frac{WT}{WT_{med}}\right)^{0.75} \times \left(\frac{PMA^{HILL}}{TM_{50}{}^{HILL} + PMA^{HILL}}\right)$$

where CL_i is the individual clearance, CL_{pop} is the population clearance, $WT_{med}$is the median weight, PMA is postmenstrual age, $HILL$ is the Hill coefficient, and TM_{50} is the maturation half-life. All volume of distribution parameters were scaled *a priori* with linear weight. Between-subject variability (BSV) was described using an exponential error model and a proportional error model was used to describe residual variability. Between-occasion variability (BOV) was tested on CL. For model evaluation, decrease in objective function value (OFV), plots of observed versus population predicted concentrations, observed versus individual predicted concentrations, conditional weighted residuals (CWRES) versus time after dose, and CWRES versus population predicted concentration were utilized. Parameter estimates were compared using three different methods for handling data below quantification limit (BQL): omitting BQL samples, setting values to half the limit of quantification (LOQ/2), and the M3 method, enabling estimation of the likelihood of BQL measurements being real BQL data.[15] While body weight and age were included *a priori* as described above, CysC was then further tested, since it is known that amoxicillin and clavulanic acid are renally cleared.[16] After this, a stepwise covariate model (SCM) building exercise was performed with a forward inclusion criterion of $p<0.01$ and backwards elimination criterion of $p<0.005$. The following covariates were tested in the SCM: primary reason for admission, measures of organ function and patient severity of illness as described by the PELOD (Pediatric Logistic Organ Dysfunction) Score, PRISM II

(Pediatric Risk of Mortality) Score[17,18], presence of surgery, presence of mechanical ventilation, cotreatment with vasopressors and nephrotoxic medications (aminoglycosides, glycopeptides, diuretics, angiotensin-converting enzyme [ACE] inhibitors, nonsteroidal anti-inflammatory drugs, tacrolimus, cyclosporin, methotrexate), fluid resuscitation (>60 mL/kg per 24 h), type of catheter used for drug administration and blood sampling and C-Reactive Protein (CRP). Given that 35% of Scr samples were BQL, Scr and CrCL could not be tested as covariates on drug clearances. The final population model was evaluated in two ways: a nonparametric bootstrap sampling procedure (n = 1000) and a visual predictive check (VPC) (n = 1000).

Clinical outcome assessment

Clinical failure in our study population was defined as premature termination of amoxicillin/clavulanic acid with change of antibiotic therapy or additional antibiotics commenced within 48 h of completion of therapy. Duration of antibiotic therapy was depending on the type of infection and the patient's clinical evolution and was determined at the discretion of the attending physician.

Assessment of dose-exposure relationship

Monte Carlo simulations (n = 1000 patients) were performed for three dosing regimens (**Table 1**).[9,19] Based on these simulations, the fraction of time during which the unbound drug concentration is above the MIC ($fT_{>MIC}$) was calculated for the first dose and over the first 48 h of treatment. The target efficacy exposure was defined as 40% $fT_{>MIC}$[20] and a target MIC of 8 mg/L for amoxicillin was chosen as worst-case scenario, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints for *Escherichia coli*.[21] As the EUCAST clinical breakpoint for amoxicillin was determined using a fixed concentration of 2 mg/L clavulanic acid, this was chosen as the target concentration for clavulanic acid (note that the CLSI susceptibility testing concentration was 4 mg/L). The mean protein binding of amoxicillin and clavulanic acid are 18 and 25%, respectively and this was used to simulate unbound concentration.[16]

Table 1. Simulated dosing scenarios

Dosing regimen[a]	Drug FDrug formulary
25 mg/kg q12h for children between 1 and 3 months	British National Formulary for Children[19]
25 mg/kg q8h for children older than 3 months	British National Formulary for Children[19]
25 mg/kg q6h[b]	Sanford Guide for Antimicrobial Therapy[9]
25 mg/kg q4h[c]	

[a]The dosing regimen was based on the amoxicillin component and a fixed ratio of amoxicillin/clavulanic acid of 5:1. All simulations included a bolus and 1-h infusion regimen. Abbreviations: q12h, every 12 h; q8h, every 8 h; q6h, every 6 h; q4h, every 4 h.
[b]Study dosing regimen.
[c]Newly tested dosing regimen.

RESULTS

A total of 50 patients were included in this study; demographic, clinical and treatment characteristics are summarized in **Table 2**. Patients younger than 2 years old accounted for 44% of the study population (n = 22).

Table 2. Demographic, Clinical and Treatment Characteristics

Characteristic[a]	Value[b]
Sex	
male	30 (60)
female	20 (40)
Age (years)	2.58 (0.08-15)
Weight (kg)	14.4 (4.07-65)
Total length of ICU stay (days)	9.5 (3-72)
PRISM II score	6.5 (0-32)
Primary reason for ICU admission	
postoperative	16 (32)
respiratory	10 (20)
gastro-intestinal	10 (20)
neurologic	7 (14)
cardiovascular	6 (12)
other	1 (2)
Reason for antibiotic treatment	
treatment of infection	33 (66)
postoperative prophylaxis	17 (44)
Mechanical ventilation[c]	29 (58)
Vasopressor treatment[c]	16 (32)
PELOD score[d]	1 (0-31)
Serum Creatinine (mg/dL)[d]	0.21(<0.17-1.89)
Plasma Cystatin C[d,e] (mg/L)	0.63 (0.33-1.23)
Serum CRP[c] (mg/L)	5.4 (0.40-28.79)

[a]Abbreviations: ICU, Intensive Care Unit; PRISM, Pediatric Risk of Mortality; PELOD, Pediatric Logistic Organ Dysfunction; CRP, C-Reactive Protein.
[b]Values are median (range) or number (percentage of total number of patients).
[c]During ICU stay.
[d]At day(s) of sampling.
[e]Based on values from 49 patients.

A total of 325 amoxicillin and 151 clavulanic acid concentrations in plasma were available for population PK analysis. A three-compartment model for amoxicillin and a two-compartment model for clavulanic acid best described the data (**Figure 1A-B**).

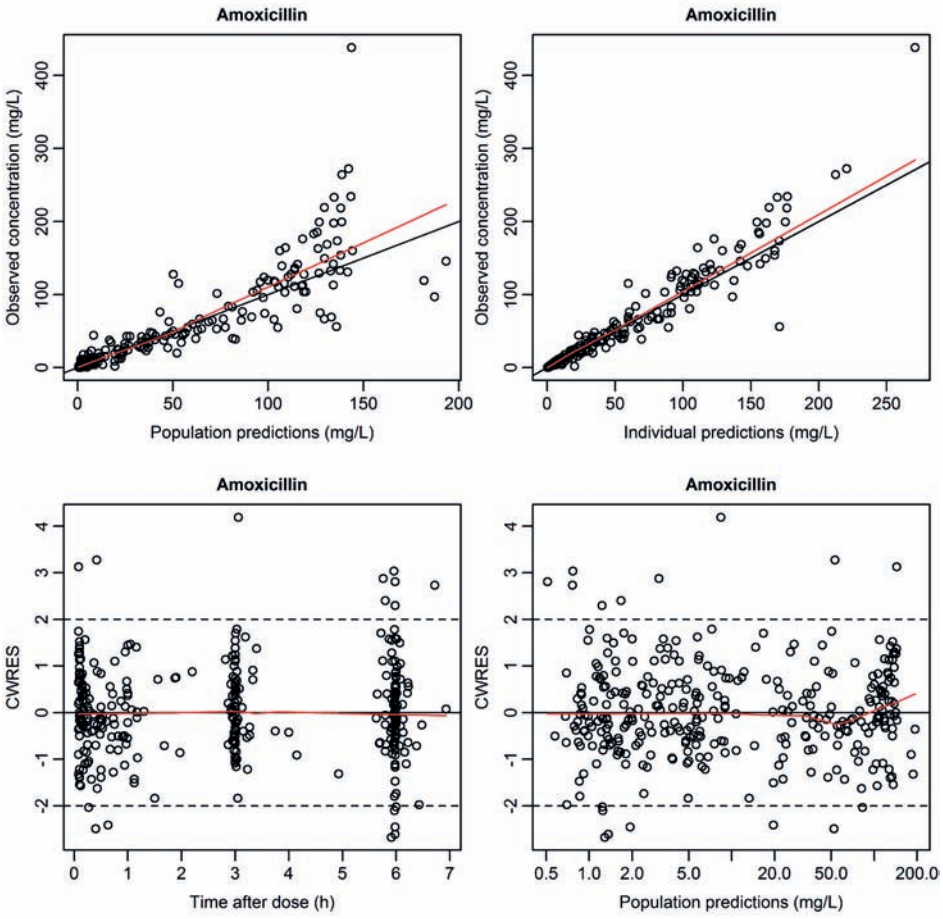

Figure 1A. Goodness-of-fit diagnostic plots for amoxicillin. The plots show observations versus population predictions and individual predictions and conditional weighted residuals (CWRES) versus time after dose and population predictions. In the observation versus prediction plots, a line of identity (black solid line) and a Loess smooth line (red solid line) were included as a reference. In the CWRES plots, dashed lines at +2 and -2 standard deviations from the mean (solid line) were included to indicate the expected region of approximately 95% of the data.

BSV was supported for amoxicillin and clavulanic acid central clearance and volume only. Central volume values for amoxicillin and clavulanic acid were highly correlated (R^2>0.99), so a single random effect with a scaling factor for clavulanic acid was used (**Table 3**). The correlation between amoxicillin and clavulanic acid clearance was also estimated. BOV was supported for amoxicillin clearance and clavulanic acid clearance. Since these values were highly correlated, a single random effect with scaling factor was also used (**Table 3**). The Hill coefficient on the maturation model was estimated to be close to 1, and no significant change in OFV was noted when it was fixed to 1. When CysC

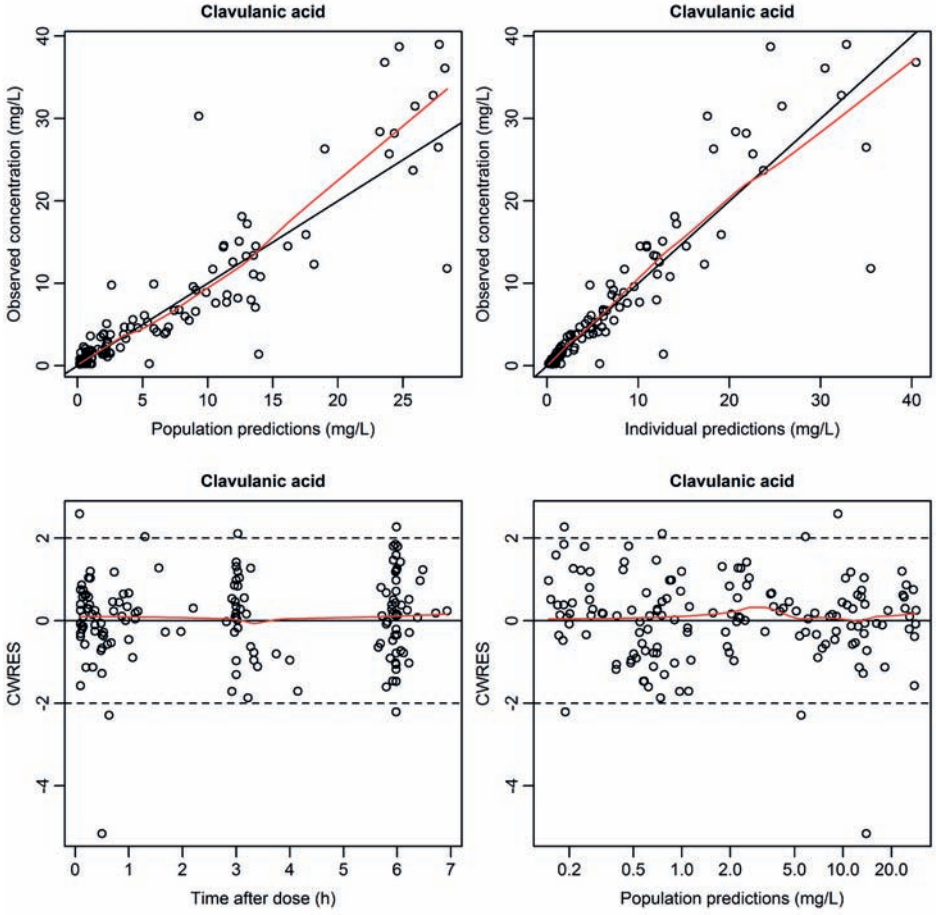

Figure 1B. Goodness-of-fit plots for clavulanic acid. The plots show observations versus population predictions and individual predictions and conditional weighted residuals (CWRES) versus time after dose and population predictions. In the observation versus prediction plots, a line of identity (black solid line) and a Loess smooth line (red solid line) were included as a reference. In the CWRES plots, dashed lines at +2 and -2 standard deviations from the mean (solid line) were included to indicate the expected region of approximately 95% of the data.

was included as a covariate on amoxicillin and clavulanic acid clearance, it significantly improved the fit of the model with drops in OFV of 6.63 points for amoxicillin, and 6.61 points for clavulanic acid. Model building to this stage was initially undertaken with the exclusion of BQL data, which comprised 9% of the observations. BQL data were then included to compare parameter estimates as described above. Given no significant parameter estimates differences were observed between BQL handling methods and the run time for the M3 method was more than 3-fold longer than the LOQ/2 method, the latter method was chosen for handling BQL. This model, with mechanistic covariates,

Table 3. Population pharmacokinetic estimates of amoxicillin/clavulanic acid

Parameter[a]	Estimate	Median Bootstrap Estimate	2.5th percentile from bootstrap[b] (n = 1000)	95th percentile from bootstrap[b] (n = 1000)
Amoxicillin				
CL(L/h/70 kg)	17.97	17.83	15.33	21.30
V1 (L/70 kg)	9.07	9.00	6.41	11.66
V2 (L/70 kg)	5.43	5.75	3.57	13.45
V3(L/70 kg)	11.24	11.00	7.01	13.78
Q1 (L/h/70 kg)	35.88	34.73	12.03	60.09
Q2 (L/h/70 kg)	5.52	5.36	1.49	8.03
θ in (CYSC/MCYSC)θ	-0.54	-0.54	-1.01	-0.14
θ in COVVASO=(1+θ)	-0.18	-0.18	-0.28	-0.07
BSV on CL (% CV)	18.9	18.1	8.10	28.6
BSV on V1 (% CV)	48.6	46.8	30.9	66.7
BOV on CL (% CV)	14.6	13.8	7.70	20.2
Residual error (% CV)	28.7	28.4	23.4	33.1
Clavulanic acid				
CL_{clav} (L/h/70kg)	12.20	12.09	10.54	14.55
$V1_{clav}$ (L/70 kg)	11.60	11.39	8.42	13.76
$V2_{clav}$ (L/70 kg)	9.85	10.22	8.05	13.87
$Q1_{clav}$ (L/h/70 kg)	6.22	6.81	3.94	23.9
θ in CYSCOV = (CYSC/MCYSC)θ	-0.37	-0.36	-0.85	-0.08
BSV on CL_{clav} (% CV)	15.6	15.6	7.8	22.6
θ in BSV on $V1_{clav}$ = θ*BSV on V1	0.77	0.77	0.40	0.97
θ in BOV on CL_{clav} = θ*BOV on CL	0.52	0.48	-0.02	1.34
Residual error (% CV)	35	34	27	40
Amoxicillin/clavulanic acid				
Hill coefficient[c]	1	1	1	1
TM_{50} (weeks)	39.90	39.10	20.66	64.29

[a]Abbreviations: CL, clearance; clav, clavulanic acid; V1, central volume of distribution; V2 and V3, peripheral volumes of distribution; Q1 and Q2, intercompartmental clearances; θ, model parameter in NONMEM code; CYSCOV, Cystatin C covariate on clearance; CYSC, plasma cystatin C value; MCYSC, median plasma cystatin C value; COVVASO, vasopressor covariate on clearance (θ=0 if no coadministration with vasopressors); BSV, between-subject variability; BOV, between-occasion variability; CV, coefficient of variation; TM_{50}, maturation half life

[b]Nonparametric bootstrap of 953 successful runs.

[c]Fixed value.

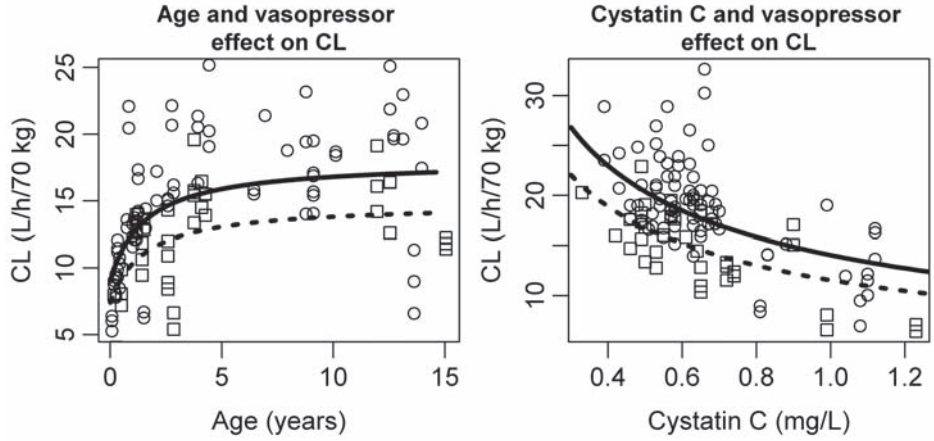

Figure 2. Impact of covariates in the final model. The left-hand plot shows weight- and cystatin C-standardised amoxicillin clearance with age split by whether patients were on vasopressors. The right-hand plot shows weight- and age-standardised amoxicillin clearance plotted against measured cystatin C split by whether patients were on vasopressors. Note there is more than one clearance value per subject, since between-occasion variability was included. Open circles, patients not on vasopressors; open squares, patients on vasopressors. Solid line, population predicted values if the patients were not on vasopressors; broken line, population predicted values if the patients were on vasopressors.

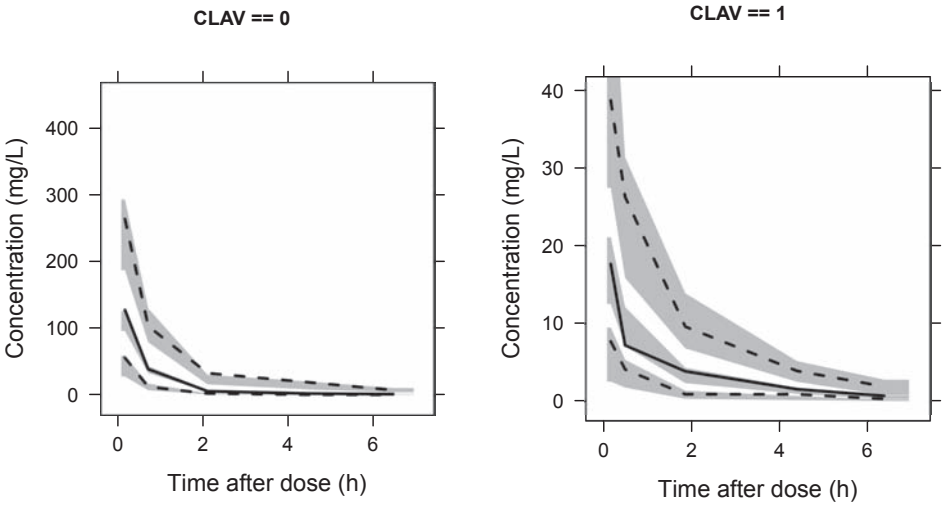

Figure 3. Stratified visual predictive check for amoxicillin (CLAV = 0) and clavulanic acid (CLAV = 1). The grey shaded areas are 95% confidence intervals of simulated 5th, 50th and 95th percentiles. The lines are 5th, 50th and 95th percentiles of raw data.

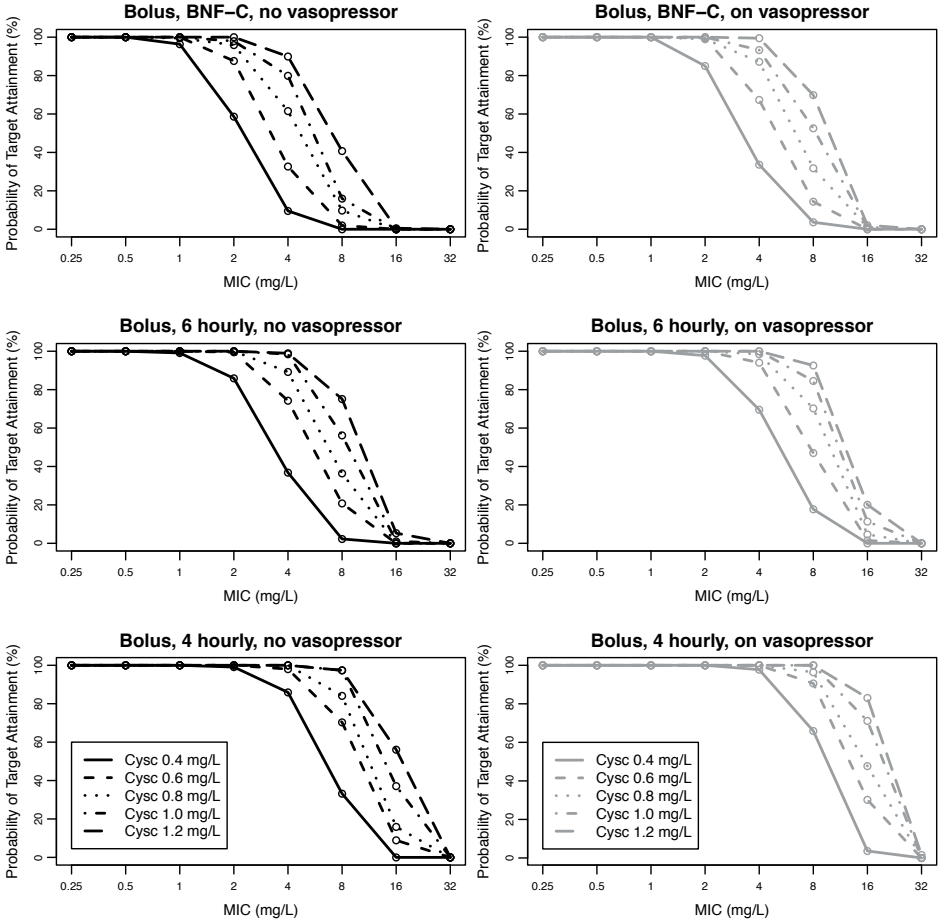

Figure 4A. Probability of Target Attainment (PTA) (n = 1000 patients) for amoxicillin according to a bolus dosing regimen, presence/absence of vasopressor therapy and plasma Cystatin C value. The three simulated dosing regimens were as follows: (i) 25 mg/kg every 12 h if under 3 months of age, otherwise every 8 h (British National Formulary for Children [BNF-C]19), (ii) 25 mg/kg every 6 h (Sanford Guide for Antimicrobial Therapy9), (iii) 25 mg/kg every 4 h (alternative dosing regimen). Amoxicillin target was defined as 40% of time above a MIC of 8 mg/L.

was then taken forward to the SCM, and vasopressor treatment on amoxicillin clearance further significantly improved the model fit for amoxicillin and therefore was retained in the final model. The impact of covariates is illustrated in **Figure 2**. The population PK parameter estimates and their precision are summarized in **Table 3**. The VPC plots are presented in **Figure 3**; the 5th, 50th and 95th percentiles of the predicted concentrations closely follow the percentiles of the observed data, suggesting a good model fit in both cases.

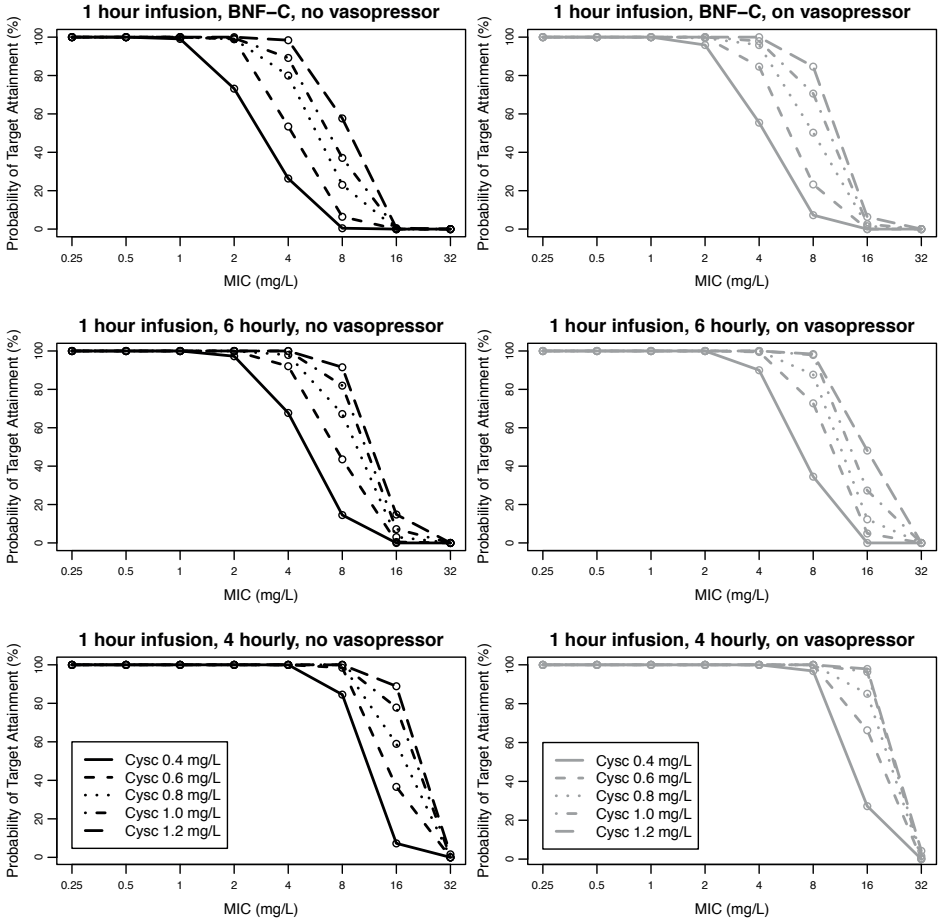

Figure 4B. Probability of Target Attainment (PTA) (n = 1000 patients) for amoxicillin according to a 1 hour infusion dosing regimen, presence/absence of vasopressor therapy and plasma Cystatin C value. The three simulated dosing regimens were as follows: (i) 25 mg/kg every 12 h if under 3 months of age, otherwise every 8 h (British National Formulary for Children [BNF-C]19), (ii) 25 mg/kg every 6 h (Sanford Guide for Antimicrobial Therapy9), (iii) 25 mg/kg every 4 h (alternative dosing regimen). Amoxicillin target was defined as 40% of time above a MIC of 8 mg/L.

Pathogens were grown in only 50% of patients and in 63.6% of patients treated for infection. The clinical failure in patients receiving amoxicillin/clavulanic acid treatment or prophylaxis was 32%; in those patients treated for infection alone, it was 34.4%. The main pathogens identified in patients with clinical failure were *Pseudomonas aeruginosa* (37.5%) (after which amoxicillin/clavulanic acid was switched to piperacillin/tazobactam) and *Enterobacteriaceae* (25%). No pathogen could be identified in 31.3% of patients.

Probability of target attainment for amoxicillin against MIC after the first dose are presented in **Figure 4 A-B**. Since no significant drug accumulation was seen, the results from the first 48 h of treatment were similar (data not shown). When doses were prescribed as a bolus according to the British National Formulary for Children (BNFc) dosing regimen, the Sanford Guide dosing regimen or the four-hourly dosing regimen (**Table 1**), the median target attainment values for clavulanic acid after the first dose were 48%, 66% and 96%, respectively; when the doses were given as a 1-h infusion, the median target attainment values were 53%, 73% and 99%, respectively.

DISCUSSION

This is, to our knowledge, the first report characterizing amoxicillin/clavulanic acid disposition in (critically ill) children using a population PK/PD modeling approach. Besides growth and maturation, renal function was found to be a significant covariate on amoxicillin and clavulanic acid clearance. This finding is in concordance with data in critically ill adults showing both clearances to be proportional to CrCL.[22] As previously discussed, we were not able to test Scr or CrCL as a covariate on drug clearances. However, the use of Scr and CrCL as markers for renal function have some major disadvantages, especially in children, as they are sensitive to changes in age, muscle mass, feeding and disease status.[23,24] Moreover, in younger children, Scr is generally underestimated when using the standardized Jaffe analysis method.[25] Finally, creatinine undergoes tubular secretion leading to an overestimation of glomerular filtration rate (GFR). CysC, a newer endogenous renal biomarker, was previously shown to be superior to plasma creatinine in estimating renal function in critically ill children.[26,27] Second, it has been shown that CysC is a good renal biomarker in children with sepsis[28] and that CysC-based GFR estimations remain accurate in children presenting with hyperfiltration (in contrast to Scr- based formulae).[24] Finally, CysC was found to better predict elimination of renally excreted drugs in adults when compared to Scr or CrCL.[29–32] This is only the second report to identify this new biomarker as a predictor of renal drug clearance in children at the expense of Scr or CrCL.[33] Treatment with vasopressors (mainly norepinephrine; 14/15 patients) was also associated with an 18% decrease in amoxicillin clearance. Although high-quality scientific evidence is lacking, available data for norepinephrine suggest a positive effect on renal blood flow in patients presenting with sepsis.[34] One may hypothesize that treatment with vasopressors should be considered an overall parameter reflecting critical illness severity, irrespective of renal function.

The observed population estimate for amoxicillin clearance is much higher than previously reported in critically ill adults (10 L/h/75 kg) and somewhat variable between

doses (BOV 14.6% CV).[22] A high mean clearance was previously reported in 15 seriously ill children aged 2 to 14 years using a noncompartmental (NCA) PK analysis (16.99 L/h/1.70m^2).[7] The elevated clearance could be explained by a state of 'augmented renal clearance' (ARC) in our study's patient population. Although the underlying pathophysiological mechanisms are yet to be revealed[35], this phenomenon has been increasingly investigated in the critically ill adult population, including its impact on the PK and PD of renally cleared antimicrobials[36], but ARC has –to the best of our knowledge– never before been described in critically ill children. The hypothesis of a 'hyperdynamic' status of our study is supported by the fact that a large proportion of measured renal biomarkers was undetectable (Scr) or low (CysC) compared to age-corrected reference values.[37] A plausible explanation, besides the analytical challenges for creatinine as described above, could be a faster renal clearance of these endogenous compounds. Moreover, as we observed trough concentrations from maintenance doses that remained very low in most patients, we could conclude that no accumulation in steady-state conditions was attained, probably due to the enhanced renal capacity. It is also worth noting that although one would expect a correlation between age and CysC below the age of 2 years[37], we were not able to identify such a relationship in our patient population. It is possible that maturational changes in CysC were masked in our study due to ARC.

Regarding volumes of distribution, the observed population estimate for amoxicillin is similar to what has been reported in critically ill adults (27.4 L/75 kg)[22] and slightly lower than that found by Jones *et al*.[7] Similar trends in clearance and volume of distribution were observed for the clavulanic acid compound.

Of particular importance, our data challenge current paediatric dosing recommendations, as they could lead to subtherapeutic treatment in severe infections (**Figure 4 A-B**). Although our study has a small sample size, clinical outcome data from our study suggest that underdosing of amoxicillin/clavulanic acid could result in clinical failure in severe infections with *Enterobacteriaceae* (EUCAST and CLSI MIC breakpoints are 8 mg/L). We have shown that, at minimum, a dosing regimen of 25 mg/kg (based on the amoxicillin component) every 4 h is warranted in those infections (given as a bolus in children with CysC above 1 mg/L and as a 1 h infusion to children with CysC under 1 mg/L). It was decided not to simulate longer infusion times in order to maximize tissue penetration and to circumvent potential drug incompatibilities, drug stability issues and drug administration errors with more-complex dosing regimens.[39] This dosing recommendation is fully in concordance with Jones *et al*., suggesting that higher/more-frequent amoxicillin/clavulanic acid dosing might be justified and needed in paediatric intensive care units.[7] Moreover, it should be highlighted that 40%$fT_{>MIC}$ is a rather conservative target, as 100 % $fT_{>MIC}$ has been associated with better outcomes in critically ill adults.[40]

With regard to clavulanic acid, no clear-cut PK/PD targets are reported. As EUCAST clinical susceptibility breakpoints were determined in the presence of 2 mg/L clavulanic acid, this was chosen as the target concentration.[21] We hypothesized that lower targets of beta-lactamase inhibitors could potentially result in higher MIC breakpoints for the combined penicillin antibiotic. This hypothesis is supported by the study from Liu et al. demonstrating that for equal piperacillin exposure, different tazobactam half-lives have a significant effect on antimicrobial outcome.[41] Although a larger interpatient variability was observed than for amoxicillin, our dosing recommendation (as above) resulted in an acceptable target attainment with no accumulation after 48h of treatment. With regard to potential toxicity of higher cumulative amoxicillin/clavulanic acid daily doses, it should be noted that, for our optimized dosing regimen, we have specifically chosen not to select higher individual doses (25 mg/kg based on the amoxicillin compound) to eliminate potential safety risks related to higher peak concentrations. Finally, we feel confident that our optimized dosing regimen will not increase idiosyncratic clavulanic acid induced liver toxicity, as it is known that the mechanism of its toxicity is immunoallergic, to some extent genetically controlled and dose independent.[42]

This research has some notable limitations. First, the studied population included a heterogeneous group of children with regard to possible differences in (suspected) infecting organism and tissue involvement/penetration. Second, total drug plasma concentrations were mathematically corrected for protein binding instead of free drug concentration measurement in plasma or drug measurement at the site of infection. However, this simplification was previously found to be acceptable for β-lactam antibiotics with low protein binding like amoxicillin and clavulanic acid.[43] Third, MIC values were not prospectively determined. Instead, a worst-case scenario using the clinical breakpoints for *E. coli* was chosen to challenge dosing regimens regardless of the infecting organism. This approach is justifiable, as β-lactam antibiotics have a wide therapeutic index and the consequences of potentially supratherapeutic dosing are therefore of less concern. Fourth, notwithstanding that a substantial number of younger patients were recruited, PK data from additional neonates and infants are needed to estimate maturation parameters more precisely on both clearances and refine dosing regimens in these age categories.

In conclusion, this is the first population PK study demonstrating that the current dosing recommendations for amoxicillin/clavulanic acid can result in subtherapeutic treatment in critically ill children, thereby risking treatment failure. Besides developmental changes, CysC as a (new) biomarker for renal function and co-treatment with vasopressors were found to be significant covariates influencing drug disposition. The findings from this study make a significant contribution to knowledge regarding how to optimize the

clinical use of amoxicillin/clavulanic acid in critically ill children. Whether these results of augmented renal clearance can be extrapolated to other renally cleared (β-lactam) antibiotics or indeed other classes of medication remains speculative and needs to be investigated in future research.

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TRANSPARENCY DECLARATIONS

None to declare.

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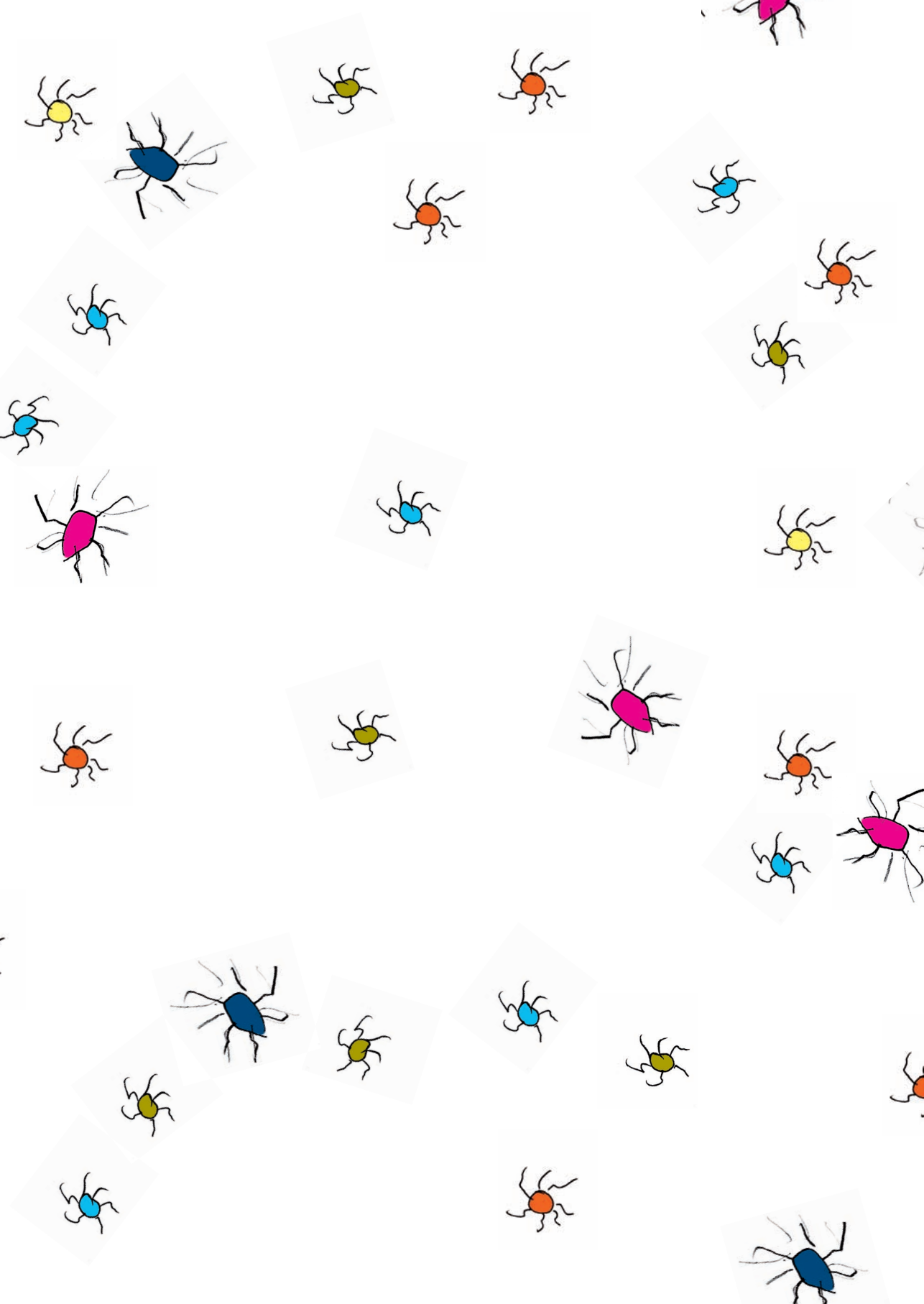

CHAPTER 4

DOSE OPTIMISATION OF PIPERACILLIN/ TAZOBACTAM IN CRITICALLY ILL CHILDREN

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ABSTRACT

Objectives: The aim of this study was to characterize the population pharmacokinetics of piperacillin and tazobactam in critically ill infants and children, in order to develop an evidence-based dosing regimen.

Patients and Methods: This pharmacokinetic study enrolled patients admitted to the paediatric ICU for whom intravenous piperacillin/tazobactam (8:1 ratio) was indicated (75 mg/kg q6h based on piperacillin). Piperacillin/tazobactam concentrations were measured by a liquid chromatography-tandem mass spectrometry method. Pharmacokinetic data were analysed using non-linear mixed-effects modelling. Clinicaltrials.gov: NCT02456974.

Results: Piperacillin and tazobactam blood samples were collected from 47 patients (median age = 2.83 years; range = 2 months to 15 years). Piperacillin and tazobactam disposition was best described by a two-compartment model which included allometric scaling and a maturation function to account for the effect of growth and age. Mean clearance estimates for piperacillin and tazobactam were 4.00 L/h and 3.01 L/h for a child of 14 kg. Monte Carlo simulations showed that an intermittent infusion of 75 mg/kg (based on piperacillin) q4h over 2 h, 100 mg/kg q4h given over 1 h or a loading dose of 75 mg/kg followed by a continuous infusion of 300 mg/kg/24h were minimally required to achieve the therapeutic targets for piperacillin (60% $fT_{>MIC}$>16 mg/L).

Conclusion: Standard intermittent dosing regimens do not ensure optimal piperacillin/tazobactam exposure in critically ill patients, thereby risking treatment failure. The use of a loading dose followed by a continuous infusion is recommended for treatment of severe infections in children >2 months of age.

INTRODUCTION

Paediatric sepsis and septic shock reportedly affect 30% of children admitted to paediatric ICU, with a 25% mortality rate.[1] Early intervention with appropriate antibiotic treatment remains a cornerstone in the pharmacological treatment of those children.

Piperacillin/tazobactam is a broad-spectrum β-lactam antibiotic commonly used in the paediatric ICU for (empirical) treatment of severe infections. Typical indications include ventilator-associated pneumonia, intra-abdominal infections and sepsis of unknown origin. Despite its use, only treatment of intra-abdominal infections in children older than 2 years is currently approved by the European Medicines Agency.[2] This means that clinical practice still represents off-label use of this drug combination in younger paediatric patients.

It is well known that the efficacy of β-lactam antibiotics most strongly relates to the time during which the unbound drug concentration (*f*T) is above the pathogen MIC of the pathogen. The target pharmacokinetic/pharmacodynamics (PK/PD) index (i.e. $fT_{>MIC}$) associated with positive clinical outcomes for β-lactams in critically ill patients is a $fT_{>MIC}$ between 50% to 100% of the dosing interval.[3] Recent studies reported the PK/PD efficacy index for the β-lactamase inhibitor (BLI) tazobactam to be the percentage of time during which the unbound concentration remains above a threshold concentration ($fT>C_T$).[4,5] $fT>C_T$ targets ranged from 35% to 85% of the dosing interval, depending on the antibiotic-BLI combination and stability of the β-lactamase. Threshold concentration targets were thought to depend on β-lactamase transcription level, with upper limits of 4 mg/L used.[5,6]

Piperacillin and tazobactam are predominantly excreted in unchanged form by glomerular filtration and tubular secretion (piperacillin: 46% to 73%; tazobactam: 65% to 80%).[7] In addition, saturable renal elimination has been identified previously in adults.[8–10] To date, the pharmacokinetics of piperacillin/tazobactam have been described in (pre)term neonates and non-ICU children, but only in a small number of children admitted to the paediatric ICU (n = 13 and n = 12 patients), between 1 and 9 years of age.[7,11–15]

Any effort to define the dose rationale in infants and young children needs to account for the effect of developmental processes, which are known to affect drug exposure and potentially treatment response.[16] Moreover, the impact of pathophysiological changes on pharmacokinetics has been widely demonstrated in critically ill adults.[17–19] The aims of this study were therefore: (i) to investigate the pharmacokinetics of intravenous piperacillin and tazobactam in critically ill infants and children and (ii) to revisit the dose

rationale of the drug combination and evaluate the efficacy of current and alternative dosing regimens in this population based on PK/PD indices.

PATIENTS AND METHODS

Study design and ethics

A prospective, pharmacokinetic study was conducted at the paediatric ICU unit of the Ghent University Hospital, Ghent, Belgium between May 2012 and March 2014. Patients between 1 month and 15 years of age admitted to the paediatric ICU in whom treatment with intravenous piperacillin/tazobactam was clinically indicated, were included. Patients were excluded if they required an extracorporeal circuit or did not have, other than the drug infusion line, an arterial or intravenous access available for blood sampling. The research was conducted in accordance with the guidelines of the Declaration of Helsinki, was approved by the institutional Ethics Committee (EC/2012/172) and was registered at Clinicaltrials.gov (NCT02456974). Written informed consent was obtained from the parents or legal representatives as well as assent from patients older than 12 years. Collected demographic and clinical variables included: body weight (WT), postmenstrual age (PMA), primary reason for admission, measures of organ function and patient severity of illness as described by the PELOD (Pediatric Logistic Organ Dysfunction) Score, PRISM II (Pediatric Risk of Mortality) Score, type of catheter used for drug administration and blood sampling, presence of mechanical ventilation, co-treatment with vasopressors and nephrotoxic medications (amikacin, ibuprofen, diclofenac, vancomycin, teicoplanin), presence of surgery, fluid resuscitation (>60 mL/kg per 24 h), and C-Reactive Protein (CRP).[20,21]

Drug dosing and administration

Piperacillin/tazobactam (Tazocin® 2 g/250 mg and Tazocin® 4 g/500 mg, Pfizer, Belgium) was prescribed in a dose of 75 mg piperacillin per kilogram body weight (maximum 4000 mg) every 6 h and administered intravenously over 5 to 30 min using a calibrated syringe driver, according to current dosing guidelines.[22] Immediately after drug administration, infusion lines were flushed with normal saline with a minimum of twice the dead space volume.

Blood sampling

Serial blood samples were obtained from 1st and/or assumed steady-state doses from an indwelling catheter other than the drug infusion line. The total number of samples collected (per individual patient) was limited by the predefined total maximum blood volume permitted for PK sampling (i.e. 2.4 mL/kg body weight).[23] A typical sampling

scheme included blood sampling just before dosing (t = 0), immediately after dosing and flush, between 5 and 70 min after the start of the infusion, at 3 h after the start of the infusion and a trough sample just prior to the next dose. All samples were immediately transferred on ice to the chemistry laboratory and centrifuged (8 min, 1885g) after which the resulting plasma was frozen at -80°C for a maximum of 3 months before assay.

Drug and biochemical assays

Piperacillin and tazobactam total plasma concentrations were quantified simultaneously using a validated UPLC-tandem mass spectrometry method.[24] The lower limit of quantification (LLOQ) was 0.5 mg/L for both compounds and the imprecision was < 15% at all levels. For the first 29 patients, only the piperacillin compound was quantified. Plasma Cystatin C (CysC) was measured using the N Latex cystatin C assay on the Behring Nephelometer II (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) (intra-assay coefficient of variation [CV]: 1.4%; inter-assay CV: 5.4%) and was standardized according to the ERM-DA471/IFCC reference material.[25] Creatinine was measured in serum (Scr) using the rate-blanked compensated Jaffe technique (Modular P and Cobas 6000, Roche Diagnostics GmbH, Mannheim, Germany).

Pharmacokinetic analysis

The pharmacokinetics of piperacillin/tazobactam was evaluated using non-linear mixed effects modelling. Data were analysed using the first-order conditional estimation method with the interaction option (FOCE-I), as implemented in NONMEM version 7.2 (ICON PLC; Ellicott City, Maryland). R (version 3.1.1) and PsN (version 3.5.3) were used for pre- and post-processing of the data as well as the creation of graphical and statistical summaries. One-, two-, and three-compartment disposition models with zero order input were tested to characterize the time course of plasma concentrations of both compounds independently using the ADVAN subroutines.[26] For piperacillin, first-order (FO), Michaelis-Menten (MM) and FO+MM elimination were also evaluated. A decrease in objective function value (OFV) of 3.84 points ($p<0.05$) or more was considered statistically significant assuming a χ^2 distribution for nested models. Goodness-of-fit included visual inspection of the following plots: observed versus population predicted concentrations, observed versus individual predicted concentrations, conditional weighted residuals versus time, conditional weighted residuals versus population predicted concentrations.

A log-normal distribution was assumed for the between-subject variability (BSV), whereas additive and proportional models (and a combination of both) were tested to describe residual variability in the data. Interoccasion variability (IOV) was tested on clearance and central volume of distribution of piperacillin.

Covariate model building

Continuous covariates were evaluated using a linear or exponential equation (equation 1):

$$P_i = P_{pop} \times \left(\frac{COV}{median\ (COV)}\right)^k \text{(1)}$$

In this equation P_i represents the individual parameter estimate of the ith subject, P_{pop} represents the population parameter estimate, COV is the covariate of interest and k the exponent which is fixed to 1 for a linear function and estimated for an exponential function.

Binary covariates were tested using the following equation (equation 2):

$$P_i = P_{pop} \times (1 + m_i) \text{ (2)}$$

where m was estimated for one of the dichotomic covariate values (e.g. males).

Body weight was *a priori* included as a covariate using a power function with a fixed exponent of 0.75 on clearance and 1 on volume parameters. Furthermore, as children below the age of 2 years were included, a Hill function based on postmenstrual age was tested to describe maturation on clearance (equation 3):

$$F_{mat} = \frac{PMA^{HILL}}{TM_{50}{}^{HILL} + PMA^{HILL}} \text{ (3)}$$

where F_{mat} represents the maturation function, PMA the postmenstrual age, Hill the Hill coefficient describing the steepness of the function, and TM_{50} the maturation half-life.[27]

The potential impact of remaining covariates was explored by visual inspection of post-hoc individual PK parameter estimates and deviations from population-predicted PK parameters (ETAs) versus covariate plots. Only clinically relevant associations were considered: gender, serum cystatin C, PELOD score, PRISM score, admission reason and co-medication related covariates for clearance, and age, gender, PELOD score, PRISM score and admission reason for volumes of distribution.

To account for the age effect in serum cystatin C values, data from Fischbach *et al.* and Randers *et al.* were used as reference (i.e. typical value) for each age ($T_{cystatin\ C}$). [28,29] An exponential decline was found, according to following equation (equation 4):

$$T_{cystatin\ C} = 1.598 \times e^{-0.624 \times age\ (years)} \text{ (4)}$$

Above the age of 1.3 years, a typical constant value of 0.8 mg/L was used.

Possible influence of serum cystatin C on clearance was subsequently evaluated using measured serum cystatin C values ($M_{cystatin\ C}$) and according to following equation (equation 5):

$$\left(\frac{M_{cystatin\ C}}{T_{cystatin\ C}}\right)^{n} \quad (5)$$

Scr could not be evaluated as potential covariates on clearance, given 39% of Scr samples were below quantification limit (BQL). Selected covariates were then separately entered into the model and evaluated by use of OFV via forward inclusion ($p<0.05$) and backwards elimination methods ($p<0.001$). In addition, a clinically relevant reduction in the magnitude of BSV on the parameter of interest, acceptable precision of the model parameters, and visual inspection of the goodness-of-fit plots were used to support the additional inclusion of additional covariates into the model.

Model evaluation

Model performance, stability and robustness were evaluated using a nonparametric bootstrap analysis (n = 1000 samples), a stratified visual predictive check (pcVPC) stratified for weight (n = 1000 simulations), and the normalised prediction distribution error (NPDE) (n = 1000 simulations).[30,31]

PTA simulation analysis

Monte Carlo simulations were performed to simulate piperacillin and tazobactam exposures for 3500 patients (**Table 1**).[22,32] The simulation dataset was created using a function described by Sumpter *et al*., in order to simulate weights based on postmenstrual age and sex, evenly distributed within the age range of our patient population (n = 250 boys and 250 girls each, for the age categories 1 to 6 monts, 6 months to 1 year, 1 to 2 years, 2 to 4 years, 4 to 8 years, 8 to 12 years, 12 to 15 years).[33]

Based on these simulations, $fT_{>MIC}$ and $fT>C_T$ were calculated for the first 48 h of treatment, as early and appropriate therapy is most critical.[34] The target efficacy exposure for piperacillin was defined as 60% $fT_{>MIC}$ and PTA was calculated for MICs between 1 to 64 mg/L.[16] A PTA ≥ 90% was defined as optimal. To evaluate proposed dosing regimens, an infection with *Pseudomonas aeruginosa* and MIC of 16 mg/L was used, according to the EUCAST breakpoint for piperacillin.[35] For tazobactam, the reference target efficacy exposure values included 40%, 60% and 80% $fT>C_T$ and PTA was calculated for C_Ts between 0.25 and 8 mg/L. Given that tazobactam is given in a fixed combination with piperacillin (ratio 8:1), only those dosing regimens with a PTA≥90% for piperacillin were appraised (**Table 1**). Mean protein binding of piperacillin and tazobactam is 30%, and this was used to simulate unbound concentrations.[36]

Table 1. Simulated dosing scenarios

Intermittent dosing regimen[a]	Infusion duration
75 mg/kg every 4 h	0.25, 0.5, 1, 2 h
75 mg/kg every 6 h	0.25, 0.5, 1, 2 h
75 mg/kg every 8 h	0.25, 0.5, 1, 2 h
100 mg/kg every 4 h	0.25, 0.5, 1, 2 h
100 mg/kg every 6 h	0.25, 0.5, 1, 2 h
100 mg/kg every 8 h	0.25, 0.5, 1, 2 h
Continuous infusion dosing regimen[a]	
LD of 75 mg/kg over 1 h, followed by CI 300 mg/kg over 24 h	
LD of 75 mg/kg over 1 h, followed by CI 350 mg/kg over 24 h	
LD of 75 mg/kg over 1 h, followed by CI 400 mg/kg over 24 h	

[a]Based on piperacillin component and a fixed ratio of piperacillin:tazobactam of 8:1.
Abbreviations: LD: loading dose; CI: continuous infusion.

Table 2. Demographic and clinical characteristics of the study population

Variable[a]	Median (range)
Gender	
Male	21 (44.7%)
female	26 (55.3%)
Age (years)	2.83 (0.17-15)
Weight (kg)	14 (3.40-45)
PRISM II score	8 (0-40)
Primary reason for ICU admission	
respiratory	11 (23.4)
gastro-intestinal	10 (21.3)
neurologic	7 (14.9)
postoperative	7 (14.9)
cardiovascular	7 (14.9)
burn	2 (4.3)
oncology	1 (2)
other	2 (4.3)
Mechanical ventilation[b]	25 (53.2)
Vasopressor treatment[b]	15 (31.9)
PELOD score[c]	1 (0-32)
Serum Creatinine (mg/dL)[c,d]	0.21(<0.17-0.55)
Plasma Cystatin C[c,d] (mg/L)	0.66 (0.38-1.13)
Serum CRP[e] (mg/L)	7.8 (0.1-147)

[a]Abbreviations: PRISM, Pediatric Risk of Mortality; PELOD, Pediatric Logistic Organ Dysfunction; CRP, C-Reactive Protein.
[b]During ICU stay.
[c]At day(s) of sampling.
[d]Below quantification limit in 14 patients.
[e]Based on values from 44 patients.

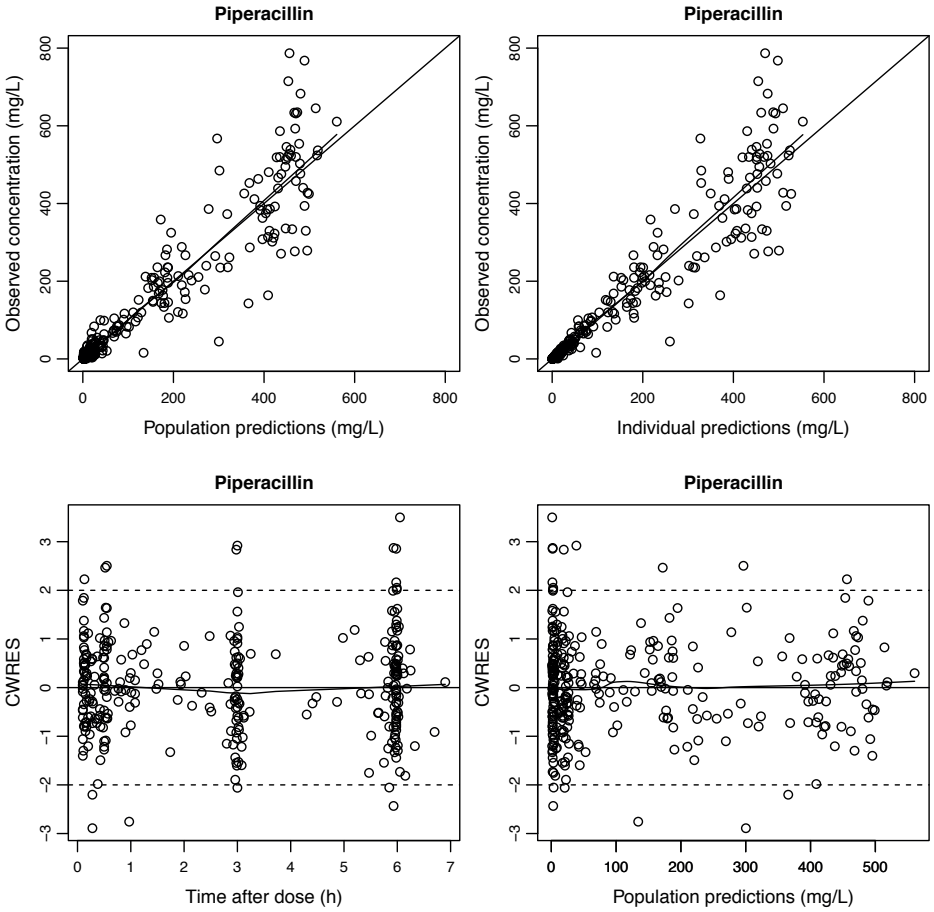

Figure 1A. Goodness-of-fit diagnostic plots for piperacillin: observations versus population predictions and individual predictions and conditional weighted residuals (CWRES) versus time after dose and population predictions.

RESULTS

A total of 47 patients were included; demographic, clinical and treatment characteristics are summarized in **Table 2**. Patients younger than 2 years accounted for 42.5% of the study population (n = 20). Of a total of 317 piperacillin and 125 tazobactam plasma samples collected, 7 piperacillin (2%) and 4 tazobactam (3%) concentrations were excluded from pharmacokinetic analysis due to sampling errors. Median number of samples available for analysis per patient was 7 for piperacillin and 6 for tazobactam.

A two-compartment model with first-order elimination best described the data of both piperacillin and tazobactam. BSV for piperacillin and tazobactam was described using

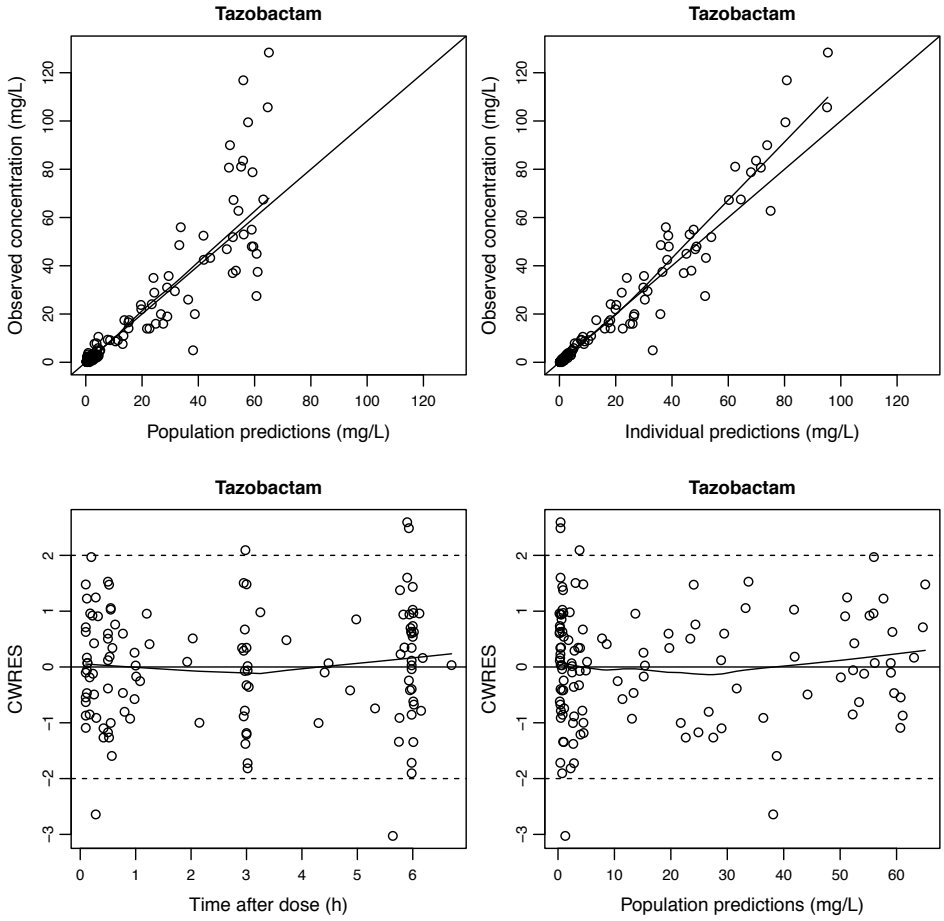

Figure 1B. Goodness-of-fit diagnostic plots for tazobactam: observations versus population predictions and individual predictions and conditional weighted residuals (CWRES) versus time after dose and population predictions.

an exponential model and was identified on clearance and all volume parameters. A proportional error model was used to describe residual variability for both compounds. Neither saturable elimination nor IOV on clearance and volume of distribution was identified on piperacillin. BSV on the central volume of distribution of the piperacillin compound was estimated to be close to a value of 0 after inclusion of allometric scaling. No significant change in OFV was noted when it was fixed to 0 for subsequent model building steps. Implementation of a concomitant vancomycin treatment covariate on piperacillin clearance resulted in a drop in OFV of 18.57 points with a marginal decrease of BSV on clearance. With only six individuals receiving vancomycin, and a potential confounding by age differences between those who received vancomycin and those

who did not (median age [range] 4.71 [3.08-11.92] years, versus 2.17 [0.17-15] years), this covariate was not included in the final model. In addition, the more parsimonious final model incorporating weight and PMA as described above performed reasonably well, with only slight deviations in the higher concentration range **(Figure 1A-B).** No other collected clinical variables were deemed necessary for further statistical covariate testing, based on visual inspection of the covariate plots.

The final covariate equations, population PK parameter estimates and their precision are summarized in **Table 3**. All structural model parameters were estimated with adequate precision, which was further confirmed with the bootstrap analysis. The pcVPC plots are presented in **Figure 2**; the 5th, 50th and 95th percentiles of the predicted concentrations closely follow the percentiles of the observed data, suggesting a good model fit in both cases. The NPDE mean and variance were not significantly different from 0 and 1, respectively ($p>0.1$) (Figure not shown).

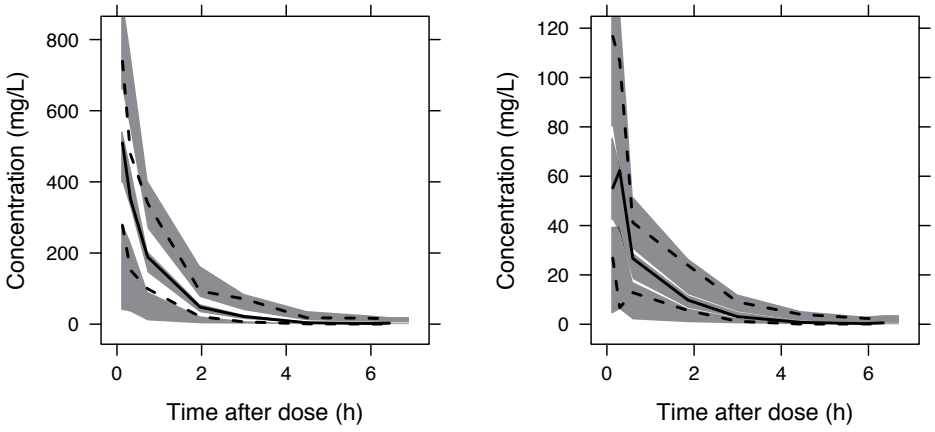

Figure 2. Stratified visual predictive check (n = 1000 simulations) for piperacillin (left panel) and tazobactam (right panel): grey shaded areas are 95% confidence intervals of simulated 5th, 50th and 95th percentiles, lines are 5th, 50th and 95th percentiles of raw data.

The PTA for piperacillin by MIC after 48 h of treatment for different dosing scenarios (**Table 1**) are presented in **Figure 3** (intermittent dosing) and **Figure 4** (continuous dosing regimens). With a MIC value of 16 mg/L, PTA for intermittent dosing regimens ranged from 5.9% (75 mg/kg piperacillin every 8 h, 15 min infusion) to 99% (100 mg/kg piperacillin every 4 h, 2-h infusion). Three intermittent dosing regimens met the PTA criterion of 90% (75 mg/kg piperacillin every 4 h, infusion over 2 h; 100 mg/kg every 4 h over 1-2 h). For all continuous dosing regimens, PTA was 100% for the time after the loading dose.

Table 3. Population Pharmacokinetic Estimates of Piperacillin/tazobactam

	Piperacillin					Tazobactam				
	Estimate	**RSE (%)**	**Bootstrap estimates (n=1000)[a]**			**Estimate**	**RSE (%)**	**Bootstrap estimates (n=1000)[a]**		
			Median	*Percentile 2.5%*	*97.5%*			*Median*	*Percentile 2.5%*	*97.5%*
Structural model parameters										
$CL_i = CL_{pop} \times \left(\frac{WT}{14}\right)^{0.75} \times \left(PMA^{HILL}/TM_{50}{}^{HILL} + PMA^{HILL}\right)$										
CL(L/h)	4.00 (0.25)[b]	8	4.04	3.50	5.50	3.01 (0.13)[b]	5	3.01	2.72	3.33
TM_{50} (weeks)	61.2	15	62.5	46.2	126	41.2	11	41.7	33.6	51.31
Hill coefficient	1.62	27	1.60	0.74	2.84	2.96	31	3.10	1.41	14.3
$Q_i = Q_{pop} \times \left(\frac{WT}{14}\right)^{0.75}$										
Q (L/h)	2.72 (0.19)[b]	14	2.68	2.06	3.78	2.11 (0.15)[b]	28	2.18	1.34	6.64
$V_i = V_{pop} \times \left(\frac{WT}{14}\right)^{1}$										
V1 (L)	1.80 (0.13)[b]	5	1.80	1.63	1.99	1.86 (0.13)[b]	12	1.82	1.19	2.15
V2 (L)	1.59 (0.11)[b]	9	1.58	1.35	1.92	1.58 (0.11)[b]	16	1.59	1.19	2.51
Between-subject variability										
BSV CL (% CV)	26.7	25	25.5	18.5	31.3	14.5	29	11.7	3.92	19.8
BSV V_1 (% CV)	0[c]	-	-	-	-	41.1	14	41.0	21.4	57.6
BSV Q (% CV)	0[c]	-	-	-	-	0[d]	-	-	-	-
BSV V_2 (% CV)	22.6	72	22.9	8.42	37.8	27	21	26.1	12.9	36.5
Residual variability										
Proportional (% CV)	31.0	14	30.5	26.5	34.6	30.5	20	30.2	24.0	36.6
Additive (mg/L)	0.0001[c]	-	-	-	-	0.0001[c]	-	-	-	-

[a]Non-parametric bootstrap: 969 runs mimization successful for piperacillin; 850 runs minimization succesful for tazobactam.

[b]Parameters per kg body weight.

[c]Fixed value.

Abbreviations: CL_i, individual clearance; CL_{pop}, population clearance; WT_{med}, median weight; PMA, postmenstrual age; TM_{50}, maturation half-life; Hill, Hill coefficient; Q, intercompartmental clearance; BSV, between-subject variability; RSE, relative standard error

PTA for tazobactam by C_T after 48h of treatment are presented in **Figure 5** (selection of intermittent dosing regimens with PTA>90% for piperacillin) and **Figure 6** (continuous dosing regimens). For a C_T below 2 mg/L, PTA was >90% for all selected intermittent dosing scenarios, regardless of the target $fT>C_T$ (12.5 mg/kg tazobactam every 4h, 1-2h infusion, 9.375 mg/kg every 4 h, 2 h infusion). For all continuous dosing regimens, PTA for a C_T of 4 mg/L was 100% for the time after the loading dose, regardless of the target $fT>C_T$.

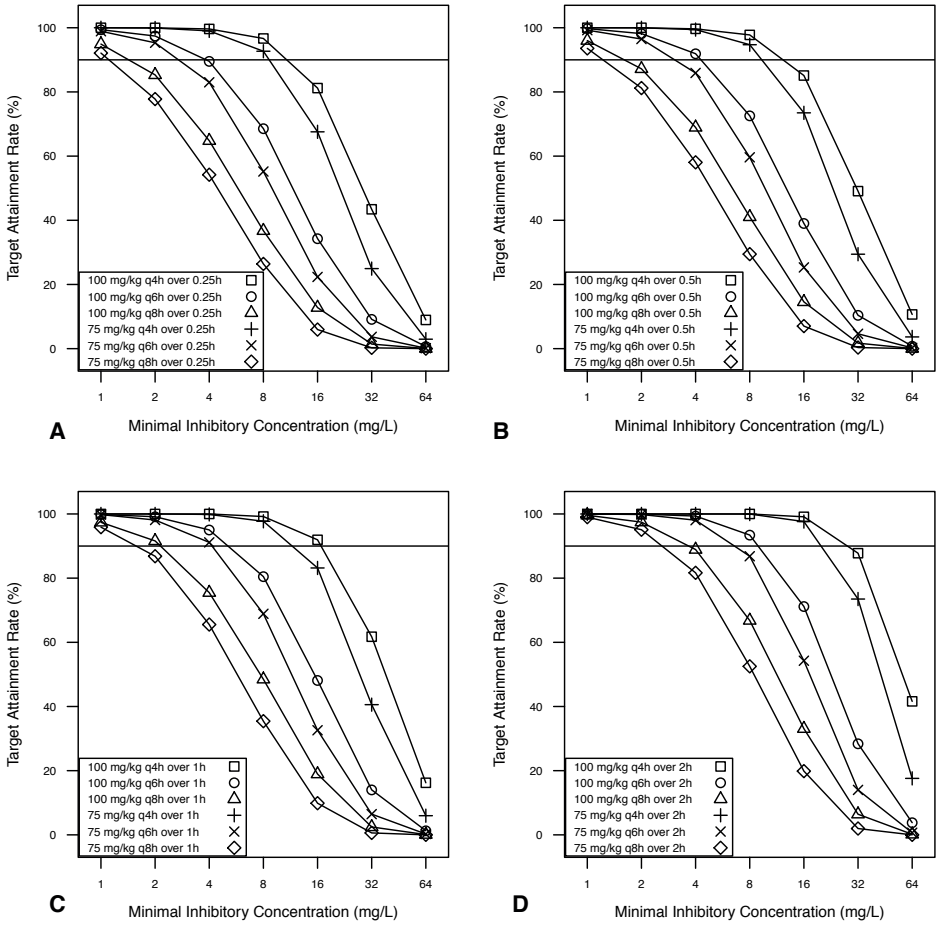

Figure 3. PTA for piperacillin (n = 3500 patients) according to following intermittent dosing regimens: (A) 75 mg/kg every 4, 6, 8 h or 100 mg/kg every 4, 6, 8 h over 0.25 h, (B) 75 mg/kg or 100 mg/kg every 4, 6, 8 h over 0.5 h, (C) 75 mg/kg or 100 mg/kg every 4, 6, 8 h over 1 h, (D) 75 mg/kg or 100 mg/kg every 4, 6, 8 h over 2 h. Piperacillin target was defined as 60% of time above a MIC of 16 mg/L. The solid horizontal line represents 90%.

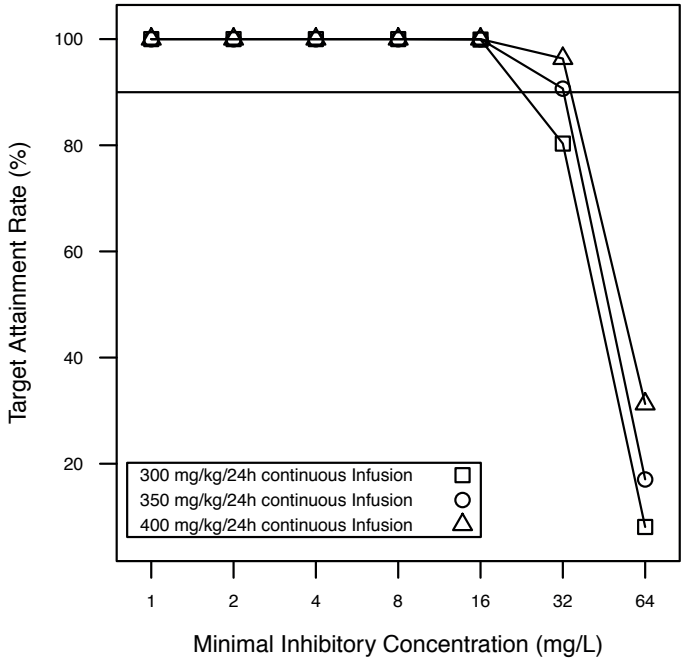

Figure 4. PTA for piperacillin (n = 3500 patients) according to following continuous dosing regimens: loading dose of 75 mg/kg over 1 h, followed by (i) a continuous infusion (CI) of 300 mg/kg/24h, (ii) CI of 350 mg/kg/24h, (iii) CI of 400 mg/kg/24h. Piperacillin target was defined as 60% of time above a MIC of 16 mg/L. The solid horizontal line represents 90%.

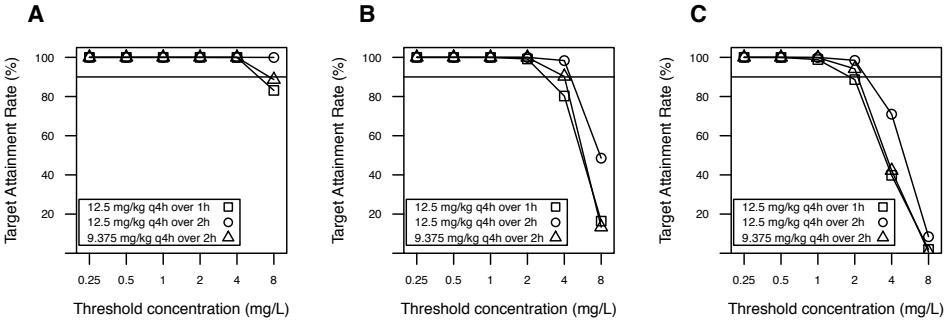

Figure 5. PTA for tazobactam (n = 3500 patients) according to following intermittent dosing regimens: (i) 9.375 mg/kg every 4 h, 2 h infusion, (ii) 12.5 mg/kg every 4 h, 1 h infusion, (iii) 12.5 mg/kg every 4 h, 2 h infusion. Tazobactam target was evaluated for (A) 40%, (B) 60% and (C) 80% of time above the threshold concentration. The solid horizontal line represents 90%.

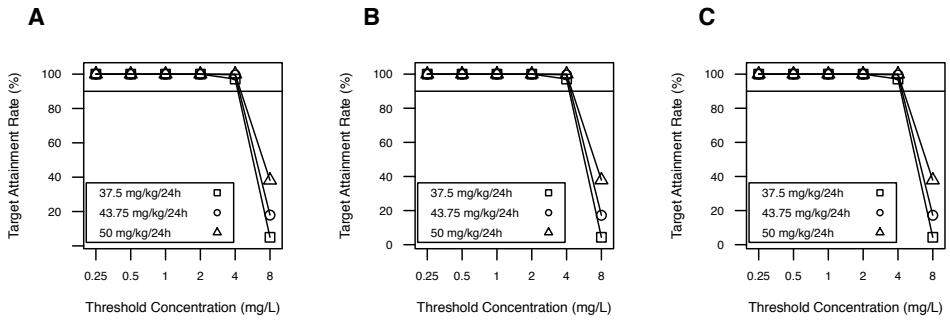

Figure 6. PTA for tazobactam (n = 3500 patients) according to following continuous dosing regimens: loading dose of 9.375 mg/kg over 1 h, followed by (i) a continuous infusion (CI) of 37.5 mg/kg/24h, (ii) CI of 43.75 mg/kg/24h, (iii) CI of 50 mg/kg/24h. Tazobactam target was evaluated for (A) 40%, (B) 60% and (C) 80% of time above the threshold concentration. The solid horizontal line represents 90%.

DISCUSSION

This is, to our knowledge, the largest study to date in which the pharmacokinetics of piperacillin and tazobactam has been characterized in critically ill infants and children (n = 47). This is also the first time that pharmacokinetic data have been collected in children between the ages of 2 months and 1 year (n = 14) and 9 and 15 years (n = 10).

Of note is the fact that we have characterized the effect of growth and organ maturation on the pharmacokinetics of both compounds, as demonstrated by the functions describing the clearance of both piperacillin and tazobactam. A similar model describing the effect of organ maturation was proposed by Tornoe *et al.*, who analysed pooled data from hospitalized children with a suspected or proven infection, and Rhodin *et al.*, who described the maturation on glomerular filtration rate.[37,38] The maturation half-life, which is the age associated with 50% maturation of clearance, and the age associated with full maturation in our study were 5.5 months and 4.8 years, respectively (**Table 3**). These estimates were significantly lower than previously reported by the forementioned authors, (maturation half-life: 2.2 months; full maturation around 2 years of age) and suggest that critical illness could cause a (temporary) impairment of the underlying renal maturation process.[37,38]

When comparing the maturation parameter estimates of piperacillin versus tazobactam in our population, it seems that maturation of tazobactam clearance was less affected when compared to piperacillin clearance, with a maturation half-life and age of full-maturation closer to Tornoe and Rhodin estimates.[37,38] Although more data from neonates and infants are needed to estimate maturation more accurately, these observations raise

questions about the impact of fixed-dose combinations of piperacillin/tazobactam in seriously ill young children.

Since both compounds are renally cleared, one cannot exclude the role of organ function on the elimination of either compound. Hence, while the relationship between markers of renal function is plausible and expected, variations in drug clearances in this group of patients were captured primarily by body weight and the maturation function, which is in agreement with findings from previous studies in critically ill children.[14,15]

Cystatin C is a low molecular weight protein which is completely filtered through the glomeruli, rendering it a promising biomarker for measuring Glomerular Filtration Rate (GFR) in children. Recently, it was found to predict elimination of the renally cleared amoxicillin/clavulanic acid with a similar covariate model as ours, in a comparable population PK study in critically ill children (n = 50 patients).[39] One could only speculate why, in this study, we were not able to identify cystatin C as a drug clearance descriptor. Potential explanations include (i) serum cystatin C may be affected by the underlying disease (septic conditions), which may mask the effect of age on organ function (i.e., GFR), (ii) too narrow variation in cystatin levels to identify a statistically significant correlation, since no patients with renal insufficiency were included, (iii) both compounds are cleared substantially more through tubular secretion (besides glomerular filtration), when compared to amoxicillin/clavulanic acid. As mentioned previously, we were not able to evaluate serum creatinine (and estimated glomerular filtration rate based on serum creatinine) as a potential covariate due to the large portion of BQL values. In this study, the Jaffe reaction was used for creatinine bio-analysis, a method which is still very popular due to its simplicity and low cost. Due to a standardisation of creatinine measurements in 2006, analyzers automatically now correct through the use of a fixed correction factor to adjust for interfering protein content in adults. Unfortunately, due to lower total protein reference ranges, this overcorrection can potentially lead to undetectable creatinine levels in infants and children.[40] No further clinically relevant covariates on PK parameters were found.

As β-lactam antibiotics are time-dependent antibiotics with $fT_{>MIC}$ the PK/PD parameter of interest, drug clearance is the most important PK parameter related with adequate exposure. The observed population estimate for piperacillin clearance (0.25 L/h/kg) is within the observed range in 47 non-ICU children (0.20-0.35 L/h/kg) and comparable to what has been observed in 16 critically ill adults with hyperfiltration (0.25 L/h/kg).[7,41] It is noticeably higher (>20%) when compared to studies in neonates (0.08-0.14 L/h/kg), non-ICU oncology children (0.20 L/h/kg), healthy adults (0.14-0.16 L/h/kg), and a cohort of 12 critically ill children (0.20 L/h/kg), but substantially lower (>20%) than observed in

another cohort of 13 critically ill children (0.30 L/h/kg).[10,11,13-15,42] The observed tazobactam clearance (0.13 L/h/kg) is lower to what has previously been observed in children of the same age.[7,15] Despite the limitations for a direct comparison of the results, disease-driven changes in drug disposition can have major impact on drug clearance. In several subpopulations of critically ill adults, augmented renal clearance (ARC) of antibiotics leading to subtherapeutic concentrations has been extensively described.[43] Despite increasing appreciation of this phenomenon, scarce data are available in children receiving β-lactam antibiotics.[14,40,44]

In our study population clearance values higher than expected were observed in some patients with observed individual piperacillin clearances up to 0.35 L/h/kg. We hypothesize that such an apparent variation in clearance results from an increase in renal blood flow, leading to hyperfiltration in those patients with sepsis. The hypothesis of ARC was also supported by the fact that a large proportion of measured renal biomarkers was undetectable (Scr) or low (CysC) compared to age-corrected reference values.[45] A plausible explanation, besides the analytical challenges for creatinine described above, could be a faster renal clearance of these endogenous compounds. Moreover, trough concentrations from maintenance doses remained very low in most patients. This phenomenon suggests that no accumulation occurs during steady-state conditions, probably due to the enhanced renal capacity. Although our study was not powered for the evaluation of efficacy, we speculate that children admitted to the ICU with lower disease severity and organ failure scores are most at risk for ARC and subsequent subtherapeutic antibiotic concentrations, as previously observed in adults.[41] Notably, children admitted to a general paediatric ward, may also experience ARC since high piperacillin clearances (upper range of 0.35 L/h/kg) have also been reported in non-ICU children with suspected or proven infection.[7] Further investigation is needed to identify patient risk factors for developing hyperfiltration in children.

Regarding the observed population estimate of volume of distribution for piperacillin (0.25 L/kg), our observation is within the observed range in non-ICU children (0.24-0.33 L/kg). It is noticeably higher (>20%) when compared to healthy adults (0.14-0.18 L/kg) but substantially lower than reported in (pre-)term neonates (0.37-0.42 L/kg), non-ICU oncology children (0.41 L/kg, two other studies in critically ill children (0.43-0.55 L/kg) and critically ill adults (0.35 L/kg).[7,11-15,41] The observed volume of distribution of tazobactam (0.24 L/kg) is lower to what has previously been observed in children of the same age (0.30-0.39 L/kg).[7,15] Also here, it is unclear whether these differences in volume of distribution are due to differences in body composition of the study population (e.g. larger total and extracellular body water content in neonates compared to infants and

children), differences in disease severity (e.g. vascular leakage), and/or different sampling and PK parameter estimation methods.

Treating infections in the seriously ill child without evidence-based dosing recommendations remains a huge challenge and may lead to an increased morbidity and mortality.[46] Our analysis challenges currently used dosing regimens (75-100 mg/kg piperacillin every 6 to 8 h, given as a short infusion), as they only yield a PTA between 5.9% to 34% for piperacillin, thereby potentially leading to subtherapeutic treatment (**Figure 3**).[14] These findings of underdosing are consistent with previously reported exposure data in critically ill children of the same age.[14,15]

For the treatment of Pseudomonas infections, no clear-cut $fT>C_T$ target values are available for tazobactam, in combination with piperacillin. Therefore, we performed a PTA analysis appraising different targets (40%-60%-80% $fT>C_T$) **(Figure 5,6).** The choice was based on the only properly designed *in vitro* study in which the pharmacodynamics of tazobactam was characterised in combination with piperacillin.[5] Further studies are required to confirm the appropriate target. Our analysis should be interpreted with caution, but it does provide insight into how differences in exposure may affect antimicrobial response.

More frequent dosing, prolonged infusions and continuous infusions have been proposed as dose optimization strategies for β-lactam antibiotic treatment.[47] However, it should be noted that, we have specifically chosen not to select higher amounts per dose for intermittent dosing regimens (max. 100/12.5 mg per kg piperacillin/tazobactam) than currently recommended. This was done to mitigate potential safety risks related to higher peak concentrations, thereby avoiding the potential for saturation of the elimination processes which determine the clearance of piperacillin. This 'same amount per dose' approach should also prevent a higher degree of reduced tazobactam clearance, as it is known that both piperacillin and tazobactam interact by a competitive inhibition at the level of the tubular anion transporter system.[48] Regarding the safety of continuous infusions, Delvallée *et al.* reported the use of a 400 mg/kg/day infusion on a paediatric haematology unit without any observed adverse events.[44]

Our simulations showed that, four hourly dosing regimens (given as a prolonged infusion), and all continuous dosing regimens met the PTA criterion for piperacillin (**Figure 3**,4). Despite the higher PTA with these prolonged and continuous infusions, we acknowledge that these dosing regimens may have important implications on drug administration practices, as intravascular access is frequently limited and drug incompatibilities with piperacillin/tazobactam often occur.[50,52] Therefore, a rational choice in

dosing regimen is advised, depending on the individual patient characteristics, site of infection and target MIC. In our opinion, prolonged and continuous infusions seem a preferable option whenever possible, especially when antibiotic therapy is started empirically or when higher $fT_{>MIC}$ targets are needed (e.g. neutropenic children).

This research has some notable limitations. First, the studied population included a heterogeneous group of children with regard to possible differences in (suspected) infecting organism and tissue involvement/penetration. Second, total drug plasma concentrations were mathematically corrected for protein binding instead of free drug concentration measurement in plasma, or drug measurement at the site of infection. However, this simplification was previously found to be acceptable for β-lactam antibiotics with low protein binding like piperacillin and tazobactam.[50] Third, MIC values were not prospectively determined in order to be able to calculate individual target drug concentrations in culture-proven infections. Instead, a worst-case scenario using the clinical breakpoints for *P. aeruginosa* was chosen as reference to explore dosing regimens by $fT>C_T$. This approach is suitable for β-lactam antibiotics and tazobactam, which are known to have a wide therapeutic index. Consequently, there should be limited concern about potentially supra-therapeutic dosing. Moreover, from our simulation studies, we concluded that there is no risk for accumulation of piperacillin and tazobactam, when using any of the alternative dosing scenarios (**Table 2**). Fourth, notwithstanding that a substantial number of younger patients were recruited, more extensive PK data from neonates and infants are needed to estimate maturation parameters more precisely on both clearances and refine dosing regimens in these age categories.

In conclusion, our study shows that current dosing recommendations for piperacillin and tazobactam can result in subtherapeutic treatment in critically ill children, thereby risking treatment failure. We proposed alternative, model-based dosing regimens that increase the PTA from 5.9% to 100 % for *P. aeruginosa* infections with a MIC of 16. A prospective, randomized controlled trial evaluating efficacy and safety for the proposed optimized dosing strategies may be required to further substantiate these results.

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TRANSPARENCY DECLARATIONS

None to declare.

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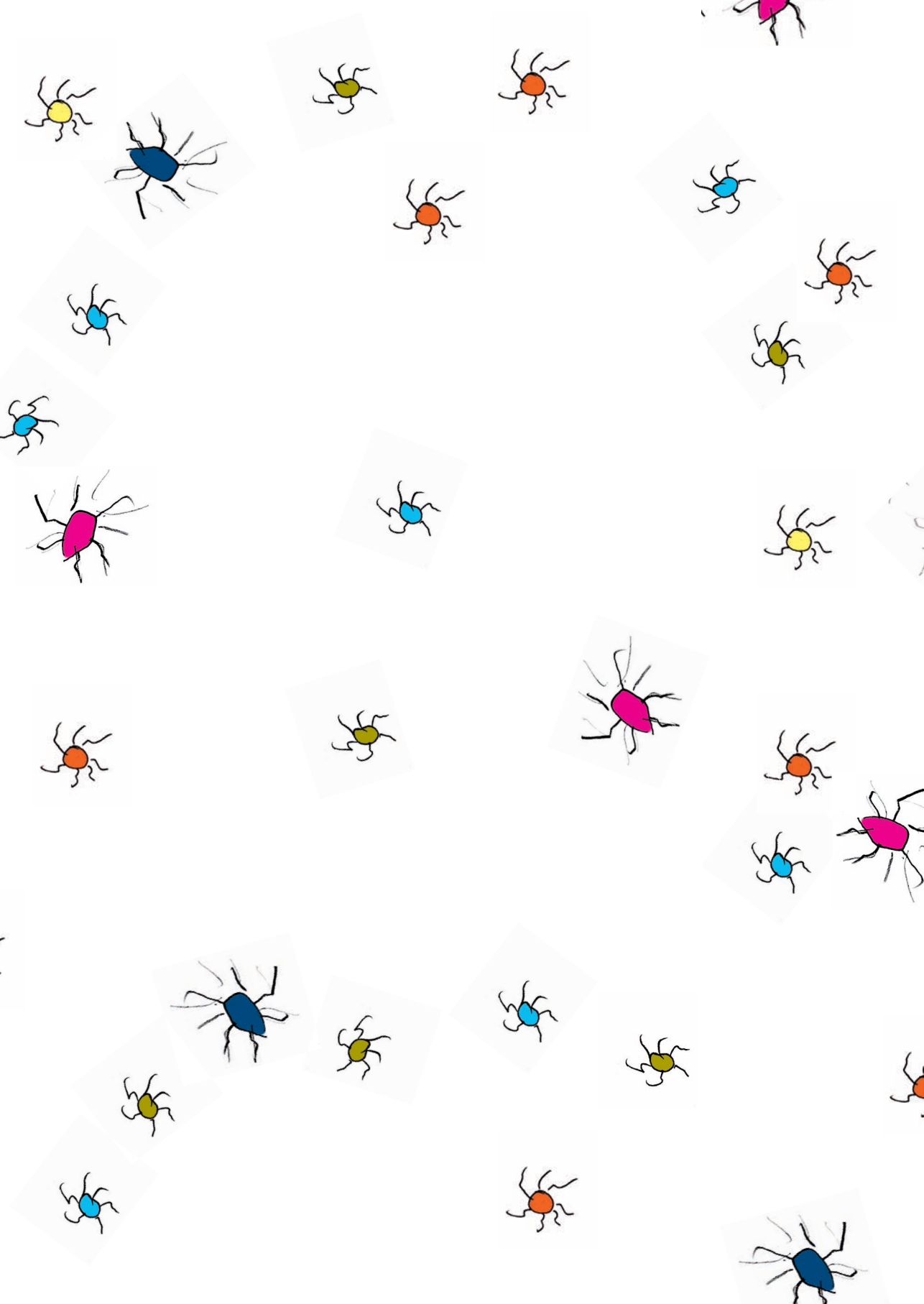

CHAPTER 5

POPULATION PHARMACOKINETICS OF CEFAZOLIN BEFORE, DURING AND AFTER CARDIOPULMONARY BYPASS TO OPTIMISE DOSING REGIMENS FOR CHILDREN UNDERGOING CARDIAC SURGERY

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ABSTRACT

Objectives: The objective of this study was to characterize cefazolin serum pharmacokinetics in children before, during and after cardiopulmonary bypass (CPB), in order to derive an evidence-based dosing regimen.

Patients and Methods: This study included children who received cefazolin before surgical incision, before cessation of CPB and after surgery. Blood samples of total and unbound cefazolin concentrations were collected before, during and after CPB. The cefazolin concentration-time profiles were analysed using population pharmacokinetic modelling and predictors for interindividual variability in pharmacokinetic parameters were investigated. Subsequently, optimized dosing regimens were developed using stochastic simulations. Clinicaltrials.gov: NCT02749981.

Results: A total of 494 total and unbound cefazolin concentrations obtained from 56 children (age 6 days to 15 years) were included. A two-compartment model with first-order elimination plus an additional compartment for the effect of CPB best described the data. Clearance (1.56 L/h), central volume (1.93 L) and peripheral volume (2.39 L) were allometrically scaled by body weight. The estimated glomerular filtration rate (eGFR) was identified as a covariate on clearance and the serum albumin concentration was associated with maximum protein binding capacity. Our simulations showed that an additional bolus dose at start of CPB improves the PTA in typical patients from 59% to >94%. Prolonged surgery and preserved renal function (i.e. drop in eGFR <25 %) had a negative impact on PTA.

Conclusions: We propose an optimized dosing regimen for cefazolin during cardiac surgery in paediatric patients to avoid treatment failure due to inadequate antibiotic prophylaxis.

INTRODUCTION

Post-operative infection in cardiac surgical patients is a cause of major morbidity and mortality and has a considerable financial impact.[1,2] In paediatric cardiac surgery, the rate of surgical site infections (SSI) is sometimes even higher, when compared with adult procedures.[1] One key prevention strategy is appropriate use of antibiotic prophylaxis. Shah *et al.*[3] recently showed that failure to administer a correct antibiotic prophylactic dose resulted in a 2-fold increase in the risk of developing an SSI in children undergoing surgery. Moreover, suboptimal prophylactic dosing likely results in the emergence of antimicrobial resistance necessitating later broad-spectrum antibiotic treatment.[4] To date, there is no consensus on optimal antibiotic dosing regimens in children undergoing cardiac surgery with cardiopulmonary bypass (CPB) and current recommendations are based on extrapolation from adults.[1]

CPB and post-operative renal function changes have been shown to have a major impact on drug disposition.[5,6] Moreover, maturational changes in drug pharmacokinetics from birth towards adulthood further complicate optimal drug treatment in the paediatric population.[7]

The efficacy of β-lactam antibiotics most strongly relates to the time during which the unbound drug concentration (*f*T) is above the MIC for the pathogen, i.e. $fT_{>MIC}$. The pharmacokinetic/pharmacodynamic target (i.e. $fT_{>MIC}$) index associated with positive clinical outcomes for β-lactams in critically ill patients is an $fT_{>MIC}$ between 50% to 100% of the dosing interval.[8] Although pharmacokinetic/pharmacodynamic targets for surgical antibiotic prophylaxis are not established, the goal should be to achieve blood and tissue concentrations that exceed pathogen MICs for the entire duration of surgery, until a few hours after skin closure.[1]

To date, only sparse data on the pharmacokinetics of unbound cefazolin are available in children during CPB with mild to moderate hypothermia (n=5 patients) and no data are available after paediatric cardiac surgery.[9] Previously, it has been shown that with traditional untested dosing schemes, antibiotic concentrations in children could be severely altered, increasing the risk of treatment failure and increased antibiotic resistance.[10,11] The primary aims of this study were: (i) to quantitatively characterize intravenous cefazolin serum pharmacokinetics, protein binding and predictors of interindividual variability in critically ill children before, during and after CPB; (ii) to evaluate the PTA for the current standard dose regimen; and (iii) to assess if further improved evidence-based practical dosing regimens can be developed for this patient population.

PATIENTS AND METHODS

Study design and ethics

A prospective pharmacokinetic study was conducted at Ghent University Hospital, Ghent, Belgium between November 2012 and May 2014. Patients between 1 day and 15 years of age undergoing cardiac surgery with CPB and receiving cefazolin as standard-care surgical antibiotic prophylaxis were eligible. Patients were excluded if they did not have, other than the drug infusion line, an arterial or intravenous access available for blood sampling. The research was conducted in accordance with the guidelines of the Declaration of Helsinki, approved by the institutional Ethics Committee and was registered at Clinicaltrials.gov (NCT02749981). Written informed consent was obtained from the parents or legal representatives and from the patients if older than 12 years. No formal power analysis was performed; the number of patients was based upon similar paediatric pharmacokinetic studies which showed adequate characterization of the pharmacokinetics. Collected demographic and clinical variables included: sex, body weight, postmenstrual age (PMA), height, body surface area, primary reason for admission, priming solution volume, laboratory chemistry at three fixed time points before, during and after CPB (serum albumin, creatinine, total protein), lowest body temperature on CPB (oesophageal, arterial, venous blood temperature), CPB pump flow rate, co-treatment with vasopressors/inotropes/vasodilators (dobutamine, adrenaline, norepinephrine, phenylephrine, milrinone, nitroprusside, nitroglycerine), type of catheter used for drug administration and blood sampling, time of skin incision, CPB start and stop time and time of skin closure.

CPB procedure

Anticoagulation was achieved by unfractionated heparin (300 IU/kg) given before initiation of CPB, in order to maintain an activated clotting time above 400 seconds (Hemotec; Medtronic, Brussels, Belgium). Saint Thomas II cardioplegic solution was used for cardiac protection (15-20 mL/kg). CPB circuits were primed with PLASMA-LYTE A (Baxter International, Deerfield, Ill), 20% albumin, mannitol (0.5 g/kg), sodium bicarbonate, heparin sodium and furosemide. Packed red cells were added when necessary to maintain a haematocrit between 25%-30% during bypass. Fresh frozen plasma was added if the post dilution calculated fibrinogen concentration was below 120 mg/dL. The CPB circuit consisted of a membrane oxygenator (D100 or D101; Liva Nova, Mirandola, Italy), polyvinylchloride tubing (Liva Nova, Mirandola, Italy), and roller pumps (S5; Liva Nova, Munchen, Germany). Moderate hypothermia (28 - 32 °C) was used in most cases. No ultrafiltration was used.

Drug dosing and administration

Cefazolin (Mylan®, Hoeilaart, Belgium) was prescribed at a dose of 25 mg/kg body weight (maximum 2000 mg), administered intravenously as a bolus within 1 h before surgical incision, at start of rewarming on CPB, 8 h after the second dose and 8 h after the third dose, according to current institutional dosing guidelines. Immediately after drug administration, infusion lines were flushed with normal saline with a minimum of twice the dead space volume.

Blood sampling

Serial blood samples (0.5 mL) were obtained before, during and after CPB from an arterial catheter (or from the arterial CPB circuit sampling port if a sample was taken just prior to the second dose). The total number of samples collected (per patient) was limited by the predefined total maximum blood volume permitted for pharmacokinetic sampling per individual patient, defined as 2.4 mL/kg bodyweight.[12] Sampling times focussed peri-operatively, pre- and immediately post-drug administration to characterize clearance and volume of distribution parameters. A full sampling scheme typically included a sample just before dosing (t=0), a sample 5 min after the first dose, a sample at skin incision, a sample immediately after arterial cannulation or on full-flow CPB, a sample just prior to aorta clamping, a trough sample just prior to the second dose, a sample just before venous decannulation, a sample just before skin closure, a sample just prior to the third dose, and a sample 5 min after the third dose. All samples were immediately centrifuged (15 min, 1885g) after which the resulting serum was frozen at -80°C before shipping to the clinical laboratory for assay. Unbound drug was separated at 37°C using a validated ultrafiltration method with an Amicon Ultra 0.5ml 30,000-molecular-weight-cutoff centrifugal filter device.[13]

Drug and biochemical assays

Cefazolin serum total and unbound concentrations were quantified using a validated HPLC-ultraviolet spectrophotometric method.[14] Total and unbound concentrations were analysed in three different batches and both concentrations per sample were measured within one day. Repeat analysis of unbound concentrations was performed on 8% of samples within one day of the first measurement. The lower limit of quantification (LLOQ) was 0.1 mg/L and the imprecision was < 10% at all concentrations. The percentage of unbound fraction (f_u%) was calculated as ultrafiltrate concentration divided by total concentration, multiplied by 100. Serum total protein (biurete), albumin (bromocresol green), creatinine (rate blanked, compensated Jaffe technique), total bilirubin (diazonium, colorimetric) and urea (kinetic urease) concentrations were measured on the Cobas 8000 (c502/c701) analyzer (Roche Diagnostics, Mannheim, Germany). Serum creatinine concentrations were afterwards recalculated towards enzymatic values,

based on the equation from Speeckaert *et al.*[15] Glomerular Filtration Rates (eGFR) were estimated using the Modified Schwartz formula.[16]

Pharmacokinetic analysis

Cefazolin pharmacokinetics was evaluated using the non-linear mixed-effects modelling software NONMEM version 7.2 (ICON, Ellicott City, MD, USA). The first-order conditional estimation method with the interaction option was used to estimate pharmacokinetic parameters and variability. R (version 3.1.1) and PsN (version 5.18.2) were used for pre- and post-processing. One, two-, and three-compartment disposition models with zero order input were tested to fit serum concentrations of bound and unbound concentrations using the NONMEM library, subroutine ADVAN6 TOL=9.[17] The effect of CPB was modelled using a separate on/off compartment. Intercompartmental clearance from this CPB compartment was fixed to the CPB pump flow rate and volume of distribution to the CPB priming volume (**Figure 1**). Clearance and volume parameters were estimated using unbound concentrations.

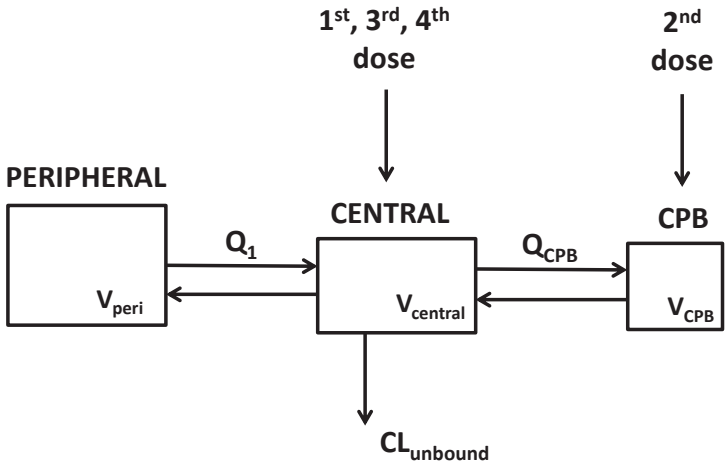

Figure 1. Schematic presentation of the structural three-compartment pharmacokinetic model of unbound cefazolin concentrations. CPB, cardiopulmonary bypass; V_c, V_p, V_{CPB}, volumes of distribution; Q_{12}, Q_{CPB}, inter-compartmental clearances; CL_u, clearance of unbound cefazolin. The study dosing regimen consisted of a dose of 25 mg/kg, given within one hour before surgical incision, at start rewarming on CPB, and 8 and 16 h after the second dose (Table 1).

The total cefazolin concentrations (C_t) and unbound concentrations (C_u) were modelled simultaneously and related to each other, taking into account saturable serum protein binding (**Figure 2**), using the following equation:

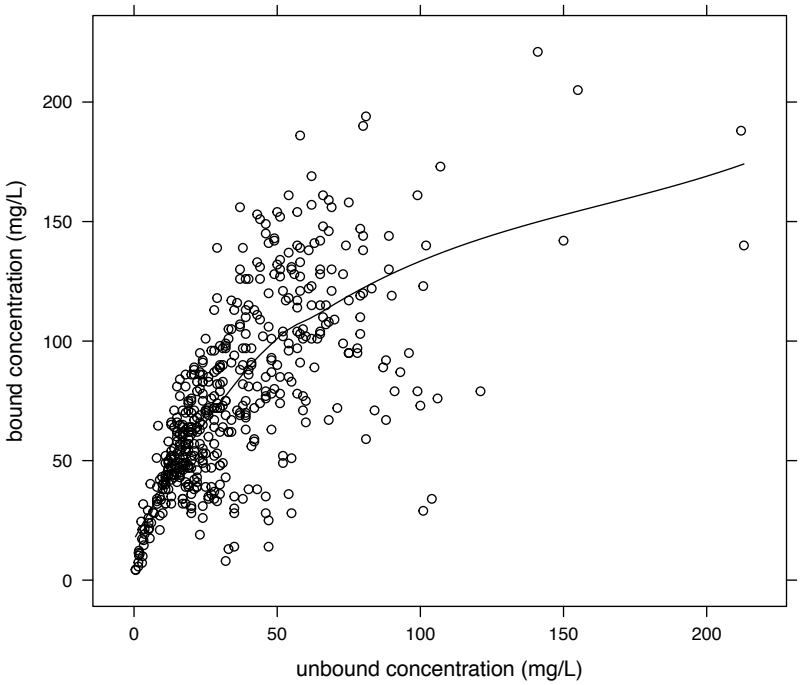

Figure 2. Bound versus unbound concentration plot. A Loess smooth line (solid line) was added to the plot.

$$C_t = \left(\frac{B_{max} \times C_u}{K_D + C_u}\right) + C_u \qquad (1)$$

where B_{max} is the maximum protein binding (mg/L) and K_D the dissociation constant (mg/L).

In the pharmacokinetic model, all observations were assumed to be from the central compartment. A decrease in objective function value (OFV) of 3.84 points ($p<0.05$) or more was considered statistically significant assuming a χ^2 distribution for nested models (1 degree of freedom). In addition, visual inspection of goodness-of-fit plots was performed for diagnostic purposes: observed versus population predicted concentrations, observed versus individual predicted concentrations, conditional weighted residuals versus time, conditional weighted residuals versus population predicted concentrations. A Wilcoxon signed-rank test was used to compare the serum unbound fraction just before and after initiation of CPB (maximum 25 min before and 5 min after initiation of CPB).

A log-normal distribution was assumed for the between-subject variability (BSV). Residual variability as additive, proportional models or a combination of both were tested.

As total and unbound concentration measurements were obtained from the same sample, correlations in residual errors associated with simultaneous total and unbound concentration samples were handled using the L2 method in NONMEM.[17]

Covariate model development

The covariate model was constructed in a stepwise fashion, through forward inclusion ($p<0.05$), and subsequent backwards elimination ($p<0.001$). In addition, a clinically relevant reduction in the magnitude of BSV on the parameter of interest, acceptable precision of model parameters, and visual inspection of the goodness-of-fit plots were used to support the inclusion of additional covariate terms into the model. Continuous covariates were evaluated as follows:

$$P_i = P_{pop} \times \left(\frac{COV_i}{median(COV)}\right)^k \qquad (2)$$

where P_i represents the individual parameter estimate of the *i*th subject, P_{pop} represents the population parameter estimate, *cov* is the covariate of interest and *k* the exponent, which could be fixed to 1 for a linear function or estimated.

Dichotomic covariates were evaluated using the following equation:

$$P_i = P_{pop} \times (1 + m_i) \qquad (3)$$

where *m* was estimated for one of the dichotomic covariate values (e.g. males).

Body weight was *a priori* included as a covariate using a power function with a fixed exponent of 0.75 on (intercompartmental) clearance parameters and 1 on volume parameters, normalized by the median body weight.[18] The potential impact of remaining clinical covariates was explored by visual inspection of *post hoc* individual pharmacokinetic parameter estimates and deviations from population-predicted pharmacokinetic parameters estimates versus covariate plots. Only clinically relevant associations were considered: sex, age, renal function, temperature and comedication covariates for clearance and age and sex for volumes of distribution. Total protein and albumin concentrations were tested on maximum binding protein (B_{max}). Specifically for the impact of age on clearance, we tested a sigmoidal maturation function based on post-menstrual age:

$$F_{mat} = \frac{PMA^{HILL}}{TM_{50}{}^{HILL} + PMA^{HILL}} \qquad (4)$$

where F_{mat} is the maturation function, PMA is post-menstrual age, Hill is the Hill coefficient, and TM_{50} is the maturation half-life.

Model evaluation

The final population pharmacokinetic model was evaluated as follows. A non-parametric bootstrap analysis (n = 1000 samples) was used to assess parameter precision. Adequate description of time course and variability was assessed using a prediction-corrected visual predictive check (pcVPC) stratified for total and unbound concentrations (n = 1000 simulations) and by assessment of the normalized prediction distribution error (NPDE) distribution (n = 1000 simulations).[19,20]

PTA simulation analysis

Stochastic simulations were performed to simulate unbound cefazolin concentration-time profiles for the study dose regimen (SDR) and for three alternative dose regimens (ADR1-ADR3) in 1000 individuals (**Table 1**).

Table 1. Simulated dosing scenarios

SDR:	25 mg/kg, 30 min before surgical incision; 25 mg/kg, at the start rewarming on CPB; 25 mg/kg, 8 h after the second dose; 25 mg/kg, 8 h after the third dose
ADR1:	30 mg/kg, 30 min before surgical incision; 30 mg/kg, at the start rewarming on CPB; 30 mg/kg, 8 h after the second dose; 30 mg/kg, 8 h after the third dose
ADR2:	30 mg/kg, 30 min before surgical incision; 15 mg/kg, at start of CPB; 15 mg/kg, at the start rewarming on CPB; 30 mg/kg, 8 h after the third dose; 30 mg/kg, 8 h after the fourth dose
ADR3:	40 mg/kg, 30 min before surgical incision; 20 mg/kg, at start of CPB; 20 mg/kg, at the start rewarming on CPB; 40 mg/kg, 8 h after the third dose; 40 mg/kg 8 h after the fourth dose

All simulations included a bolus administration. SDR, study dosing regimen; ADR, alternative dosing regimen.

Table 2. Patient study demographics stratified by body weight, used for the stochastic dose regimen simulations.

	Median (percentile)[a]		
Patient characteristics	**<6 kg**	**6-11 kg**	**>11 kg**
Body weight (kg)	4.85	7.50	17.2
eGFR pre-CPB (ml/min/1.73 m^2)	89	112	112
Serum albumin pre-CPB (g/L)	44	45	46
CPB pump flow rate (L/h)	40.9	54.8	102.5
Priming volume (L)	0.17	0.35	0.35
Time from first dose to incision (h)	0.57 (0.77)		
Time from first dose to start CPB (h)	0.97 (1.27)		
CPB duration (h)	1.33 (1.60)		
Time from first dose to the start of rewarming on CPB (h)	1.77 (2.34)		
Time from first dose to skin closure (h)	2.75 (3.31)		
Fractional albumin change before/during/post CPB	100%/-25%/-10 % (100%/-33%/-19%)		
Fractional eGFR[b] change before/during/post CPB	100%/-24%/-34% (100%/-18%/-24%)		

[a]Percentiles shown are 75th, except for fractional eGFR, which is 25th percentile.
[b]According to the modified Schwartz formula.

Dose regimen simulations were conducted, stratified by three body weight groups. The associated median patient-specific covariates and CPB/surgery characteristics for each of these weight groups, as observed in our clinical study, were used **(Table 2)**. In addition to these typical, median values, we also included a number of additional conservative subscenarios. These scenarios consisted of: (i) patients with a smaller drop in renal function [observed 25th percentile instead of the median estimated glomerular filtration rate (eGFR) fractional change from before to during and after CPB]; (ii) patients with a longer CPB and surgical procedure (observed 75th percentile of time to skin incision/CPB duration/time to skin closure); and (iii) a larger drop in serum albumin concentrations (observed 75th percentile of fractional change in albumin from before to during and after CPB).

Based on these scenarios, the simulated $fT_{>MIC}$ was calculated for the perioperative period and the period from skin closure to 24 h after skin incision. The target efficacy exposure was defined as 100% $fT_{>MIC}$ during surgery[1] and 50% $fT_{>MIC}$ after surgery.[21] PTA was calculated for MICs between 0.125 and 16 mg/L. A PTA ≥90% was defined as optimal.[22] To evaluate proposed dosing regimens, an infection with staphylococci and worst-case MIC breakpoint of 8 mg/L was used.[1,23]

Table 3. Demographic and clinical characteristics of the study population

Variable	n (%) or median (range)
Sex	
Male	28 (50)
Female	28 (50)
Age (years)	2.8 (0.013-15)
Weight (kg)	6.8 (2.7-70)
Procedure duration (h)	2.15 (1.21-8.50)
CPB duration (h)	1.33 (0.37-5.10)
CPB pump flow rate (L/h)	44.8 (18.4-249)
Lowest venous temperature on CPB (°C)	30 (16-34)
Priming volume (mL)	175 (150-1000)
eGFR[a] before CPB (ml/min/1.73 m^2)	108 (32-187)
eGFR[a] during CPB (ml/min/1.73 m^2)	80 (29-112)
eGFR[a] after CPB (ml/min/1.73 m^2)	74 (27-135)
Serum albumin before CPB (g/L)	45 (32-53)
Serum albumin during CPB (g/L)	33 (22-41)
Serum albumin after CPB (g/L)	39 (25-51)

[a]According to the modified Schwartz formula.

Table 4. Parameter estimates of the population pharmacokinetic model.

				Bootstrap estimates (n=1000)[a]		
Description	Parameter[a]	Estimate	RSE (%)	*Median*	*2.5%ile*	*97.5%ile*
Structural model parameters						
$CL_{i,\,unbound} = CL_{pop} \times \left(\frac{WTi}{6.8}\right)^{0.75} \times \left(\frac{eGFR}{91.6}\right)$						
Clearance	CL (L/h)	1.56 (11.7)[b]	5	1.56	1.40	1.73
$Q_{i,\,unbound} = Q_{pop} \times \left(\frac{WTi}{6.8}\right)^{0.75}$						
Inter-compartmental clearances	Q_{12} (L/h)	5.44 (31.3)[b]	6	5.42	4.80	6.06
	Q_{CPB} (L/h)	fixed to CPB pump flow rate[c]				
$V_{i,\,unbound} = V_{pop} \times \left(\frac{WTi}{6.8}\right)^{1}$						
Central volume of distribution	V_c (L)	1.93 (19.9)[b]	6	1.93	1.68	2.17
Peripheral volume of distribution	V_p (L)	2.39 (24.6)[b]	7	2.40	2.04	2.76
CPB volume of distribution	V_{CPB} (L)	fixed to priming solution volume[d]				
$B_{max} = B_{max,pop} \times \left(\frac{[albumin]}{39}\right)^{m}$						
Maximum binding protein	B_{max} (mg/L)	210	6	209	189	238
Exponent	m	0.571	15	0.572	0.394	0.723
$C_b = \left(\frac{B_{max} \times C_u}{K_D + C_u}\right)$						
Dissociation constant	K_D (mg/L)	53.7	11	53.8	43.7	66.9
Between-subject variability						
Clearance	CL (% CV)	33.5	29	32.7	23.7	43.0
Central volume of distribution	V_c(% CV)	31.8	38	31.2	18.7	42.9
Peripheral volume of distribution	V_p (% CV)	48.2	26	47.5	34.5	60.5
Dissociation constant	K_D (% CV)	36.6	30	35.8	25.6	46.7
Residual variability						
Unbound concentration	$C_{u,\,proportional}$ (% CV)	28.1	11	28.0	25.9	31.4
Total concentration	$C_{t,\,proportional}$ (% CV)	16.6	15	16.5	14.0	19.1

WT, patient weight

Population parameters estimated for the median values in the population for body weight of 6.8 kg, eGFR of 91.6 mL/min/1.73m^2 and serum albumin concentration of 39 g/L

[a]Non-parametric bootstrap: 988 runs minimization successful.

[b]Estimated parameters for a 70 kg person, eGFR rate of 120 mL/min/1.73 m^2 and serum albumin concentration of 39 g/L.

[c] CPB pump flow rate target value: 144 L/h/m^2 body surface area.

[d] Depending on CPB pump flow rate: <30 L/h, 150 ml ; >30 and < 48 L/h, 170 mL;>48 L/h and <120 L/h, 350 mL; and >120 L/h, 800-1000 mL.

RESULTS

A total of 56 patients were included. Demographic, clinical and treatment characteristics are summarized in **Tables 2 and 3**. Patients younger than 2 years (n=38) accounted for 67.8% of the study population. Some 497 total and 494 unbound cefazolin concentrations were available for pharmacokinetic analysis with all samples having a concentration above the lower limit of quantification (median of 9 samples per patient) (n = 130/129 total/unbound concentrations before, n = 229/228 during, n = 138/137 after CPB). The median unbound fraction was 28% (IQR = 23%-36%) and a median unbound fraction difference of 3% was measured in the repeat analysis (IQR = 2%-3%).

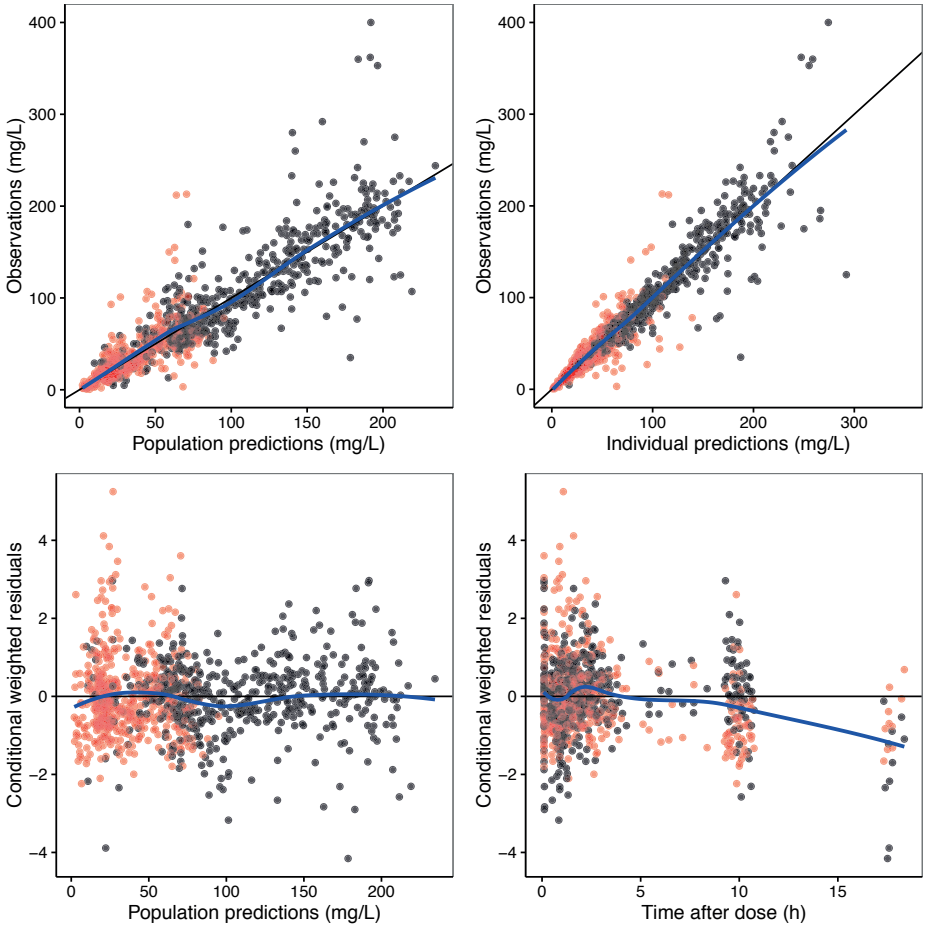

Figure 3. Goodness-of-fit diagnostic plots for unbound (red dots) and total (black dots) concentrations versus population predictions and individual predictions and conditional weighted residuals (CWRES) versus time after dose and population predictions. A line of identity (black solid line) was included in the observation versus prediction plots as a reference and a Loess smooth line (blue solid line) in all plots.

A two-compartment model with first-order elimination plus an additional compartment for the effect of CPB best described the data **(Figure 1, 3).** Only a slight bias beyond 15 h of treatment was observed in the plot of conditional weighted residuals over time, potentially due to the sparse nature of the data in this time period. The equations, population pharmacokinetic parameter estimates and their precision are summarized in **Table 4**. All structural model parameters were estimated with adequate precision (relative standard error <15%) and adequate precision was further confirmed with the bootstrap analysis. Between-subject variability (BSV) was estimated for clearance, volume parameters and the serum protein-binding dissociation constant. A proportional error model was used to describe residual variability for both total and unbound concentrations. A more parsimonious covariate model for clearance incorporating weight and GFR performed better compared with a model incorporating weight and an age-based maturation function, with one extra parameter to be estimated (see Patients and methods section). It had a lower BSV [coefficient of variation (CV) 33% versus 37% on terminal clearance parameter and improved precision on estimated parameters]. Subsequently the GFR model was chosen as the final model. Albumin was implemented as a covariate to describe saturable protein binding. No other collected clinical variables were selected for further statistical covariate testing, based on changes in objective function value and/or visual inspection of the covariate plots and clinical plausibility.

The pcVPC plots are presented in **Figure 4**; the 5th, 50th and 95th percentiles of the predicted concentrations closely follow the percentiles of the observed data, suggesting a good model fit in both cases. The NPDE mean and variance were not significantly different from 0 and 1, respectively (p>0.1) (data not shown).

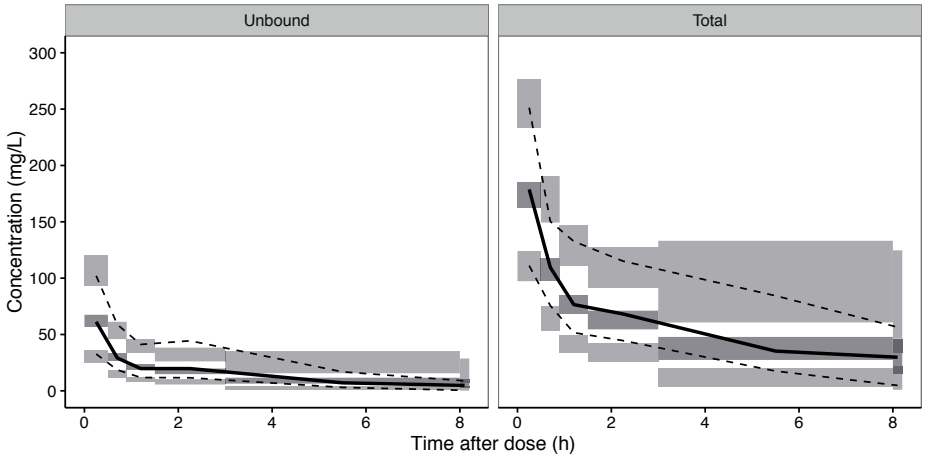

Figure 4. Prediction-corrected visual predictive check (n =1000 simulations) for unbound (left panel) and total cefazolin concentrations (right panel): grey shaded areas are 95% confidence intervals of simulated 5th, 50th and 95th percentile; lines are 5th, 50th and 95th percentile of raw data.

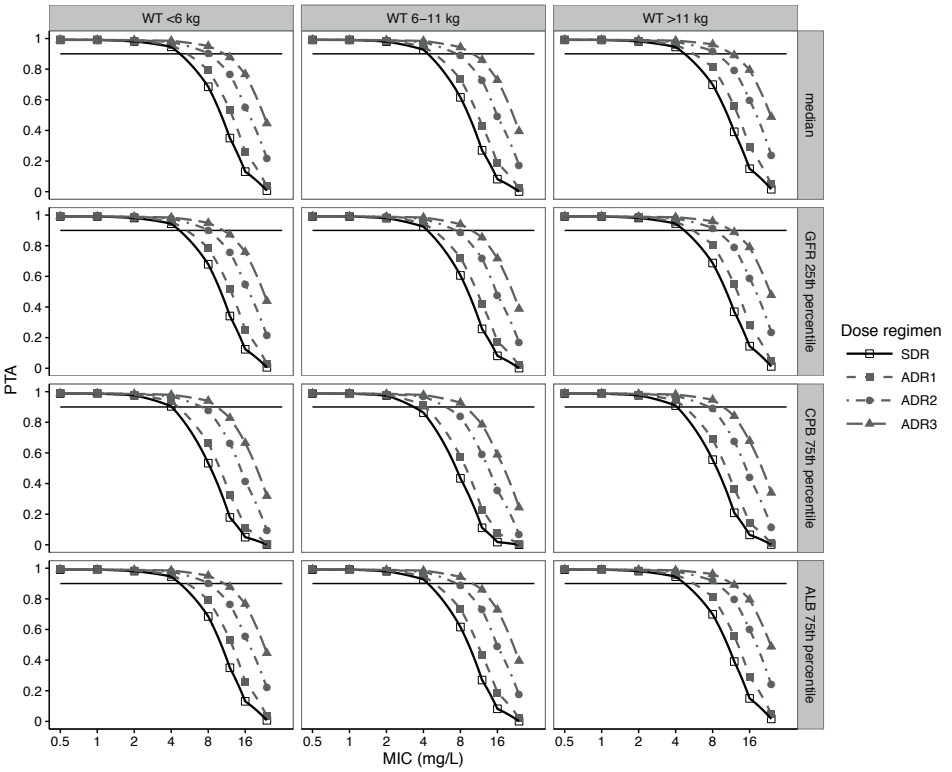

Figure 5. PTA (n = 1000 patients) for cefazolin during surgery, according to weight category and dosing scenario (Tables 1 and 3). Impact on PTA of a minor drop in eGFR (25th percentile) during and after CPB, a longer CPB/surgery duration (75th percentile) or a larger drop in albumin concentration (ALB) during and after CPB (75th percentile) were also shown. Target was defined as 100% of time above MIC.

PTA analyses for different dosing scenarios in patients with median patient characteristics (see **Tables 1, 2**) are presented in **Figure 5** (during surgery) and **Figure 6** (after surgery). PTA for the SDR was between 62% and 70% during surgery and between 89% and 98% after surgery. Also, the impact on PTA of a longer procedure duration (75th percentile of time to skin incision/CPB duration/time to skin closure), a preserved renal function (25th percentile of eGFR fractional change from before to during and after CPB) and drop in albumin concentration during and after CPB (75th percentile of albumin fractional change from before to during and after CPB) are illustrated. Prolonged surgery had a significant negative effect on PTA during surgery (maximum drop of 19%) **(Figure 5)**; the effect of a preserved renal function was smaller (maximum drop of 10%) and mainly after surgery **(Figure 6).** Changes in albumin concentration did not show any effect on PTA.

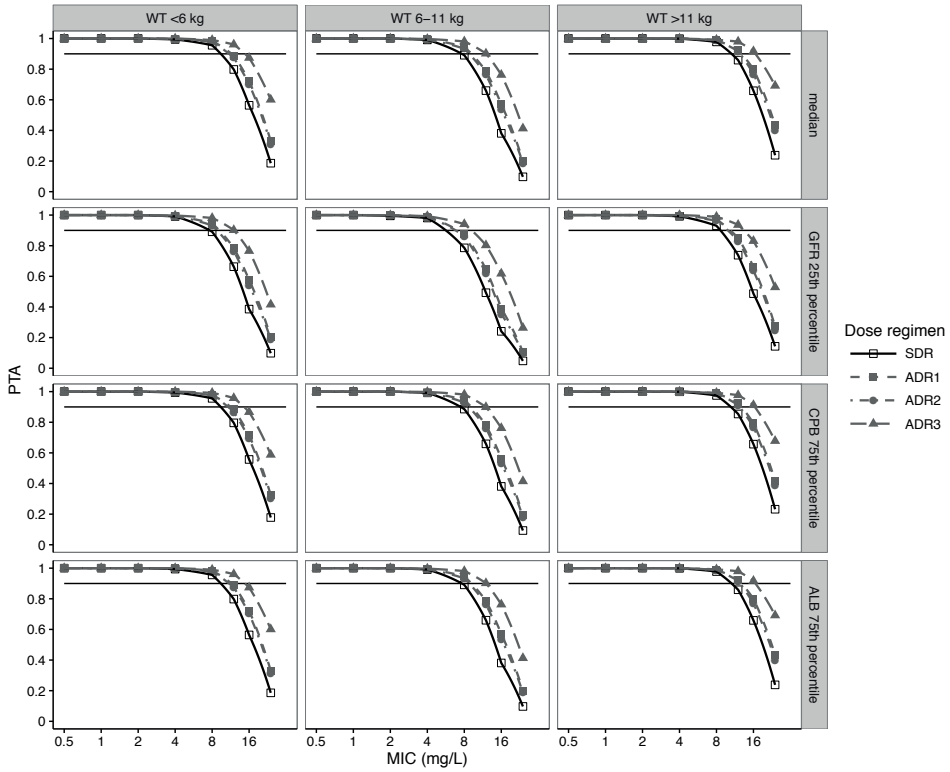

Figure 6. PTA (n = 1000 patients) for cefazolin after surgery, according to weight category and dosing scenario (Tables 1 and 3). Impact on PTA of a minor drop in eGFR (25th percentile) during and after CPB, a longer CPB/surgery duration (75th percentile) or a larger drop in albumin concentration (ALB) during and after CPB (75th percentile) were also shown. Target was defined as 50% of time above MIC.

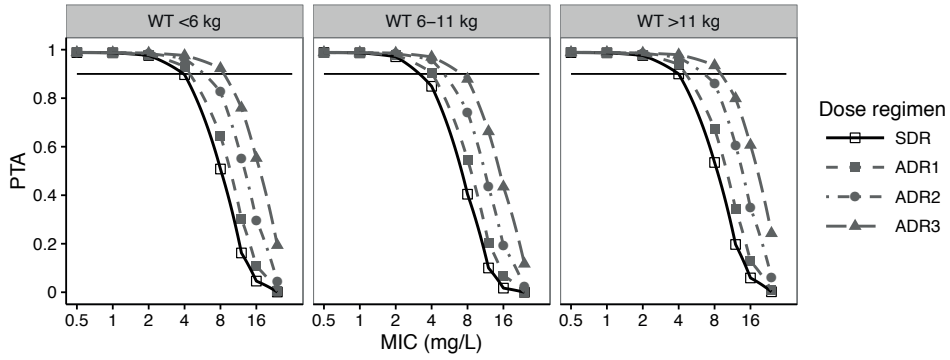

Figure 7. PTA (n = 1000 patients) for cefazolin during and after surgery, according to weight category and dosing scenario (Tables 1 and 3). Simulated patient scenarios consisted of a longer CPB/surgery duration (75th percentile) in combination with a minor drop in eGFR (25th percentile) during and after CPB. The combined target was defined as 100% above MIC during surgery and 50% of time above MIC after surgery until 24 h after skin incision.

The probability of achieving the target pharmacokinetic/pharmacodynamic index both peri- and post-operatively in conservative patient scenarios (75th percentiles on CPB and surgery duration combined with a 25th percentile on fractional eGFR change) are presented in **Figure 7**. PTA with the SDR was within the range of 40% to 54%, whilst the ADR3 dosing regimen **(Table 1)** provided the most optimal PTA between 88% to 99%.

DISCUSSION

To the best of our knowledge, this is the first population pharmacokinetic model describing the serum pharmacokinetics and protein binding of cefazolin in a large cohort of children before, during and after CPB. The developed population pharmacokinetic model was based on rich data from a relevant cohort of infants and children (n = 56 patients). Patients were recruited over a full paediatric age range (6 days to 15 years) and both total and unbound cefazolin concentrations were measured, using an informative blood sampling schedule that was not restricted to the intraoperative period.

We identified an increase in the volume of distribution during CPB, as observed by a limited drop in serum antibiotic concentrations, which is consistent with previous reports on antibiotic pharmacokinetics in children on CPB.[24,25] It is known that the fairly large ratio of priming volume to the child's circulating blood volume can have an impact on the volume of distribution of hydrophilic antibiotics (e.g. cefazolin) when initiating CPB.[5] To account for this change, a separate CPB compartment was modelled, as previously suggested by Eaton *et al*.[26] **(Figure 1)**.

The volume of distribution of the CPB compartment was estimated to be equal to the priming volume and may suggest that there was little or no adsorption of drug to the polymeric components of the circuit. This is in contrast to earlier *in vitro* findings of moderate adhesion of cefazolin to paediatric extracorporeal membrane oxygenation (ECMO) circuits.[27] Potential reasons include: (i) absence of a relevant effect due to shorter antibiotic-circuit contact times when compared with ECMO; and (ii) our data were too sparse to identify such a parameter.

We were able to quantify concentration-dependent (saturable) cefazolin serum protein binding, with serum albumin concentrations as a covariate on maximum protein-binding capacity in our paediatric population (Equation 1). This is in agreement with previous studies in neonates and adults.[28–30] Following initiation of CPB, protein binding decreased significantly (p=0.02) (median ratio unbound fraction just before versus just after CPB: 0.88). A drop in albumin concentration (median = 25%; IQR = 19%-33%) due to

haemodilution, competition with other highly bound compounds and pH changes during CPB may have contributed to this reduced protein binding.[5] As pH values were not collected, pH changes could not be tested as covariate on protein-binding affinity (K_D).

A covariate model on unbound clearance including renal function (as estimated by eGFR) was finally chosen, which is in line with previously published adult pharmacokinetic studies.[31–35] It performed better than a model including a sigmoidal maturation function on clearance, a method that is commonly used in paediatric population pharmacokinetic modelling. In our opinion, the maturation model would mainly reflect maturation on renal clearance and would be more applicable to a stable patient population, whereas our eGFR model is a more precise and direct measure of renal maturation and disease-driven alterations of renal function in a population with rapidly changing renal function. This is also in agreement with Illamola *et al.*,[36] who compared renal function and age as predictors of amikacin clearance in neonates and showed that creatinine clearance predicted more BSV.

Worsening renal function during and immediately after CPB is common[37,38] and leads to decreased renal drug elimination.[6] In our patient population, eGFR decreased by a median of 24% during CPB (IQR = 17%-38%), after which it further decreased during the first post-operative hours by a median of 10% (IQR = 0.5%-15%). The reduced serum clearance of cefazolin resulted in more unbound drug being available for redistribution into tissues and the CPB circuit. A similar trend of reduced plasma clearance was previously observed for cefazolin and other renally cleared antibiotics in children during and after CPB.[24,25]

In children undergoing CPB, only one small study (n = 12 patients weighing < 10 kg) on plasma unbound cefazolin pharmacokinetics has been published.[9] Given the differences in weight range, priming solution volume and content, CPB circuit material, CPB procedure (including hypothermia and pH management) and pharmacokinetic analysis, no valid comparisons of pharmacokinetics parameters between these studies can be made.

With regard to the potential toxicity of our optimized dosing regimens, it should be noted that we specifically chose not to evaluate doses greater than that currently recommended for children (50 mg/kg given as a bolus infusion), to avoid potential safety risks related to higher peak concentrations.[39] Dosing regimens with prolonged infusion times were not tested as they were regarded as impractical in the operation theatre setting.[40] The first alternative dose scenario (ADR1) included the same dosing times as the current paediatric and adult hospital dosing guideline; two alternatives (ADR 2 and ADR 3) included a supplemental dose at the onset of CPB. The rationale for this timing

was 2-fold: from a pharmacokinetic point of view, the start of CPB results in an increase in the distribution volume, leading to an immediate drop in antibiotic concentrations; from a more practical point of view, selecting a fixed timepoint during surgery might also reduce the risk of simply forgetting this extra dose **(Table 1).**

Optimal targets for surgical prophylaxis wtih β-lactam antibiotics are currently unknown.[1] Because only a few surgical patients develop a wound infection, we used the conservative pharmacokinetic/pharmacodynamic target indices for antibiotic treatment, given the immature immune response in younger children, the knowledge that CPB may contribute to immunoparesis and the broad therapeutic range of β-lactam antibiotics.[41,42] Due to the higher chance of microbial contamination during surgery, a more conservative target (100% $fT_{>MIC}$) was chosen for the perioperative period, compared to the immediate post-operative period (50% $fT_{>MIC}$). Target attainment was evaluated within a 24 h window after skin incision, as longer post-operative antibiotic courses do not modify the SSI risk.[1]

Our analysis challenges the current dosing regimen; for a typical (median) patient population and a MIC of 8 mg/L for staphylococcal infections, the PTA was between 62% and 70% during surgery and between 89% and 98% after surgery **(Figures 5**, **6)**. The simulation studies also revealed that it was primarily the duration of surgical (CPB) procedure that reduced the likelihood of achieving optimal cefazolin exposure during surgery **(Figure 5)**. This is in accordance with previously reported findings in adults.[43] In this context, interestingly, various studies identified operative and CPB duration as an independent risk factor for SSI after paediatric cardiac surgery.[44–47] None of these studies measured antibiotic concentrations. A potential relationship between subtherapeutic antibiotic prophylaxis and SSI was, however, confirmed in two studies in adults undergoing cardiac surgery.[48,49] Although the present study was not powered to estimate an accurate incidence, one patient developed an SSI, which is within the reported range of 1.7-15 SSI per 100 cases.[9] Deep sternal wound infections are serious complications leading to higher morbidity and mortality and maximal precautions should be taken to avoid them. Renal function, as estimated by the eGFR, also had a significant influence on PTA, mainly in the post-operative period **(Figure 6)**. These results are also in agreement with previously published data in adults undergoing cardiac surgery.[32] Although albumin concentration was identified as a covariate on protein binding and protein binding slightly decreased on initiation of CPB, varying albumin concentrations had a negligible impact on PTA.

For final dose selection, the PTA in both the peri- and post-operative period was evaluated for patients with prolonged surgery combined with a minor drop in renal func-

tion, as the risk of underdosing was considered of greater importance than potential overdosing with a compound like cefazolin, which has relatively low toxicity. In those scenarios, the combined PTA for the current dosing guideline was even lower (between 40% and 54% for a MIC value of 8 mg/L) **(Figure 7)**, thereby potentially leading to subtherapeutic treatment. Only the ADR3 with a higher initial dose (40 mg/kg) at induction and a supplemental dose at start of CPB (20 mg/kg) was deemed appropriate. One recent study investigating tissue cefazolin concentrations in infants (n = 12) undergoing CPB also suggested the use of such supplemental dose(s) as potential dose optimization, although without distinct evaluation.[9] Current international dosing guidelines on antibiotic prophylaxis for adults and children aged > 1 year recommend a single pre-incision dose (25-50 mg/kg) and a supplemental redosing interval of 4 h (i.e. two times the cefazolin half-life in adults) during prolonged surgery (or whenever there is extensive blood loss). According to this dosing scenario, and assuming that the half-life in children is equal to that in adults, only one patient in our study population would have been redosed during surgery. Evidently, this dosing regimen would result in an even lower PTA than the current SDR with fixed redosing at the start rewarming on CPB (PTA between 40% and 54% for conservative patient scenarios).

Tissue concentrations were not measured in our study. However, a fairly good tissue penetration was described in a comparable subgroup of infants on CPB and moderate hypothermia (n = 5), with remarkably similar plasma unbound and interstitial tissue concentrations (interstitial fluid to plasma unbound AUC ratio: 0.8-1.3).[9] These tissue penetration characteristics for cefazolin were comparable to what has previously been observed in several adult studies.[34,50-52] Moreover, unbound serum concentrations are also informative for antimicrobial efficacy, as blood can bathe the incision site and therefore provide antibiotic distribution at the possible site of infection.

Our analysis is based on data from paediatric patients with mild to moderate hypothermia; therefore, these dose recommendations may not apply to conditions with deep hypothermic circulatory arrest. A ≥ 50% lower tissue penetration could be expected in this subpopulation.[9]

Using creatinine-based Schwartz formula to accurately estimate GFR has some limitations, especially in young and critically ill children.[53] However, this is still a well-established, bedside method and has been applied in studies of pharmacokinetics in critically ill children as well the pRIFLE criteria for defining acute kidney injury.[54]

In conclusion, we demonstrated that the current paediatric dose recommendation of cefazolin in patients may result in subtherapeutic concentrations, especially in those

patients with preserved renal function and prolonged cardiac surgery. We provided a rational, model-based dose regimen improvement that increases the PTA in this at-risk subpopulation from 40% to >88% for infections with staphylococci. A prospective, randomized controlled trial evaluating efficacy and safety for the optimized dosing strategy is warranted.

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TRANSPARENCY DECLARATIONS

None to declare.

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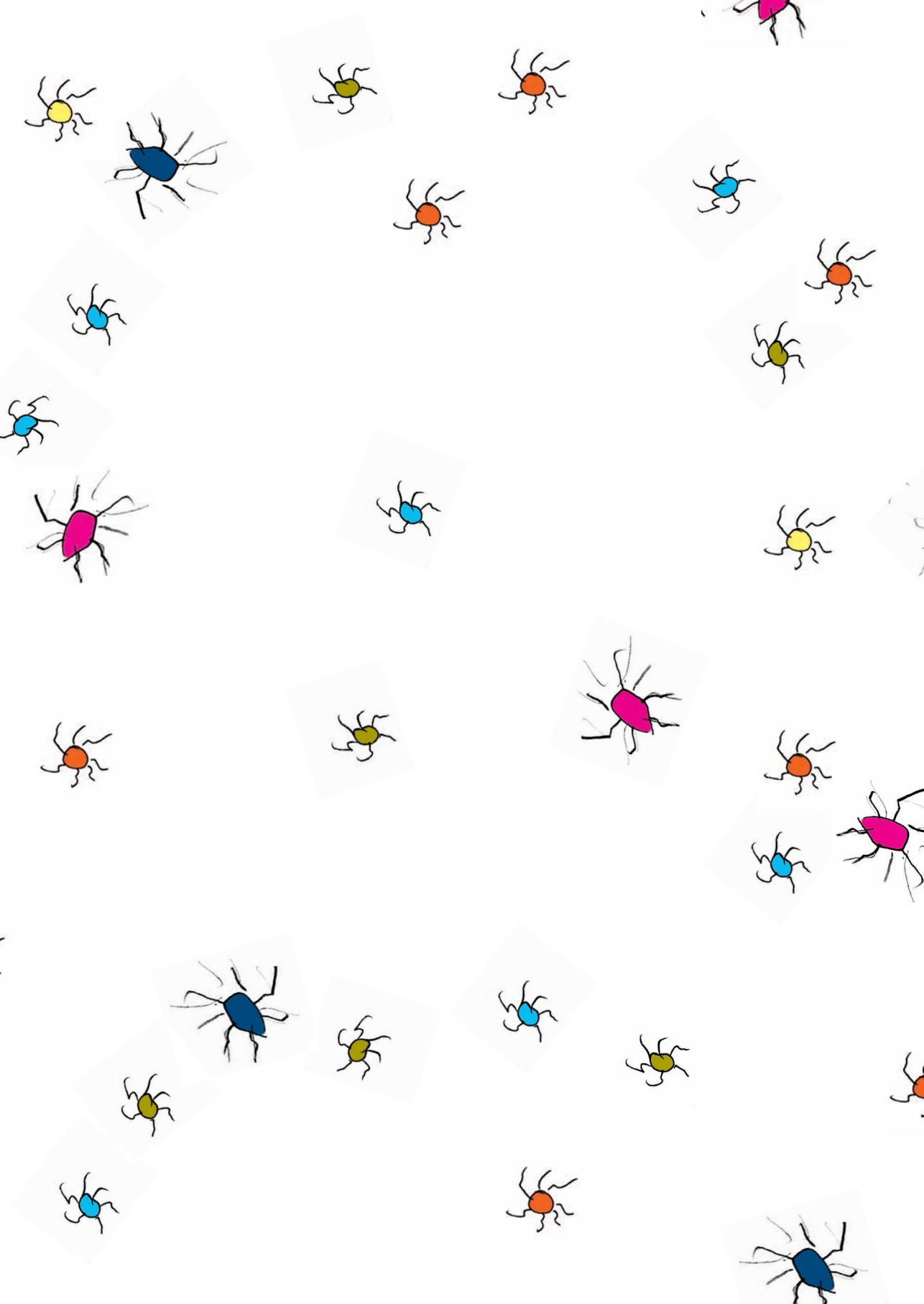

CHAPTER 6

IMPACT OF VANCOMYCIN PROTEIN BINDING ON TARGET ATTAINMENT IN CRITICALLY ILL CHILDREN: BACK TO THE DRAWING BOARD?

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ABSTRACT

Objectives: The objectives of this observational study were to investigate plasma protein binding and to evaluate target attainment rates of vancomycin therapy in critically ill children.

Patients and Methods: Paediatric ICU patients, in whom intravenous intermittent (ID) or continuous (CD) dosing with vancomycin was indicated, were included. Covariates on unbound vancomycin fraction and concentration were tested using a linear mixed model analysis and attainment of currently used pharmacokinetic/pharmacodynamic (PK/PD) targets was evaluated. Clinicaltrials.gov: NCT02456974.

Results: One hundred and eighty-eight plasma samples were collected from 32 patients. The unbound vancomycin fraction (median = 71.1%; IQR = 65.4%-79.7%) was highly variable within and between patients and significantly correlated with total protein and albumin concentration, which were both decreased in our population. Total trough concentration (ID) and total concentration (CD) were within the aimed target concentrations in 8% of patients. The targets of AUC/MIC≥400 and *f*AUC/MIC≥200 were achieved in 54% and 83% of patients, respectively. Unbound vancomycin concentrations were adequately predicted using the following equation: unbound vancomycin concentration(mg/L) = 5.38 + [0.71 x total vancomycin concentration(mg/L)] – [0.085 x total protein concentration(g/L)]. This final model was externally validated using 51 samples from another six patients.

Conclusions: The protein binding of vancomycin in our paediatric population was lower than reported in non-critically ill adults and exhibited large variability. Higher target attainment rates were achieved when using PK/PD indices based on unbound concentrations, when compared to total concentrations. These results highlight the need for protein binding assessment in future vancomycin PK/PD research.

INTRODUCTION

The emergence of MRSA strains has led to an extensive use of vancomycin in the treatment of serious infections in critically ill children.[1] Vancomycin is a glycopeptide antibiotic with a narrow therapeutic range.[2] Achievement of pharmacokinetic (PK) and pharmacodynamic (PD) indices associated with maximum bacterial killing are recommended to increase the probability of clinical cure and decrease the likelihood of toxicity.[2] Studies in adults have shown that the advocated PK/PD index of favourable clinical outcome is an AUC over a 24 h period in steady-state divided by the MIC of the suspected pathogen (AUC/MIC) of at least 400.[3] Despite its use in children, clinical studies currently lack to validate this target value.[4–6] In routine clinical practice, trough concentrations are used as a 'surrogate' parameter to optimize vancomycin dosing regimens, because AUC/MIC calculations are labour- and cost-intensive.[2,7] Both targets are based on total drug concentrations, whereas only the 'unbound' or 'free' drug exerts a pharmacological effect.[8] A more direct *f*AUC/MIC target ≥200 has been advocated as PK/PD target assuming a fixed unbound vancomycin fraction of 50%.[2,9] However, critically ill children exhibit marked variability in plasma protein concentrations (with albumin concentration ranging between 15-54 g/L), which may alter the protein binding.[10,11] To date, no studies have investigated the implications of altered protein binding on target attainment rates.

The study had three aims: (i) to document plasma protein binding and factors that modulate the protein binding of vancomycin in critically ill children; (ii) to compare target attainment rates of three different currently used targets: total (trough) concentration, AUC/MIC and *f*AUC/MIC; and (iii) to develop a prediction model for the unbound vancomycin concentration.

PATIENTS AND METHODS

Study design

This two-centre, prospective, observational study enrolled children between 12 days and 15 years of age, admitted to the ICU of the Ghent University Hospital (Ghent, Belgium) and Queen Fabiola Children's University Hospital (Brussels, Belgium) between May 2012 and April 2016 in whom intravenous vancomycin therapy was indicated, independently of the indication.

Ethics

The research was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Ghent University Hospital (EC2012/172). This study was registered at Clinicaltrials.gov (NCT02456974). Written informed consent was obtained from parents or a legally authorized representative and also from the patient themselves if older than 12 years. If patients older than 12 years were not able to give assent during the study, they were informed about their participation afterwards. Samples were only analysed if written patient informed consent was obtained; otherwise samples were destroyed.

Vancomycin treatment

Patients received a weight-based dosing regimen [either intermittent dosing (ID) or continuous dosing (CD)] (Vancomycine Mylan®, Hoeilaart, Belgium; Vamysin Teva®, Wilrijk, Belgium; Vancomycine Actavis®, Gentofte, Denmark). Vancomycin doses were prescribed according to current institutional guidelines: ID of 15 mg per kilogram body weight was administered intravenously over 60 min four times daily; CD consisted of a loading dose of 15 mg/kg over 60 min, immediately followed by a maintenance dose of 40 mg/kg over 24 h. Dosing regimens were adjusted using therapeutic drug monitoring (TDM) as part of routine clinical care.

Samples

In case of ID, blood samples were collected during first and/or assumed steady-state doses (at least after three administered doses). The total number of samples collected (per patient) was limited by the predefined total maximum blood volume permitted for PK sampling per individual patient, defined as 2.4 mL/kg bodyweight, according to the EMA guidelines.[12] A full sampling scheme (ID) per dose included a sample just before dosing, a peak sample immediately after drug infusion, a sample between 60 min and 180 min after the start of infusion, a mid-dose-interval sample 180 min after the drug infusion start time and a trough sample just prior to the next dose. For patients who received CD, samples were drawn at 12 h, 24 h and 48 h after the start of the loading dose.

Patient data

Patient data included age, gender, bodyweight, primary reason for admission, II (Paediatric Risk of Mortality) score at admission and measures of organ function, and patient severity of illness as described by the PELOD (Pediatric Logistic Organ Dysfunction) score at day(s) of sampling. Data on drugs, with a plasma protein binding higher than 80%, administered 12 h before sampling until the time of sampling were collected. Each blood sample collected for vancomycin measurement was also analysed for albumin, total bilirubin, urea and total protein concentration; other biochemical data (serum

creatinine, serum C-reactive protein (CRP)) were collected from the routine laboratory measurements.

Analytical methods

Samples were collected in lithium-heparin tubes and centrifuged (1885 g, at 20°C for 8 min) on arrival in the laboratory within 60 min after sampling. The supernatant was separated immediately and frozen at -80°C until analysis. The unbound plasma vancomycin concentration was determined using a validated ultra-filtration method.[13] Therefore samples were incubated in a capped Centrifree Centrifugal Filter Device (Millipore, Billerica, MA) at 37°C for 30 min. The filter with a molecular weight cut off of 30.000 Da was placed in a preconditioned Heraeus Labofuge 400 R Centrifuge (Thermo Fisher Scientific, Asheville, NC) and spun for 30 min (1885 g, at 37°C). Subsequently the vancomycin plasma concentrations were measured by the Architect i2000SR Plus analyser (Abbott diagnostics, Illinois, US) using a validated chemiluminescence microparticle immunoassay technique. The lower limit of quantification for vancomycin was 3.0 mg/L, with an interassay coefficient of variation lower than 6.4%. The unbound vancomycin fraction was calculated using the following equation[13]:

$$f_{unbound} = \frac{C_{ultrafiltrate}}{C_{plasma}}$$

The $f_{unbound}$ represents the unbound vancomycin fraction, $C_{ultrafiltrate}$ the vancomycin concentration in the ultrafiltrate and C_{plasma} the vancomycin concentration in plasma. Total protein (biurete), albumin (bromocresol green), creatinine (rate blanked, compensated Jaffe technique), total bilirubin (diazonium, colorimetric), urea (kinetic urease) and CRP (particle enhanced immunoturbidimetric) concentrations were performed on the Cobas 8000 (c502/c701) analyser (Roche Diagnostics, Mannheim, Germany).

Data analysis

Statistical analysis was performed using SPSS Statistics version 22 (IBM, New York, USA). Normality of the data was visually confirmed by generating Quantile-Quantile plots. The potential covariate(s) on unbound vancomycin fraction and unbound vancomycin concentration were tested sequentially by forward inclusion in a linear mixed model analysis. In such a model the responses from a subject are thought to be the sum of the fixed and random effects. These random effects only contribute to the covariance structure of the data. This model was used because more than one sample per patient was taken and the observations are therefore dependent. Evaluation of p-values and of the Akaike's Information Criterion (AIC) score were used to select the models that were the best approximate of the reality given the recorded data. The total and unbound vancomycin concentration, age, gender, bodyweight, biochemical data and co-medication

were tested as covariates on the unbound vancomycin fraction. Serum creatinine levels were in 25% of samples (45 samples) lower than the limit of quantification (<0.17 mg/dl) and therefore could not be tested as potential covariate. The same covariates minus the unbound vancomycin concentration were tested on the unbound vancomycin concentration. Concerning the co-medication, each different drug was given a score of 1 and the summations were included in the covariate analysis. A p-value of <0.05 was considered to indicate statistically significant difference. Correlation was assessed by means of a scatterplot and Spearman's rank correlation coefficient.

PK/PD target attainment analysis

Evaluated PK/PD targets included total trough concentration between 10-15 mg/L (ID), total concentration between 20-25 mg/L (CD), AUC/MIC≥400, and fAUC/MIC≥200.[2,3,7,14,15] Samples taken after TDM-guided dosing were not included in the target attainment analysis. For patients receiving ID the (*f*)AUC was calculated for total and unbound vancomycin concentrations using a non-compartmental analysis based on the log-linear trapezoidal rule with the PK Solver Excel add-in program (Microsoft Office Excel version 2013).[17] The (*f*)AUC was calculated using steady-state condition samples by the following equation[18]:

$$(f)\mathrm{AUC} = [(f)\mathrm{AUC}_{\mathrm{ss}}] \times 4$$

With $(f)\mathrm{AUC}_{\mathrm{SS}}$ being the (*f*)AUC over a 6 h dosing interval in steady-state conditions. If only samples from the first dose interval were available the AUC was calculated using the following equation with extrapolation to infinity, to be able to compare first dose and steady-state dose conditions [18]:

$$(f)\mathrm{AUC} = [(f)\mathrm{AUC}_{\mathrm{first\ dose}} + (f)\mathrm{AUC}_{6\mathrm{h}-\infty}] \times 4$$

The $(f)\mathrm{AUC}_{\mathrm{first\ dose}}$ represents the (*f*)AUC over 6 h from the first dose interval and $(f)\mathrm{AUC}_{6\mathrm{h}\text{-}\infty}$ the extrapolated (*f*)AUC from 6 h to infinity. The AUC was not calculated if the extrapolated $(f)\mathrm{AUC}_{6\mathrm{h}\text{-}\infty}$ to infinity exceeded 25% of the total (*f*)AUC and if less than four samples per dose interval were available, due to the risk for imprecise AUC calculations. For patients receiving CD the (*f*)AUC was calculated using the average of total and unbound vancomycin concentrations multiplied by a 24 h time interval. The AUC/MIC index was calculated presuming a MIC of 1 mg/L.

Model validation

The final prediction model for the unbound vancomycin concentration was validated using separate patient samples. In a Bland-Altman analysis the differences between calculated and measured unbound vancomycin concentration were plotted against the averages of calculated and measured unbound vancomycin concentration to identify outliers and consistent bias. Through a Passing-Bablok regression analysis of the

measured unbound vancomycin concentration and calculated unbound vancomycin concentration the systematic and proportional differences were evaluated. The analyses were performed using MedCalc version 16.4.3 (MedCalc, Ostend, Belgium).

RESULTS

Thirty-two patients were included resulting in a total of 188 plasma samples. All samples were analysed for total and unbound vancomycin concentration. Six samples were excluded from the analysis because there was an implausible result (unbound concentration being higher than total concentration). Demographic and clinical data, sampling characteristics and disease severity are summarized in **Table 1.** The median unbound fraction was 71.1% (IQR = 65.4%-79.7%) ranging from 49.4% until 98.1%. The median difference between the lowest and highest value of the unbound fraction within patients was 14.3% (IQR = 8.7%-18.9%) ranging from 3.1% to 41.8%. The median difference results are based on values from 31 patients, because one patient had only one sample. Saturation of plasma protein binding did not occur within the range of clinically achieved concentrations. Pathogens were isolated in nine patients and included seven Staphylococci infections, one infection with *Streptococcus pyogenes* and one infection with *Enterococcus faecalis.*

Mixed model analysis on unbound vancomycin fraction

Total protein ($p<0.001$) and albumin concentration ($p<0.001$) were found to be significant covariates on the unbound fraction.

PK/PD target attainment evaluation (Figure 1 and 2)

We evaluated 32 trough samples for patients who received ID and 6 samples for patients who received CD. The median total trough (ID) and total concentrations (CD) were 6.7 mg/L (IQR = 4.7-8.7 mg/L) and 14.5 mg/L (IQR = 10.2-18.7 mg/L), respectively. Only three trough samples (ID) achieved the target range (one after first dose and two after steady-state dose) and all of the measured total concentrations (CD) were below the target range. For 24 patients the (*f*)AUC was accurately calculated (8 were excluded as the AUC could not be precisely calculated). The median AUC/MIC and *f*AUC/MIC were 425 (IQR = 293-497) and 294 (IQR = 222-357), respectively.

Mixed model analysis on unbound vancomycin concentration

The model with total vancomycin ($p<0.001$) and total protein concentration ($p=0.001$) (AIC:790) performed slightly better than the model with total vancomycin ($p<0.001$) and albumin concentration ($p=0.008$) (AIC:792); including a third covariate did not lead to a

Table 1. Demographic, clinical, treatment and sampling characteristics of patients.

	Study group	Validation group
Demographic	Value for characteristic[b]	
Number of patients	32	6
Number of samples	182	51
Male	18 (56)	3 (50)
Female	14 (44)	3 (50)
Age (years)	4.1 (1.3-6.3)	9.0 (1.9-15.7)
Weight (kg)	17 (10-23)	30 (13-64)
Clinical[a]	Value for characteristic[b]	
Length of ICU stay (days)	17 (11-30)	17 (11-25)
PRISM II score	12 (0.25-21)	8 (2.75-8)
Primary reason for ICU admission		
neurologic	7 (22)	2 (33)
cardiovascular	7 (22)	0 (0)
respiratory	6 (19)	0 (0)
postoperative	3 (9)	1 (17)
gastro-intestinal	2 (6)	3 (50)
other	7 (22)	0 (0)
PELOD score[c,d]	1 (0-11)	0 (0-0)
Empirical start	23 (72)	5 (83)
Number of co-medication[e]	2 (1-3)	3 (1-4)
Albumin concentration (g/L)	30.6 (26.9-33.8)	36.5 (33.2-39.0)
Total bilirubin concentration (mg/dL)	0.21 (0.16-0.35)	0.46 (0.27-2.81)
Urea (g/L)	18.3 (11.4-24.5)	25.5 (19.5-32.2)
Total protein concentration (g/L)	55.8 (49.8-60.9)	62.3 (56.3-68.3)
Serum creatinine[d] (mg/dL)	0.22 (0.19-0.29)	0.34 (<0.17-0.48)
Serum CRP[d] (mg/L)	48.2 (11.4-138.1)	33.5 (25.0-79.6)
Total vancomycin concentration (mg/L)	16.6 (8.7-30.3)	17.0 (12.9 – 21.2)
Unbound vancomycin concentration (mg/L)	12.2 (6.2-21.7)	12.5 (8.0-15.3)
Unbound vancomycin fraction (%)	71.1 (65.4-79.7)	71.8 (63.1-77.1)
Treatment and Sampling	Value for characteristic[b]	
Length of vancomycin therapy (days)	6 (4-9)	9 (5-10)
Intermittent dosing		
Number of patients	29 (91)	4 (67)
Number of samples	176 (97)	34 (67)
Collected samples per dose	5 (4-5)	5 (4-5)
Continuous dosing		
Number of patients	3 (9)	2 (33)
Number of samples	6 (3)	17 (33)
Collected samples per patient	2 (2-2)	9 (6-11)

[a]Abbreviations: PRISM, Pediatric Risk of Mortality; PELOD, Pediatric Logistic Organ Dysfunction; CRP, C-Reactive Protein. [b]Values are median (IQR) or number (percentage). [c]Based on values from 26 patients for study group and all patients for validation group. [d]At day(s) of sampling. [e]Based on values from all samples of the study group and 43 samples of the validation group.

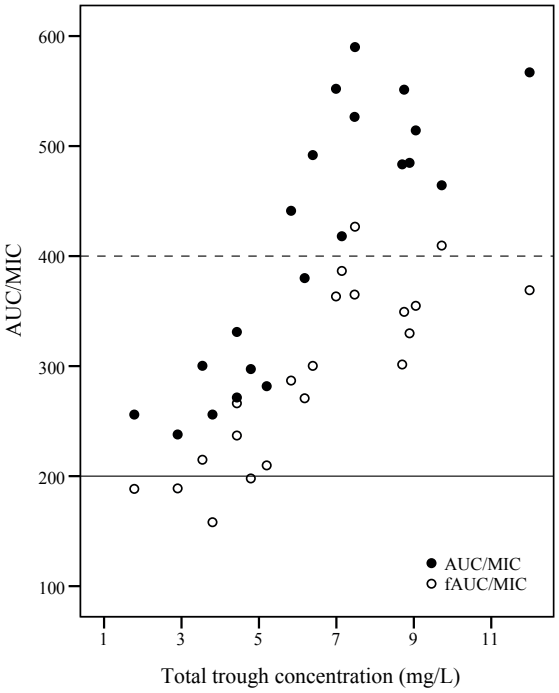

Figure 1. Correlation between total trough concentrations and (*f*)AUC/MIC for patients who received ID (n = 21). The broken line indicates the target AUC/MIC of 400, the continuous line indicates the target *f*AUC/MIC of 200, filled circles represent the calculated AUC/MIC and open circles represent the calculated *f*AUC/MIC. Spearman's rank correlation coefficients: AUC/MIC, R = 0.85 ($p<0.01$); and *f*AUC/MIC, R = 0.82 ($p<0.01$). Twelve (57%) patients reached the AUC/MIC of 400 (above the broken line) and 17 (81%) patients reached the *f*AUC/MIC of 200 (above the continuous line).

better fit of the data. The unbound vancomycin concentration in our patient population could be predicted by the following equation:

$$C_{unbound} = 5.38 + (0.71 \times C_{total}) - (0.085 \times C_{tp})$$

In this equation $C_{unbound}$ represents the unbound vancomycin concentration (mg/L), C_{total} the total vancomycin concentration (mg/L) and C_{tp} the total protein concentration (g/L).

Model validation

The prediction model for the unbound vancomycin concentration was validated using another six patients (51 samples). Demographic and clinical data of the validation group are summarized in **Table 1**. The median total vancomycin and total protein concentration were 17.0 mg/L (IQR = 12.9-21.2 mg/L) and 62.3 g/L (IQR = 56.3-68.3 g/L), respectively. Excellent agreement between measured and calculated unbound vancomycin concentration is

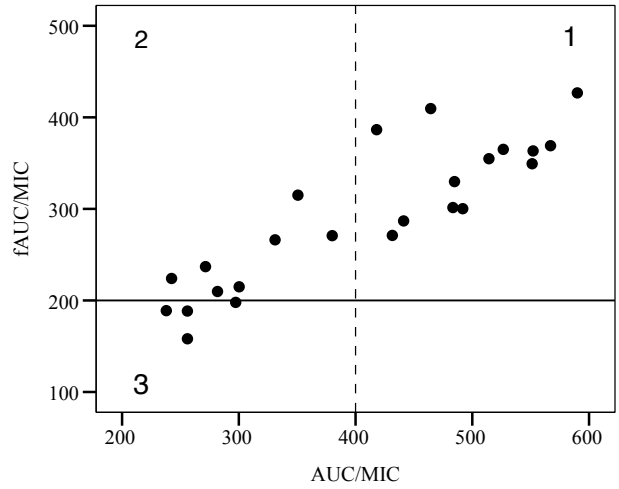

Figure 2. Target attainment based on AUC/MIC and *f*AUC/MIC for patients who received ID (*n* = 21) and CD (n = 3). The vertical broken line indicates the target AUC/MIC of 400 and the horizontal continuous line indicates the target *f*AUC/MIC of 200. Spearman's rank correlation coefficient: R = 0.88 (p<0.01). Thirteen (54%) patients reached the AUC/MIC of 400 (first quadrant), 20 (83%) patients reached the *f*AUC/MIC of 200 (first and second quadrant) and 4 (17%) patients did not reach the (*f*)AUC/MIC (third quadrant).

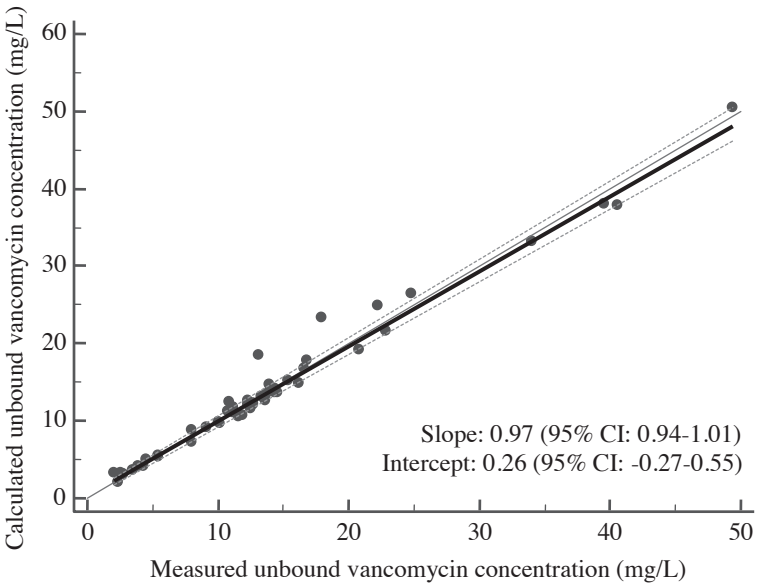

Figure 3. A Passing-Bablok regression analysis of the measured unbound vancomycin concentration and calculated unbound vancomycin concentration for 51 samples from 6 patients. The solid black line represents the Passing-Bablok regression line (Y = 0.26 + 0.97X), the two dashed grey lines represent the 95% confidence interval of the curve and the solid grey line denotes the identity line.

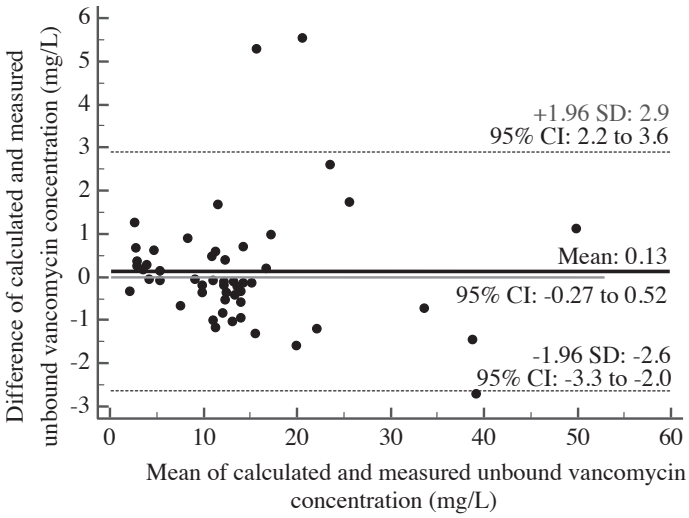

Figure 4. A Bland-Altman analysis of the differences between calculated and measured unbound vancomycin concentration were plotted against the mean of calculated and measured unbound vancomycin concentration. The solid black line indicates the mean difference, solid grey represents the line of equality and the two dashed black lines denotes upper and lower limits of agreement. SD: Standard deviation.

shown in **Figure 3** and **Figure 4** showed no proportional or systematic bias. The mean difference was 0.13 mg/L with the limits of agreement being -2.6 mg/L to 2.9 mg/L (**Figure 4**).

DISCUSSION

The present study revealed that protein binding of vancomycin in critically ill children is comparable to what has been described in critically ill adults, but is substantially lower in comparison to healthy volunteers and non-critically ill adults (50%-55%).[13,19,20,21] The lower vancomycin binding may be explained by differences in protein concentrations as we found that protein binding depended on total protein concentrations (of which albumin is the most important fraction). Both total protein (range = 21.1-74.2 g/L) and albumin concentrations (range = 14.2-45.1 g/L) were decreased in our study population and exhibited a marked variability.[10,11,22] Oyaert et al.[19] observed a similar trend of reduced protein binding in children, when compared to adults. Besides the observation of reduced protein binding a considerably high intra- and intervariability in protein binding was found.

As protein binding was altered and highly variable, our study aimed to compare target attainment rates using different proposed PK/PD targets, i.e. total (trough) concentration

and (*f*)AUC/MIC. The most commonly used PK/PD parameter, i.e. total (trough) concentration, was within the aimed target ranges in only 8% of cases. Since trough concentrations of 10-15 mg/L are believed to be a good surrogate to achieve an AUC/MIC≥400, it would be reasonable to assume that the latter target would not be achieved in the majority of patients.[2] However, our study revealed that an AUC/MIC≥400 was achieved in 54% of patients. Total trough concentrations correlated well with the calculated AUC/MIC (R = 0.85) and a total trough concentration of ~7 mg/L corresponded to an AUC/MIC of 400. This is in agreement with two previous studies in children, which predicted that achievement of an AUC/MIC of 400 (assuming MIC = 1 mg/L) corresponded with lower trough concentrations (8-10 mg/L) due to altered PK.[23,24] More importantly, our target attainment analysis showed that in the same group of patients, the *f*AUC/MIC≥200 target was reached in an even larger proportion (83%) of our study population. The *f*AUC/MIC target reflects directly the exposure to the unbound pharmacologically active concentration. Consequently, attempts to achieve the AUC/MIC target of 400, for the 29% of cases who already achieved the *f*AUC/MIC target, may not result in additional clinical benefit but may lead to unnecessary drug exposure and potential toxic effects **(Figure 2; quadrant 2).**

Despite the *f*AUC/MIC target value of 200 being a more arbitrary goal, based on the most conservative and fixed protein binding of 50%, to our knowledge there is no superior alternative target available.[9] In each case, these findings question the magnitude of underdosing as suggested in previous studies, which only used total trough concentration and AUC/MIC for target attainment analysis.[7,25,26] In clinical practice, monitoring vancomycin exposure by *f*AUC (with MIC if available), and thereby taking into account the protein binding, might be a more justified target to prevent underdosing or overexposure. Furthermore, given the high variability in protein binding in our study population, it seems not advisable to assume a fixed unbound fraction to calculate this *f*AUC/MIC ratio. In this heterogeneous population with different types of infection we were able to accurately predict the unbound vancomycin concentration in plasma based on total vancomycin and total protein concentration.

Our study has a number of limitations to consider. First, we did not determine actual vancomycin MIC values to accurately calculate the (*f*)AUC/MIC, mainly because in 72% of cases therapy was started empirically. Instead, we used a commonly used MIC breakpoint of 1 mg/L to compare target attainment with previous study results.[23,24] Second, a small number of subjects on CD were included, so no definite conclusions could be drawn with regard to target attainment. Third, tissue concentrations were not measured and higher targets may be needed to ensure adequate penetration in tissues.

In conclusion, our study demonstrates that the unbound vancomycin fraction in our population is higher than generally assumed, exhibiting high intra- and intervariability. The number of patients achieving target vancomycin concentrations varied widely depending upon the type of PK/PD target used. These results argue against currently used PK/PD indices for making quantitative exposure-response assessments in critically ill children. Further clinical and bacteriological outcome studies should be performed in specific populations to define appropriate PK/PD indices based on unbound concentrations. We provided a validated prediction tool for unbound vancomycin concentrations and offer an easy alternative for measuring unbound concentrations in clinical practice.

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TRANSPARENCY DECLARATIONS

None to declare.

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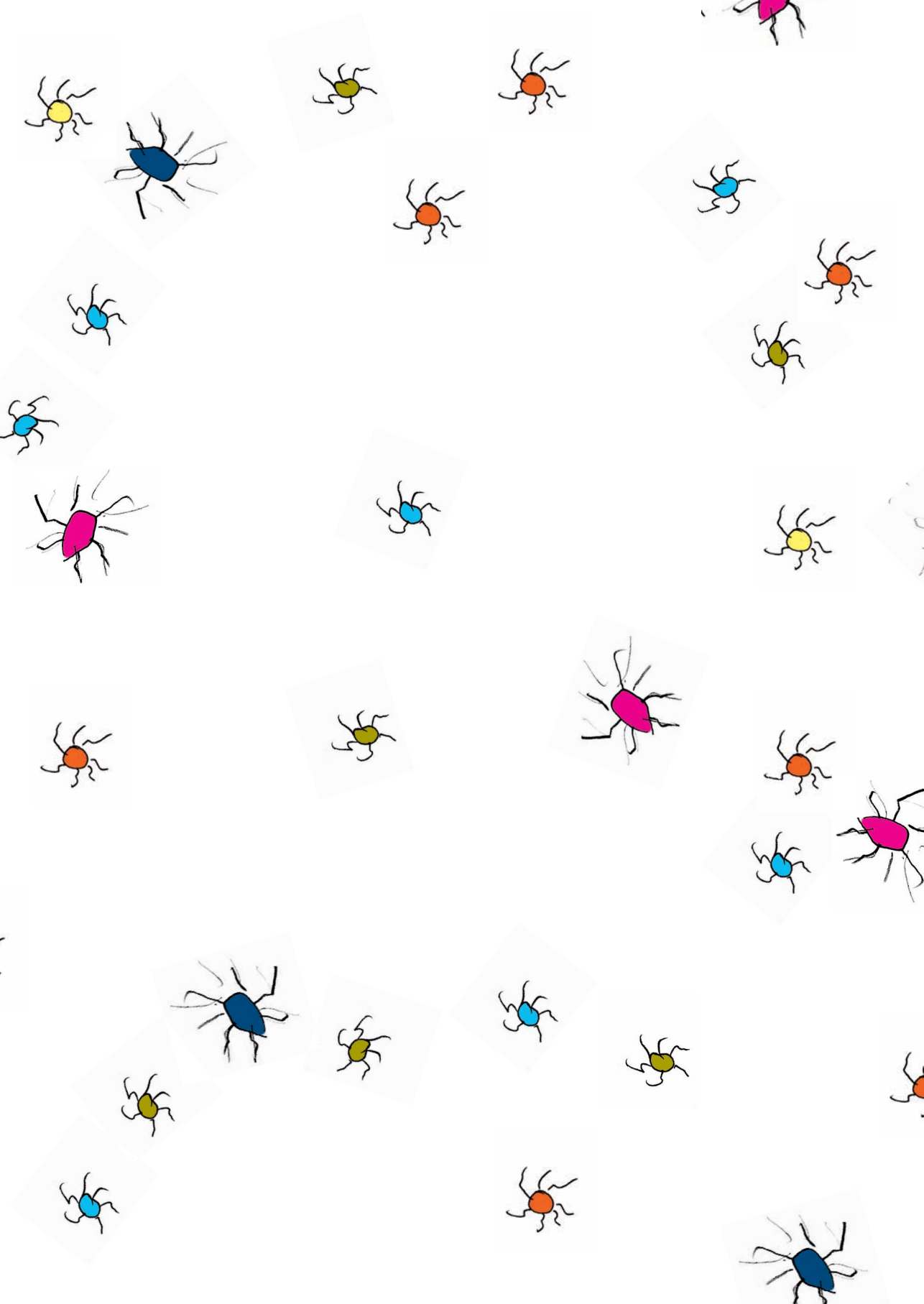

CHAPTER 7

DISCUSSION AND FUTURE PERSPECTIVES

The response to drugs may be different in neonates, infants and children when compared to adults. These differences are mainly caused by differences in PK and/or PD. From birth up to adulthood, maturational changes in body composition, drug metabolising enzymes, cardiac output, blood flow, function of eliminating organs and functionality/expression of drug receptors occur, leading to major changes in disposition and effect **(Chapter 1, Figure 1)**.[1,2] Moreover, both disease and treatment can also affect drug PK and PD **(Chapter 1, Figure 2)**.[3]

Notwithstanding the improvement in paediatric drug research 10 years after implementation of the European Regulation, drug dosing in critically ill children remains a real challenge. To date, the impact of major pathophysiological (e.g. sepsis) and treatment related changes on PK/PD are rarely studied.[4] Finally, off-patent drugs remain completely orphaned in terms of evidence-based dosing.[4,5]

Given the ethical issues and practical constraints (e.g. limited number of blood samples) trials in critically ill children are more difficult to perform.[6–9] The introduction of M&S tools in drug research (e.g. mixed effects modelling) enabled the analysis of sparse and unbalanced datasets, thereby opening new horizons for paediatric drug research.[10–13]

In this doctoral dissertation, the disposition and target attainment of four intravenous antibiotics and two BLIs were studied in critically ill children (using mixed effects modelling), with the aim to develop evidence-based dosing regimens. The antibiotics were selected based on their frequency of use in combination with their empirical use in clinical practice. Here, we critically appraise the relevance and limitations of our research and reflect on future perspectives, also in a broader international context.

What have we learnt from these studies?

In **Chapter 3**, the population pharmacokinetics of the β-lactam antibiotic amoxicillin in combination with the BLI clavulanic acid were studied in 50 critically ill children. After scaling for size and age, renal function was identified as a significant covariate on the clearance of both compounds. Treatment with vasopressors was also associated with a 18% reduced amoxicillin clearance. Stochastic simulations showed that lower CysC resulted in a lower PTA and that at least four hourly dosing of 25 mg/kg (based on amoxicillin; as a bolus infusion for CysC > 1mg/L or 1 hour infusion for CysC< 1 mg/L) was required to achieve the therapeutic target (40% $fT_{>MIC}$). This is, to the best of our knowledge, the first population PK study on amoxicillin/clavulanic acid, and the first to detect augmented renal clearance in this patient population, with serum CysC used as a novel predictor for renal drug clearance.

The PK of the piperacillin/tazobactam combination was characterized in 47 critically ill children in **Chapter 4**. To account for size and maturation, allometric scaling and a Hill function on clearance were implemented. Also here, high individual clearances were identified in some patients, which prompted to a change in regimen with more frequent dosing to ensure optimal piperacillin exposure (60% $fT_{>MIC}$). Monte Carlo simulations showed that an intermittent infusion of 75 mg/kg (based on piperacillin) given four hourly over 2 hours, 100 mg/kg given four hourly over 1 hour or a loading dose of 75 mg/kg followed by a continuous infusion of 300 mg/kg/24h were minimally required to achieve the therapeutic target.

Chapter 5 described the population PK and protein binding of cefazolin in 56 children undergoing cardiac surgery before, during and after CPB. Besides body weight which accounted for the change in size, eGFR was identified as a covariate on clearance. An increase in the volume of distribution was identified during CPB and modelled using a separate CPB compartment. Moreover, saturable plasma protein binding of cefazolin was quantified with serum albumin concentration as a covariate on maximum protein binding capacity. Prolonged surgery and preserved renal function had a negative impact on PTA. When aiming at target concentrations of 100 % $fT_{>MIC}$ during and 50% $fT_{>MIC}$ shortly after surgery, more frequent and higher dosing appeared to be needed (40 mg/kg, 30 min before surgical incision; 20 mg/kg, at start of CPB; 20 mg/kg, at the start of rewarming on CPB; 40 mg/kg, 8 h after the third dose; 40 mg/kg 8 h after the fourth dose). This is, to the best of our knowledge, the first population PK study on cefazolin in children undergoing cardiac surgery with cardiopulmonary bypass.

In **Chapter 6**, a pilot study on the role of plasma protein binding and impact on target attainment of vancomycin in 38 critically ill children was performed. Our study revealed that the unbound fraction was comparable to what has been published in critically ill adults, but substantially lower in comparison with healthy volunteers and non-critically ill adults. Furthermore, protein binding was highly variable and correlated with plasma protein concentration, which was also decreased and exhibited large variability. Target attainment rates were also compared in this study using different proposed PK/PD targets based on total and unbound concentrations. We found that trough concentrations and (*f)*AUC correlated well with each other but that target attainment rates were largely dependent on the individual target: 8% within the trough concentration range of 10-15 mg/L, 54% reaching an AUC/MIC of 400, and 83 % reaching a *f*AUC/MIC of 200. To the best of our knowledge, this was the first study to evaluate plasma protein binding and target attainment based on unbound concentrations in critically ill children.

All studies described above clearly illustrate that, besides developmental changes, co-treatment modalities (e.g. vasopressors, CPB), altered organ function (e.g. hyperfiltration) and other disease related changes (e.g. protein binding) always need to be considered as potential covariates on drug disposition. Moreover, whilst performing the clinical trials, we concomittantly evaluated how renal function **(Chapter 3, 4, 5)** and protein binding **(Chapter 5,6)** change in these patient populations. These data can be incorporated in future PK/PD research using more (semi-)mechanistic approaches (e.g. physiologically-based pharmacokinetic modelling).

The leitmotiv through the methodology section in our studies was the use of a mixed effects modelling approach for analysis and interpretation of the results. Despite the widespread acceptance of population pharmacokinetic methods in the drug approval process, relatively few population pharmacokinetic studies have been conducted among critically ill children.[3,11,14] From our studies we can conclude it was a powerful tool to analyse unbalanced (and in some patients sparsely collected) data. Through this approach we were able to propose evidence-based dosing regimens **(Chapter 3, 4, 5)** and identify covariate factors on protein binding **(Chapter 6)**.

What are the direct clinical implications on drug dosing and monitoring?

Since fatal tragedies happened in the past (e.g. gray baby syndrome due to chloramphenicol overdosing), physicians are more prudent to prescribe drugs to children, in the absence of evidence-based paediatric dosing guidelines.[15,16] As they are mostly concerned about the potential toxicity risks of drug overdosing, currently, paediatricians often tend to prescribe the "lowest known effective dose". In particular with regard to renally excreted drugs, paediatricians are traditionally more focussed on the potential impact of renal failure on drug levels than on the consequences of augmented renal clearance. However, our study results on β-lactam antibiotics in critically ill children **(Chapter 3, 4 and 5)** demonstrated that the current dosing recommendations do not always apply to this dosing paradigm. To avoid subtherapeutic treatment in our patient population, new dosing regimens were suggested using a shorter dosing interval with a higher cumulative daily dose, taking into account the PK/PD parameter of interest i.e. $fT_{>MIC}$.

On the other hand, higher doses may not always be necessary and special attention is warranted for drugs with a smaller therapeutic range than β-lactam antibiotics. In the case of vancomycin, the most commonly proposed target PK/PD parameter for beneficial clinical outcome in adults is an AUC/MIC of 400.[17] Total trough levels of 10-15 mg/L are aimed for as a proxy for an AUC of 400, assuming a MIC <= 1 mg/L.[18] Recently, Le *et al.* demonstrated a strong relationship between higher AUC and the occurrence

of nephrotoxicity.[19] Vancomycin trough levels >=15 mg/L and AUC >=800 mg.h/L were also independently associated with a 2.5 and 3.7 fold increased risk, respectively. Furthermore, ICU stay and concomitantly receiving nephrotoxic agents were found to significantly increase the absolute risk. While the above mentioned threshold values are still substantially higher than the highest AUC and trough level observed in our study, these results highlight the importance of precision dosing. From our pilot results, we could confirm previous findings which showed that, in routine clinical practice, lower target trough levels (~7-10 mg/L) were required to reach an AUC/MIC of 400 **(Chapter 6)**. Furthermore, since only the unbound drug is pharmacologically active and a conservative target of *f*AUC/MIC of 200 was reached in the majority of patients, our results question the previously claimed magnitude of underdosing.[20–22] Further clinical and bacteriological outcome studies should be performed to define appropriate PK/PD targets. Finally, we provided a validated prediction tool for unbound concentrations based on total vancomycin concentrations and offer hereby an easy alternative for measuring unbound concentrations in clinical practice.

In summary, the findings in this PhD dissertation contributed to the knowledge on factors influencing antibiotic disposition and dose optimisation in seriously ill neonates, infants and children.

What are the limitations and future perspectives of this research?

Model validation

All PK models within this PhD dissertation are internally validated, as described under the methods section of each chapter. Preferentially, the population PK/PD models should also be validated using data from another similar patient population before implementation into the clinic.[23] This external validation step is particularly important to ensure that the model reflects the parameter distributions in the overall population and is not only descriptive for the study sample of patients. Ideally, a subsequent prospective validation of the new dosing regimen is performed in another set of patients to make sure the desired target concentrations are reached and dosing regimens are safe.[11,24] As described in **Chapter 6**, external validation of the model describing the unbound vancomycin concentration was performed using data from another 6 patients.

However, it was decided to implement our revised β-lactam dosing regimens before such validation data are available, after having rigorously outweighed potential benefits and risks: (i) there is evidence that subtherapeutic antibiotic dosing leads to clinical failure and extra morbidity and mortality; (iii) β-lactam antibiotics have a broad therapeutic range and toxicity with a high dose of amoxicillin/clavulanic acid, piperacillin/

tazobactam and cefazolin is not frequently reported in adults. However, proper safety data on (intensified) dosing in (critically ill) children are currently lacking; (iii) we have specifically chosen not to select higher amounts per dose than currently recommended to mitigate potential safety risks related to higher peak concentrations; (iv) we aimed for rather conservative $fT_{>MIC}$ targets in the case of amoxicillin/clavulanic acid (40% $fT_{>MIC}$) and piperacillin/tazobactam (60% $fT_{>MIC}$); (v) the total treatment duration for surgical prophylaxis with cefazolin is limited to 24 hours.[25,26]

Extrapolation

Notwithstanding the relatively high number of younger patients included in our studies, PK data from additional neonates and infants are needed to estimate maturation parameters more precisely on clearances of amoxicillin, piperacillin, clavulanic acid and tazobactam and refine the dosing regimens in these age categories **(Chapter 3, 4)**. Furthermore, our study results (including proposed dosing regimens) may not apply to patients with characteristics that differ from our patient population. Additional studies are therefore required to study the impact of moderate to severe kidney injury, severe burn injuries, ECMO, RRT, body cooling and septic shock on antimicrobial pharmacokinetics.

Bio-analytical aspects

Differences in pre-analytical sample handling (e.g. ultrafiltration for separation of unbound and bound antibiotic) and interassay variation in antibiotic measurement, can lead to large differences in measured (unbound) concentration and may compromise the applicability of the PK/PD model in other clinical settings.[27] Stove *et al.* identified temperature as a critical variable during ultrafiltration for determining unbound vancomycin concentration and concluded that 37°C should be preferred for temperature stable compounds.[28] Oyaert *et al.* and Samardzic *et al.* demonstrated a disconcordance in vancomycin concentration measurement up to 20 % when comparing different vancomycin assays.[29,30] In our study on vancomycin plasma protein binding **(Chapter 6)**, the unbound vancomycin fraction was approximately 10% lower than described by Oyaert *et al.* in non-critically ill children, despite comparable albumin and total protein concentrations. Apart from dissimilarity in patient characteristics and sample size, pre-analytical and analytical variation may explain this difference. These methodological issues may also be applicable to our study measuring unbound cefazolin concentrations in children on CPB (**Chapter 5)**; however similar clinical studies are currently lacking.

The generalisability of our results also depends on the similarity of assays used for CysC and creatinine. In **Chapter 3**, CysC as a marker of renal function was found to be a covariate on amoxicillin and clavulanic acid clearances. Currently, this promising biomarker is not commonly used in routine practice and several commercially available measure-

ments remain unsatisfactory in terms of calibration to the European reference material of the international federation of clinical chemistry and laboratory medicine (ERM-D471/IFCC). .[31] In **Chapter 5**, serum creatinine was used to calculate an eGFR based on the modified Schwartz formula. Serum creatinine was measured using the (Isotope Dilution Mass Spectrometry traceable) compensated Jaffe method and recalculated towards enzymatic values, as this is currently the most advocated method in children.[32,33] Although these corrected creatinine values have shown a large interchangeability with enzymatically measured serum creatinine for children between 3 and 14 years, the applicability might be lower depending on the patient age and the type of creatinine assay used.[23,32]

Although serum creatinine is still frequently used as a renal biomarker, in two of our studies, we were not able to test serum creatinine as a covariate on clearance **(Chapter 3, 4)**, due to a large proportion of values remaining below the quantification limit. Analytical challenges (overcorrection of interfering protein in the compensated assay) as well as augmented renal clearance of this biomarker were suggested as potential causes. Consequently, future assessment of serum creatinin as covariate in PK studies should include a more sensitive enzymatic creatinine assay.[33] Furthermore, alternative renal biomarkers (e.g. serum cystatin C, beta-trace protein) and new GFR estimation methods (e.g. derived eGFR formula, urinary creatinine clearance) are to be further explored as covariate on drug clearance in critically ill children. [34–37]

In **Chapter 3 and 4**, total drug concentrations were measured and mathematically corrected for protein binding. Although small changes of low protein bound drugs are thought to only have a minor effect on the unbound concentration, a direct measurement of unbound drug concentrations may still be more advantageous for PK/PD studies in children with highly variable protein binding, as illustrated for vancomycin in **Chapter 6**. Also the 2015 draft EMA guideline on the use of pharmacokinetics and pharmacodynamics in the development of antibacterial medicinal products explicitly states that PK/PD indices should be expressed as a function of unbound concentrations.[38]

PK/PD relationship – risk/benefit ratio

For our dosing recommendations, antibiotic target attainment was evaluated against commonly used summary PK/PD target indices **(Chapter 3, 4, 5, 6)**, using MIC values that were not prospectively collected. Instead, commonly used **(Chapter 6)** or worst-case MIC scenarios **(Chapter 3, 4, 5)** based on EUCAST clinical breakpoints were chosen. Although this is still the most commonly used method for dose evaluation, we recognise that these indices are only simplifications of the underlying PK/PD relationship. Many drawbacks have been described and mainly relate to the lack of measurement of time-related PD effects and inaccuracy in MIC determination. Moreover, it carries

the assumption that target PK/PD indices are not affected by differences in PK, dosing regimen and bacterial susceptibility.[39,40] Therefore, the building of more sophisticated mechanistic PK/PD models is encouraged by the EMA and should be the next step in our antibiotic dose optimisation process.[38] In these models, typically, the PK is linked to the microbiological response over time (growth and killing rate of bacteria) from *in vitro* or animal infection experiments, taking into account the development of antibiotic resistance, the immune response from the host and/or the magnitude of the inoculum **(Figure 1)**.[39] Ideally, effects and side-effects over time of the antibiotic treatment are modelled using data from real patients to assess benefit-risk ratios. Measurement of bacterial killing and development of antimicrobial resistance over time, however, is still a challenge and valuable biomarkers are currently lacking. Ultimately, the impact of model-based dose optimisation on morbidity and mortality should be investigated in a blinded, randomised controlled trial design comparing both the 'old' and 'revised' dosing regimens. These kind of studies require patient recruitment on a large scale.

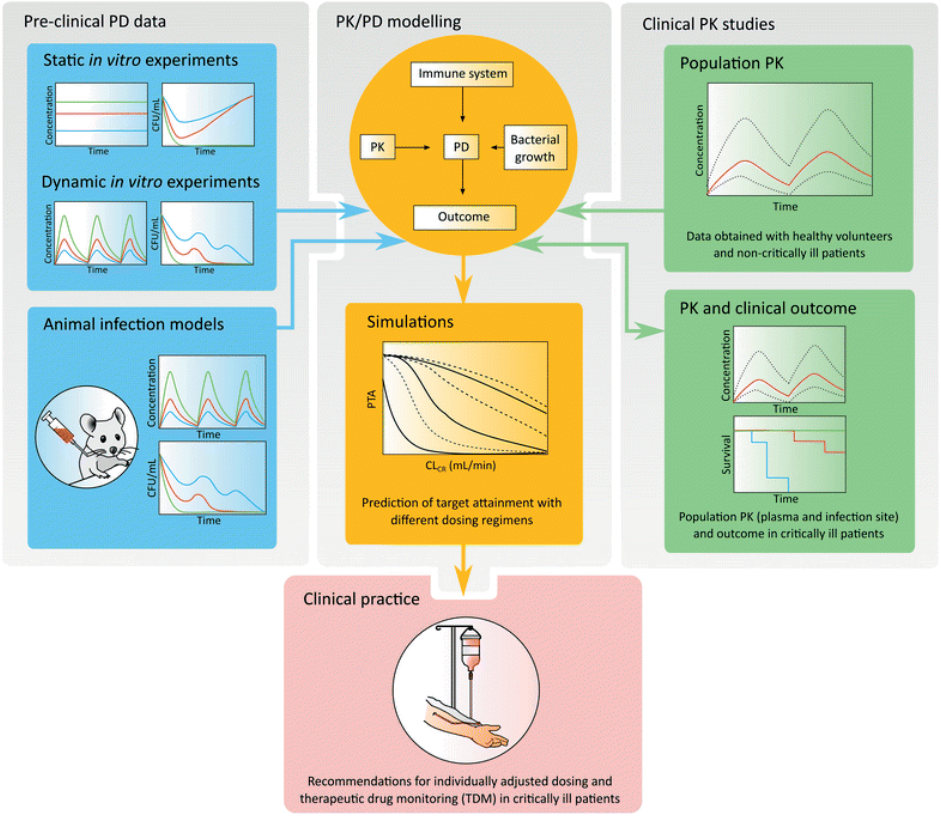

Figure 1 : Role of preclinical infection models, PK/PD modelling and clinical PK studies to optimise dosing in critically ill patients (Reproduced with permission from Tängdén T. *et al.*[41] , Copyright SpringerOpen)

In our PK/PD studies on amoxicillin/clavulanic acid and piperacillin/tazobactam **(Chapter 3 and 5)**, we used very crude $fT_{>threshold}$ measures in an attempt to evaluate the BLI dose. Hence, we are aware that the corresponding PTA results for BLI are only explorative in nature and further *in vitro* and animal PD studies are needed to define the target in infections of interest.[38,42,43] Initially, BLI dose fractionation studies are needed to identify indices with maximum activity (C_{max}/MIC, AUC/MIC, $fT_{>MIC}$). Subsequently, as described above for antibiotic components, mechanistic modelling to include the time course of bacterial killing and the conduct of clinical studies are encouraged **(Figure 1)**. In all those studies, administration of β-lactam compounds should mimic its clinical use as PK/PD indices for BLI may depend on the β-lactam antibiotic dosing regimen.

Establishing BLI targets in future studies will enable to further adapt dosing recommendations using our PK data.

Tissue penetration

We studied serum/plasma PK in heterogeneous groups of children, with regard to possible differences in tissue involvement/penetration. In patients with compromised tissue penetration, higher doses may be needed to ensure adequate treatment. Further studies should focus on these aspects. Microdialysis enables direct measurement of antibiotic concentrations in the interstitial space and has gained a lot of popularity in the last few years.[40,44] Using this technique, a microdialysis probe is first inserted in the interstitial space of the target tissue, after which, a carrier fluid is infused at a fixed rate through this probe, which is then collected outside the body. The outer wall of the probe consists of a semi-permeable membrane allowing the antibiotic to diffuse from the interstitual fluid toward the carrier fluid. Tissue antibiotic concentrations are subsequently measured at prespecified time intervals in aliquots of the dialysate. Despite the technical challenges and invasiveness of the technique, it has been successfully used to measure cefazolin skeletal muscle concentrations in children undergoing cardiac and spinal surgery.[45,46]

What is the broader context of this research?

Some future perspectives of our research deserve attention in a broader context. As encountered in our studies, performing clinical research in an ICU environment is challenging. Below, we identify priorities and discuss promising developments to improve and accelerate pharmacological research within the NICU/PICU environment.

Networking and collaboration

In the 10-year EMA report on the paediatric regulation, the lack of sustained funded research infrastructure, coordination of research activities at a network level, and awareness of existing networks to the industry were identified as some of the main hurdles to

tackle.[4] As we encountered in our studies, this is especially the case for the ICU setting, due to the high population heterogeneity and the relatively low number of admissions per single centre.

To date, few (inter)national research networks, addressing the specific pharmacological needs of critically ill children, exist. The Foundation Paediatric Intensive Care (Stichting Kinder Intensive Care) is an example of a national academic research network in the Netherlands between 8 paediatric intensive care units in which, currently, 2 medication trials were initiated. Within Europe, only the European Society on Neonatal and Paediatric Intensive care medicine Research Network (ESPNIC) is a dedicated research network recognized by the European network of paediatric research and specialist networks at the EMA (Enpr-EMA). Currently, no collaborative medication trials were initiated yet within this ESPNIC network. In the future, such sustainable collaborative research efforts should be more actively encouraged as they undoubtedly increase patient recruitment. Furthermore, they may raise more awareness amongst regulatory agencies for this vulnerable subpopulation and challenge the pharmaceutical industry to the setup of more clinically relevant research in critically ill children.

Essential for the prosperity of such multicentric collaborations, will be a more streamlined ethics board review and harmonised informed consent and assent procedure to guarantee a same level of information, protection from risks and potential benefit of the drug between trial participants.[47,48]

Ethical conduct – trial participation

Ethically performing research is of utmost importance, including the requirement of a high standard of informed consent. NICU/PICU researchers, however, may face several practical barriers to implement a valuable informed consent, as described in **Chapter 1**.

Although the informed consent procedure was conducted with maximal integrity, some of these problems were also encountered in our studies. Regularly, parents (or legal guardians) were informed and asked to participate in fraught circumstances, shortly before or after an antibiotic was administered, leaving them a relatively short timeframe to decide whether they wanted their critically ill child to participate or not. In some patients, trial participation was not considered as the treating physician felt not comfortable with asking for informed consent (e.g. highly perceived emotional parental stress, language barrier, complex family situation, child that was too sick etc). Excluding these patients holds a risk of impairing the validity of study results because of selection bias. In this context, Garde *et al.* clearly indicated room for improvement as only one third of clinical trials in critical care reported on consent rates.[49] Moreover, children included in

our studies were only asked to give assent, if they were older than 12 years of age and physically capable, at a convenient time at the discretion of the treating physician. In most patients, this was only possible a while after inclusion, when the child's clinical condition enabled to do so. Only the study described in **Chapter 5** was an exception in that sense that the study could be proposed by the treating surgeon in less stressful conditions, the day before cardiac surgery.

All these hurdles illustrate the limitations of the standard consent and assent procedure in this setting and highlight the importance of alternative strategies to be studied. Essential to improve this process in terms of voluntariness and patient recruitment, is the input of parents and children on both procedures and trial design.[50] To date, the insight in encouraging and discouraging factors for participating in drug research in the NICU/PICU is, however, rather small and sometimes conflicting.[51] Characteristics of the consent encounter, individual parent, child and study have been reported to be related to the decision making process.[50]

Regarding the timing and modalities of consent and assent, some alternative options to the standard method have been proposed. Allmark *et al.* demonstrated that a stepwise designed parental consent procedure improved the quality of the consent in a randomised, controlled trial in critically ill neonates.[52] In this approach, a selection of crucial information is given before asking for consent to enrol the patient, due to the time constraints and potential impaired ability of parental decision making. As time goes on, more detailed information and explicit choice to opt out is given, both on a continuous basis. Deferred consent is another method to deal with the emergency of decisions to be taken in an acute care setting like NICU/PICU and implies that the consent is requested after the patient is recruited. This approach is currently only allowed when, owing to the urgency of the treatment and the trial, it is impossible to obtain prior consent from the patient or legal representative, provided that an ethics committee has given its approval.[53] Preliminary research suggests that parents can appreciate this way of consenting, if appropriately timed and explained.[54–56] A waiver of consent is another method and implies that consent is not required. To date, this method has only been used in paediatric resuscitation research. Many of these studies used community consultation and public disclosure on the unit to inform parents and caregivers that children could be enrolled in a clinical trial.[57,58] Overall, there is a reluctance of caregivers and parents towards this practice. Innovations in communication technology may also contribute to solving some issues with the informed consent process. The newest method is the so-called 'dynamic consent' in which online communication platforms (e.g. video calls, webinars, websites) are used for personalised consenting.[59] Usually, it is designed to facilitate the dialogue between researchers and parents (or children) before,

during and after the study through online communication. It also enables the parents or children to refine their consent to specific parts of the study (e.g. data sharing with drug companies) or change it at any time. Finally, such platforms may offer the possibility to provide updates on study results and outcomes. Regarding alternative methods to ask for assent of minors, video game technology support is currently under development.[60] To date, studies on the use of new communication technology in a paediatric intensive care setting are lacking.

Amongst the characteristics related to the parent and child, the perceived risk and benefit for the individual child and society, additional burden for the child, anxiety, attitude towards research and illness severity were most commonly cited as influential factors to participate in clinical research.[51,61–63]

In the Ghent University Hospital consent is usually asked by the treating physician. However, during the patient recruitment in our studies, some physicians reported that they had some moral objections against this common practice. Current ethical guidelines are also cautious with regard to dependent relationships between patients or parents and the study team professional. Especially, the voluntary informed consent may be compromised, due to a potential conflict of interest.[64] Interestingly, however, it was previously reported that parents preferred their treating physician to advise on their decision, rather than to take it independently.[62] Menon *et al.* also reported that introducing the researcher to the patient's family by any member of the patient's intensive care team resulted in higher consent rates.[61] This suggests that the existing relationship with and trust in the treating care team, may increase the parents' decision rate to participate. To the best of our knowledge, no studies on ICU are available investigating the influence of the above mentioned factors on the decision process.

In order to guide regulatory agencies, ethics committees and NICU/PICU researchers on the most valuable approach to ask for consent and assent, more knowledge is needed on the wishes and preferences on trial design, decision making process of both parents and children (including capabilities) and the roles of the treating physician and research team.

Minimal risk designs

As described in **Chapter 1**, blood sampling volume limits PK/PD research to be performed in young and critically ill children. Potentially beneficial blood sampling techniques for facilitating PK studies in this protected population are currently under investigation.[14]

Microsampling, which is the collection of smaller-than-normal plasma samples for bio-analysis, may provide a solution and includes mainly dried blood spots (DBS), dry plasma spots (DPS), volumetric absorptive microsampling (VAMS) and capillary microsampling techniques. In DBS or DPS, a small volume of blood or plasma is applied on an absorbent paper which is dried after saturation has occurred. In the laboratory, the blood or plasma is eluted out of the paper and subsequently analysed. Cohen-Wolkowiez *et al.* recently reported on the feasibility of using DBS concentrations in combination with plasma samples for PK model building of piperacillin and tazobactam in infants.[65] A quite similar, optimized technique is VAMS, in which a fixed volume of blood is absorbed by the porous, hydrophilic tip of the device. After the tip is dried, it is sent to the lab for drug extraction and bio-analysis. In capillary microsampling blood is collected in a capillary tube and subsequently centrifuged in this tube before bio-analysis of the sample. Overall, microsampling in critically ill children seems promising as it would allow a significant reduction in the blood volume required, thereby reducing the risk of further upsetting fluid balance which may already be compromised.

Another non-invasive technique for measuring drug exposure is using saliva as matrix. The advantages of saliva monitoring in paediatric PK trials are acknowledged by the FDA and mainly relate to the fact that saliva sampling potentially reduces blood sampling, is easy to collect and causes minimal patient discomfort.[66] The usefulness of saliva for TDM has been studied for several drugs including mainly anti-epileptics, antiretrovirals, antipsychotics, antibiotics and antifungals.[67–71] To date, however, the majority of studies concerning antibiotics lack sufficient PK data to evaluate the suitability.[70]

Opportunistic sampling, also known as scavenged sampling or left-over sampling, salvages remaining blood, plasma or other body fluids from routine biochemical tests for measurement of drug concentrations. Several studies in children have successfully used opportunistic sampling alone or in combination with timed blood sampling to characterize the PK of anti-infective treatment.[72–76] Interestingly, this process can also be useful in the opposite direction: Germovsek *et al*. presented a PK model that allows healthcare providers to take a gentamicin TDM sample at a time that is convenient (i.e. during a routine blood test) rather than needing to take a specific "trough" sample to determine whether drug levels are low enough.[77] Finally, routinely collected TDM data can also provide useful data to build PK/PD models for the monitored drug, as illustrated for gentamicin by Medellín-Garibay *et al*.[78]

Microdosing is another promising method to minimize patient burden while efficiently studying the pharmacokinetic behaviour of a drug under development. Commonly, the given dose is 1/100th of the No Observed Adverse Effect Level and is labelled with

a microtracer [^{14}C] for quantification using the most sensitive HPLC techniques. A prerequisite is the dose linearity from the magnitude of the microdose up to therapeutic ranges. In children, only two proof-of-concept PK microdosing studies, characterising paracetamol PK were published.[79,80]

Modelling and simulation

Over the last few years, M&S is rapidly evolving in the design and data analysis of paediatric PK/PD experiments, as it may overcome most challenges encountered in paediatric drug development.[81]

One increasingly important method to optimize a clinical trial is the use of mathematical algorithms to design the study in such a way that the collected information is maximally informative.[82,83] In these algorithms, prior knowledge on the structure of the model and parameter distributions are mandatory as input (e.g. published PK/PD model from older children). Typically, in PK/PD trials, the total number of patients, sampling times and/or number of samples are optimised for, taking into account the ethical (e.g. blood sampling volume limits) and practical constraints (e.g. minimum timeframe between two blood sampling times). In our PK studies, we did not use an optimal sampling design. Instead, we used an informative sampling design in which the sampling times were based on a combination of knowledge on common PK principles and target PK/PD indices. However, we acknowledge that the benefit of these optimal design algorithms is worth exploring in future NICU/PICU study designs.

Quantitative extrapolation of PK and/or PD for first-dose estimation is recognized by ICH, FDA and EMA as another valuable study design aid to explore dosing scenarios increasing the benefit-risk ratio, before even enrolling children into the clinical trial.[81,84–86] Through the use of extrapolation, also unnecessary studies can be avoided and the number of children minimised (e.g. fewer data needed in adolescents when reasonably similar to adults).[85] Still, bridging PK, efficacy and safety data from older age categories to the youngest age categories (neonates and infants) has shown variable success, mainly depending on the modelling approach and individual compound.[87,88] Also, the usefulness of extrapolation to children with serious pathophysiological changes and/or comorbidity needs further study.

Overall, a strong trend of moving from pure "empirical" models towards more mechanistic models is noticeable in the field of M&S, with the ultimate goal to create generalisable PK/PD models that distinguish between drug and system parameters.[11] De Cock *et al.* showed that the GFR mediated clearance of 4 renally cleared antibiotics in neonates (netilmicin, tobramycin, vancomycin, gentamicin) could be predicted using a

covariate model on amikacin clearance, which is a molecule with similar physicochemical and disposition characteristics.[89] This so-called semi-physiological approach beyond compound specific observations may eventually lead to a further reduction in patient burden. However, at the other extreme, it is known that the predictive performance of full PBPK and systems pharmacology models in children is currently fairly low, due to the lack of precise system parameter estimates.[12] Therefore, more knowledge on the time course of physiological processes (e.g. maturation in renal transporter activity) and disease specific changes (e.g. sepsis) is first needed to be applicable to the paediatric intensive care setting.[12,90,91]

A final promising development in the M&S area, is the use of software combining the population PK/PD model as Bayesian prior information and the individual response to refine the model parameter predictions for a particular patient.[83] Typically, this response is measured using a biomarker e.g. antibiotic concentration using therapeutic drug monitoring. Although, prospective, randomised controlled clinical trials are needed to compare traditional dose adaptation strategy (standard of care) with PK/PD model-based dose adaptation strategy, such software programs have the potential to be used as a bedside dose adaptation tool to ensure efficacious treatment.[92–94] In this revolutionary field of personalised medicine, clinical pharmacists and pharmacologists with a sound understanding of pharmacometrics are ideally placed to take the lead.[95]

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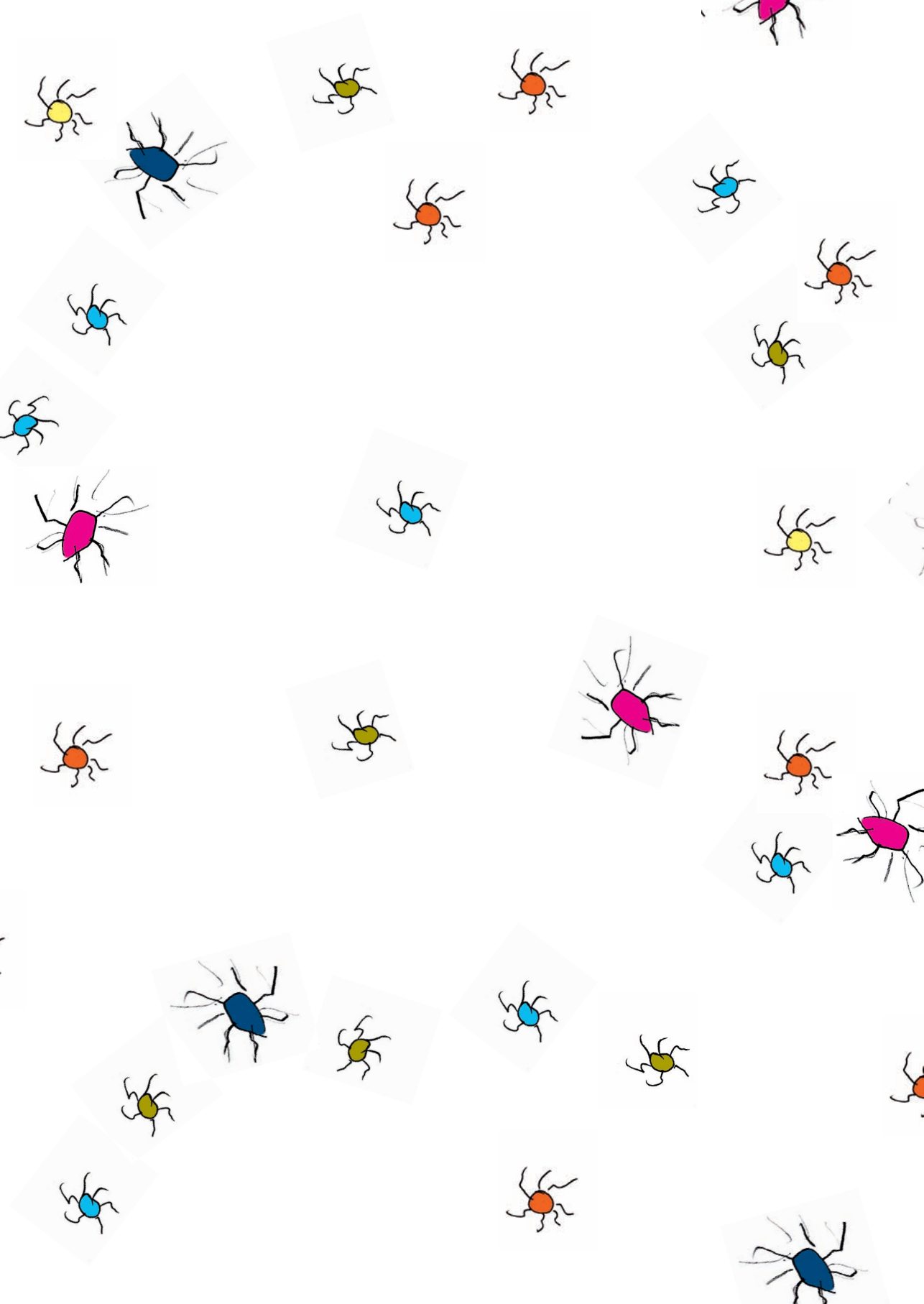

APPENDICES

SUMMARY

Bacterial infections are commonly encountered in children admitted to the ICU and require adequate antibiotic treatment. Growth, development and pathophysiological changes may alter disposition of antibiotics. Due to a lack of knowledge, most antibiotic dosing regimens are currently extrapolated from adults, relatively healthy and /or older children.

In this dissertation, the altered pharmacokinetics and target attainment of four commonly used antibiotics and two BLIs were investigated in critically ill children. It was demonstrated that current published dosing regimens of amoxicillin/clavulanic acid and piperacillin/tazobactam result in subtherapeutic concentrations in the early period of sepsis, due to augmented renal clearance, thereby risking clinical failure. Underdosing was also observed for cefazolin in children undergoing cardiac surgery with CPB. Hence, dose optimization strategies were suggested, based on population M&S. In a pilot study on vancomycin, it was found that its plasma protein binding was lower than generally assumed and depended on the plasma protein concentration. Target attainment was the highest when using a target based on unbound concentrations. Because only the unbound concentration exerts a pharmacological effect, the risk of underdosing in critically children is potentially lower than previously assumed. Finally, a validated prediction tool for unbound vancomycin plasma concentrations was developed.

In conclusion, the complex interplay between physiology and pathophysiology needs to be considered when assessing antibiotic disposition in critically ill children. Population M&S provides an effective tool to optimize antibiotic treatment in this vulnerable patient population.

Many questions are to be resolved. First, the proposed antibiotic dosing regimens require a prospective validation. Additional studies should focus on the tissue penetration of unbound antibiotic and link the PK with microbiological response and development of antimicrobial resistance over time. In the longer term, the impact of model-based dose optimisation on clinical outcome needs to be studied using a randomised, controlled trial design.

SAMENVATTING

Bacteriële infecties komen vaak voor bij kinderen opgenomen op de intensieve zorg afdeling, en vereisen een adequate antibioticatherapie. Groei, ontwikkeling en pathofysiologische veranderingen bij kinderen kunnen leiden tot een veranderde farmacokinetiek van antibiotica. Echter, tot op vandaag worden geneesmiddelen, in casu antibiotica, vaak aan kritiek zieke kinderen voorgeschreven zonder adequaat bewijs van het benodigd doseerschema. Clinici grijpen dan ook vaak terug naar 'op ervaring' gebaseerde doseringsaanbevelingen.

In dit proefschrift werd de dispositie onderzocht van vier antibiotica en twee BLIs bij kritiek zieke kinderen, alsook de kans op het bereiken van vooropgestelde doelconcentraties. Er werd aangetoond dat de huidige doseerregimes van amoxicilline/clavulaanzuur en piperacilline/tazobactam vaak leiden tot subtherapeutische concentraties in de initïele periode van sepsis, tengevolge van een verhoogde renale klaring. Een onderdosering werd ook waargenomen voor cefazoline bij kinderen die cardiochirurgie ondergingen met CPB. Om therapiefalen te vermijden, werden geoptimaliseerde doseerregimes voorgesteld, met behulp van populatie farmacokinetische methodes. In een laatste pilootstudie werd vastgesteld dat de plasma eiwitbinding van vancomycine bij kritiek zieke kinderen lager is dan algemeen werd aangenomen en afhankelijk is van de plasma eiwitconcentratie. Vancomycine doelconcentraties werden het vaakst bereikt, wanneer deze werden afgetoetst ten opzichte van een target op basis van ongebonden concentraties. Aangezien het farmacologisch effect gerelateerd is aan de ongebonden concentratie, is bijgevolg het risico van onderdosering bij kritiek zieke kinderen mogelijks lager dan voorheen werd aangenomen. Tot slot werd een gevalideerde formule ontwikkeld voor het voorspellen van ongebonden vancomycine plasmaconcentraties op basis van totale concentraties.

Samengevat kunnen we stellen dat zowel fysiologische processen van groei en ontwikkeling als pathofysiologie in rekening moeten worden genomen bij het bestuderen van de PK van antibiotica bij kritiek zieke kinderen. Populatie modellering en simulatie methodes zijn een effectieve tool om antibiotica dosering te optimaliseren in deze kwetsbare populatie.

Vele vragen dienen nog te worden beantwoord. Ten eerste moeten de nieuw voorgestelde doseerregimes prospectief gevalideerd worden. Aanvullend onderzoek moet zich bovendien toespitsen op weefselpenetratie van ongebonden antibioticum, om dit vervolgens te linken met een microbiële respons en ontwikkeling van antibioticaresistentie over tijd. Tot slot dient de impact van dosisoptimalisatie te worden onderzocht op klinische uitkomst in een gerandomiseerd, gecontroleerd studie-opzet.

DANKWOORD

Dit werk was nooit mogelijk geweest zonder de hulp van anderen.

"A leader is one who knows the way, goes the way, and shows the way" - John C. Maxwell

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"The proper route to an understanding of the world is an examination of our errors about it" - Errol Morris

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"Never spend your money before you have earned it" - Thomas Jefferson

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"Individuals play the game, but teams beat the odds." - SEAL Team saying

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"Looking after a very sick child was the Olympics of parenting" - Chris Leave

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"Gun jezelf toch een dagje lummelen" - Bond zonder Naam

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"Family is not an important thing. It is everything." - Michael J. Fox

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Griet, schat, woorden zijn niet nodig: estava escrito nas estrelas ;-). Eu gusto muito de ti!

CURRICULUM VITAE

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2. QUALIFICATIONS

2001	Pharmacist, Ghent University, graduated with honours
2002	Hospital pharmacist, Ghent University, graduated with great honours
2016	Certified clinical pharmacologist, Dutch Society for Clinical Pharmacology and Biopharmacy, The Netherlands

3. JOB DESCRIPTION

2003-present accredited hospital pharmacist staff member at Ghent University Hospital

- medication distribution pharmacist (1/4 FTE)
- clinical pharmacist (1/4 FTE)
- hospital pharmacy researcher (1/2 FTE)

4. ADDITIONAL TRAINING

2004	Certificate "Clinical Pharmacy", Université Catholique de Louvain (exam passed) (15-day course)
2004-2005	Certificate "Antibioticabeleidsdeskundige" [expert in antibiotic policies] : interuniversity course Ghent University, Catholic University of Leuven, Antwerp University (5-day course)

2005-2016	Training initiated with an eye to obtaining a clinical pharmacologist degree (Dutch Society of Clinical Pharmacology and Biopharmacy; supervisor: prof. L. Van Bortel)
2006	Course on Good Clinical Practice + EU law on clinical trials, Ghent University Hospital (exam passed)
2007	Course on paediatric drug research, Rotterdam, the Netherlands (Erasmus Winter Programme)(4-day course)
2007	5th European summer school in clinical pharmacology and therapeutics, Ghent, Belgium (4-day course)
2008	Course biostatistics, Ghent, Belgium (5-day course)
2008	Pharmmed course: criteria for first dose in men, Brussels, Belgium (1/2-day course)
2009	Mylan management course for hospital pharmacists, Waasmunster (6-day course)
2009	Training Pharmonitor (dosing recommendations from PC-pharmacokinetic calculations), UZA, Antwerp (1/2-day course)
2009	Course on pharmacokinetics: introduction to NONMEM (population kinetics), Université Catholique de Louvain, Brussels (3-day course)
2009	Course on pharmacokinetics (Leiden-Amsterdam Centre For Drug Research), Oegstgeest, the Netherlands (4-day course)
2010	Postuniversity course on biostatistics (SPSS software), Ghent University (5-day course)
2010	Fisher/Shafer introductory course on non-linear mixed effects modelling (NONMEM), Bethesda, USA (4-day course)
2012	A statistical approach to PK-PD analysis in practice. Workshop of the European Society of Clinical Microbiology and Infectious Diseases, Athens, Greece (4-day course)
2012	Introductory course on population PK-PD modeling. Centre for Human Drug Research, Leiden, the Netherlands (3-day course)
2013	Global Research in Pediatrics (GRIP) roadshow- training course: medicines in children – what you need to know, organised by European Society of Developmental, Paediatric and Perinatal Pharmacology, Salzburg, Austria (1-day course)
2013	Postgraduate course on pharmacotherapy, 24th Annual Meeting of the European Society of Paediatric and Neonatal Intensive Care, Rotterdam, The Netherlands (1- day course)
2013	Introductory course on pharmacokinetics. Kinesis Pharma, Breda, the Netherlands (3- day course)

2014	Sheiner/Rowland advanced course in pharmacokinetics/pharmacodynamics, Silz-Maria, Switzerland (5-day course)
2014	Uppsala pharmacometrics summer school, Uppsala, Sweden (scientific project selected for presentation/workshop)
2014	PKPD modelling of continuous and categorical data in NONMEM, Alicante, Spain (3-day course)
2014	Pharmacometric statistics workshop (TACCA Training), Dublin, Ireland (3-day course)
2015	Global Research in Pediatrics (GRIP) roadshow: Basic concepts of pediatric pharmacokinetics/pharmacodynamics, organized by the GRIP network, Brussels, Belgium (1-day course)
2015	Global Research in Pediatrics (GRIP) roadshow - training course: Medicines in children, organized by the European Society of Developmental, Paediatric and Perinatal Pharmacology (ESDPP) and GRIP network, Belgrade, Serbia (1-day course)
2015	Comprehensive interuniversity course in pharmacokinetics : Fundamental principles and application to contemporary drug development. Leuven University, Leuven, Belgium (3-day course)
2015	Management course, organised by the Flemish Society for Hospital Pharmacists, Westerlo, Belgium (1 day course)
2016	Good Clinical Practice certificate, Ghent University Hospital (exam passed) (1/2 day course)

5. CLINICAL-SCIENTIFIC EXPERIENCE

2005	Clinical internship at the paediatric intensive care unit, Texas Children's Hospital, Houston, USA (2 months)
2007-2008	Clinical internship at the phase I research DRUG unit as part of the clinical pharmacology training (80hrs)
2008-2009	Clinical internship at the geriatric department and emergency department as part of the clinical pharmacology training (20hrs)
2014-2015	Scientific Internship in population PK-PD modelling at the division of Pharmacology, Leiden Academic Centre for Drug Research, Leiden, the Netherlands (supervisor: prof. dr. O. Della Pasqua) (10,5 months)

6. TEACHING EXPERIENCE

2008-2011	"Geneesmiddelendocumentatie en informatieverstrekking in het ziekenhuis: inleiding en workshop" [Drug information in the hospital : introduction and workshop] to hospital pharmacy students (person in charge: Prof. H. Robays; 2 hrs/academic year; Ghent University)
2008-2011	"Inleiding tot de klinische farmacie in het ziekenhuis" [Introduction to hospital clinical pharmacy services] to pharmacy students (person in charge: Prof. H. Robays; 2hrs/academic year; Ghent University)
2010-2012	"Medicatie bij kinderen: alles wat u wil weten over concentraties en toedieningswijze" [Paediatric drug therapy: everything you want to know about concentrations and drug administration] to bachelor nursing (2,5hrs/academic year; Vesalius Hogeschool, Ghent)
2012-present	"Pharmacogenetics and influence on pharmacokinetics and pharmacodynamics of medicinal products" (lecture for clinical pharmacology trainees, Heymans Institute for Clinical Pharmacology; 1,5h/2 yearly)
2012	"Rationeel, evidence-based geneesmiddelengebruik bij kinderen" [Rational, evidence-based drug therapy in children] (lecture as part of an interuniversitary course for pediatrician fellows; 2h/academic year)
2012-present	Tutor and examination of medical students in various clinical pharmacology topics (person in charge: Prof. dr. T. Christiaens; 6-15 hrs/academic year; Ghent University)
2012-present	"Klinische farmacokinetiek, therapeutic drug monitoring en farmacogenetica" [Clinical pharmacokinetics, therapeutic drug monitoring and pharmacogenetics] interuniversity course master in hospital pharmacy (coordinator: Prof. dr. I. Spriet; 4h/academic year)
2013-present	"Rationeel, evidence-based geneesmiddelengebruik bij kritiek zieke kinderen" [Rational, evidence-based drug therapy in children] (lecture as part of an Bachelor after Bachelor program in Intensive Care and Emergency Medicine, KAHO Sint-Lieven, Aalst, Belgium; 2h/academic year)
2013-present	"Rationeel, evidence-based geneesmiddelengebruik bij kinderen" [Rational, evidence-based drug therapy in children] (lecture as part of Postgraduate training in Pediatric Nursing, HOGent, Ghent, Belgium; 3h/academic year)

7. MASTER THESIS SUPERVISION

2007 "Medicatiefouten op een afdeling intensieve zorgen pediatrie" [Medication errors on a pediatric intensive care unit] – master after master in hospital pharmacy (daily supervisor) (promotor: prof. apr. H. Robays) (Leen Ronsyn)

2008 "Medicatiefouten op een afdeling medium-care pediatrie" [Medication errors on a pediatric medium-care unit] – master after master in hospital pharmacy (daily supervisor) (promotor: prof. apr. H. Robays) (Peter Declercq)

2008 "Beleid Totale Parenterale Nutritie op een afdeling intensieve zorgen pediatrie" [Evaluation of the Total Parenteral Nutrition prescription on a paediatric intensive care unit] - master after master in hospital pharmacy (daily co-supervisor with apr. J. De Cloet) (promotor: prof. apr. H. Robays) (Evelien Haegeman)

2009 "Medicatiefouten op een afdeling intensieve zorgen pediatrie na implementatie van een elektronisch voorschrift" [Medication errors on a paediatric intensive care unit after implementation of a computerized physician order entry system] – master after master in hospital pharmacy (daily supervisor) (promotor: prof. apr. H. Robays) (Kim Pieters)

2009 "Onderzoek naar de kwaliteit van therapeutic drug monitoring van antibiotica op de afdelingen pediatrie" [Evaluation of the quality of therapeutic drug monitoring of antibiotics in children] – master in pharmaceutical sciences (daily supervisor) (promotor: prof. apr. H. Robays) (Nele Goerlandt)

2009 "Medicatiefouten op een afdeling intensieve zorgen neonatologie" [Medication errors on a neonatal intensive care unit] – master after master in hospital pharmacy (daily supervisor) (promotor: prof. apr. H. Robays) (Maïté Vandaele)

2011-2012 "Dexmedetomidine als analgosedativum op intensieve zorgen" [Dexmedetomidine used as analgosedative drug on intensive care] – master in medical sciences (co-promotor) (promotor: prof. dr. P. De Paepe)(Sofie Herregods)

2012 "Effecten van hypoalbuminemie en hyperfiltratie op de dosering van antibiotica bij kritisch zieke kinderen" [Effects of hypoalbuminemia and hyperfiltration on antibiotic dosing in critically ill children] – master in pharmaceutical sciences (daily supervisor) (promotor: prof. apr. H. Robays) (Nicky Boeykens)

2013-2014 "Cefazolin disposition in children undergoing cardiac surgery with cardiopulmonary bypass"- master in medical sciences (co-promotor) (other promotors prof. dr. P. De Paepe – prof. dr. K. Francois) (Kim Vanderburght – Karen Jacobs)

2013-2014 "Hyperfiltration in the paediatric intensive care unit" - master in medical sciences (co-promotor) (other promotors prof. dr. P. De Paepe – dr. A. de Jaeger) (Benjamin Leenknegt – Karlien Roelandt)

2013-2014 "Dispositie van cefazoline en vancomycine bij kritisch zieke kinderen" [disposition of cefazolin and vancomycin in critically ill children] – master after master in hospital pharmacy (daily supervisor) (promotor: prof. apr. A. Somers) (Sarah Desmet)

2015-2017 "Dispositie van teicoplanine en meropenem bij kritiek zieke kinderen"[disposition of teicoplanin in critically ill children] – master after master in hospital pharmacy (daily supervisor) (promotor: prof. apr. A. Somers) (Margot Wollaert)

2016-2017 "Dispositie van ciprofloxacine en amikacine bij kritiek zieke kinderen" [disposition of ciprofloxacin and amikacin in critically ill children] – master in medical sciences (co-promotor) (promotor prof. dr. P. De Paepe) (Kamilya Jakipbayeva)

2016-2017 "Prospectieve validatie van model-based cefazoline doseringsregime bij kinderen die cardiochirurgie ondergaan met cardiopulmonaire bypass" [prospective validation of model-based cefazolin dosing regimen in children undergoing cardiac surgery with cardiopulmonary bypass] – master in medical sciences (co-promotor) (other promotors prof. dr. P. De Paepe – prof. dr. K. Francois) (Gerrit van Vliet)

8. SCIENTIFIC AWARDS – PROJECT FUNDING

2006 Amgen Scientific Award. Best Poster Award "Crushing the tablet? Development of guidelines and information database." Flemish Society for Hospital Pharmacists

2011-2016 Clinical Research Fund Ghent University Hospital
(funding personnel cost ½ FTE)

2013 Amgen Scientific Award. Best Poster Award "Penicillin dosing regimens are inadequate in critically ill children". Flemish Society for Hospital Pharmacists

2014-present	Funding for work package 'Altered drug disposition and effect in paediatric critical illness' within the interuniversity/university hospital research project funded by the Agency for Innovation by Science and Technology (IWT/SBO130033) (WP leader: Prof. dr. P. De Paepe)
2014	Travel grant, Research Foundation Flanders, Belgium (K218614N)
2014	Grant for long research stay, Research Foundation Flanders, Belgium (V4.144.14N)

9. SCIENTIFIC OUTPUT

(chronologically)

PUBLICATIONS

Bauters T., Nguyen B., Buyle F., Schelstraete P., De Cock P., de Jaeger A., Verrijckt A., Robays H. Clinical pharmacy and pediatrics: why focus on antibiotics? Pharm World Sci. 2006;28(1):3-5

De Smet J., Boussery K., De Cock P., De Paepe P., Remon J.P., Van Winckel M., Van Boxlaer J. A bio-analytical hydrophilic interaction LC-MS/MS method for the simultaneous quantification of omeprazole and lansoprazole in human plasma in support of a pharmacokinetic omeprazole study in children. J. Sep Sci. 2010;33(6-7):939-47 (Impact Factor: 2,746)

De Cock P., Claus B., Robays H. CPOE and prevention of prescribing errors. Hospital Pharmacy Europe 2010 (A2 publication)

Boussery K., De Smet J., De Cock P., Vande Velde S., Mehuys E., De Paepe P., Remon J-P., Van Bocxlaer J.F.P., Van Winckel M. Pharmacokinetics of two formulations of omeprazole administered through a gastrostomy tube in patients with severe neurodevelopmental problems. Br J Clin Pharmacol 2011; 72(6):990-996 (Impact Factor: 3.578)

De Cock P., Smits A., De Cock R., Cosaert K., Allegaert K. Geneesmiddelenformulering, klinische farmacologie en de zuigeling: over de relevantie van pediatrische formuleringen. Percentiel 2012;17:24-29. (A3 publication)

De Cock P., Standing JF, Barker CI, de Jaeger A, Dhont E, Carlier M, Verstraete AG, Delanghe JR, Robays H, De Paepe P. Augmented renal clearance implies a need for increased

amoxicillin-clavulanic acid dosing in critically ill children. Antimicrob Agents Chemother 2015;59(11):7027-35. (Impact Factor: 4.415)

De Cock P., Mulla H, Desmet S, De Somer F., Mcwhinney B., Ungerer J. , Moerman M., Commeyne S., Vande Walle J., Francois K., Van Hasselt J., De Paepe P. Population pharmacokinetics of cefazolin before, during and after cardiopulmonary bypass to optimize dosing regimens for children undergoing cardiac surgery. J Antimicrob Chemother 2016 doi: 10.1093/jac/dkw496 (Impact Factor: 4.919)

De Cock P., Desmet S., de Jaeger A., Biarent D., Dhont E., Herck I., Vens D., Colman S., Stove V., Commeyne S., Vande Walle J., De Paepe P. Impact of vancomycin protein binding on target attainment in critically ill children: back to the drawing board? J Antimicrob Chemother 2016 doi: 10.1093/jac/dkw495 (Impact Factor: 4.919)

Vandamme E., Lemoyne S., van der Gucht A., De Cock P., van de Voorde P. LAT gel for laceration repair in the emergency department: not only for children? Eur J Emerg Med. 2017;24(1):55-59. (Impact Factor: 2.026)

Nellis G., Metsvaht T., Varendi H., Toompere K., Lass J., Mesek I., Nunn A., Turner M., Lutsar I. Collaborators: Graham S., Duncan J., Shah U., Mulla H., Pandya H., McElnay J., Millership J., Yakkundi S., Rieutord A., Storme T., Vaconsin P., Resch B., De Cock P., Jekova N., IVore E., Sarafidis K., Vegso A., O'Callaghan N., Agostino R., Kyiluna D., Tameliene R., Kornelisse R., Bratlid D., Pereira A., Ognean M., Bajcetic M., Paro D., Valls E., Nydert P., Bucher H., Cordina M. Potentially harmful excipients in neonatal medicines : a pan-European observational study. Arch Dis Child 2015;100(7):694-9. (Impact Factor: 3.231)

De Cock P., van Dijkman S., de Jaeger A., Willems J., Carlier M., Verstraete A., Delanghe J., Robays H., Vande Walle J., Della Pasqua O., De Paepe P. Dose optimisation of piperacillin/tazobactam in critically ill children. J Antimicrob Chemother 2017 doi: 10.1093/jac/dkx093 (Impact Factor: 4.919)

De Cock P., Dhont E., De Paepe P. Challenges of appropriate antimicrobial dosing in ICU children. (submitted) (A3 publication)

van Dijkman S., De Cock P., Di Ioro V., Smets K., Decaluwe W., Allegaert K., Robays H., Vande Walle J., De Paepe P., Della Pasqua O. Optimal trial design in drug development programs for newborns: dexmedetomidine pharmacokinetics in mechanically ventilated neonates as an illustrative example. (submitted)

Abstracts – poster presentations

Vandenbroucke J., De Cock P., Robays H. Influence of a computerised program on the quality of prescribing and preparing cytotoxic drugs. Poster presentation, Annual Scientific Meeting, Flemish Society for Hospital Pharmacists (VZA), Brussels, Belgium 2003
Claus B., Somers A., Bauters T., Buyle F., De Cock P., Van Hooreweghe M., Robays H. Development and implementation of a database for systematic registration of interventions by clinical pharmacists. Poster presentation, euroDURG meeting, Ulster, United Kingdom 2005

De Cock P., Spriet I. ,Troosters F., De Vroe C., Verschueren E., Van Hee A., Herbots E., Glorieux I. , Coppens H. , Desloovere C., Goris E., Bollen M., Chapelle F. Crushing the tablet? Development of guidelines and information database. Poster presentation, European Society for Clinical Pharmacy conference, Vilnius, Lithuania 2006 (Winner Amgen Award 2006)

Claus B., Somers A., Bauters T., Buyle F., De Cock P., Robays H. Pharmaco-economic modelling of interventions by clinical pharmacists. Poster presentation, annual scientific meeting of the Flemish Society for Hospital Pharmacists (Klapperdag, VZA), Affligem, Belgium 2007

De Cock P., Van Winckel M., Verrijckt A., de Jaeger A., Van Hooreweghe M. , Robays H. Tacrolimus-clarithromycin interaction in a paediatric liver transplant with severe diarrhea : A case report. Poster presentation, yearly conference of the European Society for Clinical Pharmacy, Edinburgh, United Kingdom, 2007 (published in: Pharm World Sci. 2007, 31(1): 130)

De Cock P., Van Bortel L., Raes A. , Vandewalle J., de Jaeger A., Robays H. Safety of short-acting nifedipine in children : A literature review. Pharm World Sci. 2009, 31(1): 130. Poster presentation, yearly conference of the European Society for Clinical Pharmacy, Edinburgh, United Kingdom, 2007 (published in: Pharm World Sci. 2007, 31(1): 130)

De Cock P., Ronsyn L., de Jaeger A., Somers A., Robays H. Direct observation approach for detecting medication administration errors in a Belgian paediatric intensive care unit. Poster presentation, conference of the European Society for Developmental, Perinatal and Paediatric Pharmacology (ESDP), Rotterdam, The Netherlands 2008

De Cock P., Six R., Desmedt M., Haegeman E., Schouppe G., Robays H. Crushing the tablet : an observational study of administration practices in a teaching hospital. Poster

presentation, Annual Scientific Meeting of the Flemish Society for Hospital Pharmacists (VZA), Ghent, Belgium, 2008

Van Winckel M., De Smet J., Boussery K., De Cock P., Huyghebaert N., De Paepe P., Remon J-P., Van Boxlaer J. Pharmacokinetic study of omeprazole Multi-Unit Pellet System (Losec MUPS) versus extemporaneous bicarbonate formulation in patients with cerebral palsy. Poster presentation, European Society for Developmental, Perinatal and Pediatric Pharmacology conference, Rotterdam, the Netherlands, 2008

Van Winckel M., De Smet J., Boussery K., De Cock P., Huyghebaert N., De Paepe P., Remon J-P., Van Boxlaer J. Pharmacokinetic study of omeprazole Multi-Unit Pellet System (Losec MUPS) versus extemporaneous bicarbonate formulation in patients with cerebral palsy. Poster presentation, International Pediatric Pharmacy and Clinical Pharmacology Symposium, Orlando, USA, 2009

De Cock P., Declercq P., De Vriendt M., de Jaeger A., Robays H. Direct observation approach for detecting medication administration errors in a Belgian paediatric medium-care unit. Poster presentation, Belgian Society for Fundamental and Clinical Physiology and Pharmacology, Ghent, Belgium, 2009

De Cock P., Haegeman E., Goerlandt N., Vanhaesebrouck P., Stove V., Verstraete A., Schelstraete P., Robays H. Vancomycin dosing and monitoring in children: compliance with hospital guidelines in a Belgian teaching hospital. Poster presentation, International Union of Basic and Clinical Pharmacology Meeting (World Pharma Conference), Copenhagen, Denmark 2010 (published in Br J Clin Pharmacol 2013;76:835-836)

De Cock P. Haegeman E., Goerlandt N., Vanhaesebrouck P., Stove V., Verstraete A., Schelstraete P., Robays H. Quality analysis of aminoglycoside treatment in children at a Belgian university hospital. Poster presentation, International Union of Basic and Clinical Pharmacology Meeting (World Pharma Conference), Copenhagen Denmark 2010 (published in Br J Clin Pharmacol 2013;76:835-836)

van Dijkman S., Di Iorio V., De Cock P., Robays H, De Paepe P., Della-Pasqua O. Optimal design for a dexmedetomidine pharmacokinetic trial in mechanically ventilated neonates with single organ respiratory failure. Poster presentation, Population Approach Europe (PAGE) meeting, Athens, Greece 2011

De Cock P., Haegeman E., Goerlandt N., Schelstraete P., Robays H. A prospective qualitative analysis on administration and blood sampling of antibiotics in hospitalized chil-

dren. Poster presentation, European Society for Developmental, Perinatal and Pediatric Pharmacology conference, Oslo, Norway 2011

De Cock P., Haegeman E., Goerlandt N., Schelstraete P., Robays H. A pilot qualitative analysis of vancomycin dosing and monitoring in children at a Belgian teaching hospital. Poster presentation, Mededelingendag Dutch Society of Pharmacology and Biopharmacy, Utrecht, the Netherlands 2012

De Cock P., de Jaeger Annick, Dhont E., Carlier M., Stove V., Verstraete A., Robays H., De Paepe P. Penicillin dosing regimens are inadequate in critically ill children. Poster presentation, European Society for Developmental, Perinatal and Pediatric Pharmacology conference, Salzburg, Austria, 2013 (Amgen award winner 2013)

De Cock P., Boeykens N., de Jaeger A., Robays H., De Paepe P. Glomerular hyperfiltration in critically ill children: are we missing anything? Poster presentation, Yearly Conference of the Flemish Society for Hospital Pharmacists, Schelle, Belgium, 2014

De Cock P., van Dijkman S., de Jaeger A., De Paepe P., Della-Pasqua O. Population piperacillin pharmacokinetics in critically ill children. Poster presentation, Uppsala Pharmacometrics Summer School, Uppsala, Sweden, 2014

Desmet S., De Cock P., Coene L., de Jaeger A., Dhont E., Stove V., Robays H., De Paepe P. Vancomycin therapy in critically ill children: an urgent plea for pharmacokinetic-pharmacodynamic research integrating protein binding. Poster presentation, Yearly Conference of the Flemish Society for Hospital Pharmacists, Schelle, Belgium, 2015

De Cock P., Mulla H., Jacobs K., Vanderburght K., Desmet S., McWhinney B., Ungerer J., Desomer F., Moerman A., Robays H., Francois K., De Paepe P. Cefazolin pharmacokinetics before, during and after cardiopulmonary bypass in children undergoing cardiac surgery. Poster presentation, Population Approach Europe meeting (PAGE), Hersonissos, Crete, 2015

De Cock P., Standing J., Barker C., de Jaeger A., Dhont E, Carlier M, Verstraete A, Delanghe J, Robays H, De Paepe P. Augmented renal clearance implies a need for increased amoxicillin-clavulanic acid dosing in critically ill children. Poster presentation, Belgian Society for Infectious Diseases and Clinical Microbiology, La Hulpe, Belgium, 2015

De Cock P., Mulla H., Jacobs K., Vanderburght K., Desmet S., McWhinney B., Ungerer J., Desomer F., Moerman A., Robays H., Francois K., De Paepe P. Cefazolin pharmacokinet-

ics in children undergoing cardiac surgery with cardiopulmonary bypass. Poster presentation, Belgian Society for Infectious Diseases and Clinical Microbiology, La Hulpe, Belgium, 2015

Desmet S., De Cock P., de Jaeger A., Biarent D., Dhont E., Vens D., Stove V., Commeyne S., De Paepe P. The impact of vancomycin protein binding on target attainment in critically ill children. Poster presentation, Belgian Society for Infectious Diseases and Clinical Microbiology, La Hulpe, Belgium, 2015

De Cock P., van Dijkman S., de Jaeger A., Willems J., Carlier M., Verstraete A., Delanghe J., Robays H., Vande Walle J., Della Pasqua O., De Paepe P. Piperacillin-tazobactam pharmacokinetics in critically ill children: implications on adequate dosing regimens. Poster presentation, Population Approach Europe meeting (PAGE), Lisbon, Portugal, 2016

Desmet S., De Cock P., de Jaeger A., Biarent D., Dhont E., Vens D., Stove V., Commeyne S., De Paepe P. The impact of vancomycin protein binding on target attainment in critically ill children. Poster presentation, 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, the Netherlands, 2016

Desmet S., De Cock P., de Jaeger A., Biarent D., Dhont E., Vens D., Stove V., Commeyne S., De Paepe P. The impact of vancomycin protein binding on target attainment in critically ill children. Poster presentation, Student's Research Symposium Ghent University, Ghent, Belgium, 2016

De Cock P., Mulla H., Jacobs K., Vanderburght K., Desmet S., McWhinney B., Ungerer J., Desomer F., Moerman A., Robays H., Francois K., De Paepe P. Cefazolin pharmacokinetics before, during and after cardiopulmonary bypass in children undergoing cardiac surgery. Poster presentation, Student's Research Symposium Ghent University, Ghent, Belgium, 2016

Liu X., Smits A., Yu T., Wead S., De Cock P., Neely A., Dewandel I., Kagan R., Healy D., Allegaert K., Sherwin C. A comparative pharmacometric analysis of amikacin use in pediatric patients with burn injuries verses those with oncology conditions. Poster presentation, World Conference on Pharmacometrics, Brisbane, Australia, 2016

Capiau A., De Cock P., De Keulenaer J., Somers A. Intraperitoneal administration of antibiotics to treat endocarditis: a good choice? Poster presentation, yearly Conference of the Flemish Society for Hospital Pharmacists, Schelle, Belgium, 2017

Wollaert M., De Cock P., Desmet S., McWhinney B., de Jaeger A., Vande Walle J., De Paepe P. Teicoplanin therapy in critically ill children. A plea for more dedicated research on optimal pharmacokinetic-pharmacodynamic indices. Poster presentation, yearly Conference of the Flemish Society for Hospital Pharmacists, Schelle, Belgium, 2017

Liu X., Smits A., Wang Y., Wead S., Kagan R., Healy D., De Cock P., Allegaert K., Sherwin C. Dosing optimization of amikacin in pediatric patients with burn injuries and those with oncology conditions. Poster presentation, yearly Conference of the American Society for Clinical Pharmacology and Therapeutics, Washington, USA, 2017

De Cock P., Wollaert M., Desmet S., McWhinney B., de Jaeger A., Vande Walle J., De Paepe P. Observational study on target attainment and protein binding of teicoplanin in critically ill children. Poster presentation at the Mededelingendag Dutch Society of Pharmacology and Biopharmacy, Nijmegen, the Netherlands, 2017

Oral communications

Improving patient's safety with intrathecal or epidural drug administration. Yearly conference of the European Society of Clinical Pharmacy, Vilnius, Lithuania 2006

Penicillin dosing regimens are inadequate in critically ill children. Research Day, Ghent University, Ghent, Belgium 2014

"SAFEPEDRUG work package 5: "Altered drug disposition and effect in paediatric critical illness". International Advisory Board Meeting. Vilvoorde, Belgium 2014

"SAFEPEDRUG work package 5 : Altered drug disposition and effect in paediatric critical illness: progress." International Advisory Board Meeting. Ghent, Belgium 2015

Implications of augmented renal clearance on amoxicillin-clavulanic acid dosing in critically ill children. European Society for Developmental, Perinatal and Paediatric Pharmacology, Belgrade, Serbia, 2015

Vancomycin therapy in critically ill children: an urgent plea for pharmacokinetic-pharmacodynamic research integrating protein binding. International Conference on Therapeutic Drug Monitoring and Clinical Toxicology, Rotterdam, The Netherlands, 2015

Implications of augmented renal clearance on amoxicillin-clavulanic acid dosing in critically ill children. Research Day Ghent University, Ghent, Belgium 2016

Piperacillin-tazobactam pharmacokinetics-pharmacodynamics implies a need for higher dosing in critically ill children. 44th Annual Congress Belgian Society for Pediatrics, Brussels, Belgium, 2016

Piperacillin-tazobactam pharmacokinetics-pharmacodynamics : an urgent plea for revising dosing recommendations. 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, the Netherlands, 2016

Bypassing the impact of cardiopulmonary bypass on cefazolin disposition in children using a model-based dosing regimen. PKUK meeting, London, UK, 2016

Bypassing the impact of cardiopulmonary bypass on cefazolin disposition in children using a model-based dosing regimen. Student Research Symposium, Ghent University, Ghent, Belgium, 2017

Population pharmacokinetics of cefazolin before, during and after cardiopulmonary bypass in children undergoing cardiac surgery with cardiopulmonary bypass. Joint Conference of the International Society for Anti-infective Pharmacology and European Society of Clinical Microbiology and Infectious Diseases- PK/PD of anti-infectives study group. Vienna, Austria, 2017

An observational study on protein binding and target attainment of teicoplanin in critically ill children. Accepted for oral presentation at the 16th European Society for Developmental, Perinatal and Paediatric Pharmacology Congres, Leuven, Belgium 2017

Book chapters

Allegaert K., Christiaens D., De Cock P., Degomme P., de Jaeger A., Fontaine J ., Fonteyne C., Gillis P., Govaerts M ., Lebrun F., Mulder A., Van Gorp V., Verlooy J., Veyckemans F. Compendium on analgosedative drugs, in Handbook on 'Acute pain management in children' 2009, editor: Belgian Society of Paediatricians

Co-author Handbook : '10 years of clinical pharmacy services in the Ghent University Hospital', 2010, editor : H. Robays, Ghent University Hospital

Allegaert K., De Cock P., van den Anker J. Pediatric Formulations: a roadmap. Chapter 6: The clinical relevance of pediatric formulations. American Academy of Pharmaceutical Scientists; Advances in the Pharmaceutical Sciences Series, vol. 11. Bar-Shalom, Daniel, Rose, Klaus (Eds.) ISBN 978-1-4899-8011-3

De Cock P., Allegaert A., Linakis M., Sherwin C. Antibiotic pharmacokinetic/pharmacodynamic considerations in the critically ill. Chapter 13: Antibiotic dosing in paediatric critically ill patients. Springer publishing (in press)

10. OTHER

2005-present	Member of the Dutch Society of Clinical Pharmacology and Biopharmacy (the Netherlands)
2007-present	Member of the Pharmacotherapeutical Group and Expert Group on Paediatric Drug Prescribing (Ghent University Hospital)
2009-present	Member of the expert group on acute pain management in children from the Belgian Society of Paediatricians
2009-present	Scientific principal investigator NEODEX study (multicentric PK-PD study in 4 Belgian recruitment centres) (NCT01266252)
2011-present	Member of the multidisciplinary expert group on 'Acute Pain in Children' (Ghent University Hospital)
2012	National principal investigator for the European Study of Neonatal Excipient Exposure (ESNEE) (5 study sites)
2012-present	Scientific principal investigator ADIC trial (two-center PK/PD study)
2013-present	Member of the European Society for Developmental, Perinatal and Paediatric Pharmacology
2013-present	Member of the Antibiotic Policy Group Ghent University Hospital
2013-present	Invited reviewer for the scientific peer-reviewed journals : Archives of Disease in Childhood (Impact Factor: 3.231), International Journal of Clinical Pharmacy (Impact Factor: 1.339), Current Therapeutic Research, Journal of Antimicrobial Chemotherapy (Impact Factor: 4.919) and Clinical Pharmacokinetics (Impact Factor: 4.892)

2014-present	Member of the European Society of Clinical Microbiology and Infectious Diseases
2014-present	Member of the European Society of Clinical Microbiology and Infectious Diseases PK/PD of Anti-Infectives Study group
2014-present	Member of the European Society for Intensive Care Medicine
2014-present	Member of the European Society for Paediatric and Neonatal Intensive Care
2014-present	Team leader SAFEPEDRUG studynurse team (co-lead with dr. P. De Bruyne; project leader Prof. dr. J. Vande Walle)
2015-present	Scientific principal investigator PADECMO study (two-center PK/PD study)