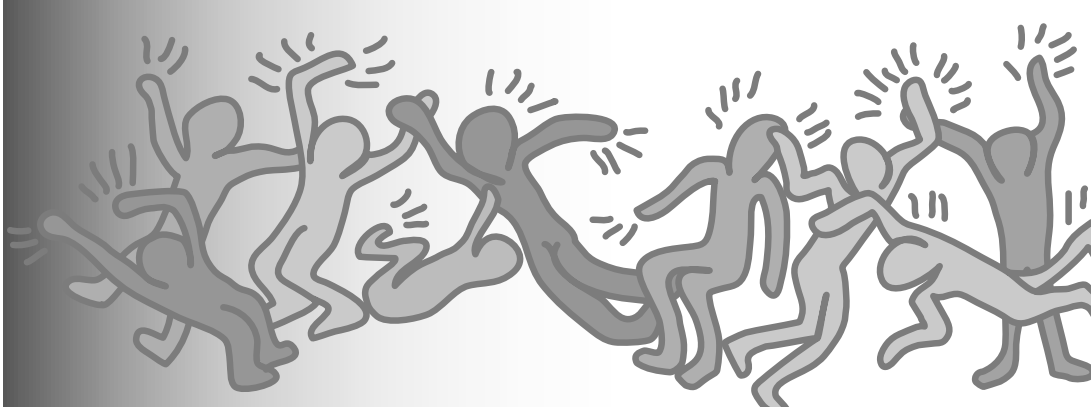
Section III

Developmental Changes in Renal Function (GFR and Tubular Processes) in Preterm and Term Neonates by Describing the Pharmacokinetics of Cefazolin



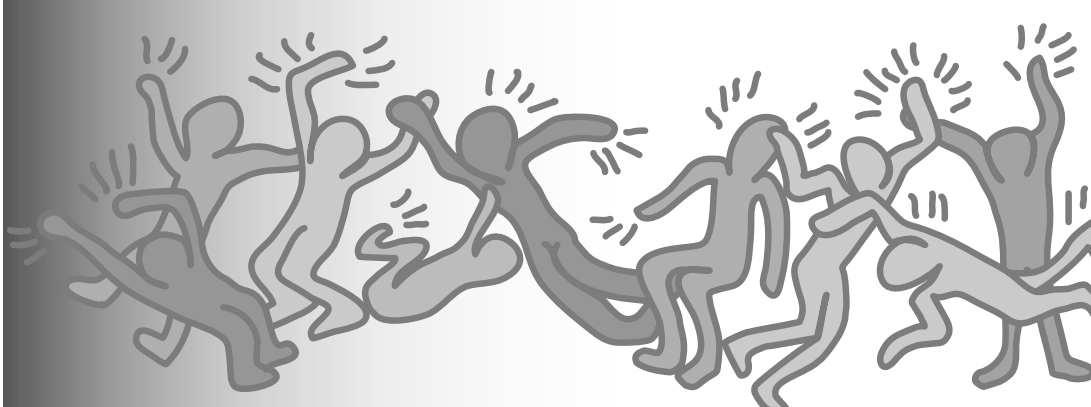
Chapter 6

Population Pharmacokinetic Modeling of Total and Unbound Cefazolin Plasma Concentrations as a Guide for Dosing in Preterm and Term Neonates

Roosmarijn F.W. De Cock[#], Anne Smits[#], Karel Allegaert, Jan de Hoon, Veroniek Saegeman, Meindert Danhof, Catherijne A.J. Knibbe

[#] Both authors contributed equally to the manuscript

J Antimicrob Chemother 2014 Feb 2 [Epub ahead of print}



Abstract

Objectives

Cefazolin is frequently administered for antimicrobial prophylaxis and treatment of infections. In neonates, pharmacokinetic observations are limited and dosing regimens variable. The aim of this study was to describe the pharmacokinetics of cefazolin in neonates based on total and unbound concentrations to optimize cefazolin dosing.

Methods

Thirty-six neonates [median birth bodyweight (bBW) 2720 (range 540-4200)g, current bodyweight (cBW) 2755 (830-4200)g, postnatal age (PNA) 9 (1-30) days] receiving intravenous cefazolin (50mg/kg/8h) were included. Based on 119 total and unbound plasma concentrations, a population pharmacokinetic analysis with a covariate analysis was performed. Monte Carlo simulations were performed aiming for unbound concentrations above a minimal inhibitory concentration of 8 mg/L (>60% of time) in all patients.

Results

A one-compartment pharmacokinetic model was developed in which total and unbound concentrations were linked by a maximal binding capacity B_{max} of 136 mg/L and a dissociation constant for cefazolin protein binding K_D of 46.5 mg/L. Current bodyweight was identified as covariate for volume of distribution (Vd), bBW and PNA for clearance (Cl) and albumin plasma concentration for B_{max}, explaining 50%, 58% and 41% of interindividual variability in Vd, Cl and B_{max}, respectively. Based on Monte Carlo simulations, a bodyweight and PNA adapted dosing regimen was proposed resulting in similar exposure across different weight and age groups.

Conclusions

A neonatal pharmacokinetic model taking into account total and unbound cefazolin concentrations with saturable plasma protein binding was identified. As current bodyweight and PNA were the most important covariates, these may be used for individualized dosing in neonates.

6.1. Introduction

Based on a European survey, 15% of antimicrobial use for surgical prophylaxis in children is covered by first generation cephalosporins [1]. In a United States point prevalence survey in pediatric (PICU) and neonatal intensive care unit (NICU) patients, cefazolin was used in respectively 17.6% and 1.2% of patients on the day of survey [2]. Indications for cefazolin administration in neonates are mainly prophylactic (72%), to a lesser extent therapeutic (17%) (e.g. coagulase-negative staphylococcal sepsis) [3] or empiric (11%) [2]. While the pharmacokinetics (PK) of cefazolin have been described in adults, information on cefazolin PK in early life is limited [4-6]. Cefazolin is highly bound to human serum albumin and this binding displays saturation [7-9]. Only the unbound cefazolin distributes to the extravascular compartments and undergoes renal elimination. Neonates have a proportionally large total body water volume, immature renal function and low albumin level [10-12]. This population specific physiology likely affects cefazolin disposition.

Efficacy of cefazolin relates to the time *unbound* cefazolin concentrations exceed the minimal inhibitory concentration (MIC) for a given pathogen ($T_{>MIC}$) [13]. In neonates, often regarded as vulnerable and even immunocompromised patients, effective cefazolin therapy requires at least 60% of $T_{>MIC}$ [14].

Up to now, neonatal cefazolin clearance values described in literature are based on total cefazolin concentrations only, necessitating a cefazolin PK analysis integrating both total and unbound drug concentrations in neonates. Moreover, currently used cefazolin dosing regimens for neonates are variable (Table S1) [15-21].

Therefore, the aim of this study was to describe the pharmacokinetics of cefazolin in preterm and term neonates on the basis of both total and unbound cefazolin concentrations. Based on the final pharmacokinetic model, Monte Carlo simulations were performed to illustrate exposure to cefazolin in (pre)term neonates following currently used dosing regimens. Subsequently, a model-based dosing regimen was developed for preterm and term neonates.

6.2. Methods

6.2.1. Ethics, study population and drug dosing

The patients included in this study are based on a previously published cohort of 39 neonates and young infants, all admitted to the Neonatal Intensive Care Unit of the University Hospitals Leuven Belgium [8]. The study was approved by the ethical board of the hospital, registered at ClinicalTrials.gov (NCT01295606) and parental written informed consent was obtained. Inclusion was feasible if cefazolin (Cefazolin Sandoz®, Sandoz, Vilvoorde, Belgium) was administered intravenously as routine surgical prophylaxis. At induction of surgery, a cefazolin 50 mg/kg dose was administered over 30 minutes. According to the local standard of care (depending on foreign body implantation or contamination risk of the procedure), additional 50 mg/kg cefazolin dose(s) could be administered every 8 hours up to a maximum of 48 hours. As in the present analysis only neonates with postnatal age (PNA) 1-30 days were included, three patients (PNA 48, 51 and 108 days) were excluded from the original dataset [8]. Clinical characteristics were extracted from the medical files. Albuminaemia (g/L), indirect serum bilirubin concentrations (mg/dL) and serum creatinine (mg/dL) registered in a time interval of 24 h before or after the first cefazolin administration were collected. Plasma free fatty acids concentrations were determined in samples at the end of the study. Clinical characteristics of the study population are presented in Table 1.

6.2.2. Blood sampling

Blood samples were collected in lithium-heparin tubes at fixed time points, i.e. at 0.5, 2, 4 and 8 h after the first cefazolin administration and subsequently at 8 h intervals prior to each scheduled cefazolin administration, to determine total and unbound cefazolin concentrations. However, the number of samples collected from each patient was limited since the predefined total volume of blood available for sampling per patient was maximized to 1 mL/kg bodyweight. Blood samples (0.6 mL/sample) were immediately centrifuged (5 minutes, 4500 rpm at 4 °C) and the resulting 0.3 mL plasma was stored at -20°C in two aliquots of 0.15 mL.

6.2.3. Drug assay

Total and unbound cefazolin concentrations were determined by High Performance Liquid Chromatography after solid-phase column extraction. The initial method was developed in our laboratory [22] and adapted for measurement of cefazolin in small

Table 1: Clinical characteristics of the patients included in the study. Data are presented as median (range) or incidence.

Patient characteristics	Median (range)
Number of patients	36
Number of samples	119
Birth bodyweight (g)	2720 (540-4200)
Current bodyweight (g)	2755 (830-4200)
Postnatal age (PNA, days)	9 (1-30)
Gestational age (weeks)	37 (24-40)
Postmenstrual age (PMA, weeks)	38 (25-41)
Albumin (g/L)	34.5 (28.2-43.7)
Creatinine (mg/dL)	0.46 (0.26-1.03)
Free fatty acids (mmol/L)	0.08 (0-0.84)
Indirect bilirubin (mg/dL)	2.91 (0.1-11.13)
Gender (male/female)	22 / 14

volume plasma samples [8]. The lower limit of quantification for cefazolin was 0.1 μg/mL, with a coefficient of variation lower than 20%. Intra-assay precision and accuracy averaged 3.9 and 5.5% respectively. Inter-assay precision and accuracy averaged 5.7 and 6.8%, respectively, which is in line with FDA analytical recommendations [23, 24].

6.2.4. Biochemical assays

Albumin, indirect bilirubin and creatinine (enzymatic) were quantified on Roche Modular P (Roche Diagnostics, Basel, Switzerland). Free fatty acids were determined with a kit from DiaSys (DiaSys, Diagnostic Systems, Holzheim, Germany).

6.2.5. Population pharmacokinetic analysis

Model development

The population pharmacokinetic analysis was performed using the non-linear mixed effect modeling software NONMEM version 6.2 (Globomax LLC, Hanover, MD, USA) using the first-order conditional estimation method with the interaction option (FOCE-I). Tools like S-Plus version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P Consultants BV, Leiden, The

Netherlands), PsN and R (version 2.10.1) were used to visualize and evaluate the model.

The model building process was performed in a stepwise manner: (i) choice of the structural model, (ii) choice of the statistical sub-model, (iii) choice of the covariate model, (iv) model evaluation. Different diagnostic tools were used to discriminate between the different models [25]. A decrease in objective function (OFV) of 3.9 points or more was considered statistically significant ($p<0.05$ based on X2 distribution, for nested models). Furthermore, the goodness-of-fit plots were evaluated. Finally the total number of parameters, visual improvement of individual plots, correlation matrix, confidence intervals of parameter estimates, ill-conditioning [26] and shrinkage [27] were assessed.

Structural and statistical sub-model

A one and two compartment pharmacokinetic model was fitted to both total and unbound cefazolin concentrations using NONMEM VI, subroutine ADVAN6, TOL=3. Unbound cefazolin concentrations were related to total cefazolin concentrations by the following equation, taking into account non-linear protein binding [28].

$$C_{unbound} = \frac{1}{2} \bullet (C_{total} - B_{max} - K_D) + \sqrt{(C_{total} - B_{max} - K_D)^2 + 4 \bullet K_D \bullet C_{total}}$$

(Equation 1)

In this equation $C_{unbound}$ represents the unbound cefazolin concentrations, C_{total} the total cefazolin concentrations, B_{max} the maximum protein binding and K_D the dissociation constant.

For the statistical sub-model, the inter-individual variability was assumed to follow a log-normal distribution. For the intra-individual variability and residual error, a proportional, additive and a combined error model were tested.

Covariate analysis

The following covariates were evaluated in the covariate analysis: birth bodyweight (bodyweight at day of birth, bBW, gram), current bodyweight (bodyweight at day of blood sampling, cBW, gram), postnatal age (PNA, days), gestational age (GA, weeks), postmenstrual age (PMA, weeks, combination of GA and PNA in weeks), al-

buminaemia (g/l), creatininaemia (mg/dL), free fatty acids (mmol/L), indirect bilirubin (mg/dL) and gender. Potential covariates were separately implemented into the model using a linear or power equation (equation 2):

$$P_i = P_p \bullet (\frac{Cov}{Cov_{Median}})^k \quad \text{(Equation 2)}$$

In this equation P_i represents the individual parameter estimate of the *ith* subject, P_p equals the population parameter estimate, Cov is the covariate and k is the exponent which was fixed to 1 for a linear function or was estimated for a power function. Covariates were considered statistically significant if the objective function decreased 7.8 points (p-value <0.005) or more. The covariate causing the largest reduction in objective function was chosen as a basis to sequentially explore the influence of additional covariates. The choice of the covariate models was further evaluated as discussed under Model development, whereby the results of the Model validation were also considered.

Model validation

The stability of the final pharmacokinetic model was evaluated by a bootstrap analysis, in which the model building dataset was resampled 1000 times, in S-plus, version 6.2.1. (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P Consultants BV, Leiden, The Netherlands). To evaluate the accuracy of the model the normalized prediction distribution error (NPDE) method was performed. To perform this analysis the dataset was simulated 1000 times after which each observed concentration was compare to the simulated concentrations using the NPDE package in R [29, 30].

6.2.6. Monte Carlo simulations

To evaluate $T_{>MIC}$, the Clinical and Laboratory Standards Institute (CLSI) 2012 [31] MIC interpretative criteria for susceptibility to cefazolin corresponding with the 5 bacterial species isolated most frequently from neonatal blood cultures from our department were used. Therefore, all positive blood culture results (n=137) from our unit, for the period January - October 2012, were retrospectively collected. Identification of bacterial isolates was done by use of MALDI Biotyper (Bruker Daltonics, Bremen, Germany). *Staphylococcus* species contributed for 94.4% of the top 5 isolates. Consequently, the CLSI MIC interpretative criterion for susceptibility to cefazolin of *Staphylococcus* species (8 mg/L) was used as target MIC (Table 2) [31].

As effective cefazolin therapy is reported to require at least 60% of $T_{>MIC}$ [15], the probability of attaining unbound cefazolin concentrations during 60% of the dosing interval [14] above 8 mg/L was evaluated on the basis of Monte Carlo simulations using the final pharmacokinetic model. These Monte Carlo simulations were performed in 1000 individuals to evaluate the exposure to cefazolin in (pre)term neonates following the currently used dosing regimen in this study and the dosing regimen proposed by the Dutch Children's Formulary [15]. The covariates identified in the final pharmacokinetic model were sampled from the original dataset taking into account their correlation. Albumin was randomly generated according to the observed distribution in these 36 neonates. For the simulations, cefazolin doses were administered over 30 minutes every 8 hours until 48 hours after the first dose. To evaluate the results of the Monte Carlo simulations, 4 different groups (Group 1: PNA ≤ 7 days, cBW ≤ 2000g, Group 2: PNA ≤ 7 days, cBW > 2000g, Group 3: PNA > 7 days, cBW ≤ 2000g, Group 4: PNA > 7 days, cBW > 2000g) were created. Based on these results, a new model-based dosing regimen was proposed.

Table 2: The 5 bacterial species isolated most frequently from neonatal blood cultures (n=137) in the Leuven neonatal intensive care unit for the period January 2012 until October 2012. Corresponding CLSI MIC values are reported.

Isolate	Contribution to all positive blood cultures (%)	Contribution to top-5 isolates (%)	CLSI MIC values (mg/L)		
			Susceptible	Intermediate	Resistant
1) S. epidermidis	51.82	65.74			
2) S. hominis	9.49	12.04			
3) S. aureus	6.57	8.33	≤ 8	16	≥ 32
4) S. capitis	6.57	8.33			
5) E. coli	4.38	5.56	≤ 2	4	≥ 8

S.: Staphylococcus, E.: Escherichia, CLSI: Clinical and Laboratory Standards Institute, MIC: Minimal Inhibitory Concentration.

6.3. Results

6.3.1. Patients

The pharmacokinetic analysis was based on 119 plasma concentrations of cefazolin obtained in 36 (pre)term neonates with PNA 1-30 days. Median total and unbound cefazolin plasma concentrations, were respectively 101.09 (range 17.44-404.22) mg/L and 41.15 (range 5.34-261.38) mg/L. Median unbound fraction was 0.40 (range 0.14-0.73). Clinical characteristics are presented in Table 1.

6.3.2. Population pharmacokinetic analysis

Structural and statistical sub-model

A one compartment model was selected as structural model because a two compartment model was not superior over a one compartment model. The final one compartment pharmacokinetic model, taking into account total and unbound

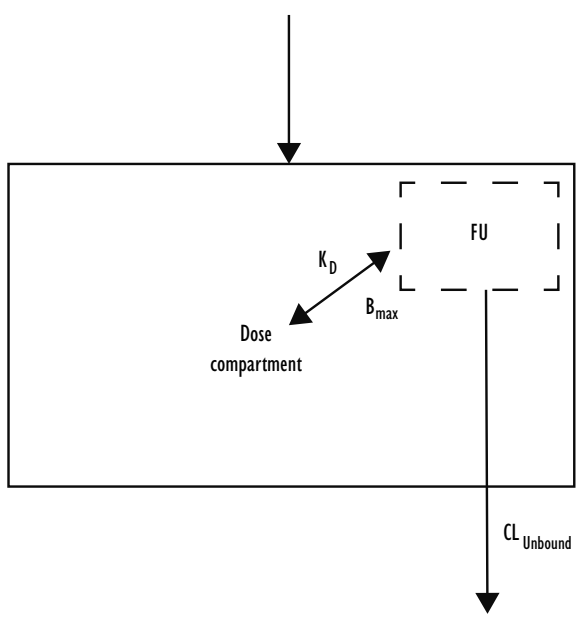


Figure 1: Schematic representation of the pharmacokinetic model using both total and unbound concentrations of cefazolin. K_D = Dissociation constant, B_{max} = Maximum protein binding, FU = unbound fraction of cefazolin, $CL_{unbound}$ = Clearance of unbound cefazolin.

cefazolin concentrations, was parameterized in terms of clearance, volume of distribution (Vd), maximum protein binding B_{max} and the dissociation constant K_D (Figure 1). By the determination of B_{max} and K_D, unbound cefazolin concentrations could be calculated from total concentrations (equation 1). Initially, a separate proportional error was estimated for total and unbound cefazolin concentrations. Since these errors were not significantly different ($p>0.05$), the model was simplified by estimating one proportional error for both total and unbound concentrations.

Table 3: Model-based population pharmacokinetic parameter estimates and the values obtained after the bootstrap analysis.

Parameter	Simple model without covariates	Final pharmacokinetic covariate model	Bootstrap final pharmacokinetic model
	Value (CV%)	Value (CV%)	Value (CV%)
Fixed effects			
CL (L/h) = CLp	0.229 (11.7)	-	-
CLp in CL = CLp x (bBW/median)^m x (1+(PNA/median) x n)	-	0.185 (12.8)	0.187 (13.3)
m	-	1.37 (16.4)	1.41 (17.3)
n	-	0.496 (38.5)	0.524 (44.5)
V (L) = Vp	0.812 (3.0)	-	-
Vp in V = Vp x (cBW/median)	-	0.863 (3.55)	0.860 (3.63)
B_{max} (mg/L) = $B_{max}p$	143 (14.5)	-	-
$B_{max}p$ in B_{max} = $B_{max}p$ x (ALB/median)	-	136 (12.6)	141 (14.5)
Kd (mg/L) =Kdp	53.2 (22.9)	46.5 (20.9)	49.5 (24.1)
Interindividual variability (ω^2)			
ω^2 CL	0.535 (33.6)	0.163 (35.1)	0.149 (38.0)
ω^2 V	0.14 (29.1)	0.0259 (38.6)	0.0258 (43.2)
ω^2 Bmax	0.102 (41.0)	0.0367 (54.0)	0.0368 (56.7)
Residual variability (σ^2)			
σ^2 (proportional)	0.0332 (22.1)	0.0351 (21.5)	0.0342 (22.5)

CL= clearance, CLp = population value for clearance for an individual with birth bodyweight of 2720g and postnatal age of 9 days, V = Volume of distribution, Vp = population value for volume of distribution for an individual with a current bodyweight of 2755g, B_{max} = maximum protein binding, $B_{max}p$ = population value for maximum protein concentration for an individual with an albumin concentration of 34.5 g/L, Kd = Dissociation constant of the drug, Kdp = population value of dissociation constant of the drug, bBW = birth bodyweight, cBW = current bodyweight, PNA = postnatal age, ALB = concentration of albumin

Covariate Model

Current bodyweight was found as most important covariate on Vd. Initially, current bodyweight was implemented on Vd using a power function with an estimated exponent of 0.94. However since the 95% confidence interval of this parameter included 1, a linear relationship between current bodyweight and Vd was used ($p>0.05$). Implementation of current bodyweight on Vd caused a significant drop in objective function (OFV) of 46 points ($p<0.005$). Although for clearance, PMA was identified as most important covariate, a combination of the covariates birth bodyweight and PNA was preferred over PMA alone. First of all, both analyses resulted in a comparable improvement of the model (i.e. same reduction in objective function (ΔOFV 32 points, $P< 0.005$). Secondly, the combination of birth bodyweight and PNA allows to make a distinction between the antenatal (birth bodyweight) and postnatal (PNA) maturation component of cefazolin clearance. Birth bodyweight was implemented on clearance using a power function with an estimated exponent of 1.37, while PNA was implemented using a linear function with an estimated slope of 0.496 (Table 3). The model was further improved (ΔOFV 12 points, $P< 0.005$) by introducing albumin on B_{max} using a linear function (Table 3).

The parameter estimates of the simple and the final pharmacokinetic model and the values obtained from the bootstrap analysis are provided in Table 3. In Figure 2, the observed *versus* predicted concentrations are plotted for the total and unbound concentrations showing that the model adequately describes the data. In Figure S1, the inter-individual variability in clearance, Vd and B_{max} is plotted against the relevant covariates for the simple and the final pharmacokinetic model. A significant part of the interindividual variability is explained (Figure S1). This is also reflected by the decrease in the estimates of the interindividual variability when comparing the simple and the final pharmacokinetic model which results in a decrease of 50% of the inter-individual variability on Vd, 58% on clearance and 41% on B_{max} (Table 3). In Figure 3 the observed and population predicted bound and unbound cefazolin concentrations are plotted from which B_{max} and the value for the unbound concentration for which the binding was half-maximal (K_D) can be derived. Variation in population predicted bound and unbound cefazolin concentrations are explained by differences in current bodyweight, birth bodyweight and PNA of the subjects (Figure 3).

The number of binding sites on the albumin molecule was derived from $B_{max,}$ which was corrected for molecular weight of albumin (67000 g/mol) and cefazolin (454.5 g/mol) (Equation 3), and the median albumin concentration (34.5 g/L) (Equation 4) and proved 0.6.

$$B_{max} = 0.136g/L \bullet (\frac{67000g/mol}{454.5g/mol}) = 20g/L \quad \text{(Equation 3)}$$

$$Number\ of\ binding\ sites = (\frac{20g/mol}{34.5g/mol}) = 0.6 \quad \text{(Equation 4)}$$

Model Validation

The results of the bootstrap analysis (Table 3) show that the median estimated values based on the resampled dataset are within 10% of the values obtained in the final model. The NPDE histograms are following the normal distribution, indicating the accuracy of the final pharmacokinetic model (Figure 2). Furthermore no trend was seen between the NPDE *versus* time or *versus* predicted concentrations (figures not shown). The number of ill-conditioning (74.6) was far below the critical number of 1000 indicating that the final pharmacokinetic model was not overparameterized. Finally, η-shrinkage expressed as a percentage was identified to be 9.8% for clearance, 21.2% for Vd and 30% for B_{max}.

6.3.3. Monte Carlo simulations

Concentration-time profiles following the currently used dosing regimen, the dosing regimen proposed by the Dutch Children's Formulary and the new model based-dosing regimen (Table 4) were predicted based on Monte Carlo simulations using the final pharmacokinetic model (Figure 4). In Figure S2, box plots illustrate the median and interquartile ranges (5% and 95%) of the individual predicted concentrations at 60% of the dosing interval after the first dose and after the fourth or sixth dose. This illustrates that less than 10% of the individual predicted concentrations at 60% of the dosing interval are below a MIC of 8 mg/L. Relatively high cefazolin peak concentrations are reached, particularly in neonates in group 1, 2 and 3 following the dosing regimen used in the current study and in group 3 following the dosing regimen proposed by the Dutch Children's Formulary (Figure 4, S2). Therefore, a new dosing regimen was advised based on the dosing regimen proposed by the Dutch Children's Formulary but including a lower dose for group 3 (Table 4). Using this dosing regimen, 0%, 1.2%, 0.7% and 1.0% of the individuals of group 1, 2, 3 and 4, respectively, would be exposed to concentrations below 8 mg/L at 60% of the dosing interval (Figure S2B).

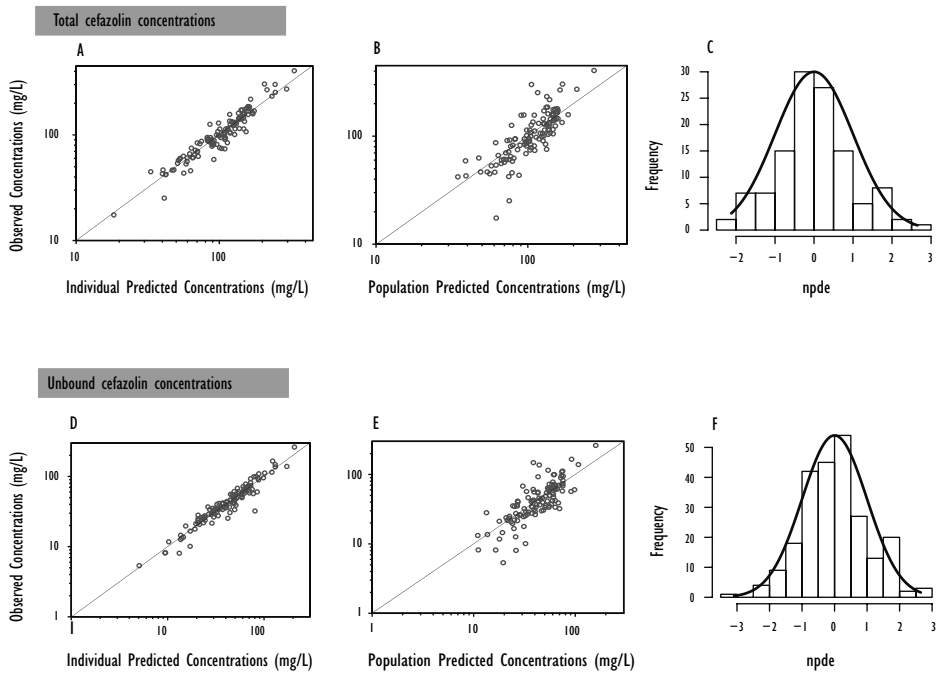


Figure 2: Observed *versus* individual predicted concentrations (a,d) and population predicted concentrations (b,e) for total (upper panels) and unbound (lower panels) cefazolin concentrations. The histograms show the distribution of the normalized prediction distribution error (NPDE) methods for total (c) and unbound (f) cefazolin concentrations.

6.4. Discussion

Neonatal cefazolin PK data are outdated since they are mainly based on total drug concentrations collected in a limited number of subjects. We aimed to characterize cefazolin pharmacokinetics and its covariates based on both total and unbound drug concentrations. In our study, the median cefazolin clearance value (coefficient of variation, %) for a neonate with a birth bodyweight of 2720 g and PNA 9 days was 0.185 (12.8) L/h (i.e. 0.068 L/kg/h). This is slightly higher than the earlier reported values of 0.53-1.10 mL/kg/min (i.e. 0.032-0.066 L/kg/h) in 11 neonates receiving 30 mg/kg cefazolin intravenously. Since only the unbound cefazolin is pharmacologically active and total drug concentrations only partially reflect unbound concentrations (Figure 3), we would like to emphasize that unbound concentrations need to be measured instead of using estimated unbound concentrations based on a fixed protein binding percentage. Especially in highly protein bound drugs this is of relevance.

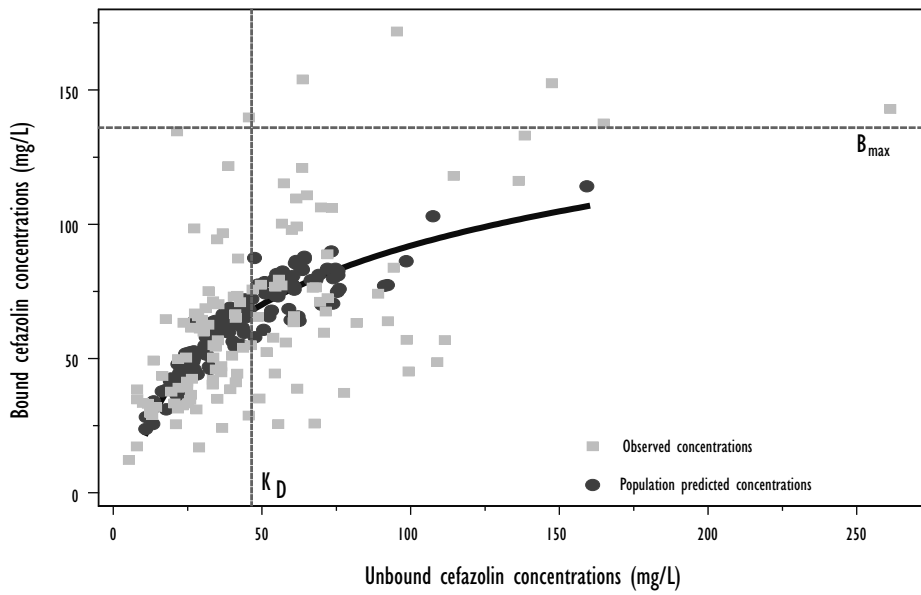


Figure 3: The relationship between the observed (*square*) and model-based predicted (*circle*) bound and unbound cefazolin concentrations (mg/L) in 36 (pre)term neonates. B_{max} (protein binding defined as the maximum estimated concentration bound to albumin) and K_D (dissociation constant defined as the unbound concentration which corresponds to 50% of the maximum binding capacity) are illustrated.

Postnatal age and birth bodyweight were the most important covariates of neonatal cefazolin clearance. This is in line with expectations, taking into account the elimination of cefazolin by renal route. Renal clearance displays maturation during early life and covariates birth bodyweight and PNA can hereby respectively reflect the prenatal and postnatal maturation [32]. Furthermore, age and bodyweight were earlier documented as clearance predictors of other beta-lactams in neonates [33-36]. We can only hypothesize on factors affecting the remaining unexplained cefazolin clearance variability within the neonatal population. Possibly, maturation of the renal tubular activity is a contributing factor. Also for other beta-lactams (e.g. amoxicillin, flucloxacillin) the presence of other elimination pathways, in addition to glomerular filtration rate (GFR), such as tubular secretion or non-renal clearance routes was suggested earlier [33, 37]. Since only the unbound drug can be eliminated and since compound specific clearance depends on compound specific protein binding, we hereby want to stress that the mean (± standard deviation) protein binding of flucloxacillin (74.5±3.1%) and in particular amoxicillin (11.7±2.7%) is lower compared to cefazolin [34, 38] Therefore, results of amoxicillin and flucloxacillin may not be directly applied to cefazolin.

Table 4: Dosing recommendations for cefazolin in preterm and term neonates according to dosing regimens used in the current study, the Dutch Children's Formulary and a new model-based proposed dosing regimen. For concentration-time profiles of these dosing regimens for neonates with different clinical characteristics we refer to Figure 4.

Guideline	PNA (days)	cBW (g)	Dose (mg/kg)	Interval (h)
Used in the current study	-	-	50	8
Dutch Children's Formulary	≤ 7 days	≤ 2000g	25	12
	≤ 7 days	> 2000g	50	12
	7-28 days		50	8
Proposed dosing regimen	≤ 7 days	≤ 2000g	25	12
	≤ 7 days	> 2000g	50	12
	7-28 days	≤ 2000g	25	8
	7-28 days	> 2000g	50	8

PNA = postnatal age, cBW = current bodyweight

The number of binding sites for cefazolin on the albumin molecule based on this analysis was calculated to be 0.6 (equation 3 and 4), which corresponds well with the number of binding sites for cefazolin on albumin previously found in literature (0.7) [7, 39, 40].

We documented relatively high cefazolin plasma concentrations based on a 50 mg/kg/8h cefazolin dosing regimen, administered to all study patients. This is likely due to the absence of any bodyweight and/or age- adapted dosing. Simulation of the dosing regimen proposed by the Dutch Children's Formulary resulted in lower cefazolin concentrations. However, based on Figure 4 and S2, the dose administered to neonates in group 3 when using the Dutch Children's Formulary, still needs further reduction. A new bodyweight- and age-based dosing regimen is suggested, derived from the dosing regimen proposed by the Dutch Children's Formulary, but with a dose reduction for group 3 in order to reach similar exposure in all four groups (Table 4). With this new model-based dosing regimen the target of 8 mg/L for 60% of the dosing interval was reached for >90% of the patients (i.e. 100%, 98.8%, 99.3% and 99% of the individuals of group 1, 2, 3 and 4, respectively).

When compared to the dosing regimen used in this study, a total daily dose reduction of 67%, 33% and 50% for patients in respectively group 1, 2 and 3 is proposed resulting in similar exposure in all groups. The proposed dosing regimen is hereby more in line with some of the recommendations presented in Table S1. As a consequence of cefazolin dose reduction, albumin binding places become available

Table S1: Overview of cefazolin dosing regimens for neonates and young infants. The dosing regimen used in the current study as well as the dosing regimen provided by the Dutch Children's Formulary and different handbooks are presented. Data are adapted to mg/kg/dose.

Reference	Age		Weight	Cefazolin dose and interval
NICU UZ Leuven				50 mg/kg/dose, q8h
Dutch Children's Formulary[15]	< 1 week PNA		< 2000 g	25 mg/kg/dose, q12h
			> 2000 g	50 mg/kg/dose, q12h
	1-4 weeks PNA			50 mg/kg/dose, q8h
Neonatal and pediatric pharmacology, Yaffe and Aranda 2011[16]	0-4 weeks PNA		< 1200 g	20 mg/kg/dose, q12h
	<1 week PNA		1200-2000 g	20 mg/kg/dose, q12h
			> 2000 g	20 mg/kg/dose, q12h
	≥ 1 week PNA		1200-2000 g	20 mg/kg/dose, q12h
			> 2000 g	20 mg/kg/dose, q8h
The Harriet Lane Handbook 2012[18]	≤ 1 week PNA			20 mg/kg/dose, q12h
	> 1 week PNA		≤ 2000 g	20 mg/kg/dose, q12h
			> 2000 g	20 mg/kg/dose, q8h
Neofax 2011[19]	≤ 29 weeks PMA,	0-4 weeks PNA		25 mg/kg/dose, q12h
		> 4 weeks PNA		25 mg/kg/dose, q8 h
	30-36 weeks PMA,	0-2 weeks PNA		25 mg/kg/dose, q12h
		> 2 weeks PNA		25 mg/kg/dose, q8 h
	37-44 weeks PMA,	0-1 week PNA		25 mg/kg/dose, q12h
		> 1 week PNA		25 mg/kg/dose, q8h
	≥ 45 weeks PMA, all			25 mg/kg/dose, q6h
Nelson's Textbook of Pediatrics 2007[17]	< 1 week PNA			20 mg/kg/dose, q12h
	> 1 week PNA			13-20 mg/kg/dose, q8h
The Sanford guide to antimicrobial therapy 2012-2013[20]	≤ 29 weeks PMA,	0-4 weeks PNA		50 mg/kg/dose, q12h
		> 4 weeks PNA		50 mg/kg/dose, q8h
	30-36 weeks PMA,	0-2 weeks PNA		50 mg/kg/dose, q12h
		> 2 weeks PNA		50 mg/kg/dose, q8h
	37-44 weeks PMA,	0-1 week PNA		50 mg/kg/dose, q12h
		> 1 week PNA		50 mg/kg/dose, q8h
	≥ 45 weeks PMA, all			50 mg/kg/dose, q6h
Redbook 2012[21]	≤ 1 week PNA		≤ 2000 g	25 mg/kg/dose, q12h
			> 2000 g	25 mg/kg/dose, q12h
	> 1-4 weeks PNA		≤ 2000 g	25 mg/kg/dose, q12h
			> 2000 g	25 mg/kg/dose, q8h

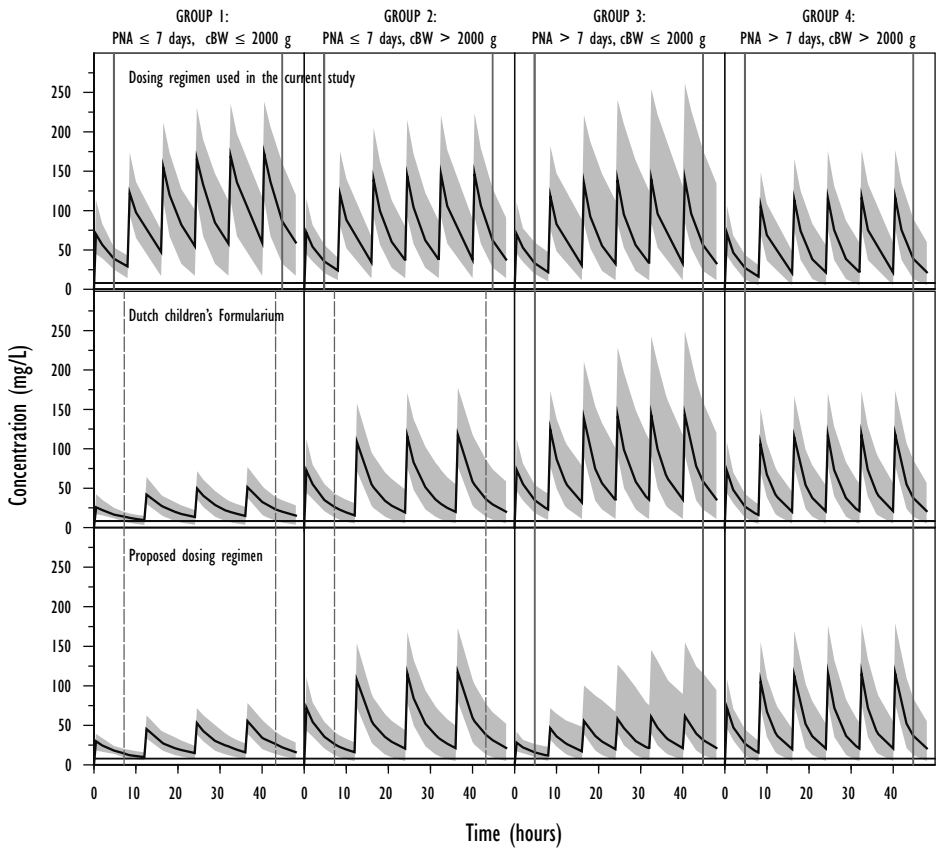


Figure 4: Concentration-time profiles based on 1000 Monte Carlo simulations using the final pharmacokinetic model following the dosing regimen used in this study (upper row), the dosing regimen proposed by the Dutch Children's Formulary (middle row) and the new model-based proposed dosing regimen (bottom row) in 4 different groups based on current bodyweight and postnatal age. The black line represents the median of the simulated profiles and the grey area represents the 90% confidence interval of the simulated values. The black horizontal line corresponds to the minimal inhibitory concentration of 8 mg/L. The grey vertical lines indicate the time at which 60% of the dosing interval is reached (4.8 and 44.8 hours) for a dosing interval of 8 hours. The grey vertical dotted lines indicate the time at which 60% of the dosing interval is reached (7.2 and 43.2 hours) for a dosing interval of 12 hours. PNA = Postnatal age, cBW = Current bodyweight.

for other endogenous (e.g. bilirubin) or exogenous compounds competing for the same albumin binding places. In neonates, frequently showing hyperbilirubinaemia (increased bilirubin production and decreased glucuronidation) and/or receiving multi drug therapies, this is a relevant and population specific advantage. Recent PK reports of other beta-lactam antibiotics commonly used in neonatal intensive care units also suggested dose adaptations compared to previously used regimens. To further illustrate this, a reduction in drug dose and interval for amoxicillin [33] and an increase of initial dose with subsequent dose reduction depending on the micro-biological isolate, for flucloxacillin [37] were suggested in neonates. This emphasizes the need for population specific PK studies in neonates. Since study methodologies can differ, a correct definition of the aimed PK target is required to achieve reliable dosing evaluations in this specific population [14, 41]. In general, we have to be aware that total daily dose reduction of an antimicrobial may lead to increased bacterial resistance and ineffectiveness [42]. Prospective validation of the new dosing regimen is therefore necessary, but this was not the intention of the present study.

The strength of our analysis is the measurement of both total and unbound cefazolin concentrations in a relevant neonatal cohort. Additionally, the final pharmacokinetic model can be used to optimize dosing regimens for other pathogens in different settings by changing the target MIC value and/or the $T_{>MIC}$. However, there are some limitations. First, the MIC values used were not prospectively determined. Secondly, the success of antibiotic prophylaxis depends not only on selection of the antimicrobial drug and drug dosing but also on the correct, well-timed drug administration and subsequent tissue distribution. Direct measurement of drug concentrations in the surgical site tissues [43, 44] may provide additional information to include in PK models, but is very challenging in this population [45].

We conclude that total and unbound cefazolin concentrations in neonates could be described by a one compartment PK model which includes saturable protein binding. Birth bodyweight and PNA were defined as the most important covariates contributing to cefazolin clearance variability. A new model-based neonatal cefazolin dosing regimen was proposed, however prospective validation of this dosing regimen is needed.

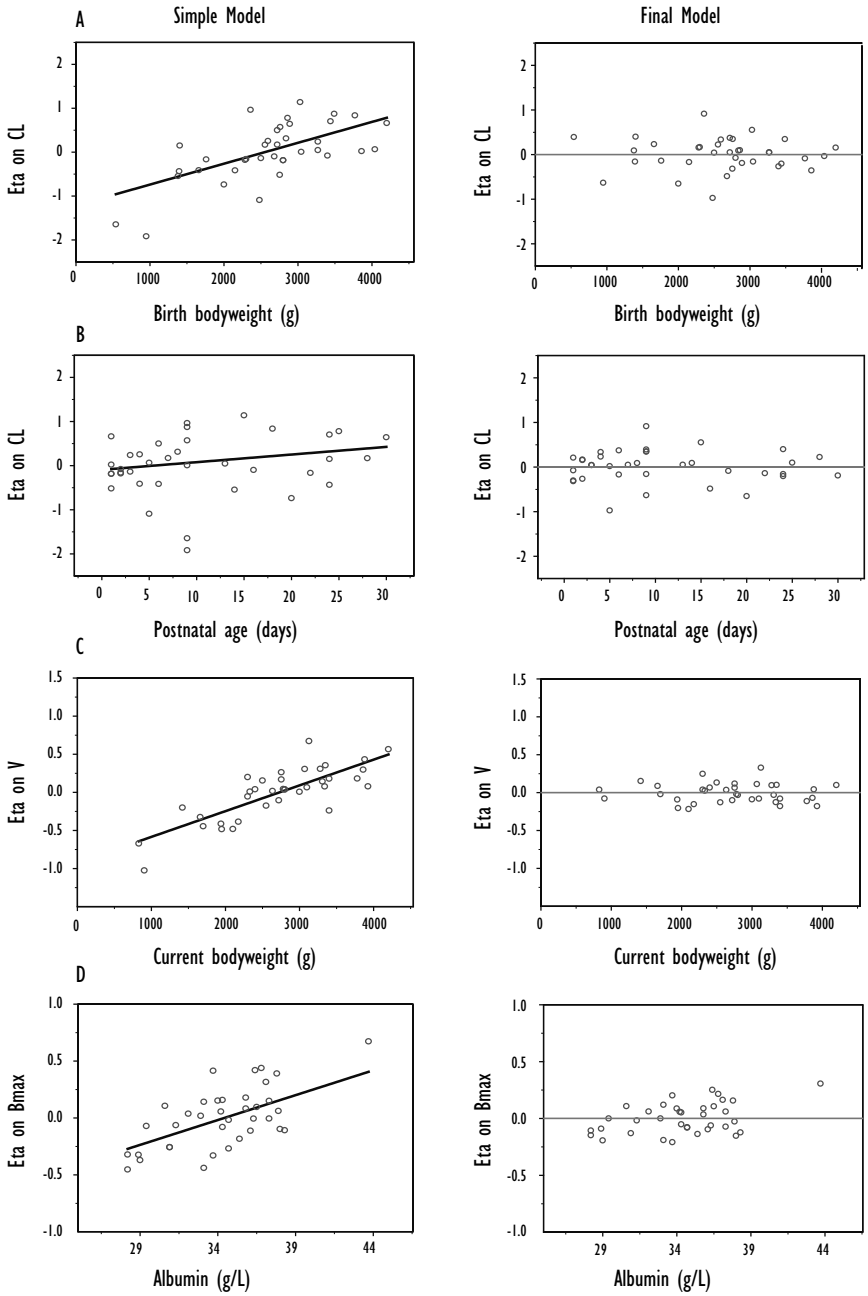


Figure SI: Interindividual variability (ETA) in a) clearance *versus* birth bodyweight, b) clearance (Cl) *versus* postnatal age, c) volume of distribution (V) *versus* current bodyweight, d) Maximum protein binding (B_{max}) *versus* albumin for the simple (left) and final covariate model (right).

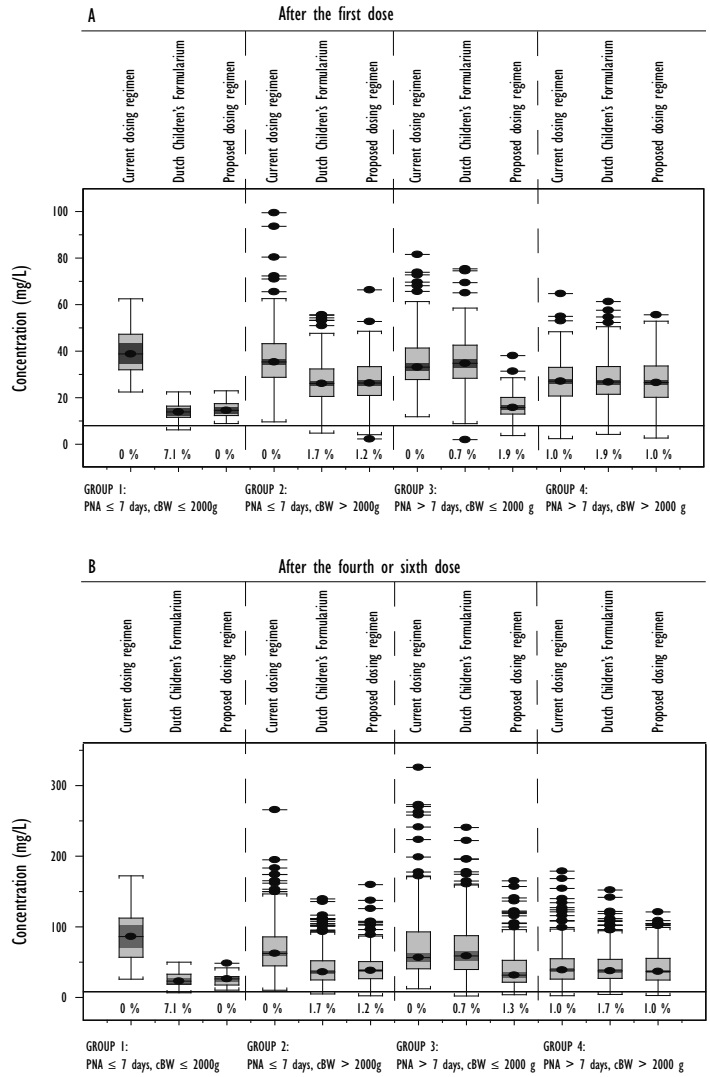


Figure S2: Individual predicted concentrations based on Monte Carlo simulations in 1000 individuals *versus* 4 different groups based on current bodyweight (cBW) and postnatal age (PNA). Plot A represents the individual predicted concentrations at 60% of the dosing interval after the first dose which corresponds to 4.8 or 7.2 hours after the first dose for a dosing interval of 8 or 12 hours respectively. Plot B represents the individual predicted concentrations at 60% of the dosing interval after 4 or 6 doses which corresponds to 44.8 or 43.2 hours based on a dosing interval of 8 or 12 hours, respectively. The black horizontal line corresponds to the minimal inhibitory concentration of 8 mg/L. For each group 3 boxplots are shown following the dosing regimen applied in this study (left), the dosing regimen suggested by the Dutch Children's Formulary (middle) and the new model-based proposed dosing regimen (right). Box plots illustrate median, interquartile range (5-95%) and outliers. The percentage of individuals with a concentration below 8 mg/L at 60% of the dosing interval is indicated for each dosing regimen per group.

Acknowledgements

This study was performed within the framework of Top Institute Pharma project number D2-104. Karel Allegaert was supported by the Fund for Scientific Research, Flanders (Belgium) (FWO Vlaanderen) by a Fundamental Clinical Investigatorship (1800214N). The authors also would like to thank LAP&P Consultants for their technical support with NONMEM and colleagues of the Center for Clinical Pharmacology (especially Rene Verbesselt and Aida Kulo) for the drug assay of the samples.

Funding

The work was partly supported by the Fund for Scientific Research, Flanders (Belgium) (FWO Vlaanderen) by a Fundamental Clinical Investigatorship (grant number 1800214N) to Karel Allegaert.

References

1. Amadeo B, Zarb P, Muller A *et al.* European Surveillance of Antibiotic Consumption (ESAC) point prevalence survey 2008: paediatric antimicrobial prescribing in 32 hospitals of 21 European countries. J Antimicrob Chemother 2010; 65: 2247-52.
2. Grohskopf LA, Huskins WC, Sinkowitz-Cochran RL *et al.* Use of antimicrobial agents in United States neonatal and pediatric intensive care patients. Pediatr Infect Dis J 2005; 24: 766-73.
3. Hemels MA, van den Hoogen A, Verboon-Maciolek MA *et al.* A seven-year survey of management of coagulase-negative staphylococcal sepsis in the neonatal intensive care unit: vancomycin may not be necessary as empiric therapy. Neonatology 2011; 100: 180-5.
4. Deguchi Y, Koshida R, Nakashima E *et al.* Interindividual changes in volume of distribution of cefazolin in newborn infants and its prediction based on physiological pharmacokinetic concepts. J Pharm Sci 1988; 77: 674-8.
5. Pacifici GM. Pharmacokinetics of cephalosporins in the neonate: a review. Clinics (Sao Paulo) 2011; 66: 1267-74.
6. Sakata Y. The pharmacokinetic studies of cephalothin, cefazolin and cefmetazole in the neonates and the premature babies. Kurume Med J 1980; 27: 275-98.
7. Decroix MO, Zini R, Chaumeil JC *et al.* Cefazolin serum protein binding and its inhibition by bilirubin, fatty acids and other drugs. Biochem Pharmacol 1988; 37: 2807-14.

8. Smits A, Kulo A, Verbesselt R *et al.* Cefazolin plasma protein binding and its covariates in neonates. Eur J Clin Microbiol Infect Dis 2012; 31: 3359-65.
9. Vella-Brincat JW, Begg EJ, Kirkpatrick CM *et al.* Protein binding of cefazolin is saturable in vivo both between and within patients. Br J Clin Pharmacol 2007; 63: 753-7.
10. Kearns GL, Abdel-Rahman SM, Alander SW *et al.* Developmental pharmacology--drug disposition, action, and therapy in infants and children. N Engl J Med 2003; 349: 1157-67.
11. De Cock RF, Allegaert K, Schreuder MF *et al.* Maturation of the glomerular filtration rate in neonates, as reflected by amikacin clearance. Clin Pharmacokinet 2012; 51: 105-17.
12. Notarianni LJ. Plasma protein binding of drugs in pregnancy and in neonates. Clin Pharmacokinet 1990; 18: 20-36.
13. Derendorf H, Lesko LJ, Chaikin P *et al.* Pharmacokinetic/pharmacodynamic modeling in drug research and development. J Clin Pharmacol 2000; 40: 1399-418.
14. de Hoog M, Mouton J, van den Anker J. New dosing strategies for antibacterial agents in the neonate. Semin Fetal Neonatal Med 2005; 10: 185-94.
15. Nederlands Kenniscentrum Farmacotherapie bij kinderen. Kinderformularium. http://www.kinderformularium.nl (27 Nov 2013, date last accessed).
16. Yaffe SJ, Aranda JV. Neonatal and Pediatric Pharmacology. Therapeutic principles in practice. Philadelphia: Wolters Kluwer, Lippincott Williams and Wilkins, 2011.
17. Kliegman RM, Behrman RE, Jenson HB *et al.* Nelson's Textbook of Pediatrics. Philadelphia: Saunders Elsevier, 2007.
18. Tschudy M, Arcara KM. The Harriet Lane Handbook. Philadelphia: Elsevier Mosby, 2012.
19. Young TE. Neofax. Montvale: Thomson Reuters, 2011.
20. Sanford JP. Sanford guide to antimicrobial therapy 2012-2013, 23th Edition of Belgian/Luxemburg version. Sperryville: J.C. Sanford, 2013.
21. Pickering LK. Red Book: 2012 Report of the Committee on Infectious Diseases. Elk Grove Village: American Academy of Pediatrics, 2012.
22. Allegaert K, van Mieghem T, Verbesselt R *et al.* Cefazolin plasma protein binding saturability during pregnancy. Methods Find Exp Clin Pharmacol 2009; 31: 25-8.
23. Shah VP, Midha KK, Dighe S. Analytical methods validation. J Pharm Sci 1992; 81: 309-12.
24. U.S.Department of Health and Human Services,FDA,Center for Drug Evaluation and Research, Center for Veterinary Medicine. Guidance for industry, Bioanalytical method Validation 2001. http://www.fda.gov/cder/guidance/index.htm (8 Dec 2013, date last accessed).
25. Krekels EH, van Hasselt JG, Tibboel D *et al.* Systematic evaluation of the descriptive and predictive performance of paediatric morphine population models. Pharm Res 2011; 28: 797-811.
26. Montgomery DC, Peck EA. Introduction to Linear Regression Analysis. In: New York: Wiley, 1982; 301-2.
27. Karlsson MO, Savic RM. Diagnosing model diagnostics. Clin Pharmacol Ther 2007; 82: 17-20.

28. Zandvliet AS, Copalu W, Schellens JH *et al.* Saturable binding of indisulam to plasma proteins and distribution to human erythrocytes. Drug Metab Dispos 2006; 34: 1041-6.
29. Brendel K, Comets E, Laffont C *et al.* Metrics for external model evaluation with an application to the population pharmacokinetics of gliclazide. Pharm Res 2006; 23: 2036-49.
30. Comets E, Brendel K, Mentre F. Computing normalised prediction distribution errors to evaluate nonlinear mixed-effect models: the npde add-on package for R. Comput Methods Programs Biomed 2008; 90: 154-66.
31. Clinical and Laboratory Satndards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility testing; Twenty-Second Informational Supplement. M100-S22. CLSI, Wayne, PA, USA, 2012; 32: 74.
32. Smits A, Annaert P, Allegaert K. Drug disposition and clinical practice in neonates: Cross talk between developmental physiology and pharmacology. Int J Pharm 2013; 452: 8-13.
33. Pullen J, Stolk LM, Nieman FH *et al.* Population pharmacokinetics and dosing of amoxicillin in (pre)term neonates. Ther Drug Monit 2006; 28: 226-31.
34. Pullen J, Driessen M, Stolk LM *et al.* Amoxicillin pharmacokinetics in (preterm) infants aged 10 to 52 days: effect of postnatal age. Ther Drug Monit 2007; 29: 376-80.
35. Charles BG, Preechagoon Y, Lee TC *et al.* Population pharmacokinetics of intravenous amoxicillin in very low birth weight infants. J Pharm Sci 1997; 86: 1288-92.
36. Huisman-de Boer JJ, van den Anker JN, Vogel M *et al.* Amoxicillin pharmacokinetics in preterm infants with gestational ages of less than 32 weeks. Antimicrob Agents Chemother 1995; 39: 431-4.
37. Pullen J, de Rozario L, Stolk LM *et al.* Population pharmacokinetics and dosing of flucloxacillin in preterm and term neonates. Ther Drug Monit 2006; 28: 351-8.
38. Pullen J, Stolk LM, Degraeuwe PL *et al.* Protein binding of flucloxacillin in neonates. Ther Drug Monit 2007; 29: 279-83.
39. Briand C, Sarrazin M, Peyrot V *et al.* Study of the interaction between human serum albumin and some cephalosporins. Mol Pharmacol 1982; 21: 92-9.
40. Tsuji A, Nishide K, Minami H *et al.* Physiologically based pharmacokinetic model for cefazolin in rabbits and its preliminary extrapolation to man. Drug Metab Dispos 1985; 13: 729-39.
41. Van den Anker JN. Population pharmacokinetics and dosing of amoxicillin in (pre)term neonates. Ther Drug Monit 2006; 28: 816-7.
42. Bradley JS, Garonzik SM, Forrest A *et al.* Pharmacokinetics, pharmacodynamics, and Monte Carlo simulation: selecting the best antimicrobial dose to treat an infection. Pediatr Infect Dis J 2010; 29: 1043-6.
43. Adembri C, Ristori R, Chelazzi C *et al.* Cefazolin bolus and continuous administration for elective cardiac surgery: improved pharmacokinetic and pharmacodynamic parameters. J Thorac Cardiovasc Surg 2010; 140: 471-5.
44. Pevzner L, Swank M, Krepel C *et al.* Effects of maternal obesity on tissue concentrations of

prophylactic cefazolin during cesarean delivery. Obstet Gynecol 2011; 117: 877-82.

45. Schmidt S, Barbour A, Sahre M *et al*. PK/PD: new insights for antibacterial and antiviral applications. Curr Opin Pharmacol 2008; 8: 549-56.