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CLINICAL PHARMACOLOGY IN NEONATES: TOWARDS IMPROVED PREDICTABILITY

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Introduction

Clinical pharmacology in neonates: Towards improved predictability

CHAPTER 1

Introduction and research aims

This chapter is in part based on

Perinatal pharmacology: Applications for neonatal neurology. *Eur J Paediatr Neurol* 2011; 15: 478-86

Drug disposition and clinical practice in neonates: Cross talk between developmental physiology and pharmacology. *Int J Pharm* 2013; 452: 8-13

Introduction

General concepts of clinical pharmacology

In clinical care, drugs are administered to patients with the intention to achieve a therapeutic effect, preferably without disproportional adverse effects. Clinical pharmacology aims to predict these drug-specific (side)effects based on pharmacokinetics (PK, concentration/time relationship) and pharmacodynamics (PD, concentration/effect relationship). One hereby takes into account characteristics of both the drug and the patient. The pharmacokinetic processes generally considered are drug *absorption*, *distribution*, *metabolism* and *excretion*. Although not exclusive, drug metabolism is mainly hepatic and excretion primarily renal (glomerular filtration and/or tubular secretion). Pharmacodynamics in part depends on the number, affinity and type of drug receptors/targets as well as post-receptor effects, but also outcome definitions and reference values used to interpret pharmacodynamic parameters [1,2]. These general principles of clinical pharmacology also apply to neonates, but their specific characteristics require not only a more integrated, but rather challenging approach. This is because safe and effective pharmacotherapy in neonates likely cannot be based on simple linear extrapolation of adult concepts or adult drug doses based on weight but needs integration of the impact of maturational aspects on PK and PD (i.e. developmental pharmacology). Interestingly, maturational (often non-linear) changes in humans are most prominent during the first year of life, more specifically during the neonatal period. To further illustrate this, body weight doubles in the first 3-4 months of life and increases 3 times in the first year of life. This is accompanied by an extensive increase in growth velocity, most pronounced in the last trimester of pregnancy and the first trimester of postnatal life [3]. Besides clinical characteristics (age, weight) reflecting the rapid and dynamic character of growth and development in early life, also co-medication and comorbidity contribute to the extensive inter-individual variability observed in neonatal PK and PD.

Developmental pharmacokinetics: Intriguing variability in early life

Pharmacokinetics (ADME) describes the processes involved when a drug is administered to the body, often cited as '*what the body does to the drug'*. In the next paragraphs, we further focus on the different PK aspects and provide examples of relevance for the clinician taking care of newborns.

Absorption

In (pre)term neonates admitted to a neonatal intensive care unit (NICU), many compounds are administered by intravenous route. However, in case of extravascular administration, the process of *absorption* (from the site of administration to the blood compartment) reflects and quantifies the ability of a specific drug formulation to overcome or cross barriers (e.g. physical, chemical, biologic) to subsequently appear in the systemic circulation. Developmental changes in intestinal motility, gastric emptying, gastric pH, biliary function and absorptive surfaces of organ(systems) involved in drug absorption (e.g. gastro-intestinal tract, lung, skin) can influence the absorption, and consequently the bioavailability of a drug [1]. Maturational differences in the activity of intestinal drug metabolizing enzymes or transporters further contribute to variability in bioavailability. Albani and Wernicke described that oral phenytoin therapy in infants required unexpectedly high doses (18 mg/kg body weight) to achieve and maintain therapeutic serum concentrations, due to impaired bioavailability, and that the absorption rate and extent is age-dependent [4].

In neonates, the absorption rate following oral administration is generally slower because of delayed gastric emptying. This has consequences for the time needed to achieve peak plasma concentrations. Compared to infants or children this peak will therefore often be delayed [1,5]. The clinical relevance subsequently depends on the compound evaluated, and the effect aimed for. Following oral administration of an analgesic drug, the peak concentration and the maximal analgesic effect may be obtained later in a neonate compared to the adult. In contrast, in a setting of treatment for neonatal opioid withdrawal syndrome, delayed and prolonged absorption may contribute to the effectiveness of opioid weaning.

A specific issue in neonates is the unanticipated higher absorption following (unintended) cutaneous application due to a proportionally higher body surface area (m^2/kg) and a more permeable skin [6]. Application of iodide containing disinfectant potentially results in acquired hypothyroidism during a time interval where the subsequent effects (e.g. neurocognitive) of transient hypothyroidism are more pronounced (maturational PD). Topical use of local anesthetics as (lipophilic) ointments to alleviate pain will potentially result in methemoglobinaemia in neonates because of the higher systemic absorption and the reduced capacity to metabolize these compounds (i.e. deficiency in the methemoglobin reductase enzyme). These observations reinforce the relevance to consider systemic exposure following cutaneous application in neonates [6].

Distribution

Distribution refers to the shift of compounds from the systemic circulation to various (deep) body compartments, tissues or cells. Together with clearance (Cl), the distribution volume (V_d) predicts the concentration/time profile while both are reflected in the terminal elimination half life ($t_{1/2}$, h) of a compound. Since both Cl and V_d values will appear throughout different chapters in this thesis, we feel it appropriate to define both concepts in this general introduction section. Clearance of a drug, expressed in L/h or a weight-corrected derivative (e.g. mL/kg/h) refers to the rate of net removal of a compound and is either based on metabolism or excretion (cfr. infra). Volume of distribution, usually expressed in L or L/kg, is a mathematical or 'apparent' volume into which an administered amount of drug would be dispersed if the concentrations throughout this volume were equal to that in the serum. As mentioned earlier, the terminal elimination half-life reflects both Cl and V_d. By definition, terminal elimination half-life is the time needed to have a decrease of 50% compared to the initial concentration [7]. In case of first-order elimination, $t_{1/2} = 0.693 \times V_d/Cl$. This explains why $t_{1/2}$ of aminoglycosides in neonates, known to be patients with a decreased clearance, will further prolong in conditions resulting in an increase of V_d, like during sepsis [8].

The pattern of drug distribution will in part depend on patient-specific (e.g. body composition, systemic and regional blood flow, membrane permeability) and compound-specific (e.g. lipophilic or hydrophilic drug, molecular size, ionization, extend of protein binding) characteristics. Variability in organ perfusion and permeability of membranes (e.g. increase in capillary leak) are often associated with disease states and will further influence drug distribution [1]. We hereby would like to highlight two important covariates of distribution that need to be taken into account in neonates, i.e. body composition and drug binding capacity related to circulating plasma proteins [9]. Changes in body composition are most prominent in neonates: the total body water content (/kg) (80-85%) is markedly higher compared to values at the end of infancy (60-65%). Subsequently, the total body water content stabilizes throughout childhood. For the extracellular water, a similar trend is seen, starting at 40% and decreasing to about 25-30% at the end of infancy. The body fat content (/kg) at birth is low (10-15%) and increases to 20-25% at the end of infancy, with a subsequent decrease back to 10-15% until adolescence [5]. Consequently, water soluble and lipophilic drugs will result – when administered in a mg/kg approach – in lower and higher plasma concentrations during distribution, respectively. These findings might also be of clinical relevance. The peak plasma

concentrations of aminoglycosides, water soluble antibiotics frequently administered to neonates, will not only be delayed, but also blunted compared to older children or adults, when a similar mg/kg dose is applied. Therefore, this maturational aspect needs to be taken into account in neonatal aminoglycoside dosing regimens. A similar (/kg) dose of propofol will result in a higher peak concentration in a newborn compared to older children or adults, because of the lower body fat content [10]. Together with a reduced clearance, this can contribute to faster and more pronounced accumulation.

Plasma protein binding of drugs is typically reversible, with drug-protein binding associations generally due to hydrophobic forces or ionic interactions [11]. The amount and type of circulating plasma proteins (e.g. albumin, alfa 1-acid glycoprotein) influences drug disposition (PK) as well as drug action (PD), since only the unbound drug can be distributed throughout the body and only the unbound drug can have a pharmacological effect [12]. Neonates often display hypoalbuminemia in the first days of life, resulting in a reduction of total albumin binding capacity available [11]. Furthermore, competitive binding at the albumin sites between endogenous (e.g. bilirubin, free fatty acids) and exogenous compounds (e.g. ibuprofen, diazepam, phenytoin, propofol) can result in an increase in the free fraction of an exogenous compound or an increase in free bilirubin, potentially resulting in kernicterus despite perceived 'safe' bilirubin plasma concentrations. Additionally the presence of fetal albumin, characterized by a reduced binding affinity for weak acids has been suggested [1]. Alfa-1 acid glycoprotein, an acute phase protein, is lower in neonates and will increase following surgical trauma. Alfa-1 acid glycoprotein binds local anesthetics like bupivacaine. An increased free fraction may result in seizures during continuous epidural infusion in infants, resulting in the recommendation not to continue bupivacaine beyond 24h in infants [9].

Clinical implications of alterations in drug protein binding are most relevant for drugs which are highly protein bound, have a narrow therapeutic index and of which dose is not titrated based on effect [13]. In literature, drug protein binding can be expressed as *unbound* drug fraction or *bound* drug fraction. The unbound drug fraction equals the unbound plasma concentration of the drug divided by the total plasma concentration of the drug. The bound drug fraction equals the bound concentration divided by the total concentration and is also calculated by 1- the unbound fraction [11].

Metabolism

Metabolism or biotransformation, are enzymatic reactions frequently resulting in the formation of a more hydrophilic derivative of a given endogenous substance (e.g. bilirubin) or exogenous compound (e.g. paracetamol, propofol), which can subsequently be excreted easier. Besides elimination of the parent compound, drug metabolism can also result in active metabolites with specific pharmacodynamic (side)effects (e.g. codeine or tramadol metabolites and analgesia). The most important site for drug metabolism, although not exclusive, is the liver. Drug metabolism mechanisms are classified into phase I and phase II reactions. The former primarily involves non-synthetic modification (e.g. oxidation, reduction, hydrolysis) of the drug while the latter consists of conjugation with another usually more water-soluble moiety (e.g. glucuronidation, sulphation, acetylation) [14]. The most important group of enzymes involved in phase I processes are the cytochrome P450 (CYP) iso-enzymes with CYP3A4 representing 30-40% of the total CYP content. CYP3A4 activity is very low before birth but increases rapidly thereafter. Between the age of 6-12 months, 50% of adult levels are reached. During infancy, CYP3A4 activity appears to be even slightly higher than that of adults [1,15]. The largest group of enzymes involved in phase II reactions are uridin diphosphate glucuronosyltransferase (UGT) iso-enzymes responsible for glucuronidation. Data on ontogeny of UGT activity are scant and mainly based on studies with morphine or paracetamol as probes [16,17].

In general, drug metabolism is low in neonates and phenotypic activity of drug metabolising enzymes is even considered as a major contributor to the overall pharmacokinetic differences between neonates, children and adults. However, this does not exclude extensive interindividual variability within the neonatal population. The majority of drug metabolism is performed by the 23 iso-enzymes belonging to 1 of 3 groups distinguished by Hines *et al* [18], based on extensive *in vitro* research. Although somewhat simplified, ontogeny of drug metabolizing enzymes can be classified as: (1) group 1 [enzymes expressed at their highest levels early in fetal life (first trimester) and subsequently remaining at high concentrations, or decreasing during gestation, but silenced or only low expression up to 2 years after birth (e.g. CYP 3A7)], (2) group 2 [enzymes expressed at constant levels throughout gestation with only minimal postnatal changes (e.g. CYP3A5 or CYP2C19)] and finally, (3) group 3 [enzymes not (or only at low levels) expressed during fetal life (second or third trimester), but display a perinatal onset or significant increase in expression within the first 1-2 years after birth (e.g. CYP3A4, CYP2D6)] [18]. *In vivo* research not only allows to mirror these maturational patterns

described in *in vitro* experiments, but also allows to further investigate the impact of disease characteristics: besides age-related maturation (both postmenstrual and postnatal age), it is documented that phenotypic enzyme activity also depends on comorbidity (e.g. asphyxia, cardiopathy), genetic polymorphisms and environmental factors (e.g. maternal smoking, co-medication, repeated drug administration) [19].

Excretion

Besides metabolic elimination (mainly hepatic), drugs can be eliminated by direct excretion (mainly renal) of the parent compound and/or its metabolites. A thorough understanding of the developmental changes in renal elimination function is needed to estimate renal clearance capacity. The rate of renal clearance is expressed as the sum of the glomerular filtration rate (GFR) (unidirectional diffusion) and the rate of tubular secretion (bidirectional active transport) minus the rate of tubular reabsorption (bidirectional active and passive processes) [20]. At birth, anatomic and functional immaturity of the kidney limits the glomerular and tubular functional capacities, which results in inefficient drug elimination and delayed clearance for many compounds eliminated by renal route. During this stage of human life, renal drug clearance almost entirely depends on GFR which increases during the postnatal period. At birth, GFR is low (2-4 ml/min/1.73 m² in term neonates, and even 0.6-0.8 ml/min/1.73 m² in preterm neonates) compared to adults. During the first 2 weeks of life GFR augments and reaches adult values (6L/h/70 kg) by the first year of life [21].

Similar to drug metabolism, overall renal clearance (i.e. the combined result of GFR and tubular functions) in neonates is low, but more predictable and its assessment (diuresis, creatinaemia) is more readily available. The most important factors that influence the renal elimination capacity are gestational age (GA), postnatal age (PNA), prenatal drug exposure (e.g. betamethasone), perinatal asphyxia or postnatal exposure to drugs such as ibuprofen, caffeine, dopamine or furosemide. The maturation of renal elimination capacity itself is driven by gestational age (nephrons are being formed from the 4th up to the 34-36th gestational week in an uneventful pregnancy) [20] and hemodynamic changes, i.e. a decrease in vascular resistance and increase in cardiac output and renal blood flow after birth (related to postnatal age) [14]. At present, the age-dependent functional development of renal tubular processes is less well explored compared to the age-dependent morphological development of the nephrons themselves [20]. It is generally accepted that renal tubular functions are not yet fully developed at birth, in part due to both the immaturity of the active transport processes as well as the smaller renal tubular mass and size compared to later life [22,23]. However, robust data on

e.g. ontogeny of renal drug transporters and relation between regional blood flow and metabolic activity of renal tubular transporters are still very limited.

Developmental pharmacodynamics: Questioning the endpoint when evaluating the effect

Pharmacodynamics refers to the exposure – effect relation of a drug, also described as '*what the drug does to the body'*. While knowledge about the impact of developmental changes on drug disposition, metabolism and excretion (PK) is increasing, information regarding ontogeny (age-related maturation) on drug effects (PD) is still more limited and mostly related to the number, affinity and type of receptors or the availability of natural ligands [9]. However, examples from clinical and animal data on ontogeny of receptors e.g. opioid receptors and expression of adrenergic receptors, resulted in strong evidence for changes in drug response during development, in addition to but independent from pharmacokinetic alterations [24]. Another example is dopamine, a catecholamine with peripheral as well as central nervous system effects, acting through a family of dopamine receptors with different subtypes (e.g. D1-D5). Via dopamine receptors in the kidney, dopamine increases renal blood flow, diuresis and sodium excretion. Data of several animal species documented a different PD response to low doses of dopamine between mature (renal vasodilatation) and newborn (vasoconstriction due to α-adrenergic receptor stimulation) animals [24-26]. The natriuretic response to D1 agonists in the newborn seemed hereby blunted. Whether this is based on differences in receptor affinity, receptor density, coupling to second messengers or intracellular mechanisms is at present unknown. Interestingly, human studies indicating increased urine output in very low birth weight neonates receiving dopamine were published [26]. More research on the mechanism of action, (side)effects and indications to use dopamine in neonates are needed.

Besides maturational changes in opioid, adrenergic and dopamine receptors also gamma-aminobutyric acid (GABA) mediated effects during the first weeks of life are different compared to older age. Although GABA is the principal *inhibitory* neurotransmitter in the adult mammalian brain [27], it is the main neurotransmitter of *excitation* in the first postnatal weeks of life. Changes in the $GABA_A$ receptor subunit composition, resulting in a decrease in intracellular chloride concentration, occur at 1-2 weeks after birth in rats. It is not yet fully known when this occurs in humans [9,28].

Immaturity can also result in an altered risk of drug toxicity, even a decreased risk. To illustrate this, infants appear to be less susceptible to renal toxicity induced by

aminoglycosides compared to older patients. A reduced intracellular accumulation of these compounds in the tubular epithelial cells of the renal cortex due to reduced endocytosis capacity via the multi-ligand receptor megalin after glomerular filtration, is considered to be the underlying mechanism of this PD characteristic [24,29].

Surrogate markers, clinical scores and long term outcome

In addition to developmental changes influencing final PD effects in neonates, correct measurement, evaluation and interpretation of exposure – effect relationships in this specific patient population is also of relevance. For many drugs commonly administered to neonates, this aspect remains poorly investigated. Following administration of a sedative or an anti-arrhythmic drug, the short-term effect will likely be obvious based on clinical examination of the patient and/or inspection of cardiac monitoring, respectively. For other drugs e.g. antibiotics, direct short-term effects are less obvious, which makes evaluation of the exposure-effect relationship more complex. In such cases, surrogate markers are used e.g. peak/MIC (minimal inhibitory concentration for a given pathogen) for aminoglycosides, T>MIC for cephalosporins and AUC/MIC>400 for glycopeptides. Unfortunately, target values aimed for when applying these criteria are derived from adult studies and not systematically investigated in neonates. Because of specific diseases, pathogens involved and the maturation of the immune system in neonates, it is urgently needed to validate the above mentioned PD targets in this population [30].

Even validated population specific assessment tools may have their limitations when used in the clinical setting. In order to evaluate and interpret drug effects in the absence of verbal communication, scoring systems e.g. pain score (analgesics), Finnegan score (opioid withdrawal), sedation and relaxation scores (sedatives) are tools to guide the clinician taking care of neonates. However, these instruments also have their limitations. First, scoring systems reflecting short-term phenotypic effects, will not inform us on the long-term effects of drugs administered. Nevertheless, long-term outcome measures are often even more important. Second, scores based on clinical assessments remain to a certain extent indirect and (inter)subjective. Furthermore, these observations might differ from the direct physiologic or neurobiologic effects occurring after administration of a compound. To illustrate this, small volumes of sucrose reduce behavioral responses and composite pain scores (pain expression) in neonates receiving painful procedures [31], while the nociceptive brain activity in these children (nociception) is not significantly different from those receiving placebo (sterile

water) [32]. Therefore, the impact of oral sucrose on clinical scores after painful events in neonates should not be interpreted as pain relief [32]. The same even holds true for vital signs without robust data on safe blood pressure.

Strategies to improve predictability of drug exposure: the search for covariates

One important general message emerges from the topics discussed above: variability is the core finding of neonatal pharmacology. Due to the highly dynamic period of neonatal life, many covariates influence PK/PD in neonates, resulting in a large inter- and intra-individual variability in drug exposure and effects. Consequently, in addition to median values of PK estimates or outcome parameters, the range and its contributing covariates are of utmost importance [33].

The main objective throughout this doctoral thesis is to further explore the covariates explaining PK/PD variability of frequently used drugs in neonates. Such an effort may reduce the observed variability, and in the meanwhile, can improve drug exposure predictability in an individual patient. One of the methodological approaches frequently applied to explore covariates of neonatal PK, (but also PD and PK/PD) is population pharmacokinetic modeling.

Population pharmacokinetic modeling

Two different methods can be used to describe concentration-time and concentration-effect data collected in a population: *the classical, standard two-stage approach* and *the population approach* using non-linear mixed effect modelling [34].

In the classical approach, pharmacokinetic parameters (e.g. Cl, V_d) are estimated for each individual, based on individual concentration-time profiles. This necessitates a large number of samples collected at pre-defined fixed time points in each individual, hereby avoiding as much as possible variability [24,34]. Subsequently, the individual structural parameters are treated as variables and combined to calculated summary (mean, median) measures. If the number of observations for each study patient is not equal or if the responses are highly variable, weighting is needed to a certain extent [35]. Variability can theoretically be estimated, but is often overestimated [35]. Additionally, no firm discrimination of between-patient and within-patient variability or residual variability can be determined using the classical approach [34]. In addition to the large number of samples needed, this is considered as a disadvantage of the classical approach. In contrast, the population pharmacokinetic approach using non-linear mixed effect modelling allows a sparse (i.e. limited number of samples) and

random (i.e. unbalanced, allowing chaos in the time frame to collect samples) sampling design, which is preferable and feasible in an intensive care setting.

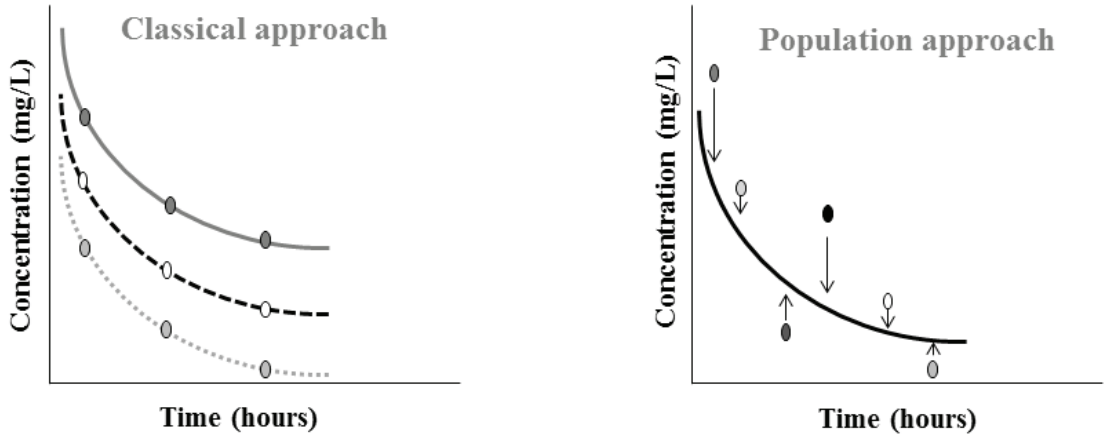

Figure 1: Presentation of concentration-time profiles (theoretically) achieved using a classical pharmacokinetic (PK) approach (different samples at fixed time points to create individual trend lines) versus the population PK approach (sparse and unbalanced sampling to create a one population trend line with subsequent identification of covariates explaining between- and within-patient variability).

As a result of this sparse sampling strategy, population PK allows inclusion of a broader spectrum of a heterogeneous cohort of participants who receive the drug in clinical settings [24]. In essence, the primary goal of a population PK analysis is to describe the overall pharmacokinetic parameters of the study group. As a second goal, it aims to quantify both between- and within-patient variability as well as to define covariates (e.g. clinical characteristics) explaining this variability [36]. This means that population PK is a perfect tool to characterize the impact of development and age on drug PK. In addition to the logistic advantages mentioned above, this methodological approach fits in the care for vulnerable and highly variable populations such as neonates and children [24,34]. Therefore, population PK became a standard method in neonatal PK/PD studies.

Non-linear mixed effects models describe data by integration of both fixed and random effects. Fixed effects predict the impact of a covariate (e.g. weight) on the between-subject variability of a parameter (e.g. Cl, V_d), while the random effects describe the remaining between-subject variability, not predictable from the fixed effect average [35].

During the model building process using non-linear mixed effect modelling, 3 different steps can be distinguished. In a *first* step, the most appropriate structural model (e.g. a one or two compartment model) is determined depending on the available data (and using fixed effect parameters such as Cl and V_d for PK). This structural model describes the overall trend in the data [34,35]. In a *second* step, a statistical submodel is defined (random effects), hereby aiming to reduce the remaining variability not explained by the structural model [34]. In a *third* step, a covariate analysis is performed, resulting in a covariate submodel. Relationships between covariates (e.g. age, weight) and parameters of the structural model (Cl, V_d) are explored and the nature of these relationships (i.e. linear, exponential) is defined [34]. Covariates which significantly improve the explained variability are added to the model. Discrimination between different models is based on the objective function (i.e. a number that evaluates the probability of achieving the observed data taking into account the model and its parameters) [35]. This final model needs an evaluation or internal model validation. Both the stability and the accuracy of the model are tested to verify if the final model adequately describes the data which are used to build the model. The former can be done by a bootstrap analysis, the latter by e.g. visually comparing observed versus predicted concentrations [34].

Population PK models can be used to simulate which concentrations (and/or effects) would be achieved in case different drug doses are given to patients. It is considered as a tool to evaluate and to optimize drug dosing regimens since covariates explaining inter-individual PK variability may be integrated in new drug dosing regimens. As such, drug exposure predictability can improve for an individual patient. However, each dosing regimen adaptation needs further validation, either external (is the model able to adequately describe external datasets) and/or prospective (is the model able to adequately describe prospectively collected datasets using a model-based dosing regimen). Based on this research strategy, PK knowledge in neonates has improved last decades, as nicely illustrated in the overview of population PK studies performed up to the age of 2 years, by Marsot *et al*, [37] and additionally offers opportunities to facilitate future drug evaluation [36]. Nevertheless, unexplained neonatal PK variability remains extensive for many drugs. For newly developed drugs, companies are encouraged to conduct PK/PD studies in neonates and children. Although this initiative, driven by law, will further improve PK/PD knowledge of new compounds, neonates remain a therapeutic 'orphaned' population [38]. This is reflected by the limited contribution of studies conducted in neonates found in public registers, but also by the fact that many neonates do not benefit from the drugs studied since a relevant percentage is even not used in NICUs, or

remains off label [38]. For old, but often frequently used drugs in neonates (e.g. antibiotics, propofol) covariates explaining PK/PD variability as well as optimal dosing regimens, also remain unknown. This resulted in the origin of the general research objective of this doctoral thesis.

Research aims

General objective

The aim of this doctoral thesis is to further improve drug exposure predictability in neonates based on PK and/or PD studies of frequently used drugs in this population. The compounds studied are vancomycin, amikacin, propofol and cefazolin. We hereby aim to integrate different aspects of clinical pharmacological research in neonates using the research sequence presented in Figure 2, which reflects the 3 main parts of this thesis:

PART 1: Identification of clinical pharmacology-related problems and exploration of covariates contributing to inter-individual variability in drug exposure in neonates.
PART 2: Integration of these covariates to improve drug dosing and drug exposure prediction
PART 3: Prospective validation of model-based dosing regimens in neonates

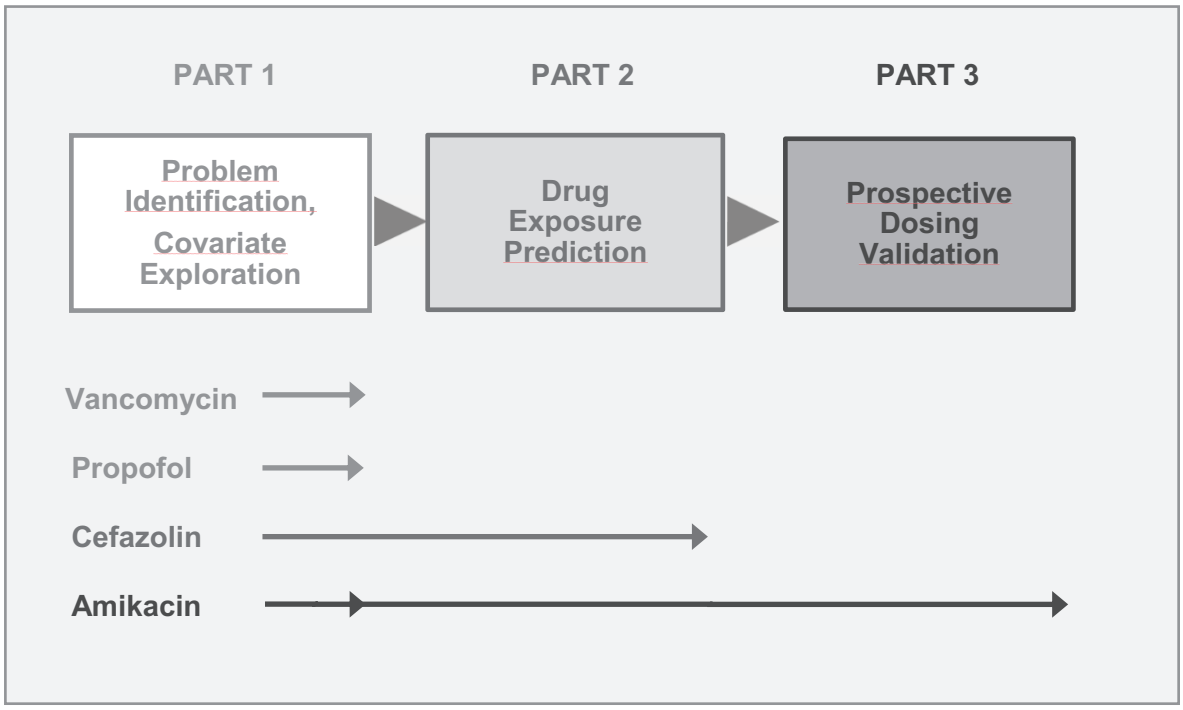

Figure 2: Schematic overview of the 3-step general objective of this thesis. Arrows along the compounds indicate the part in which the compound-specific objectives are investigated.

Compound-specific objectives

Part 1: Problem identification and covariate exploration

Vancomycin

Vancomycin, a glycopeptide antibiotic, is used for more than 50 years to treat late onset sepsis in neonates. However, PK variability in this population is extensive and there is no clear consensus on the optimal vancomycin dosing regimen. Specific objective investigated in **chapter 2**:

- To evaluate neonatal vancomycin exposure achieved using 2 published dosing regimens and to explore covariates of (sub)optimal exposure.

Amikacin

Amikacin, an aminoglycoside, is used to treat (suspected) neonatal sepsis. Its efficacy relies on peak concentrations and the possibility to reach therapeutic levels at the infection site. However, reports of amikacin quantification in deep neonatal body compartments are limited and amikacin quantification in bronchial epithelial lining fluid is absent. Specific objective investigated in **chapter 3**:

- To describe amikacin concentrations in bronchial epithelial lining fluid in neonates.

Propofol

Propofol is a short acting anaesthetic. Compared to adults, propofol clearance in neonates is lower, with postmenstrual age (PMA) and postnatal age (PNA) as major covariates. There is preliminary evidence that propofol mainly undergoes hydroxylation with only limited glucuronidation in neonates. Covariates explaining the inter-individual variability in neonatal propofol metabolism need further exploration. Specific objectives investigated in **chapter 4**:

- To explore urinary metabolites and its covariates after intravenous propofol bolus in neonates.
- To explore if hyperbilirubinaemia can improve predictability of neonatal propofol clearance.
- To document the ED_{50} propofol bolus dose (i.e. the dose effective for 50% of the patients) for (semi)elective endotracheal intubation in neonates, to explore propofol PD as a safety analysis and to define PD covariates.

Part 2: Drug exposure prediction

Cefazolin

Cefazolin, a first-generation cephalosporin, is mainly used as prophylactic agent for surgical procedures. In plasma, cefazolin is bound to albumin. As explained in the introduction, protein binding influences drug disposition drug action since only the unbound drug is pharmacologically active. However, neonatal cefazolin PK data are limited and mainly based on total plasma concentrations collected in a limited number of neonates. Specific objectives investigated in **chapter 5**:

- To determine cefazolin plasma protein binding and its covariates in neonates.
- To explore cefazolin protein binding and its covariates across different human populations.
- To perform a population PK analysis of total and unbound cefazolin concentrations as a guide to improve cefazolin dosing in neonates.

Part 3: Prospective dosing validation

Amikacin

Over the last 14 years, our research group improved predictability of amikacin disposition in neonates based on extensive pharmacokinetic analysis and dosing optimalisation. Subsequently, routine measurement of amikacin peak levels was stopped. However, the current dosing regimen (based on drug exposure prediction), introduced in 2011 still needs prospective validation. Specific objective investigated in **chapter 6**:

- To prospectively validate the currently used amikacin dosing regimen in our neonatal department, including its feasibility in clinical practice.

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PART I: Problem identification and covariate exploration

Variability in drug exposure in neonates

CHAPTER 2

Disposition of intravenous vancomycin in neonates

This chapter is based on

Prospective validation of neonatal vancomycin dosing regimens is urgently needed. *Curr Ther Res Clin Exp* 2014, 76:51-57

Abstract

Introduction Although vancomycin is frequently used to treat neonatal late-onset sepsis, there is no consensus on the optimal dosing regimen. Because many neonates needed dosing adaptation due to suboptimal trough values, the vancomycin dosing regimen in our neonatal department was changed in 2012. We aimed to document the need for validation of neonatal vancomycin dosing by exploring serum trough levels achieved using 2 published dosing regimens [previous regimen (2011): based on postmenstrual age and serum creatinine, new regimen (2012): based on postmenstrual age and postnatal age] and to identify covariates associated with suboptimal vancomycin trough levels (<10 mg/L).

Methods Routine therapeutic drug monitoring serum trough levels quantified after initiation of intravenous vancomycin therapy and clinical covariates were retrospectively collected. Median vancomycin trough levels of both dosing regimens were compared using the Mann-Whitney U test. The impact of continuous and dichotomous covariates on achieving a suboptimal trough level was explored using the Van Elteren test (stratified Mann-Whitney U test) and Mantel-Haenszel test (stratified χ^2 test) respectively. Covariates significant in monovariate analysis were subsequently included in a logistic regression analysis.

Results In total, 294 observations [median current weight 1870 g (range 420 – 4863 g) and postmenstrual age 35.07 weeks (range 25.14 – 56.00 weeks)] were included. Using the previous and new dosing regimens, 66.3% and 76.2% of trough levels, respectively, were below 10 mg/L. Overall, suboptimal vancomycin trough values were significantly associated with lower weight (birth weight, current weight) and age (gestational age, postmenstrual age).

Conclusions The majority of vancomycin trough levels in neonates achieved using 2 published dosing regimens did not reach the target of 10 mg/L. This illustrates the urgent need for prospective validation of neonatal vancomycin dosing regimens. We anticipate that dosing regimens integrating covariates reflecting general physiological maturation, renal maturation as well as disease characteristics could improve vancomycin exposure in neonates.

What is already known on this topic

- Targets to evaluate vancomycin exposure in neonates are derived from studies in adults.
- Extensive variability in vancomycin pharmacokinetics is documented in neonates.
- Many neonatal vancomcyin dosing suggestions are available, but they all lack prospective validation.
- Due to these multifaceted problems of vancomycin therapy in neonates (and children), the drug is on the revised priority list for studies into off-patent paediatric medicinal products of the European Medicines Agency'.

What this study adds

- 66.3 to 76.2% of early vancomycin trough levels, obtained using 2 published neonatal dosing regimens, do not reach 10 mg/L.
- Weight and age, both reflecting ontogeny, were major covariates associated with vancomycin serum trough levels in neonates.
- We demonstrated that prospective validation of vancomycin dosing approaches in neonates is of major priority to achieve safe and effective therapy.

Introduction

According to the Neonatal Research Network of the National Institute of Child Health and Human Development, 21% of very low birth weight infants experience at least one episode of late-onset sepsis (LOS), a major cause of morbidity and mortality in this specific population. Gram-positive bacteria are the most common isolated pathogens (70%) causing LOS, with coagulase-negative staphylococci (CONS) accounting for 48% of the isolates [1]. Vancomycin, a glycopeptide antibiotic, is frequently used to treat these pathogens. However, an optimal vancomycin dosing regimen for neonates is not available and prospective validation of published dosing guidelines is lacking.

In adults, a 24 hour area under the concentration-time curve divided by the minimum inhibitory concentration for a given pathogen (AUC_{0-24}/MIC) ≥400 is considered to be the best predictor of vancomycin efficacy [2,3]. During routine clinical care, vancomycin serum trough concentrations are used as a surrogate marker for AUC, aiming to achieve trough levels above 10 mg/L during intermittent intravenous administration [3]. In neonates, there is no strong correlation between serum trough levels and vancomycin efficacy. Consequently, serum vancomycin target levels for this special population are derived from adults. However, neonates differ from adults based on their specific physiology and diseases, resulting in population-specific pharmacokinetics (PK). Furthermore, neonates require population-specific pharmacodynamic (PD) targets because they have an immature innate immune system but otherwise are at risk for (maturational) toxicity.

The fact that we have been using vancomycin in neonatal care for more than half a century, but are still searching for the optimal dosing regimen and efficacy targets confirms the complexity of neonatal vancomycin pharmacology. These deficits can also be noticed in daily clinical care. First of all, clinicians are confronted with a diversity of dosing regimens presented in commonly used handbooks (Table 1) [4-12]. Secondly, subtherapeutic vancomycin trough levels are still frequently observed in neonates.

Since many neonates displayed vancomycin trough levels below the target value (needing subsequent dosing adaptation) when using a previously published postmenstrual age (PMA) and serum creatinine-based dosing regimen [13], we decided to introduce the PMA and postnatal age (PNA)-based Neofax® dosing approach in the UZ Leuven NICU in 2012 as new

vancomycin dosing regimen [4]. To illustrate the need for prospective validation of neonatal vancomycin dosing regimens, we explored serum trough levels achieved using both dosing approaches and, by pooling all observations, we aimed to identify covariates associated with vancomycin serum trough levels below 10 mg/L in neonates and young infants.

Table 1: Intermittent vancomycin dosing regimens for neonates as retrieved in reference handbooks [4-12]. Data are adapted to mg/kg/dose. PMA: postmenstrual age, PNA: postnatal age, GA: Gestational age, bacteremia [a], meningitis [b].

Reference	PMA (weeks)	PNA (days)	Current weight (g)	Creatinine (mg/dL)	Dose (mg/kg)	Interval (h)
Neofax 2011[4] and The Harriet Lane Handbook 2012 [5] and The Sanford guide 2012-2013 [6]	≤ 29	0 - 14 > 14			10 [a], 15 [b]	18 12
	30 - 36	0 - 14 > 14			10 [a], 15 [b]	12 8
	37 - 44	0 - 7 > 7			10 [a], 15 [b]	12 8
	≥ 45	any PNA			10 [a], 15 [b]	6
BNF for children 2011 [7]	< 29				15	24
	29 - 35				15	12
	> 35				15	8
Neonatal Formulary 2011 [8]	< 29 GA	0 - 7 > 7			15 15	24 12
	29 - 35				15	12
	36 - 44				15	8
	> 44				15	6
Dutch Children's Formulary [9]		< 7			10	12
		7 - 28			10	8
Nelsons's texbook of Pediatrics 2007 [10]		≤ 7	< 1200 1200 - 2000 > 2000 any weight		15 7.5 - 11.3 15 15	24 12 - 18 12 12 [b]
		> 7	< 1200 1200 - 2000 > 2000		15 5 - 7.5 15	24 8 - 12 8
		7 - 28	any weight		10 - 15	8 [b]
Red book 2012 [11]				< 0.7 0.7 - 0.9 1 - 1.2 1.3 - 1.6 > 1.6	15 20 15 10 15	12 24 24 24 48
Neonatal and pediatric pharmacology 2011 (Drug formulary for the newborn) [12]		< 7	< 1200 1200 - 2000 > 2000		15 10 - 15 10 - 15	24 12 - 18 8 - 12
		> 7	< 2000 > 2000		10 - 15 15 - 20	8 - 12 8

Methods

Study population, data collection and ethics

Vancomycin Therapeutic Drug Monitoring (TDM) observations of neonates and young infants treated with intravenous vancomycin, mainly for (suspected) late-onset sepsis (i.e. >72h after birth), in the NICU of the University Hospitals Leuven Belgium, between June 2011 and December 2012, were considered for inclusion in this retrospective study. This patient population consists of (pre)term neonates, inborn or transferred, in need of specialized care related to prematurity, infections, perinatal asphyxia, congenital diseases (e.g. surgery for cardiopathy, congenital diaphragmatic hernia, esophageal atresia) or other diseases. Clinical characteristics at birth [gestational age (GA) (weeks), birth weight (BW) (g)], as well as characteristics at the moment of TDM [PMA (weeks), PNA (days), current weight (CW) (g), concurrent treatment with ibuprofen (yes/no) or dopamine (yes/no), respiratory support (continuous positive airway pressure or mechanical ventilation) (yes/no), mechanical ventilation (conventional or high frequency) (yes/no), patent ductus arteriosus (PDA) (yes/no), positive blood culture (yes/no), serum creatinine concentration (mg/dL), serum albumin concentration (g/L) and serum vancomycin concentration (mg/L)] were extracted from the patient files. The daily nursing progress reports were used to collect data regarding vancomycin prescription (dose and interval). Results were excluded if data regarding vancomycin prescription could not be obtained or in case of an administration or sampling time error. We aimed to document early vancomycin exposure (i.e. after 24h of treatment initiation), therefore only first trough levels were included. The ethical board of our hospital approved the study protocol.

Vancomycin indication, administration and TDM assay

Vancomycin (Vancocin®, Elly Lilly, Brussels, Belgium), combined with amikacin, is used as standard therapy for (suspected) late-onset sepsis in the UZ Leuven NICU and administration occurs as an intravenous infusion over 60 minutes. Add-on therapy of vancomycin for other indications (e.g. severe early-onset sepsis, prophylaxis) is limited. The previous vancomycin dosing regimen (based on PMA and creatinine, Table 2a) was used between June 2011 and June 2012 [13]. The new dosing regimen (based on PMA and PNA, Table 2b) was introduced in June 2012 [4]. Since we noticed no improvement in trough levels during clinical practice, we felt it to be inappropriate to continue with this new regimen. Therefore, only data up to

December 2012 were available for inclusion. As part of routine clinical care, blood samples for TDM were collected at the end of the dosing interval, in most cases 24 h after treatment onset. Serum vancomycin assay was performed either with a particle enhanced turbidimetric inhibition immunoassay method (Siemens Dimension, Dade Behring) (June 2011 – October 2012) or with an enzyme multiplied immunoassay technique (Cobas c702 , Roche Diagnostics) (November 2012 – December 2012). In November 2012, the assay was changed throughout the entire hospital for unrelated (i.e. no clinical) reasons. The hospital laboratory has a quality system conform ISO15189. This implies clinical interchangeability of results is verified when changing from one assay to another. To avoid censoring of values below the lower limit of quantification (LLOQ, 2 mg/L), these concentrations were replaced by LLOQ/2 (i.e. 1 mg/L) as suggested in literature [14]. An enzymatic technique (Cobas c702 module, Roche Diagnostics) was used to quantify serum creatinine levels [15]. A colorimetric method (bromcresol green) was used to quantify serum albumin concentrations.

Table 2: The 2 vancomycin dosing regimens evaluated in this study. **a**) Previous dosing regimen (2011) based on postmenstrual age (PMA) and serum creatinine, published by Anderson et al [13]. **b**) New dosing regimen (2012, Neofax®), based on PMA and postnatal age (PNA) and limited to sepsis indication [4].

a)

PMA (weeks)	Creatinine (mg/dL)	Dose (mg/kg)	Interval (h)
< 29		15	24
29-35	< 0.6 > 0.6	15	12 24
>35	< 0.6 > 0.6	15	8 12

b)

PMA (weeks)	PNA (days)	Dose (mg/kg)	Interval (h)
≤ 29	0-14 > 14	10	18 12
30-36	0-14 > 14	10	12 8
37-44	0-7 > 7	10	12 8
> 45	All	10	6

Statistical analysis

Comparison of continuous clinical characteristics as well as median vancomycin serum trough level between observations of both dosing regimens was determined using the Mann-Whitney U (MWU) test. Comparison of dichotomous covariates was done by χ^2 test.

On the total dataset, the impact of continuous and dichotomous covariates on achieving suboptimal trough levels (< 10 mg/L) was explored using the Van Elteren test (stratified MWU test) and Mantel-Haenszel test (stratified χ^2 test), respectively. Stratification was done for dosing regimen. Covariates significantly associated with suboptimal vancomycin trough levels in monovariate analysis were entered in a logistic regression analysis. Spearman correlation was used to evaluate relations between continuous variables before inclusion in the logistic regression analysis. A p-value <0.05 was considered statistically significant.

Vancomycin serum trough levels and clinical characteristics were presented as median and range or incidence. Statistical analyses were performed using MedCalc12 (MedCalc Software, Mariakerke, Belgium) and the coin package in R version 3.0.2 (R Foundation for Statistical Computing,Vienna, Austria).

Results

Dataset

In total, 593 TDM observations were obtained in 223 patients. Sixty-one observations were excluded based on criteria summarized in Figure 1. Another 238 observations, collected after dosing adjustments were also excluded. The final dataset comprised 294 vancomycin TDM observations: 193 observations of the previous (2011) dosing regimen, 101 of the new (2012) dosing regimen. Both cohorts had comparable clinical characteristics, but differences for serum albumin and creatinine were documented (Table 3a). Taking into account the 294 vancomycin treatment episodes, indications to start vancomycin were (suspected) sepsis [87.7%, of which 8.8% were early-onset cases (≤72 hours after birth) and 78.9% were late-onset cases], presence of foreign body material e.g. thoracic drain, pacemaker (2.4%), prophylaxis (e.g. perforation umbilical catheter, disconnection ventricular-external drain) (2.7%), (sub)cutaneous wound infection (3.4%), pneumonia (3.1%) or unknown (0.7%). Incidences of indications stratified by dosing regimen and by age at initiation of therapy are presented in Table 3b.

Sixteen vancomycin trough values were below the lower limit of quantification. Median vancomycin concentration of samples achieved using the same dosing regimen (Neofax®), but with different vancomycin quantification assays used during the study period, were compared and did not differ significantly (5.9 mg/L Behring versus 5.5 mg/L Roche, MWU test, p=0.773).

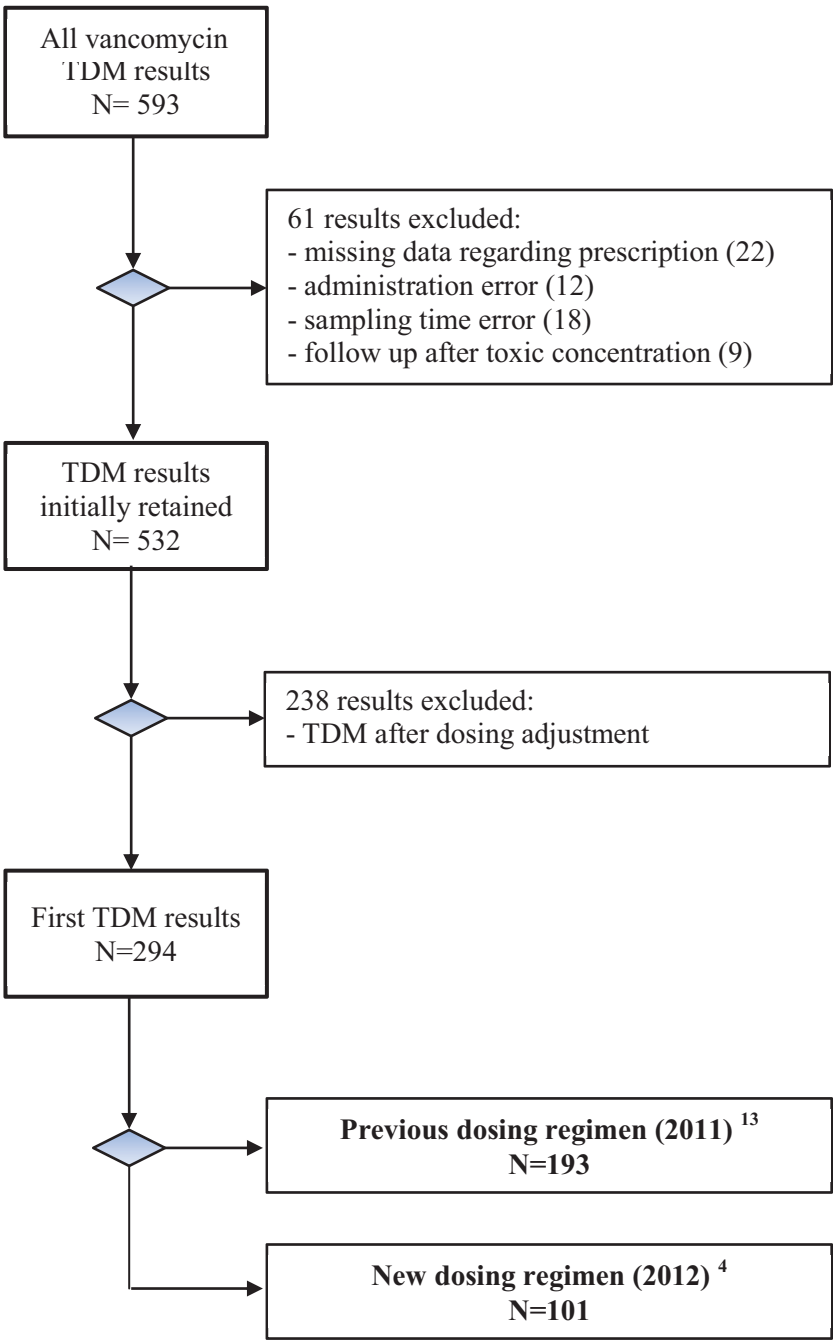

Figure 1: Flowchart of included vancomycin therapeutic drug monitoring (TDM) observations.

Previous versus new dosing regimen

The previous dosing regimen (Table 2) resulted in a significantly higher median (range) vancomycin trough concentration compared with the new regimen [7.8 (1–37.8) mg/L versus 5.8 (1–20.1) mg/L] (Figure 2). In the previous regimen, 128/193 (66.32%) of the observations were <10 mg/L and 65/193 (33.68%) were ≥10 mg/L. In the new regimen, 77/101 (76.24 %) of the observations were <10 mg/L and 24/101 (23.76%) reached levels ≥10 mg/L.

Figure 2: Vancomycin serum trough concentrations (mg/L) achieved by the 2 vancomycin dosing regimens used in our neonatal department are presented as boxplots (Mann Whitney U test, $p<0.05$)

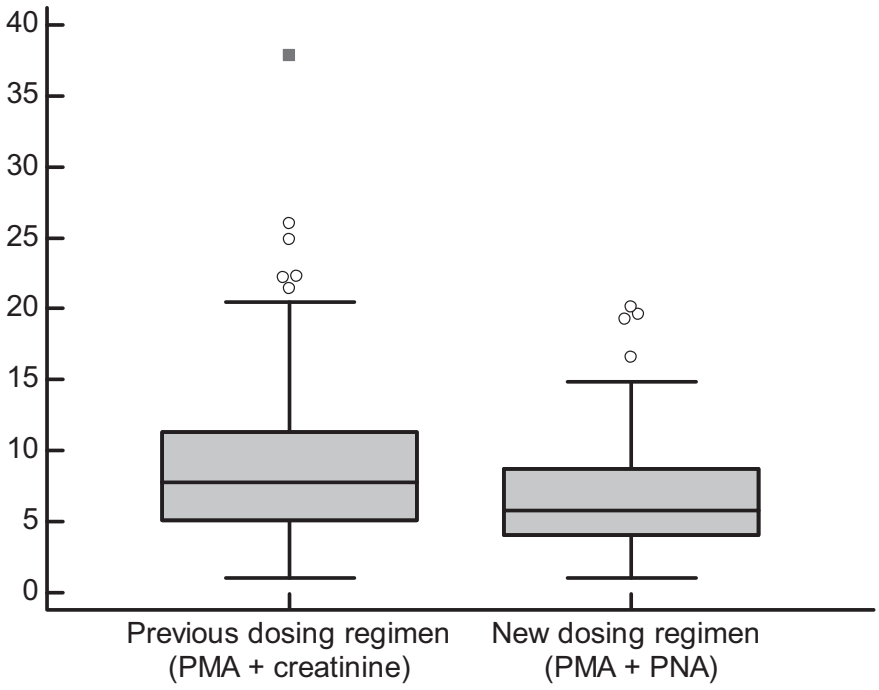

Table 3a: Clinical characteristics of included vancomycin trough concentrations achieved by the previous versus the new dosing regimen. Data are presented as median and range (continuous covariates) or incidence (dichotomous covariates). To explore continuous and dichotomous covariates between both cohorts, Mann-Whitney U test and χ^2 test were used respectively. n= number of observations; A p-value <0.05 was considered statistically significant.

Continuous covariates	**Previous dosing regimen [13] (n=193)**	**New dosing regimen [4] (n=101)**	**p-value**
Gestational age (weeks)	32.86 (24.57-41.43)	32.14 (24.86-41)	0.9862
Postnatal age (days)	13 (1-169)	12 (2-121)	0.4445
Postmenstrual age (weeks)	34.71 (25.14-49.86)	35.29 (25.43-56)	0.5950
Birth weight (gram)	1540 (420-4680)	1850 (440-4150)	0.3821
Current weight (gram)	1818 (500-4715)	2060 (420-4863)	0.9237
Albuminaemia (g/dl)	31.95 (17.40-50.40), N=164	31 (12.90-39.70), N=89	0.0290
Creatininaemia (mg/dl)	0.43 (0.14-1.18), N=178	0.49 (0.13-1.19), N=93	0.0429
Dichotomous covariates	**Previous dosing regimen [13] (n=193)**	**New dosing regimen [4] (n=101)**	**p-value**
Patent ductus arteriosus	12/153	5/80	0.8581
Concurrent ibuprofen	10/193	6/101	0.9985
Concurrent dopamine	22/193	11/101	0.9494
Positive blood culture	64/192	30/101	0.6163
Respiratory support	130/193	71/101	0.7020
Invasive respiratory support	80/193	42/101	0.9183

Table 3b: Indications to start vancomycin therapy. Data are presented as incidences (%) for both dosing regimens included in the study and are further stratified by early (i.e. ≤72h after birth) versus late (>72h after birth) age at start of therapy. Differences in incidences between both dosing regimens were explored using χ^2 test. A p-value <0.05 was considered statistically significant. EOS: Early-onset sepsis, LOS: late-onset sepsis. n= number of observations.

Vancomycin indication	Previous dosing regimen [13] (n=193)	New dosing regimen [4] (n=101)	p-value
Early (≤ 72h after birth)	**26/193 (13.47%)**	**15/101 (14.85%)**	0.8830
▪ Foreign body material	4 (2.07%)	3 (2.97%)	0.9388
▪ Prophylaxis	2 (1.04%)	2 (1.98%)	0.8939
▪ (Suspected) EOS	16 (8.29%)	10 (9.90%)	0.8059
▪ (Sub)cutaneous wound infection	4 (2.07%)	0	0.3541
Late (> 72h after birth)	**167/193 (86.53%)**	**86/101 (85.15%)**	0.8830
▪ Prophylaxis	3 (1.55%)	1 (0.99%)	0.8939
▪ (Suspected) LOS	150 (77.72%)	82 (81.19%)	0.5880
▪ (Sub)cutaneous wound infection	4 (2.07%)	2 (1.98%)	0.7031
▪ Pneumonia	8 (4.15%)	1 (0.99%)	0.2564
▪ Unknown	2 (1.04%)	0	0.7799

Clinical characteristics associated with (sub)optimal trough levels

Overall, 205/294 (69.73%) of vancomycin trough levels were <10 mg/L, while 89/294 (30.27%) reached levels ≥10 mg/L. Clinical characteristics of both groups (i.e. trough level <10 versus ≥10 mg/L) are presented in Table 4. Lower age (GA, PMA), lower weight (BW, CW) and higher PNA were significantly associated with suboptimal trough levels and these covariates were considered for inclusion in a logistic regression analysis. High correlation coefficients (r) were documented between PMA and GA (r =0.83), and between BW and CW (r =0.89). Since PMA combines GA (representing prenatal maturation) and PNA (representing postnatal maturation), GA was retained for inclusion instead of PMA. Since vancomycin is usually not administered in the first days of life, CW was chosen instead of BW. The final covariates entered in the logistic regression analysis were GA, PNA, CW and dosing regimen. Results of the analysis are presented in Table 5.

Table 4: Clinical characteristics of all included vancomycin trough concentrations as well as for the subgroups with trough concentrations < or ≥10 mg/L. Data are presented as median and range (continuous covariates) or incidence (dichotomous covariates). To explore the impact of continuous and dichotomous covariates on achieving vancomycin trough concentrations < or ≥10 mg/L, the Van Elteren test (stratified Mann-Whitney U test) and Mantel-Haenszel test (stratified χ^2 test) was used respectively. Stratification was done for dosing regimen. To explore the impact of dosing regimen on achieving trough concentrations < or ≥10 mg/L, standard χ^2 test was used (°). In all tests, degrees of freedom (df) were all equal to 1. n= number of observations; k= test statistic Van Elteren test; χ^2 = test statitistic Mantel-Haenszel test. A p-value <0.05 was considered statistically significant (*).

Continuous covariates	**All TDM data (n=294)**	**TDM <10 mg/L (n=205)**	**TDM ≥10 mg/L (n=89)**	**k**	**p-value**
Gestational age (weeks)	32.29 (24.57 – 41.43)	31 (24.57-41)	35.71 (24.86-41.43)	23.95	<0.0001*
Postnatal age (days)	13 (1 – 169)	15 (1-169)	10 (1-102)	8.25	0.0041*
Postmenstrual age (weeks)	35.07 (25.14 – 56)	33.28 (25.14-56)	38.28 (25.43-53.28)	14.11	0.0002*
Birth weight (gram)	1575 (420 – 4680)	1435 (420-4150)	2380 (440-4680)	26.51	<0.0001*
Current weight (gram)	1870 (420 – 4863)	1567 (487-4715)	2535 (420-4863)	21.03	<0.0001*
Albumin (g/dl)	31.60 (12.90 – 50.40)	32 (12.9-50.4)	31.15 (18.4-42.9)	1.38	0.2399
Creatinine (mg/dl)	0.43 (0.13 – 1.19)	0.43 (0.23-1.18)	0.43 (0.13-1.19)	2.55	0.1103
Dichotomous covariates	**All TDM data (n=294)**	**TDM <10 mg/L (n=205)**	**TDM ≥10 mg/L (n=89)**	**χ^2**	**p-value**
Patent ductus arteriosus	17/233 (7.3%)	13/172	4/61	0.0001	0.9939
Concurrent ibuprofen	16/294 (5.4%)	11/205	5/89	0.027	0.8688
Concurrent dopamine	33/294 (11.2%)	23/205	10/89	0.037	0.8478
Positive blood culture	94/293 (32.1%)	72/205	22/88	2.655	0.1032
Dosing regimen (previous/new)	193 (65.6%) / 101(34.4%)	128/77	65/24	2.628	0.1044 °
Respiratory support	201/294 (68.4%)	136/205	65/89	1.1052	0.2931
Invasive respiratory support	122/294 (41.5%)	77/205	45/89	3.8257	0.0505

Table 5: Logistic regression analysis with vancomycin serum trough levels <10 mg/L (=1) or ≥ 10 mg/L (=0) as dependent variable, based on 294 vancomycin serum trough levels. OR= Odds Ratio, SE= Standard error. Degrees of freedom was equal to 1 for all covariates. A p-value <0.05 was considered statistically significant (*).

Covariates	Coefficient B	SE	p-value	OR	OR 95% CI
Constant	2.8523	1.6130	0.0770	17.327	
Gestational age	-0.0486	0.0616	0.4296	0.9525	0.8443 to 1.0747
Postnatal age	0.0220	0.0088	0.0113*	1.0222	1.0050 to 1.0397
Current weight	-0.0005	0.0003	0.1049	0.9995	0.9990 to 1.0001
Dosing regimen	0.6363	0.3008	0.0344*	1.8895	1.0478 to 3.4075

Discussion

Up to 70% of vancomycin serum trough levels in neonates and young infants, achieved using 2 published dosing regimens for intermittent intravenous vancomycin administration, were below the target level of 10 mg/L. This finding illustrates the urgent need for optimization with subsequent prospective validation of suggested vancomycin dosing regimens (Table 1) in this specific population.

We documented that weight (BW, CW) and age (GA, PMA, PNA) - both reflecting ontogeny - were major covariates associated with vancomycin serum trough levels in neonates (Table 4). This can be explained by the fact that developmental changes in physiology are most prominent in early life and influence drug disposition (pharmacokinetics) [16,17]. Especially small (low BW, CW) and immature (low GA, PMA) babies showed vancomycin trough concentrations below 10 mg/L. Their higher body water content, resulting in a larger distribution volume for hydrophilic drugs (e.g. vancomycin) compared to older neonates, infants and adults, can in part contribute to the low TDM values observed. Besides changes in body composition with increasing age, also renal function (i.e. the combination of glomerular filtration and renal tubular functions), and consequently renal drug clearance, displays maturation. Although the maturation of glomerular filtration is well described and relates to conditions at birth (e.g. BW, GA) and conditions after delivery (e.g. PNA, ibuprofen

administration, perinatal asphyxia) [18,19], the role of renal tubular functions on neonatal drug clearance, and more specific on vancomycin clearance, is at present not yet unveiled.

The same holds true for the impact of specific diseases on vancomycin disposition in neonates. To illustrate this, the vancomycin trough value of 37.8 mg/l (outlier on Figure 2) was documented in a girl with GA 39 weeks and PNA 4 days, during the rewarming phase after hypothermia therapy for severe perinatal asphyxia. Since C-reactive protein increased while receiving amikacin and amoxicillin, vancomycin was added on day 3. Serum creatinine was normal and amikacin trough level was only slightly elevated (4.1 mg/L). Vancomycin prescription, administration and TDM sampling times were in line with our local procedures, but an error in drug handling prior to administration cannot be excluded. Although asphyxia itself can impair renal function and hypothermia can reduce renal blood flow (and consequently renal drug clearance) [20,21] the impact of these events on neonatal vancomycin disposition is at present unknown.

Therefore, we anticipate that optimized neonatal vancomycin dosing regimens should take into account covariates representing maturation but also disease characteristics [22] and co-administration of drugs influencing renal function, but that these covariates are not yet well considered in the currently proposed dosing regimens (Table 1).

Besides the above mentioned patient-specific characteristics, also the absence of optimal vancomycin efficacy targets, drug-specific characteristics and quantification assays used can contribute to variability in neonatal vancomycin exposure and can complicate the development of adequate dosing regimens [23,24]. First, there is no clear relationship between clinical response and indices of systemic vancomycin exposure in neonates. Based on studies in adults, an AUC_{0-24}/MIC ratio ≥400 has been recommended to achieve effectiveness. In clinical practice, vancomycin trough concentrations are used as surrogate marker and should be kept above 10 mg/L to correspond with an AUC_{0-24}/MIC ratio ≥400, if the MIC is <1 mg/L [3,24,25]. This assumption is derived from adults receiving 12-hourly vancomycin dosing. Moreover, trough concentrations depend on dose frequency [26]. In neonates, it is unknown what the optimal trough targets should be. Although some authors recommend to monitor directly AUC, the optimal parameter for vancomycin efficacy in neonates and young children remains unresolved [26-29].

It should be emphasized that the staphylococcal targets (CONS) for vancomycin use in neonates and their corresponding local MIC values are not comparable with the adult setting

in which vancomcyin is predominantly used to cure methicillin-resistant *Staphylococcus aureus* (MRSA) infections [30]. Second, vancomycin is bound to albumin and immunoglobulin A in plasma and only the unbound drug is pharmacologically active. However, data in neonates concerning the extent of protein binding as well as the disposition of vancomycin in deep body compartments are limited. We would like to stress that these pharmacokinetic aspects need further research in order to improve insight into the behaviour of vancomycin in neonates. Finally, currently used analytical methods for vancomycin quantification contribute to variability in TDM results and limit the transferability of vancomycin pharmacokinetic models [31], and subsequently model-derived dosing regimens, to other centres [31,32]. Therefore, the introduction of a more precise method, such as liquid chromatography-tandem mass spectroscopy (LC-MS/MS), which is considered to be the gold standard reference method, should be considered [33,34]. The high specificity, sensitivity and accuracy of LC-MS/MS makes it more suitable for e.g. pharmacokinetic studies compared with immunoassays, which in general suffer from non-specific interference from related compounds or matrix effects [33,35,36] or, in case of vancomycin, its crystalline degradation products. High instrument costs, greater technical complexity, speed and turnaround of sample analysis, are considered as the main disadvantages of LC-MS/MS. However, careful choice of sample preparation method and internal standard, and validation of assays should be able to avoid the majority of pitfalls [33]. Bijleveld *et al* [36] recently reported that LC-MS/MS documented slightly lower vancomycin concentrations than FPIA (Fluorescence Polarization Immunoassay). However, the applicability of their LC-MS/MS was only tested in 3 neonatal patients [36]. Therefore, paired analysis of neonatal vancomycin plasma concentrations using immunoassay versus LC-MS/MS in a large neonatal cohort is currently not yet available, but could be of relevance to optimize neonatal vancomycin dosing.

During the past decade, several neonatal vancomycin dosing regimens have been proposed in literature. The previous dosing regimen used in our unit seemed to be slightly better than the Neofax® regimen, but both were unable to reach sufficient median vancomycin trough levels. Nevertheless, as soon as preliminary results of our study were available, we decided to re-introduce the previous approach (based on PMA and serum creatinine) until prospectively validated improved dosing appear. Our observations are, to a certain extent, in line with Badran *et al* [37], who documented that only 51% of neonates attained a predefined vancomycin trough level between 5-10 mg/L using the Neofax® vancomycin dosing regimen and 33% of their trough concentrations were below 5 mg/L [37].

We are aware that our analysis is only based on trough levels quantified after initiation of therapy, since we aimed to achieve drug levels in the target range within a short time. We consider our covariate analysis as exploratory. More precise and predictive analyses require a population pharmacokinetic modelling approach in which available pharmacokinetic data can be used for the exploration of the most optimal vancomycin PD target in neonates, as well as for Monte Carlo simulations exploring different vancomycin administration modes (e.g. loading dose in intermittent dosin) to achieve early targeted vancomycin exposure. However, this is beyond the intention of the current study. Nevertheless, the large study size and the comparison of 2 'recently' published vancomycin dosing regimens to document the emergence of prospective validation of neonatal vancomycin dosing are relevant strengths. We hereby also would like to highlight the importance of international collaborative initiatives to improve neonatal vancomycin therapy like the NeoVanc research project (http://cordis.europa.eu/project/rcn/110198_en.html) which, besides the development of a new age-appropriate vancomycin formulation, aims to document the best vancomycin PD target and dosing regimen for neonates.

We conclude that 66.3% and 76.2% of vancomycin trough levels in neonates achieved using 2 published dosing regimens did not reach the target of 10 mg/L. This is a relevant, but just one of the problems related to vancomycin treatment of neonates. As future perspectives, prospective validation of vancomycin dosing regimens, but also further exploration of PK [e.g. protein binding, impact of renal (tubular) functions on clearance] and PD (e.g. optimal exposure targets) aspects of vancomycin in neonates are urgently needed.

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CHAPTER 3

Disposition of intravenous amikacin in neonates

This chapter is based on

Quantification of amikacin in bronchial epithelial lining fluid in neonates *Antimicrob Agents Chemother* 2011, 55:3990-3993

Abstract

Introduction Amikacin efficacy is based on peak concentrations and the possibility to reach therapeutic levels at the infection site. This study aimed to describe amikacin concentrations in the epithelial lining fluid (ELF) through bronchoalveolar lavage (BAL) in newborns.

Methods BAL fluid was collected in ventilated neonates treated with intravenous (IV) amikacin. Clinical characteristics, amikacin therapeutic drug monitoring serum concentrations and the concentrations of urea in plasma were extracted from the individual patient files. Amikacin and urea BAL fluid concentrations were determined using liquid chromatography with pulsed electrochemical detection (LC-PED) and capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D), respectively. ELF amikacin concentrations were converted from BAL fluid concentrations through quantification of dilution (urea in plasma/urea in BAL fluid) during the BAL procedure.

Results Twenty two observations in 17 neonates [postmenstrual age 31.9 (range 25.1-41) weeks, postnatal age 3.5 (range 2-37) days] were collected. Median trough and peak amikacin serum concentrations were 2.1 (range 1-7.1) mg/L and 39.1 (range 24.1-73.2) mg/L, the median urea plasma concentration was 30 (8-90) mg/dL. The median amikacin concentration in ELF was 6.5 mg/L, the minimum measured concentration was 1.5 mg/L and the maximum (peak) was 23 mg/L. The highest measured ELF concentration was reached between 6-14.5 hours after IV amikacin administration, and an estimated terminal elimination half-life was 8-10 hours.

Conclusions The median and highest (peak) ELF amikacin concentrations observed in our study population were, respectively, 6.5 and 23 mg/L. Despite the frequent use of amikacin in neonatal (pulmonary) infections, this is the first report of amikacin quantification in ELF in newborns.

What is already known on this topic

- Knowledge of amikacin disposition in deep body compartments of neonates is limited to observations in cerebrospinal fluid.
- Epithelial lining fluid (ELF) is considered as the site (compartment) of antibiotic activity against infections of the lung caused by extracellular pathogens.
- Aminoglycosides cross the blood-alveolar barrier by non-saturable, passive diffusion.

What this study adds

- This is the first report of amikacin disposition in bronchial ELF in neonates.
- Quantification of amikacin concentrations in neonatal bronchial ELF is feasible, with urea as endogenous marker to correct for the bronchoalveolar lavage-related dilution.
- The peak amikacin ELF concentration in neonates is reached 6-14.5h after intravenous administration, which is delayed compared to observations in adults.

Introduction

Aminoglycosides like amikacin are frequently administered in the treatment of suspected or proven Gram-negative infections in neonates, often in combination with penicillins. Due to a concentration-dependent killing combined with a postantibiotic effect, the bactericidal efficacy of amikacin relates to its peak serum concentration. Consequently, therapeutic peak levels at the infection site will define the effectiveness of antibiotic therapy. Renal side effects and ototoxicity relate to the trough serum amikacin concentration, based on the saturation of renal and cochlear cell-binding sites. The pharmacokinetics (PK) of amikacin displays extensive interindividual variability, which makes it difficult to achieve an effective and safe administration in the individual patient, including the neonate [1]. Based on maturational differences in body composition and renal immaturity in early life, differences in both distribution volume (V_d, L/kg) and clearance (CL, mL/kg/min) of this hydrophilic drug have been observed. Because of the higher water content in preterm infants, and thus a higher distribution volume for hydrophilic drugs, a relatively higher amikacin dose is necessary in this population [2,3].

Since pulmonary infections are a major cause of neonatal morbidity and mortality, antibiotic levels in bronchial secretions and bronchial and alveolar epithelial lining fluid (ELF) are of specific interest. Measuring antibiotic concentrations in the lung is not easy and is usually represented by ELF concentrations. Keeping the anatomy of the blood-bronchial barrier in mind, one can imagine that to reach the ELF, the antibiotic must pass through the epithelial lining cells linked by tight junctions [4]. Consequently, biochemical characteristics like the degree of protein binding, the lipophilicity and diffusibility of the antibiotic will influence antibiotic concentrations in interstitial fluid and in ELF. In adults, we are aware of studies to assess the blood-alveolar barrier after parenteral administration of amikacin. Dull *et al* [5] showed that, after intramuscular (IM) administration, the highest amikacin serum concentration correlated significantly with the highest bronchial secretion concentration of that individual and that elimination of amikacin from serum and bronchial secretions occurred at approximately the same rate with a peak concentration that is blunted in the alveolar compared to the blood compartment [5]. Since data in neonates are lacking, the aim of this study is to describe amikacin concentration in the bronchial epithelial lining fluid (ELF) of newborns.

Methods

Clinical characteristics and drug administration

From March 2009 to June 2009, bronchoalveolar lavage (BAL) fluid samples were prospectively collected in ventilated neonates, to whom amikacin was administered for clinical indications. All the patients were enrolled in the Neonatal Intensive Care Unit (NICU) of the University Hospitals of Leuven, Belgium, following approval by the Medical Ethical Committee of the hospital (B32220084581, S51291) and signed parental consent. The latter was specifically obtained to collect and analyze the BAL fluid samples and to integrate the results with individual clinical characteristics [postmenstrual age (PMA), postnatal age (PNA), birth weight, amikacin therapeutic drug monitoring (TDM) and urea plasma measurements] and treatment [amikacin dosing regimen and duration between intravenous (IV) administration and BAL procedure].

Bronchoalveolar lavage aspirates were collected using an endotracheal suctioning method as described earlier in literature [6]. Samples were collected when bronchial suctioning was performed based on perceived clinical need to perform bronchial suctioning.

The amikacin dosing regimen used in the study, was implemented in 2002 based on the suggestions of Langhendries *et al* [1,7]. In this regimen, dosing depends on PMA as follows: PMA of <28 weeks, 20 mg/kg/42 h; PMA of 28 to 30 weeks, 20 mg/kg/36 h; PMA of 31 to 33 weeks, 18.5 mg/kg/30 h; PMA of 34 to 37 weeks, 17 mg/kg/24 h; and PMA of >37 weeks, 15.5 mg/kg/24 h, with an additional dosing interval increase of 6 h if ibuprofen was co-administered or if neonates had suffered asphyxia or hypoxia. Amikacin (Amukin, 50mg/mL pediatric vial; Bristol Myers Squibb Belgium) was given as an IV infusion over 20 min via syringe driver (SIMS; Graseby, Watford, United Kingdom).

Amikacin assay in serum and BAL fluid

Serum

Blood samples for TDM were collected by arterial line or venous puncture just before ("trough") and 1 h after the initiation of administration ("near peak") of the second dose of amikacin, approximately 40 min after the 20-min IV infusion [1,7]. Amikacin serum concentration was determined using a fluorescence polarization immunoassay (TDxFLx, Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA) following sample

collection and are expressed in mg/L [1]. Drug recovery from extraction was 100% (standard deviation [SD]=2.6%) over a tested expected concentration range of 3 to 35 mg/L. The precision was assessed at 5, 15 and 30 mg/L. These concentrations yielded, respectively, a within-run coefficient of variation (CV) of 2.1, 1.4, and 1.8%, a between-day CV of 0, 1.5, and 1.7%, and a total CV of 3.2, 2.6, and 2.5%. The minimal quantifiable concentration was 0.8 mg/L as defined by a CV of <20% (information from Abbott Laboratories). The CV was typically <5% based on an internal quality assessment covering a concentration range of up to 50 mg/L [8]. For quantification of concentrations between 50 to 200 mg/L, a manual dilution (4-fold dilution with dilution buffer TDX) is needed.

BAL fluid

Measurements of concentrations in deep bronchial secretions were performed on the supernatant after it was processed as described by Santré *et al* [9]. Following collection, samples were frozen (-20°C) until completion of the study. After thawing, secretions were diluted in an equivalent sterile water volume and then centrifuged after incubation at 37°C for 18 h to provide better viscosity.

Supernatant was collected to determine the amikacin concentration. Quantification was performed by liquid chromatography with pulsed electrochemical detection (LC-PED) based on the method described by Brajanoski *et al*. for determination of amikacin in cerebrospinal fluid (CSF) [10]. A few modifications were done: as column, a reversed-phase C_{18} Hypersil BDS (100 mm by 2.1 mm; 3 µm particle size) was used, and the flow rate was 0.3 mL/min. The quantification limit for amikacin base was found to be 0.06 mg/L. Good linearity was obtained for amikacin base in the concentration range from 0.06 mg/L to 4.0 mg/L, with a correlation coefficient greater than 0.995. The precision (relative standard deviation [RSD], n=3) on the peak area of a 1.0 mg/L amikacin base reference solution was 0.1%. The recovery was found to be 99.1 % and 100.9 % for BAL fluid spiked with amikacin base at concentrations of 0.2 mg/L and 2.0 mg/L, respectively.

Since BAL fluid only in part reflects bronchial epithelial lining concentrations, we used urea concentration in plasma and in the BAL fluid to correct the bronchial epithelial lining amikacin concentrations for the BAL procedure-related dilution. When the urea concentration in plasma and the urea quantity in a lavage sample are known, V_{ELF} (ELF volume) can be calculated, using following equation: V_{ELF} = (Volume $_{BAL\ fluid}$ x [urea $_{BAL\ fluid}$]/[urea $_{plasma}$],

where [urea $_{BAL\ fluid}$] is the urea concentration in BAL fluid and [urea $_{plasma}$] is the urea concentration in plasma. As urea is a free low-molecular-weight substance that diffuses readily through the alveolar capillary membrane barrier, it may be assumed that [urea $_{plasma}$] = [urea $_{ELF}$] (Figure 1). To calculate amikacin concentration in ELF, [amikacin $_{ELF}$], the following equation can be used: [amikacin $_{ELF}$] = [amikacin $_{BAL\ fluid}$] x ([urea $_{plasma}$]/[urea $_{BAL\ fluid}$]) [4,11].

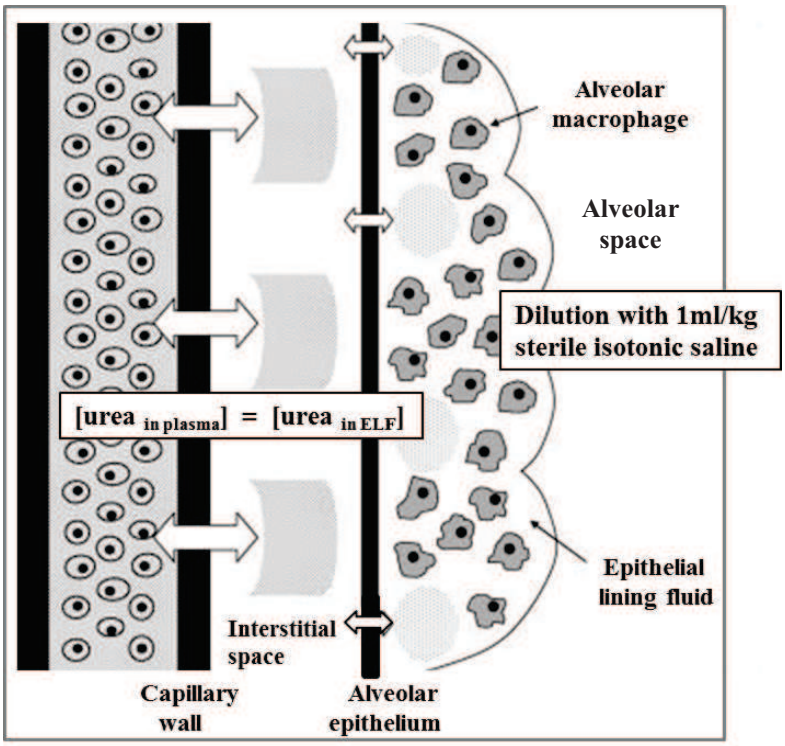

Figure 1: Schematic diagram of the blood-alveolar barrier (adapted from Kiem et al [4]) which is composed of 2 membranes: the capillary wall and the alveolar wall. They are separated by the interstitial space. Amikacin need to diffuse across the alveolar capillary wall, the interstitial fluid, and the alveolar epithelial cells to reach ELF. Urea readily diffuses through the blood-alveolar barrier.

Urea determination in plasma and BAL fluid

Plasma

Urea determination in plasma was performed by a modular urea/blood urea nitrogen (BUN) Cobas system (Roche/Hitachi, IN, USA). The measuring range in plasma is 5 to 400 mg/dL urea with 5 mg/dL as detection limit. Precision was determined using human samples and

controls in an internal protocol. Repeatability (n=21) showed a CV of 0.8% at a concentration of 198 mg/dL. Intermediate precision yielded a CV of 3.4% at a concentration of 31 mg/dL.

BAL fluid

Samples were collected as mentioned above and kept in the freezer until analysis by capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D) was performed. Since coelution of urea and sodium was observed in the CE-C^4D system, urease was added to the sample to hydrolyze urea and quantify it as ammonium [12]. BAL fluid was centrifuged for 2 min at a speed of 14500 rpm using a Mini-Spin Plus (Eppendorf, Hamburg, Germany). A volume of 100 µL of supernatant of the BAL fluid sample was pipetted into a 1.5 mL Eppendorf vial. A total of 100 µL of 0.1 mg/mL lithium hydroxide as an internal standard and 100 µL of 0.2 mg/mL urease (type IX from jack beans; 50000-100000 units/g of solid) were added and completed to 1000 µL with water. The mixture was incubated for 20 min at 37^{o}C by using a Thermomixer comfort system (Eppendorf, Hamburg, Germany). Next, the solution was centrifuged for 10 min at room temperature at a speed of 14500 rpm. The supernatant (100 µL) was transferred into a microsample vial and directly injected in CE-C^4D instrument for quantification. The background electrolyte (BGE) for analysis of ammonium contained 30 mM malic acid, adjusted to pH 4.1 by L-arginine, and 10 mM 18 Crown-6 ether. The 10 mM 18 Crown-6 ether was added to the BGE to enhance the separation of ammonium from potassium ions, two cations of similar size, by partial complexation. A voltage of 30 kV was applied on a fused silica capillary with 75 µm internal diameter and total length of 65 cm (41 cm to C^4D). The experiments were performed on a P/ACE MDQ capillary electrophoresis instrument (Beckman Coulter, Inc. Fullerton, CA, USA), coupled with an eDAQ C^4D system (eDAQ, Denistone East, Australia). The eDAQ C^4D detector was employed at a peak-to-peak amplitude of 100 V and the frequency was 1200 kHz. This method provided conditions without interference of matrix components and the recovery was found to be 97.9% and 99.3% for BAL fluid spiked with urea at concentrations of 1.8 mg/L and 7.2 mg/L, respectively. For the CE-C4D method, the intraday precision is 0.7% (n=6) and the interday precision is 1.4 % (n=18, representing 3 days with 6 analyses/day).

Results

Twenty two observations were collected in 17 neonates: 19 in male and 3 in female neonates. Median postmenstrual age (PMA) at inclusion was 31.9 (range 25.1-41) weeks, median postnatal age (PNA) 3.5 (range 2-37) days, and median birth weight was 1715 (range 550-3540) grams. All observations were collected in ventilated neonates, with 19 collected during conventional mechanical ventilation and 3 collected during high-frequency oscillation. The median oxygenation index was 3.6 (range 1.2-7.9). Median creatinaemia and serum urea were, respectively, 0.6 (range 0.4-1) mg/dL and 26.5 (range 8-90) mg/dL. The median trough and peak amikacin concentrations in serum were 2.1 (range 1-7.1) mg/L and 39.1 (range 24.1-73.2) mg/L, respectively (Figure 1).

Median time of bronchoalveolar lavage was 13.5 (range 1.5-23.5) h after amikacin administration. Amikacin ELF concentrations of 16 samples were available. During analysis, one observation with an ELF amikacin concentration of 89 mg/L was excluded since this was an extreme outlier. In this patient, BAL fluid sampling occurred at 18 h after amikacin administration. Median amikacin concentration in ELF was 6.5 mg/L, the minimum measured concentration was 1.5 mg/L, and the maximum was 23 mg/L. The highest measured concentration in our study was reached between 6 and 14.5 hours after iv amikacin administration, and a subsequent estimated elimination half-life was 8 to10 h (Figure 2). Figure 2 illustrates the amikacin ELF concentrations for different time points.

Discussion

To prevent antibiotic resistance, antibiotics need to be administered based on pharmacokinetics (PK) and pharmacodynamics (PD). Therefore, it is useful to measure the concentrations of antibiotics at infection sites, because the distribution of antibiotics may be different among a variety of tissues, in part depending on disease characteristics, maturational changes or tissue characteristics [4]. Amikacin is a commonly administered aminoglycoside to treat neonatal bacterial infections, but data about its concentrations in neonatal bronchial secretions are not yet described. In this study we showed that maximum (peak) and median concentrations of, respectively, 23 and 6 mg/L can be reached in the epithelial lining fluid, after intravenous administration of amikacin in neonates (Figure 2).

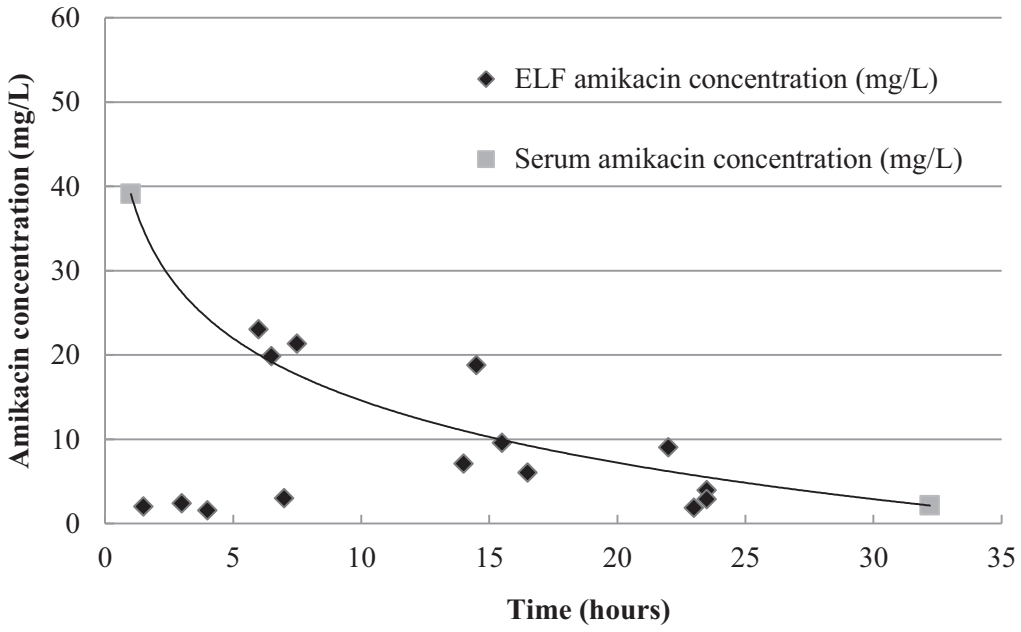

Figure 2: Amikacin disposition in bronchial epithelial lining fluid (ELF) and serum. The X- axis shows the time (hours) after the start of intravenous amikacin administration. The Y-axis shows the amikacin concentration (mg/L). For ELF, each symbol represents a unique observation. For serum, median peak and trough concentrations of the study population are represented, with an accompanying trend line assuming a one-compartment model with instantaneous input and first-order output [1,3].

To compare our data with already published evidence, we performed a systematic literature search on aminoglycoside [amikacin [5,9,13-15], tobramycin [14-19], gentamicin [20,11], and netilmicin [21]] quantification in BAL fluid after parenteral (iv or im) administration of the antibiotic drug. Twelve studies were selected (Table 1), and they were all conducted in adults (11/12 studies) or children (1/12 studies), not in neonates. A literature search, using the same terms, was repeated in July 2014 but no additional papers could be included. Five of 12 studies used amikacin, either in a once-daily (n=1), twice-daily (n=1), or three times a day dosing regimen (n=2). One study (n=1) compared once- versus twice- daily dosage. Even within the adult population variability in sample material (sputum, bronchial secretions, BAL fluid) and antibiotic dosage regimen was found. The anatomic site of sample collection is of importance since earlier reports mentioned that aminoglycoside concentrations in the whole lung tissue, sputum, and bronchial secretions approximate respectively 50%, 20-60% and 20% of serum levels [11]. It is also known that bronchial secretions cannot be used to predict ELF concentrations of aminoglycoside [21]. The introduction of BAL as sampling procedure resulted

in more standardized measurements. When considering the different dosage schedules, higher maximum serum and bronchial amikacin concentrations can be detected with a once-daily iv drug administration regimen than with a twice-daily or continuous infusion regimen [15]. Based on the data retrieved, Valcke *et al* registered the highest peak aminoglycoside concentration in ELF (14.7 mg/L) after iv administration of 450 mg netilmicin once daily in adults with pneumonia [21]. Our study was performed only in neonates after an extended antibiotic interval and with the BAL procedure to collect samples.

To reach (higher) therapeutic aminoglycoside concentrations in bronchial secretions, one can assume that aerosol-delivered drug administration directly into the bronchial tree is preferable to iv administration. No data can be found on neonates. Nebulised amikacin in mechanically ventilated adults with Gram-negative ventilator-associated pneumonia resulted in amikacin ELF concentrations more than 10-fold the MIC_{90} for micro-organisms responsible for nosocomial pneumonia [22]. Specific characteristics of nebulised antibiotic administration are the heterogeneous drug disposition within different anatomical parts of the lung and major interindividual variability in achieved drug concentrations. Aerosol-delivered drug administration also results in low systemic drug absorption. This is important in neonates, since amikacin is frequently used to treat (suspected) systemic infections.

When comparing amikacin disposition in other deep, extravascular compartments in neonates, only one report can be found concerning amikacin quantification in cerebrospinal fluid (CSF) with a median value of 1.1 mg/L. Besides serum, EFL, and CSF, further research on amikacin disposition in different body compartments is necessary for optimal treatment of neonatal infections.

Although the endotracheal suctioning method used to collect BAL samples in neonates is part of the routine medical care in ventilated neonates, the quantification of amikacin in ELF is technically more complex. We showed that amikacin concentrations in ELF are relatively low, but detectable. As mentioned above, we used the urea concentration in plasma and in the BAL fluid to correct the ELF amikacin concentrations for the BAL procedure-related dilution. Urea, as an endogenous marker, is small, relatively nonpolar and its concentration is considered the same in ELF as in serum, implying complete distribution. Significant diffusion of urea across the epithelium during the BAL procedure does not occur [5,23]. In literature, albumin and secretory component of immunoglobulin A (scIgA) are also presented as

denominators for BAL fluid constituents, but they are less appropriate markers. This is in part attributed to variation in concentrations, related to ontogeny and/or disease states, of these markers [24-27].

In addition to already mentioned factors (protein binding, lipophilicity and capacity for passive diffusion of the drug) influencing the penetration capacity of antibiotic drugs through the blood-bronchial barrier, it has been postulated that inflammation, infection and disease severity also influence this passage. Canis *et al* investigated the pharmacokinetics of once-daily iv amikacin (35 mg/kg/day) administration during the first treatment day in children (mean age 9.8 years) with cystic fibrosis. Amikacin sputum concentrations ranged from 5.1 to 19.9 mg/L at 1 hour. The highest concentrations were obtained at 2 h (mean 10.9 ± 7.5 mg/L) [13]. Our study aimed to describe population specific amikacin kinetics in ELF, but was not powered to elaborate on the potential impact of covariates on amikacin bronchial disposition. Based on the reported range in PK serum estimates within the neonatal population, we assume that further exploration of these covariates within the neonatal population will be extremely difficult and of limited add-on value.

We conclude that amikacin concentrations in neonatal epithelial lining fluid after intravenous administration, can be quantified. During analysis, urea has to be used to correct concentrations for the BAL procedure dilution. The median and highest (peak) ELF amikacin concentrations found in our study population were respectively 6.5 and 23 mg/L. Despite its frequent use in neonatal (pulmonary) infections, this is the first report of amikacin quantification in ELF in newborns.

Table 1: Aminoglycoside disposition in bronchoalveolar fluid (or bronchial secretions or sputum) after parenteral administration. n:number of patients, (ct) IV: (continuous) intravenous, IM: intramuscular, conc:concentration, h:hour, VAP:ventilator-associated pneumonia. All concentrations are converted to mg/L. If no peak values are available, detail of data are provided between square brackets. [a] Mean ± SEM, [b] Mean ± SD, [c] Highest individual value

Reference	Population	n	Aminoglycoside, mode of administration	Dosage regime	Sample	Peak serum conc (mg/L)	Peak bronchial conc (mg/L)
Dull [5]	Adult men, noninfected	14	Amikacin, IM	7.5 mg/kg, 2x/day	Bronchial secretions	23.7 ± 2.9 [a] at 1.5-2h (n=4)	5.20 ± 1.5 [a] at 1.5-2h (n=3)
Santré [9]	Adults, ventilated, pneumonia	10	Amikacin, IV	a) 15 mg/kg, 1x/day b) 7.5 mg/kg, 2x/day	Bronchial secretions	a) 46.5 ± 9.3 [b] b) 20.2 ± 9.3 [b]	a) 13.6 ± 9.3 [b] at 3-4h b) 4.8 ± 2.6 [b] at 3-4h
Canis [13]	Children, Cystic fibrosis	18	Amikacin, IV	35 mg/kg 1x/day	Sputum	121.4 ± 37.3 [b]	10.95 ± 7.5 [b] at 2h (n=13)
Mombelli [15]	Adults, tracheostomy or ventilated, acute bronchitis	13	a) Amikacin, ct IV (n=6) b) Tobramycin, ct IV (n=7)	Loading dose: a) amikacin 4 mg/kg b) tobramycin 1 mg/kg Followed by: a) 7-12 mg/kg/ 8 h b) 2-3.5 mg/kg /8 h	Bronchial secretions	a) 12.8 ± 3.3 [b] [at 5-7h] b) 3.6 ± 1 [b] [at 5-7h]	a) 2 ± 1 [b] [last 4h of infusion] b) 0.71 ± 0.7 [b] [last 4h of infusion]
Levy [14]	8-21 year patients, Cystic fibrosis	10	a) Amikacin, IV b) Tobramycin, IV	a-b or b-a a) 750 mg/m²/day in 3x b) 180 mg/m²/day in 3x	Sputum	a) 18.5 ± 2.5 [a] at 1.16h b) 3.10 ± 0.25 [a] at 1.16h	a) 1.4 ± 0.2 [a] at 1.16h b) 0.45 ± 0.05 [a] at 1.16h
Boselli [16]	Adults, ventilated, VAP	12	Tobramycin, IV	7-10 mg/kg, 1x/day	BAL fluid	22.4 ± 5.9 [b] at 0.5h	ELF 2.7 ± 0.7 [b] at 0.5h BAL 0.4 ± 0.3 [b] at 0.5h
Carcas [18]	Adults, pneumonia	16	Tobramycin, parenteral	8h intervals	BAL fluid	6.90 ± 1.44 [b] at 0.5h	ELF 2.33 ±0.5 [b] at 0.5 h
Braude [17]	Adults	9	Tobramycin, IV	1-1.7 mg/kg/dose	BAL fluid	6.3 [c] at 1h	BAL value 0.30 [c] at 1h
Mazzei [19]	a) adults (PK) b) adults (BAL)	12 10	Tobramycin IM	a1) 150 mg single dose (n=6) a2) 300 mg single dose (n=6) b1) 300 mg 1x/d, 1 day (n=5) b2) 300 mg 1x/d, 4days (n=5)	BAL fluid	a1) 71 ± 2.4 [b] at 0.75h a2) 12.2 ± 1.6 [b] at 0.75h	b1) 5.3 ± 2.9 [b] [at 6h] b2) 5.5 ± 2.1 [b] [at 6h]
Odio [20]	Adults, noninfected	5	Gentamicin IM	2 mg/kg	Bronchial secretions	Mean 6.75 at 1h	1.83 (range 0.1-4.3) at 1 h
Panidis [11]	Adults, Ventilated, VAP	24	Gentamicin, IV	240mg 1x/day	BAL fluid	13.39 ± 0.91 [a] at 0.5h	4.24 ± 0.42 [a] at 2h (n=6)
Valcke [21]	Adults, Ventilated, pneumonia	20	Netilmicin, IV	450 mg 1x/day	a) Bronchial secretions, b) BAL fluid	36 ± 1.32 [a] at 0.5 h	a) 2 ± 0.26 [a] at 2 h b) 14.7 ± 2.22 [a] at 2 h

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CHAPTER 4

Disposition of intravenous propofol in neonates

This chapter is based on

Urinary metabolites after intravenous propofol bolus in neonates.

Eur J Drug Metab Pharmacokinet 2013, 38: 97-103

Is indirect hyperbilirubinemia a useful biomarker of reduced propofol clearance in neonates?

Biomark Med 2012, 6: 283-289

Exploratory dose-finding study in neonates receiving a single intravenous propofol bolus for (semi-) elective endotracheal intubation: preliminary analysis *(manuscript in preparation)*

Abstract

Introduction Propofol, is metabolised by hydroxylation with a limited contribution of glucuronidation in early life. Clearance variability in neonates is in part explained by postmenstrual age (PMA) and postnatal age (PNA). The aims of this study were to further explore propofol pharmacokinetics (PK) (i.e. covariates of metabolism and clearance), and to perform a preliminary dose-finding study with pharmacodynamic (PD) assessment of propofol for endotracheal intubation in neonates.

Methods In 32 neonates receiving an intravenous propofol bolus, urine was collected during 24 hours to determine propofol metabolites. The impact of clinical covariates on the urinary metabolic profile of propofol was examined. To assess the impact of hyperbilirubinaemia on propofol clearance, indirect serum bilirubin was introduced in a previously published propofol PK model based on 25 neonates. Non-linear mixed effect modeling was used for this analysis. In the prospective dose-finding study (n=35), propofol ED_{50} doses were calculated based on the method of Dixon, with simultaneous assessment of clinical observation scores, vital signs as well as cerebral oxygenation.

Results Median total propofol metabolite recovery was 40.95 (2.01-129.81) % with a propofol glucuronide/quinol glucuronides ratio of 0.44 (0.01-5.93). Late PNA (≥ 10 days) resulted in a higher urinary PG fraction. Covariates PMA and PNA explained 67% of the inter-individual variability in propofol clearance compared to 45% by PMA and bilirubin. Using a propofol dose range of 0.5-4.5 mg/kg, and aiming for successful intubation [as well as extubation in case of INSURE (intubation, surfactant, extubation) cases], median ED_{50} range for preterm neonates <10 days PNA was 0.480-1.287 mg/kg. Clinical recovery was not yet fully attained 21 minutes after propofol administration. A median decrease in mean arterial blood pressure between -29.41% and -39.09% from baseline was documented**.** Variability in blood pressure and in peripheral and cerebral oxygenation, could not be explained by weight, age or propofol blood concentrations (at 3 and 12h after propofol bolus).

Conclusion Age 10 days (PNA) is pivotal in early life propofol metabolism. Also for neonatal propofol clearance, age (PMA and PNA) remains the key to explain variability. Compared to literature, lower (mg/kg) propofol doses can result in a sedative effect sufficient for intubation, while clinical recovery takes more time and is accompanied by a moderate decrease in blood pressure and a short decrease in peripheral and cerebral oxygen saturation.

What is already known on this topic

- Ontogeny, reflected by postmenstrual (PMA) and postnatal (PNA) age, only in part explains variability in propofol metabolism and clearance in neonates.
- Controversy exists concerning the safety of propofol (especially its hypotensive effect) to recommend its routine use for procedural sedation in neonatal intensive care units.
- The timing of dose-finding studies with drugs frequently used in neonates, but lacking appropriate and validated dosing regimens, is a question applicable to most compounds administered to neonates since off-label use is common practice.

What this study adds

- Propofol glucuronidation in neonates is mainly driven by PNA, while hyperbilirubinaemia was not a useful biomarker of both metabolism and clearance.
- Propofol ED_{50} values for endotracheal intubation are provided for preterm neonates <10 days of age.
- Using initial and total propofol dose ranges of 0.5-2 and 0.5-4.5 mg/kg, respectively, only a moderate decrease in blood pressure was noticed.
- Continuous vital sign measurements are of add-on value and are feasible for evaluation of pharmacodynamic effects. However, validated reference values as well as threshold values are urgently needed to adequately interpret the available data and to assess safety in neonates.

Introduction

Current recommendations indicate that (semi-)elective procedures (e.g. endotracheal intubation, chest tube removal) in neonates should be performed after premedication. Optimal premedication should eliminate pain, discomfort and physiological instability. Furthermore, it should provide conditions for safe and efficient performance of the planned procedure with fast recovery of the sedative effect and without adverse effects [1]. At present, treatment strategies (drug selection and dosing) vary [2,3].

Propofol (2,6 di-isopropylphenol), a short acting anaesthetic, is one of the frequently used drugs in neonates for procedural sedation. After intravenous (iv) administration, it is characterized by rapid distribution to the subcutaneous fat and central nervous system, with subsequent redistribution and metabolic elimination. The main routes of propofol metabolism are glucuronidation [through UDP-glucuronosyltransferase (UGT)1A9 resulting in propofol glucuronide (PG)] and hydroxylation [through cytochrome P450 (CYP)2B6 resulting in 1- or 4-quinol with subsequent glucuronidation to 1- and 4-quinol glucuronide (1-QG and 4-QG) or sulphation]. In adults, also CYP2C9 contributes to the metabolism of propofol [4]. Due to a reduced glucuronidation capacity in early life, a significantly lower contribution of PG metabolite (34% versus 77%) and significantly higher contribution of QG metabolite (65% versus 22%) on urinary propofol metabolite profile were observed in neonates compared to adults [5,6]. However, the impact of various covariates explaining inter-individual variability in neonatal propofol metabolism remains to be established. Therefore, we first aimed to describe urinary propofol metabolite profile during early life and to define covariates of neonatal propofol biotransformation, based on 24 hours (h) urine collections.

The differences in maturation of metabolism, more specifically glucuronidation, throughout age are also reflected by the extensive interindividual variability in propofol clearance (range: 3.7-78.2 ml/kg/min) within the neonatal population [7]. In a previous study, postmenstrual age (PMA) was identified as the most important covariate to explain this variability. An additional impact of postnatal age (PNA) ≥10 days (dichotomous) on propofol clearance was identified. This probably reflects ontogeny of glucuronidation activity, which is activated in the first month of life [7,8]. Besides ontogeny (reflected by age and weight), the influence of other covariates such as disease characteristics [9,10] on neonatal drug metabolism should be

considered. Since the presence or absence of jaundice is a relevant disease characteristic in early life, and since both bilirubin and propofol undergo metabolic elimination though conjugation, we aimed - in the second part of this chapter- to document if indirect bilirubin further improves predictability of propofol clearance in neonates and if it can serve as a clinically useful biomarker of reduced propofol clearance in this population.

Determination of new covariates of propofol clearance can help to optimize individual dosing and safe administration. However, besides insight into the pharmacokinetic (PK) behavior of a fast acting drug like propofol, also pharmacodynamics (PD), including safety, is needed to develop adequate dosing regimens. At present, propofol PD data in neonates are limited. Ghanta *et al* [11] documented a shorter time until sleep or muscle relaxation and shorter time to achieve successful intubation when using propofol compared to a morphine/atropine/suxamethonium regimen in preterm neonates needing semi-elective intubation. Furthermore, our team reported a modest and short-lasting decrease in heart rate and in peripheral oxygen saturation, a short-lasting decrease in cerebral tissue oxygenation index and a slight increase in fractional tissue oxygen extraction after a single bolus propofol (3 mg/kg) administration in neonates. In contrast, a relevant and long-lasting (up to 60 min.) impact on mean arterial blood pressure (MABP) was seen [12]. Significant hypotension (defined as MABP <25 mmHg using oscillometric measurement) was documented by Welzing *et al* [13] investigating propofol 1 mg/kg in preterm (gestational age 29-32 weeks, PNA <8h) neonates undergoing an INSURE procedure [(semi-) elective *in*tubation, intratracheal administration of *sur*factant followed by immediate *e*xtubation [14,15]]. This resulted in preliminary termination of their study [13]. Although the hypotensive effects of propofol were also described by other authors [16-18], reports describing the absence of profound impact on MABP can also be found [11,19]. Due to the gaps in knowledge on propofol dosing and PD effects in newborns, we aimed - as a final step - to combine an exploratory dose-finding approach with the collection of PK and PD data in neonates receiving an iv single propofol bolus for short pre-intubation sedation. The primary objective was to define the ED_{50} (i.e. the effective dose for 50% of patients) for successful INSURE procedure or successful intubation in non-INSURE conditions. In case of intubation for an INSURE procedure the combined outcome of successful intubation and successful extubation needs to be taken into account. This necessitates a balanced approach in defining the optimal dose. Additionally, we aimed to explore the propofol PD data as a safety analysis, to define PD covariates, to link PD with propofol plasma concentrations, and to compare our data with previous reports [11-13].

4.1. Urinary metabolites and its covariates after intravenous propofol bolus in neonates

Methods

Clinical characteristics, ethics, procedural sedation, and sampling

Neonates were included after approval of the study by the ethical board of the University Hospitals Leuven, Belgium, and after informed written consent was signed by the parents (internal study number S33070). Neonates to whom propofol (1-3 mg/kg iv bolus, Diprivan®, AstraZeneca, Belgium) was prescribed for procedural sedation to facilitate endotracheal intubation or elective chest tube removal were considered for inclusion only if a urinary bladder catheter was present for medical reasons.

After iv bolus administration of propofol, urine was collected for 24 h in four consecutive 6 h aliquots. For every collection period, the urine volume was registered and a 5 ml urine sample was stored at -20°C until analysis. Just before the start of the procedure, propofol was administered in addition to the analgesics already administered by continuous (fentanyl or tramadol) or intermittent (acetaminophen) infusion [5].

Clinical characteristics recorded at inclusion were PMA (weeks), PNA (days), body weight (kg), congenital cardiopathy (yes/no) and clinical diagnosis requiring intubation and/or chest tube placement (i.e. cardiac, respiratory or other conditions). Serum creatinine (mg/dL) and indirect serum bilirubin concentrations (mg/dL, in a time interval of 24 h before or after iv propofol bolus administration) were extracted from clinical files. Subsequent dichotomous partitioning of indirect serum bilirubin concentrations was based on fixed cut-off values (as reported earlier to explore the impact of bilirubin on paracetamol clearance in neonates) [9,20] according to PNA, in order to adapt for the normal postnatal transient increase with subsequent decrease of bilirubinaemia in neonatal life (Table 1).

Drug assay

The glucuronides of propofol and its quinol metabolites in urine were determined by high performance liquid chromatography (HPLC) after a dual-step solid phase extraction (SPE) combined with ultraviolet (UV) and fluorescence detection. The method was based on earlier published techniques [21,22] and applications [23-25] but further modifications and improvements were made. To five- or tenfold diluted urine with PBS (phosphate buffer saline) + 0.1% BSA,

Table 1 : Cut-off values for dichotomous partitioning of indirect serum bilirubin adapted from Allegaert 2011 [9] and Palmer 2008 [20], as applied to search of covariates of paracetamol clearance, a drug that also undergoes glucuronidation. Bilirubin conversion used: µmol/L x 0.0585 = mg/dL.

Postnatal age (days)	Indirect bilirubin threshold in µmol/L and mg/dL	
0-1	115 µmol/L	6.728 mg/dL
2-5	155 µmol/L	9.068 mg/dL
6-12	120 µmol/L	7.020 mg/dL
13-19	80 µmol/L	4.680 mg/dL
20-26	45 µmol/L	2.633 mg/dL
>27	10 µmol/L	0.585 mg/dL

20 µL of a mixture of two internal standards [Phenyl-beta-D-glucuronide (PDG) and nitrophenyl-beta-D-glucuronide (NP-DG); 200 and 40 µg/mL respectively] and 300 µL of 50 mM potassium dihydrogenphosphate buffer pH 7.0 were added.
Calibration curves were prepared by addition of standard dilutions of PG, 1-QG and 4-QG to PBS +0.1% BSA in the range of 1.56 to 25 µg/mL. Calibrators and samples were applied to the first SPE column (Oasis MAX 30 mg^{-1} mL, Waters Corporation, Milford, MA, USA). Elution of the glucuronides from the columns was performed by two times 0.5 mL of a mixture of acetonitrile and methanol (75/25, v/v) + 2% formic acid. The eluates were lyophilized and the residues were dissolved in 0.2 mL of PBS + 0.1% BSA and 0.3 mL of 0.15 M phosphoric acid. For further purification, this mixture was applied to an Oasis HLB 30 mg^{-1} mL column (Waters Corporation, Milford, MA, USA). Elution of the glucuronides from this column was performed with two times 0.5 mL methanol + 2% ammonia. After lyophilisation, the residues were dissolved in a mixture of acetonitrile and 0.1 M ammonium acetate buffer pH 5 (85/15, v/v).

Aliquots of 20 to 70 µL were injected on the Atlantis HILIC 5µ column, 250 x4.6 mm I.D (Waters Corporation, Milford, MA, USA). Chromatographic separation was performed with a mobile phase consisting of a mixture of acetonitrile and 0.1 M ammonium acetate buffer (90/10, v/v) at a flow rate of 1 mL/min. Detection was performed simultaneously with UV and fluorescence at 265 nm and 270/310 nm respectively. The peaks of PG, 4-QG, 1-QG, NP-DG and PDG were detected at 10.30, 11.81, 13.35, 15.46 and 19.51 minutes, respectively.

Recovery (mean ±SD) of PG, 4-QG, 1-QG, NP-DG and PDG was 75.4 ± 10.8, 75.8 ± 7.0, 80.1 ± 5.6, 75.4 ± 5.3 and 35.2 ± 13.0% respectively. The internal standard NP-DG has no fluorescence properties, but could be used with high recovery after the dual-SPE extraction only with UV detection. The second internal standard PDG has UV and fluorescence properties, but could be used with high recovery only after the MAX columns. In this study PG was detected and quantified by UV and 4-QG and 1-QG by fluorescence detection.

Intra-assay precision and accuracy were lower than 15% over the entire calibration range from 1.56 to 25 µg/mL for all propofol glucuronides. Inter-day precision was measured by the variation of slopes of the calibration curves: 0.013 ± 0.002 (CV%=16.6, n=27) for PG, 0.060 ± 0.010 (CV%=18.0, n=29) for 4-QG and 0.031 ± 0.004 (CV%=12.9, n=29) for 1-QG. The lower limit of quantification (LLOQ) for PG, 4-QG and 1-QG was set at 0.75 µg/mL using UV detection and at 0.25 µg/mL using the fluorescence detection for 4-QG and 1-QG.

Data reporting, exclusion criteria and statistics

Urine propofol metabolite concentrations (µg/mL) were converted based on their molecular weight (molecular weight propofol=178.27, PG=354.39, and 1-QG or 4-QG=370.38) to calculate total urine mg propofol equivalent elimination and the proportional contribution of each of the metabolites to overall renal propofol excreted over the 24 h period. Total urinary recovery of propofol equivalents (%) was calculated as [(total urinary mg propofol equivalents)/(mg propofol administered)]×100. Urinary PG/QG ratio was determined as PG metabolite (mg propofol equivalents) divided by QG metabolite (mg propofol equivalents). Patients were excluded from the analysis when 24 h urine collection failed or when metabolite recovery exceeded 130% due to HPLC related technical interference. To avoid censoring of data below LLOQ, these concentrations were set to LLOQ/2 as suggested in literature [26].

Statistical analysis was performed using Medcalc®12 software (Mariakerke, Belgium). Urinary metabolite observations and clinical characteristics were reported by median and range when non-normal distribution (Kolmogorov-Smirnov test) was documented. Spearman correlation was used to explore the impact of continuous variables on PG/QG ratio. To determine the impact of different continuous (PMA, PNA, body weight, propofol dose, creatinaemia) and dichotomous variables (PNA early/late, hyperbilirubinaemia yes/no, cardiopathy yes/no) on subgroups of study patients, Mann-Whitney U test (MWU) and Chi-square test (χ^2) were used, respectively. A p-value <0.05 was considered significant.

Results

Descriptive statistics

Data of 40 patients were collected, of whom 32 were available for analysis and 8 neonates were excluded (4/8 had an incomplete 24 h urine collection and in 4/8 cases propofol recovery exceeded 130%). Male/female distribution was 25/7. Propofol was administered for endotracheal intubation (n=19) or chest tube removal (n=13). Clinical characteristics of the included study population are provided in Table 2. Median total urinary recovery of propofol equivalents after single iv bolus administration of propofol in the 24 h urine collection was 40.95 (2.01-129.81)%. The contribution of PG and QG to overall propofol metabolite elimination was 30.50 (0.80-85.60)% and 69.50 (14.40-99.20)% respectively, resulting in a PG/QG ratio of 0.44 (0.01-5.93).

Covariate analysis

PG/QG ratio did not correlate significantly with PNA (rho=0.292, p=0.105), PMA (rho=0.050, p=0.787), body weight (rho=-0.022, p=0.906) or creatinaemia (rho=-0.176, p=0.335). A significant correlation of %PG (PG metabolite in urine/propofol dose administered) with PNA was revealed (rho=0.433, p=0.013), but not between %QG (QG metabolite in urine/propofol dose administered) and PNA (rho=0.038, p=0.839). PG/QG ratio differed significantly between neonates with early PNA, compared to late PNA. Analysis with PNA 10 days as cut-off point for early neonatal life (MWU test, p=0.010) was hereby more significant compared to PNA 7 days (MWU test, p=0.013). There was no significant difference in urine propofol metabolite profile (PG/QG ratio) between neonates with or without cardiopathy (MWU test, p=0.843), also hyperbilirubinaemia was no determinant explaining inter-individual variability in urine metabolite profile (MWU test, p=0.817). PG/QG ratio did not differ with gender (MWU test, p=0.438). Differences in clinical characteristics between neonates with PNA <10 days compared to PNA ≥10 days are presented in Table 3, differences in PG/QG ratio, %PG and %QG are presented in Figure 1.

Table 2: Clinical characteristics, reported by median and range or number of cases, of included patients (n=32) in this study.

Clinical characteristics	Value
Postmenstrual age (PMA, weeks)	36.5 (28 - 43)
Postnatal age (PNA, days)	10 (1 - 32)
Body weight at inclusion (kg)	2.675 (1.070 – 3.965)
Propofol dose administered (mg/kg)	2.094 (0.990 – 4.505)
Creatinaemia (mg/dl)	0.420 (0.190 – 1.340)
Preterm (PMA <37 weeks)	16
Term (PMA ≥37 weeks)	16
Early neonatal life (PNA <10 days)	15
Late neonatal life (PNA ≥10 days)	17
Indirect hyperbilirubinaemia	7
Clinical diagnosis	
Cardiac	*11, of whom*
Coarctatio aortae	5
Univentricular heart	2
Abnormal pulmonary venous return	1
Critical aortic valve stenosis	1
Persistent ductus arteriosus	2
Respiratory	*20, of whom*
Pneumothorax	4
Congenital diaphragmatic hernia	6
Meconium aspiration	1
Other causes of respiratory failure	9
Other	
Pierre Robin sequence	1

In an attempt to further define reliable covariates of propofol metabolism in neonates, the population was divided in 2 groups, according to low (≤10%) versus high (>10%) %PG metabolite recovery in urine (Table 4). Again, incidences of early and late neonatal life differed significantly between both groups. Significance was more pronounced with PNA 10 days (χ^2 test; p=0.022) as cut off point for early neonatal life compared to PNA 7 days (χ^2 test, p=0.043). The incidence of cardiopathy (χ^2 test, p=0.773) or hyperbilirubinaemia (χ^2 test, p=0.681) did not differ significantly between both groups.

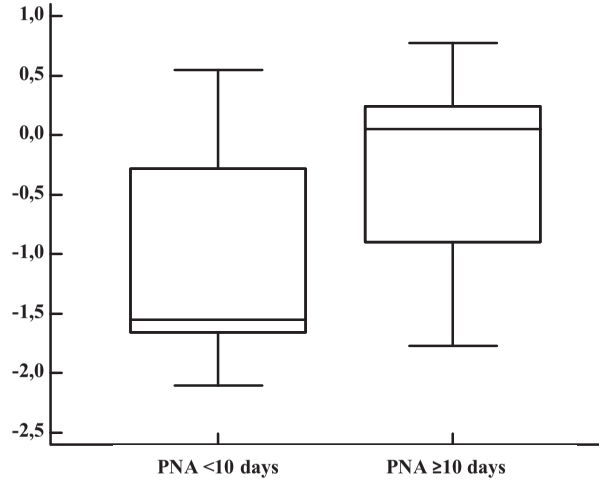

Figure 1a

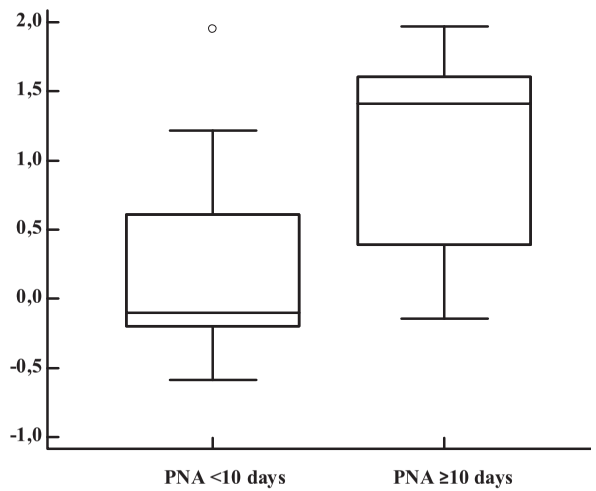

Figure 1b

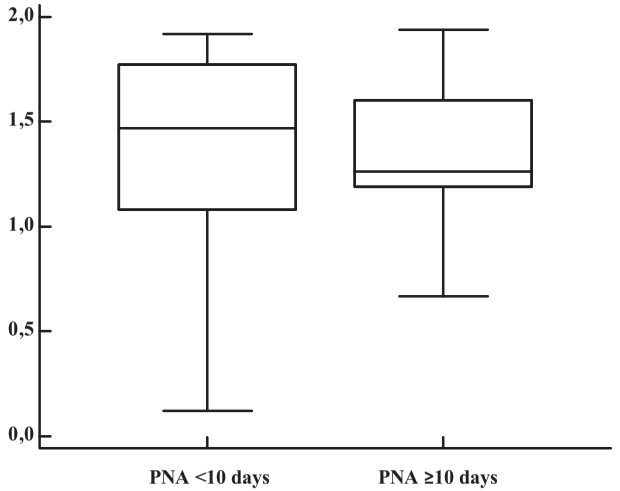

Figure 1c

Figure 1: Comparison of PG/QG ratio, %PG and %QG between patients with PNA < 10 days and patients with PNA ≥ 10 days. Data are represented as log values in box-and-whisker plots. **1a**: Comparison of log PG/QG (MWU, p=0.011), **1b**: Comparison of log %PG (MWU, p=0.002), **1c**: Comparison of log %QG (MWU, p=0.748). PNA: postnatal age, PG: propofol glucuronide, QG: quinol glucuronides, PG/QG represents propofol glucuronide (mg propofol equivalents)/ quinol glucuronides (mg propofol equivalents), %PG represents the ratio of PG metabolite (mg propofol equivalents) on propofol dose administered (mg), %QG represents the ratio of QG metabolite (mg propofol equivalents) on propofol dose administered (mg).

Table 3: Clinical characteristics, reported by median and range or number of cases, of study patients with postnatal age (PNA) < 10 days compared to patients with postnatal age ≥ 10 days. Mann-Whitney U test (continuous variables) and Chi-square test (dichotomous variables) were used for comparison of both groups. * Statistical significant at p<0.05.

Clinical characteristics	PNA <10 days (n=15)	PNA ≥10 days (n=17)	P-value
Postmenstrual age (PMA, weeks)	36 (28-40)	39 (28-43)	0.131
Postnatal age (PNA, days)	2 (1-8)	17 (10-32)	<0.001*
Body weight (kg)	2.850 (1.070-3.900)	2.500 (1.130-3.965)	0.895
Propofol dose (mg/kg)	2.105 (1.015-4.505)	2.083 (0.990-4.140)	0.584
Creatinaemia (mg/dl)	0.660 (0.260-1.050)	0.330 (0.190-1.340)	0.001*
Hyperbilirubinaemia (yes / no)	3 / 12	4 / 7	0.025*
PG/QG ratio	0.028 (0.008-3.512)	1.117 (0.017-5.928)	0.010*
% propofol glucuronide (%PG)	0.791 (0.260-89.320)	25.803 (0.715-93.615)	0.002*
% quinol glucuronides (%QG)	29.607 (1.323-83.029)	18.375 (4.650-86.894)	0.769

Table 4: Clinical characteristics, reported by median and range or number of cases, of all included study patients (n=32) are presented according to low (≤10%) or high (>10%) %PG metabolite retrieved in urine. Mann-Whitney U test (continuous variables) and Chi-square test (dichotomous variables) were used for comparison of both groups. %PG represents the ratio of PG metabolite (mg propofol equivalents) on propofol dose administered (mg), PG: propofol glucuronide. * Statistical significant at p<0.05.

Clinical characteristics	%PG ≤10% (n=20)	%PG >10% (n=12)	P-value
Propofol dose (mg/kg)	2.070 (0.990-4.505)	2.393 (1.724-4.140)	0.206
Body weight (kg)	2.430 (1.070-3.900)	2.945 (1.130-3.965)	0.243
Postmenstrual age (PMA, weeks)	36 (28-42)	38 (28-43)	0.284
Postnatal age (PNA, days)	5.5 (1-32)	15 (1-21)	0.105
Creatinaemia (mg/dl)	0.590 (0.190-1.050)	0.350 (0.200-1.340)	0.220
Early / late neonatal life (PNA 7 days)	12 / 8	2 / 10	0.043*
Early / late neonatal life (PNA 10 days)	13 / 7	2 / 10	0.022*

Discussion

Based on 24 h urine collections in neonates after single iv propofol bolus, we documented a median total propofol metabolite (converted to propofol equivalents) recovery of 40.95 (2.01-129.81)% with only limited contribution of PG metabolite [median 30.50 (0.80-85.60)%] compared to QG metabolite [median 69.50 (14.40-99.20)%]. This resulted in a median PG/QG ratio of 0.44 (0.01-5.93). These observations are in line with earlier published results in a cohort of 8 neonates [5] and confirm overall low glucuronidation capacity in early life [27]. However, this study aimed to further unveil covariates of propofol metabolism in the neonatal age range.

PMA, body weight, cardiopathy and indirect hyperbilirubinaemia did not significantly influence urinary propofol metabolite profile. In contrast, PNA (dichotomous 7 days as well as 10 days) was a significant covariate of PG/QG ratio. Late PNA more frequently resulted in high urinary PG fraction. Analysis with PNA 10 days as cut-off point for early neonatal life was hereby more significant compared to PNA 7 days. The PNA of 10 days is pivotal in early life propofol metabolism. The present study hereby also validates the importance of PNA on propofol glucuronidation. This differs from the hydroxylation pathway since %QG (eliminated/administered dose) does not increase with PNA. To further illustrate the clinical impact, we recalculated clearance values for different PMA and an additional value for neonates with PNA ≥10 days (Table 5). It was earlier documented that introduction of age 10 days in a PK model, predicting neonatal propofol clearance, improved the model [8].

Table 5: Propofol clearance values calculated for neonates with different postmenstrual age (PMA) based on clearance equation [$CL_{std} \cdot (PMA/38)^{11.5}$], with standardized propofol clearance (CL_{std}) at 38 weeks postmenstrual age (PMA) = 0.029 L/min. For neonates with postnatal age (PNA) ≥10 days, equation [$CL_{std} \cdot (PMA/38)^{11.5}$ +0,03] was used, as described by *Allegaert et al* [8]. The results of these calculations illustrate the important role of 10 days PNA in early life propofol biotransformation.

PMA (weeks)	Clearance (L/min)	Clearance for PNA ≥10 days (L/min)
26	<0.001	0.030
30	0.002	0.032
34	0.008	0.038
38	0.029	0.059
42	0.092	0.122

Interestingly, no impact of indirect bilirubinaemia on propofol metabolism was found. Although propofol and bilirubin both undergo hepatic elimination, indirect hyperbilirubinaemia does not seem to influence the metabolic profile of propofol. Taking earlier propofol clearance results [8] and the present urine data into account, age (PMA+PNA) remains the most important clinical parameter when administering propofol to neonates. An additional analysis on the previously published PK model, to explore if bilirubin (instead of or in addition to PNA) could further improve propofol clearance, will be described in the next section of this chapter (section 4.2).

When comparing our neonatal propofol data with reports of propofol metabolism in adults, some similarities (e.g. large variability) but also some differences (e.g. covariates) can be found, taking into account the discrepant methodology (i.e. *in vitro* versus *in vivo*, single propofol bolus versus continuous infusion) [28] often seen in adult studies. The clinical practice to administer propofol (off-label) in neonates is limited to (single) iv bolus. In line with neonates, an extensive inter-subject variability (median PG/QG ratio 3.46, range 2.57-13.3) of propofol biotransformation following iv bolus administration, is also observed in adults [6]. This variability can in part be explained by polymorphisms, gender and advanced age [29]. Urinary glucuronide and sulfate conjugates of 4-hydroxypropofol were found on average in 60, 47, 25 and 24% of total propofol metabolites in urine of 4 unidentified patients, 6 male Caucasian volunteers, 8 Japanese patients and 6 male Caucasian patients respectively [30]. Until now, evidence exploring the role of genotype is not consistent. *In vitro* study of human liver microsomes revealed CYP2B6 as principal determinant of inter-individual variability (19-fold) of propofol hydroxylation [29]. Loryan *et al.* documented no significant effects of CYP2B6 and UGT1A9 single nucleotide polymorphisms (SNPs) on adult propofol metabolism [31]. However, Kansaku *et al.* recently described that CYP2B6 and UGT1A9 genotype were determinant factors of propofol pharmacokinetics and pharmacodynamics [29,31]. Since PNA explained only 8.5% (R^2) of propofol metabolism in our cohort, quantification of the impact of polymorphisms on neonatal propofol metabolism would be interesting. However, since both iso-enzymes (CYP2B6 and UGT1A9) display ontogeny, the impact of polymorphisms might be even much lower in neonates compared to adults [32]. Nevertheless, this will probably depend on the ontogenic patterns of possible polymorphisms and still needs to be studied.

Not age, but gender had significant impact on the formation of propofol metabolites in adults after single iv bolus. In particular, mean values of weight-corrected area under the plasma

concentration-time curve for all propofol glucuronides were significantly higher (1.7-2.1-fold) in women [31]. In neonates, gender was not yet a determinant of propofol metabolism.

The strengths of the present analysis are the relevant study size (n=32), the urine collections during 24 h and the modified propofol quantification technique used. Urine collection up to 24 h provides optimal metabolite recovery and allows comparison with adult data [6]. Our urinary metabolites were determined by HPLC after a dual-step solid phase extraction (SPE) combined with UV and fluorescence detection, which is more specific than analyses used in the past (i.e. HPLC without previous purification of the samples or HPLC with only one detection mode). Furthermore, this is the first report of the analytical modifications used to quantify urine propofol metabolites in neonates.

However, we are aware of limitations of the study. In adults, Hiraoka *et al* defined the kidneys as the major site of extrahepatic propofol metabolism, contributing for one third to total body propofol clearance [33,34]. The presence and impact of renal metabolism in neonatal propofol elimination is unknown and cannot be determined based on urinary metabolite measurements only. However, plasma propofol metabolite data nor organ-specific arterial-venous propofol concentrations are available for neonates. The current data in neonates reflect 'whole body' propofol metabolism. Drug metabolite ratios can be affected by urine pH [35]. In neonates, data on the impact of urine pH or even tubular functions on propofol elimination are unknown. In adults, tubular reabsorption of propofol and its metabolites is described. In early life, overall renal tubular functions are less effective and renal drug clearance almost completely depends on glomerular filtration rate (GFR). At birth, GFR is low and increases during the first two weeks of life, to reach adult values at the age of 8-12 months. These maturational aspects need to be considered when evaluating neonatal urinary drug elimination. To estimate GFR in neonates, serum creatinine is a frequently used marker. Since we revealed no significant correlation between creatinaemia and PG/QG ratio, we assume that metabolite profile does not depend on renal function. However, further investigation is needed.

In conclusion, based on 24 h urine collections in neonates after single iv propofol bolus, we observed a median total metabolite (converted to propofol equivalents) recovery of 40.95 % and a PG/QG ratio of 0.44 (0.01-5.93) We confirmed PNA 10 days as pivotal time point of propofol metabolism in early life. This is in concordance with earlier reported propofol clearance studies in neonates. To define new determinants of inter-individual variability of

neonatal propofol metabolism, further research needs to be encouraged. Finally we want to emphasize that this was the first report of the analytical modifications used to quantify urine propofol metabolites in neonates.

4.2. Is indirect hyperbilirubinaemia a useful biomarker of reduced propofol clearance in neonates ?

Methods

Reported study population and propofol assay

The analysis was based on 235 arterial propofol concentration-time points collected in 25 neonates, up to 24 h after single bolus intravenous (IV) administration of propofol (3 mg/kg, 10 seconds) for procedural sedation (elective chest tube removal or placement, endotracheal intubation) [8].

The propofol assay was based on a reversed phase high performance liquid chromatography (HPLC). The chromatographic system consisted of a waters 600E pump, combined with a Waters autosampler 717 plus and a fluorescence detector (hitachi F-1000) with excitation and emission wavelengths set at 270 and 310 nm, respectively. For further details on bio-analytical techniques (including its variability) and ethical consent procedures, we refer to the initial publication [8].

Serum bilirubin assay

Total bilirubin concentration was determined using a colorimetric DPD (2,5-dichlorophenyl diazonium tetrafluoroborate) method on Roche/ HITACHI – MODULAR (BIL-T Cobas, Roche Diagnostics, Mannheim, Germany). The measuring range was 1.71-513 µmol/L with 1.71 µmol/L as lower limit of detection. Intra- and interday coefficients of variance (CV) were 1.3% and 1.9% respectively. *Direct bilirubin* concentration was determined using Roche/ HITACHI - MODULAR P (D-BIL Cobas, Roche Diagnostics, Mannheim, Germany) based on a colorimetric assay (Jendrassik-Grof procedure). The measuring range was 1.71-171 µmol/L with 1.71 µmol/L as detection limit and with calculated extended upper range up to 342 µmol/L. Intra-and interday CV were 0.6% and 2.0%, respectively. Conversion factors for bilirubin quantification are µmol/L x 0.0585= mg/dL; mg/dL x 17.1= µmol/L. Indirect serum bilirubin was calculated as total serum bilirubin minus direct serum bilirubin.

Population pharmacokinetic analysis

Previously developed model

As previously published [8], a three-compartment model adequately described the propofol pharmacokinetics. The covariates PMA, PNA, gestational age, body weight, gender and renal

function (serum creatinine) were evaluated in the model-building process using forward inclusion and subsequent step-wise backward deletion to confirm the contribution of each covariate. In the final model, all covariates associated with a significant increase in objective function after elimination (i.e. PMA and PNA) were maintained. In the final model, reported standardized propofol clearance (Cl_{std}) at 38 weeks PMA was 0.029 L/min using the equation [Cl_{std} . $(PMA/38)^{b}$] with a power scaling parameter *b* of 11.5. The addition of a fixed value in neonates with a PNA of ≥ 10 days further improved the model and resulted in the equation [Cl_{std} . $(PMA/38)^{11.5}$ + 0.03] L/min [8]. For further details we refer the reader to the initial publication [8].

Current covariate analysis

The model performance was assessed after introduction of unconjugated bilirubin into the previously developed model with PMA as the primary covariate for clearance. Indirect serum bilirubin concentrations (as collected in a time interval of 24 h before or after iv propofol bolus administration for clinical reasons) were retrospectively extracted from clinical files. Subsequent dichotomous partitioning of indirect serum bilirubin was based on fixed cut-off values (Table 1) according to postnatal age, to adapt for the normal postnatal transient increase with subsequent decrease in neonatal life, as explained in section 4.1 [9,20]. Hyperbilirubinaemia was implemented as dichotomous or continuous covariate (both age normalized) into the equation for clearance parameterized as a linear fraction or reduction. Discrimination between different covariate models was made by comparison of the objective function. A value of $p < 0.005$, representing a decrease of 7.8 points in the objective function (χ^2 distribution), was considered statistically significant. In the backward deletion a more stringent p-value ($p < 0.001$) was applied. In addition, goodness-of-fit plots were used for diagnostic purposes.

Data analysis

The current PK analysis was performed using non-linear mixed effect modeling [36] (NONMEM, GloboMax LLC, Hanover, MD, USA, version 6.2) by use of the first-order conditional estimation (Method1) with η-ε interaction. S-plus (Insightful software, Seattle, WA, USA, version 6.2) was used to visualize the data.

Results

Of the 25 neonates, with median weight of 2930 (range 680-4030) g, PMA of 38 (range 27-43) weeks and PNA of 8 (range 1-25) days, serum indirect bilirubin concentrations were available in 23 patients, of which 8 cases had hyperbilirubinaemia, defined as values above age-dependent normal indirect bilirubin values (Table 1, Figure 2).

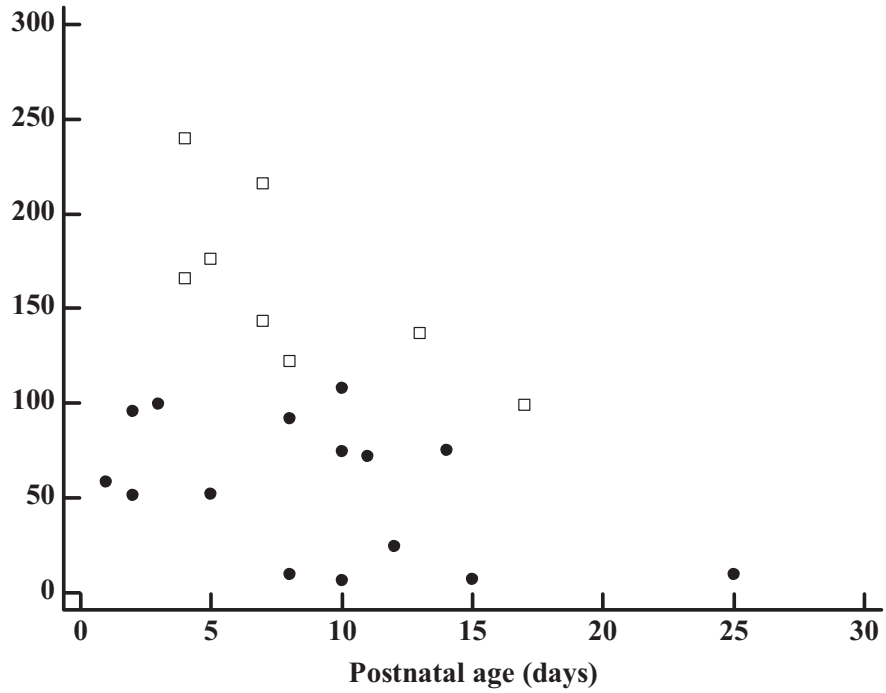

Figure 2: Individual indirect serum bilirubin values, collected in a time interval of 24 hours before or after propofol administration for 23 out of 25 study patients. The data illustrate the postnatal dependent fluctuation in indirect bilirubinaemia. Hyperbilirubinaemia cases are presented by □, cases showing no hyperbilirubinaemia are presented by ●.

Comparison of propofol clearance between cases with or without indirect hyperbilirubinaemia is represented in Table 6. The model using PMA and age normalized dichotomized bilirubin, implemented as a fraction or reduction resulted in a higher objective function compared to the model using PMA and PNA (Table 7). This was also reflected in the goodness of fit plots in which in particular observed versus population predicted concentrations worsened. Evaluation of bilirubin implemented into the model as an age normalized continuous variable did not improve the model. Finally implementation of bilirubin on other pharmacokinetic parameters

proved not statistically significant. While the covariates PMA and bilirubin explained 45% of the inter-individual variability, the covariates PMA and PNA explained 67% of the inter-individual variability of propofol clearance. Introduction of bilirubin, into the PMA+PNA model did not further improve the model ($p > 0.05$).

Table 6: Comparison of propofol clearance (L/min) between cases with and without indirect hyperbilirubinaemia. Data are represented by minimum, 25th percentile, median, 75th percentile and maximum clearance values for both groups.

Propofol clearance value	Cases without hyperbilirubinaemia (L/min)	Cases with hyperbilrubinaemia (L/min)
Minimum	0.0011	0.0012
25th percentile	0.0299	0.0024
median	0.0767	0.0090
75th percentile	0.1029	0.0642
Maximum	0.1286	0.0869

Discussion

Since raised bilirubin is considered as an indicator of deficient hepatic glucuronidation capacity in neonates, we hypothesized that indirect hyperbilirubinaemia could be a predictive biomarker and useful bedside tool to anticipate further reduced propofol clearance in neonates. However, introduction of dichotomous bilirubin values in our propofol PK model, with PMA and PNA as known covariates for clearance, did not further improve clearance predictability.

Besides body weight, propofol clearance variability in humans mainly relates to phenotypic variability in phase 1 and phase 2 iso-enzymes. Propofol undergoes both phase 1 (hydroxylation) as well as phase 2 (glucuronidation) metabolism. In vitro studies revealed the CYP450 isoform CYP2B6 as the principal determinant of inter-individual (19-fold) variability of propofol hydroxylation [30,37,38]. However, median in vitro CYP2B6 expression itself increases with age (0.6 pmol/mg microsomal protein in fetal and neonatal hepatic samples; 1.6 pmol/mg for infant to adolescent samples and 4 pmol/mg in adult samples) [32]. Glucuronidation of propofol occurs by UGT1A9.

Table 7: Model-based pharmacokinetic parameters estimates for the different models.

Parameters	PMA+PNA model	PMA+bilirubin (fraction)	PMA+bilirubin (reduction)
Objective function (points)	-269.026	-240.408	-241.021
Fixed effects			-
CL= CLp x (PMA/median)^{o} + p (if PNA >10)	0.0289	-	-
o	12	-	-
p	0.0305	-	-
CL= CLp x (PMA/median)^{o} x p (if dichotomous bilirubin =1)	-	0.0494	-
o	-	6.51	-
p	-	0.299	-
CL= CLp x (PMA/median)^{o} – p (if dichotomous bilirubin =1)	-	-	0.046
o	-	-	8.38
p	-	-	0.00635
V1	1.42	1.41	1.78
Q2	0.0391	0.0381	0.0364
V2	15.9	15.9	15.7
Q3	0.0842	0.0875	0.0962
V3	1.18	1.21	1.27
Interindividual variability			
CL	0.582	1.07	1.15
V1	0.581	0.486	1.2
Q2	0.354	0.278	0.344
Residual variability			
Proportional	0.0403	0.0404	0.0403

CL= Clearance (L/min) ; CLp= Population value for clearance (L/min); o:Power scaling parameter; p: Plus clearance constant if postnatal age ≥ 10 days; PMA: Postmenstrual age; PNA: Postnatal age; Q2: Intercompartmental clearance between central and peripheral 1 (L/min); Q3: Intercompartmental clearance between central and peripheral 2 (L/min); V1: Central volume (L); V2: Peripheral volume 1 (L); V3 = Peripheral volume 2 (L).

In neonates, phenotypic maturation in glucuronidation activity mainly reflects age-dependent phase 2 iso-enzyme maturation [27,39]. This impact of ontogeny on glucuronidation, was also

confirmed by Knibbe *et al* who defined body weight as most predictive parameter for glucuronidation capacity of morphine under the age of 3 years with a large group of preterm and term neonates in the dataset [40,41]. Although the iso-enzymes involved for conjugation of bilirubin (UGT1A1) and propofol (UGT1A9) are different, they display a similar maturational pattern. Adult activity levels of UGT1A1 and UGT1A9 are reached at 3-6 months [42] and 4 months [43] after birth, respectively. Besides ontogeny it is to be anticipated that disease characteristics also play a contributing role in phenotypic drug (metabolic) elimination clearance. Based on the link between phenotypic glucuronidation and propofol clearance, a focused search on the applicability of indirect bilirubinaemia as biomarker for further reduced propofol clearance was reasonable.

Biomarkers (defined as characteristics that can be measured and evaluated as indicators of normal biologic and pathogenic processes) are increasingly being integrated into clinical practice [44], since, to a certain extent, they reflect disease status. In this study, we focused on raised indirect bilirubinaemia, a characteristic frequently present in the neonatal population. Furthermore, serum bilirubin is routinely quantified in clinically icteric newborns. However, we failed to document that indirect hyperbilirubinaemia is a useful biomarker of reduced propofol clearance within this age group. In adults, Song *et al* reported that there was no influence of obstructive jaundice on propofol pharmacokinetics compared with patients without obstructive jaundice [45]. We have to be aware that the mechanism of jaundice in obstructive pathology (resulting in elevated direct serum bilirubin) differs from neonatal jaundice (mainly elevated indirect serum bilirubin).

We claim that our negative findings are probably due to the fact that jaundice in the first days of life is the phenotypic result of an imbalance between (indirect) bilirubin synthesis (e.g. hemolysis) and bilirubin clearance (e.g. conjugation by the iso-enzyme UGT1A1) capacity [46]. Consequently, jaundice can be present in the setting of increased synthesis despite effective conjugation capacity, while in the absence of bilirubin synthesis, deficient conjugation capacity will remain subclinical. Besides this claimed explanation, one may also suggest that hyperbilirubinaemia affects propofol clearance through an increase in free propofol concentration, since both compounds bind to human serum albumin (HSA). However, Zhou and Liu described no overlap in HSA binding sites for bilirubin and propofol [47], instead of, for example cefazolin (see chapter 5) and ibuprofen displaying competition for albumin binding places [48,49]. In addition, neonates frequently display hypoalbuminaemia during the first days of life, possibly resulting in a significant increase in free propofol concentration.

The main aim of evaluating biomarkers and other covariates of neonatal propofol clearance in PK models is to increase individual clearance predictability. This knowledge can be introduced in anaesthetic applications such as target controlled infusion systems [50]. At present, only 67% of propofol clearance variability in neonates can be explained by PMA and PNA. Since indirect bilirubinaemia has no major influence, a search for other covariates and/or biomarkers is warranted, although we are unsure about the markers to focus on. Propofol is a high extraction ratio drug and, as determined in adults, clearance depends on the liver blood flow. While there are no good data on hepatic blood flow in relation to age, hepatic blood flow in infants is suggested to be comparable to adult values [51]. This means that rather than hepatic blood flow, maturational aspects (immature metabolizing enzymes) limit propofol clearance in neonates. Considering polymorphisms of metabolizing enzymes (CYP2B6, UGT1A9) [32], there is only a limited impact of these polymorphisms in adults. Since both iso-enzymes display ontogeny, the impact of these polymorphisms in neonates are assumed to be much lower. As mentioned earlier (section 4.1), this still needs to be studied. [30,32,37-39].

Conclusion

Although only based on observations collected in 25 neonates, we conclude that ontogeny itself, reflected by PMA and PNA, is a more relevant clinical predictor of reduced propofol clearance in neonates than hyperbilirubinaemia, a specific disease characteristic potentially reflecting deficient conjugation capacity. Such observations are also of clinical relevance since propofol has become a popular intravenous drug for induction and maintenance of anesthesia, even in neonates. Based on the current observations, propofol doses should be reduced in early (PNA of <10 days) life, independent of the presence or absence of indirect hyperbilirubinaemia [52].

4.3. Exploratory dose finding study in neonates receiving a single intravenous propofol bolus for (semi-) elective endotracheal intubation: preliminary analysis

Methods

Study population, inclusion criteria and ethics

Neonates admitted to the Neonatal Intensive Care Unit (NICU) of the University Hospitals Leuven who need short procedural sedation for (semi-) elective intubation were considered for inclusion, after informed written consent of the parents. Patients considered for inclusion had to be hemodynamically stable and did not receive sedative or analgesic agents (with exception of paracetamol) during the previous 24 hours. INSURE (intubation-surfactant-extubation) procedures in the UZ Leuven NICU are mainly performed in neonates below 34 weeks of gestation, with a spontaneous respiratory drive, but characterized by an oxygen need >30% $Fi0_2$ while receiving nasal continuous positive airway pressure (nCPAP) and evidence of respiratory distress syndrome. The study (EudraCT nr 2012-002648-26) was registered at ClinicalTrials.gov and approved by the ethical board of our hospital. At present, the data collection of the 50 patients is completed. This chapter contains results of the first 35 patients as a preliminary safety analysis.

Clinical characteristics at birth [gestational age (GA, weeks), birth weight (BW, grams), Apgar score at 1 and 5 minutes, gender (male/female)] and at moment of propofol administration [postnatal age (PNA, in days and in hours after birth), postmenstrual age (PMA, weeks), current weight (CW, grams), propofol indication (INSURE versus non-INSURE), initial propofol dose (mg/kg) and total propofol dose (mg/kg)] were collected from the patient medical files. In case of an INSURE procedure, surfactant dose, time to surfactant administration, time to extubation and the need for reintubation (within 12 hours after the procedure) were also recorded.

Drug administration

Propofol (Diprivan 1%, AstraZeneca, Brussels, Belgium) is used as routine sedative agent for (semi-)elective intubation, including INSURE procedures, in the UZ Leuven NICU. Propofol is administered as intravenous bolus, immediately followed by NaCl 0.9% 1 ml/kg during 30 seconds.

Efficacy

Dose finding approach

Based on available pharmacokinetic (PK) data on propofol in neonates, PMA and PNA < or ≥ 10 days are defined as major covariates of propofol clearance [8]. Therefore, neonates included in the current study were stratified according to PMA [group 1: <28 weeks, group 2: 28-31 6/7 weeks, group 3: 32-36 6/7 weeks and group 4: ≥37 weeks] and PNA (<10 days versus ≥10 days) as presented in Table 8. Eight stratums were hereby considered.

Table 8: Stratification of included study patients in 4 groups and 8 strata.

Group	Postmenstrual age (PMA)	Postnatal age (PNA)	Stratum
Group 1	< 28 weeks	PNA < 10 days	1
		PNA ≥ 10 days	2
Group 2	28- 31 6/7 weeks	PNA < 10 days	3
		PNA ≥ 10 days	4
Group 3	32-36 6/7 weeks	PNA <10 days	5
		PNA ≥ 10 days	6
Group 4	≥ 37 weeks	PNA < 10 days	7
		PNA ≥ 10 days	8

The first patient in each stratum received an initial propofol dose of 1 mg/kg. If sedation and relaxation of the patient, perceived by the treating physician, was unsatisfactory, additional propofol (i.e. second dose always 1 mg/kg, if still unsatisfactory titration up to satisfactory clinical condition was achieved) was administered. In order not to interfere with routine clinical practice, the decision to give additional propofol as well as the decision to start the intubation was made by the treating physician. In the UZ Leuven NICU, endotracheal intubation mainly occurs by nasal route.

The initial propofol dose for the next patient in the same stratum was based on the outcome of the previous patient, using the up-and-down method [53]. The minimum and maximum predefined initial doses were 0.5 and 4 mg/kg, respectively. Successful outcome was defined as an endotracheal intubation with satisfactory sedation and relaxation (assessed by the treating physician) without the need for additional propofol (and extubation within 1 hour after propofol administration in case of INSURE procedure). If additional propofol was

needed, the initial dose for the next patient in the same stratum was increased with 0.5 mg/kg. If no additional propofol was needed, a decrease (-0.5 mg/kg) in initial dose was applied for the next patient in the same stratum.

Intubation procedure

The number of intubation attempts, the time to successful intubation (i.e. time from propofol administration until the clinician who performs the intubation considers the tube to be in the correct endotracheal position based on clinical inspection of the patient and auscultation) and the physician performing the (final) successful intubation (registrar, fellow, neonatologist) were recorded. The UZ Leuven NICU is a university training center. When the tube is not in the correct position after 1-2 attempts, intubation is performed by the supervising neonatologist.

Propofol whole blood concentrations

Blood sampling

Blood samples for quantification of propofol concentration were collected at 3 hours (h) and, if possible, at 12 h after propofol administration. Only neonates with an arterial line were included for blood sampling or alternatively samples were taken by venous puncture, when sampling for medical reasons was necessary. Blood samples (300-600 µL/sample) were collected in oxalate tubes (BD Vacutainer) and a maximum total blood volume of 1 mL/kg collected in each individual was respected.

Drug assay

Blood samples were stored at 4°C, for a maximum period of 4 weeks, until processing. To 1 volume of whole blood, 0.1 volume of the internal standard (thymol, 5 µg/mL in 50:50 methanol:water) and 2 volumes of acetonitrile were added. Subsequently, samples were vortexed 2 times for 15 seconds, before being centrifuged (20816 *g*) at 4°C for 10 min. The supernatant was transferred to a new recipient and stored at -20°C until analysis. At the day of analysis, the supernatant was thawed, vortexed and centrifuged (20816 *g*) at 4°C for 10 min. 150 µl of each supernatant was transferred into a micro-insert for HPLC (high performance liquid chromatography)-vials and injected directly into the HPLC-system. The HPLC-system consisted of a Waters 600E pump, combined with a Waters 717plus autosampler (8°C) and a Waters 2475 multi λ fluoresence detector (ex/em: 270/310 nm). The injection volume was 35 µL. Chromatographic separation of propofol (RT: 9 min) and thymol (RT: 6 min) over a total

run time of 12 min, was performed on a Gemini® C18 column (3 µm, 4.6 mm x 150 mm, Phenomenex, Utrecht, The Netherlands) protected with a Gemini® C18 SecurityGuard® cartridge (3 µm, 4 mm x 3 mm, Phenomenex, Utrecht, The Netherlands) both maintained at 30°C. The mobile phase consisted of a mixture of acetonitrile and water (0.1% v/v formic acid) (70:30, v/v) which was delivered to the system isocratically at a flow rate of 0.6 mL/min. Calibration curves of propofol were constructed at 10X concentrations in a mixture of methanol in water (50% v/v). 20 µL of each concentration was transferred to 180 µL of whole blood, after which they were processed in the same manner as whole blood samples. Calibration curves were found to be linear in the range of 0.005-20 µg/mL. Quality control samples were prepared at concentrations of 0.05, 0.5 and 5 µg/mL, processed and stored at -20°C. Intra- and interday coefficients of variation were lower than 10% and the LOD and LLOQ valued 0.0014 µg/mL and 0.0048 µg/mL, respectively.

Propofol pharmacodynamics

Relaxation and sedation scores

Relaxation and sedation scores were collected by 1 of 2 observers using predefined scoring systems. The scores were only used for retrospective evaluation of sedation and relaxation status of the patient, without interfering with decisions taken by the treating physician during the intubation procedure. Scores were evaluated every 2 minutes, from 5 minutes before up to 21 minutes after propofol administration. Additionally, at the moment of propofol administration (time=0) and 1 minute thereafter, scores were collected. Relaxation was evaluated by clinical evaluation of the tone in arms and legs. Four degrees of relaxation, adapted from Naulaers *et al* [54,55] were considered (grade 1: hypertonic, grade 2: normal tone, grade 3: mildly hypotonic, grade 4: hypotonic) with effective relaxation defined as a score > grade 2. The degree of sedation was assessed as the motor response to external stimuli. As a stimulus 'heel-rubbing' as described by Grunau *et al* [56] was used. Four degrees of sedation were considered (grade 1: moves spontaneously, grade 2: moves when touched, grade 3: moves when stimulated, grade 4: no reaction to stimulus) [54,55], with effective sedation defined as a score > grade 2.

Intubation condition score

The intubation conditions at the moment of the final intubation were retrospectively scored by the treating physician who finally performed the intubation. The intubation condition score (ICS) according to Viby-Mogensen (Table 9) [1] was hereby used. The score was only

documented for retrospective evaluation of the final intubation conditions and good condition was defined as a total ICS ≤ 10, without taking the subscores into account.

Table 9: The Viby-Mogensen Intubation Condition Score (ICS) used in this study [1]

Intubation Condition Score [1]					
	1	**2**	**3**	**4**	**Total**
Laryngoscopy	Easy	Fair	Difficult	Impossible	
Vocal cords	Open	Moving	Closing	Closed	
Coughing	None	slight	Moderate	Severe	
Jaw relaxation	Complete	Slight	Stiff	Rigid	
Limb movement	None	Slight	Moderate	Severe	
Total					

Vital signs and cerebral oxygenation

Vital signs [heart rate (HR, beats per minute, bpm), mean arterial blood pressure (MABP, mmHg), peripheral oxygen saturation (SaO_2, %), respiration rate (RR, breaths per minute) and perfusion index (PI, %, a relative assessment of the pulse strength at a specific monitoring site, calculated as the ratio between pulsatile and non-pulsatile signals by the pulse oxymeter [57])] were measured using IntelliVue MP70 (Philips, The Netherlands) with Nellcor Pulse Oxymeter sensor from 2 minutes before up to 12 hours after propofol administration. Data were recorded simultaneously and continuously on a personal computer with a sampling rate of 2 Hertz using Rugloop® (RUG, Gent, Belgium) and subsequently converted using Rugloop Converter in Excel. MABP was measured invasively if an indwelling arterial line was present. If not, manual blood pressure was measured at least every 5 minutes as safety parameter after propofol administration, but was not used for further analysis.

Near infra-red spectroscopy (NIRS) determined regional cerebral oxygen saturation ($rScO_2$, %) was used as a reliable estimator for changes in regional cerebral oxygenation [58]. An INVOS 5100 near infrared spectrometer (Somanetics Corp., Troy, Michigan, USA) was used to measure this parameter. The cerebral neonatal OxyAlert NIRSensor (Covidien) was attached to the fronto-parietal left side of the neonatal skull. To investigate the balance between oxygen delivery and oxygen consumption, the cerebral fractional tissue oxygen extraction (cFTOE) was calculated as [(SaO_2-$rScO_2$)/SaO_2]. An increase of cFTOE might indicate a reduced oxygen delivery to the brain with a constant oxygen consumption of the

brain or higher oxygen consumption than oxygen delivery. A decrease of cFTOE suggests a decrease of oxygen extraction of the brain due to less oxygen use or a constant oxygen consumption of the brain with an increased oxygen delivery to the brain [59,60].

Data analysis and statistics

Descriptive statistics

Clinical characteristics were reported for the 4 patients groups (as defined in Table 8) by median (range) or incidence. To explore continuous and dichotomous covariates between the patient groups, the Kruskal-Wallis test and Fisher exact test was used, respectively. For the patients receiving propofol for INSURE indication, time until in-and extubation were compared between 'success' versus 'failure' outcome (Mann-Whitney U test). A p-value <0.05 was considered statistically significant. Analysis was performed using SPSS (IBM statistical software, version 20.0, Armonk, New York, IBM Corp.) and Medcalc (Medcalc Statistical Software version 13.1.7, Ostend, Belgium).

Covariates of observed propofol concentrations

Whole blood propofol concentrations (µg/mL) at 3 h and 12 h after propofol administration were presented as median and range. To explore covariates of variability in propofol concentrations at 3 h and 12 h after propofol administration, univariate regression (continuous covariates) and Mann Whitney U test (dichotomous covariates) were used. Covariates significant in univariate analysis were entered in a multiple regression analysis.

Propofol pharmacodynamics

Signal processing of vital signs

Data analysis was performed using MATLAB (MATLAB Release 2013a, The MathWorks, Inc., Natick, Massachusetts, USA). Data were filtered using a median filter with a length of 10 samples. Remaining artefacts were detected manually and replaced by 'not a number' symbol (NaN). For each measured variable, its baseline value was computed as the median of the segment comprised by the 2 minutes prior to propofol administration. In addition, the MABP (mmHg) was corrected for PMA (weeks), using the following formula cMABP = MABP-PMA, where cMABP represents the corrected MABP.

To explore individual changes in the included parameters, the minimum value for MABP, HR, SaO_2, $rScO_2$ and PI after propofol administration was computed as % change from baseline value using the following formula: $\Delta X[\%] = 100*[\min(X)-\text{Baseline}(X)]$

/Baseline(X), where X represents the measured variable, min(X) represents the minimum value of X, and Baseline(X) represent the Baseline value computed for X. For the FTOE the absolute minimum and maximum value was computed.

The extent and duration in fluctuations of vital signs was taken into account by calculating the area under the curve (AUC). The AUC for each measured variable was computed using trapezoidal numerical integration. This integration is calculated as the sum of all the data in a given signal X multiplied by the sampling period, which is the inverse of the sampling frequency. For AUC calculation of a given signal X related to a predefined threshold value (TH), trapezoidal integration was used by integrating the signal $\Delta X=X-TH$, and replacing all the positive numbers in ΔX by 0 before integration. Using this approach, the AUC for cMABP, HR, SaO_2 $rScO_2$ and PI was computed using 0 mmHg, 100 bpm [61], 85% [62], 65% [63,64] and 0.44% [57] as threshold, respectively. Since at present, no cut-off value for cFTOE in neonates is available, AUC and AAC (area above the curve) were calculated form the baseline value for each individual.

Data were graphically presented for the 4 patient groups as defined in Table 8. Both a median trend line with interquartile range (p25-p75) as well as individual trend lines from the continuous measurements were plotted.

To explore covariates of AUC cMABP, AUC SaO_2 and AUC $rScO_2$ after propofol administration, univariate linear regression (for continuous covariates) and Mann Whitney U test (dichotomous covariates) were performed using clinical characteristics and propofol concentrations at 3 h and 12 h. Covariates significant in univariate analysis could subsequently be included in a multiple regression analysis.

Propofol ED_{50} calculation

An up-and-down dose-response design was used to determine the propofol ED_{50} dose (mg/kg). The ED_{50} was calculated separately in each stratum with an effective sampling size of at least N=6, using the Dixon-Massey method [53] for small sample size. The effective sample size (N) is the number of trials reduced by one less than the number of similar responses at the beginning of the series. The ED_{50} is the average of the N doses with a correction factor added, as presented in equation 1:

$$ED_{50} = \sum X_i/N + d(A + C)/N \qquad \text{Equation 1}$$

The X_i's are the initial propofol doses of the final N trials. The correction factor is a weighted function [the weight equals the interval between dose levels (d= 0.5) divided by N] of two tabulated values, A and C. Value A is obtained as a function of the difference in ineffective (i.e. failed outcome) and effective (i.e. successful outcome) responses and value C is a function of the number of similar responses in the beginning of the series. The respective values for A and C were obtained as provided by Dixon (Table 10) [53]. If there are no similar responses at the beginning of the series and there is no difference in ineffective and effective responses, the ED_{50} is simply the observed average of the used dose levels. Analyses have been performed using SAS software, version 9.2 of the SAS System for Windows.

Table 10: Values for A and C to determine ED_{50} as described by Dixon [53]. n□ = number of ineffective responses, n■= number of effective responses. C=0 for a series whose first part is a single □ or ■.

		C for test series whose first part is			
n□ - n■	**A**	□□	□□□	□□□□	□□□□□
5	10.8	0	0	0	0
4	7.72	0	0	0	0
3	5.22	0.03	0.03	0.03	0.03
2	3.20	0.10	0.10	0.10	0.10
1	1.53	0.16	0.17	0.17	0.17
0	0	0.44	0.48	0.48	0.48
-1	-1.55	0.55	0.65	0.65	0.65
-2	-3.30	1.14	1.36	1.38	1.38
-3	-5.22	1.77	2.16	2.22	2.22
-4	-7.55	2.48	3.36	3.52	3.56
-5	-10.3	3.5	4.8	5.2	5.3
n■ - n□	**- A**	**■■**	**■■■**	**■■■■**	**■■■■■**
		-C for test series whose first part is			

Results

Descriptive statistics

Study population

Data were prospectively collected in 35 patients. Clinical characteristics for the 4 patient groups are provided in Table 11. Continuous registration of vital signs was available for 34/35 patients, blood pressure was measured invasively in 28/35 patients. In 30 cases, the indication

for propofol administration was an INSURE procedure, in 5 cases a non-INSURE condition (e.g. renal biopsy, respiratory failure, clipping patent ductus arteriosus) was documented.

Efficacy

Intubation procedure

Successful intubation was achieved after the first intubation attempt in 60% of the patients, 25.7% was intubated after the second attempt and 14.3% of patients after the third attempt. Of those who were intubated after the first attempt, intubation was performed by registrars in 42.9%, by fellows in 4.8% and by neonatologists in 52.4% of cases. Overall, in 31.4% of patients final successful intubation was achieved by registrars, in 2.9% by fellows and 65.7% of final intubations were performed by neonatologists.

In 57.1% of cases the initial propofol dose was sufficient, in 25.7% 1 additional dose was administered (dosing according to protocol), in 5.7% 2 additional doses and in 11.4% up to 3 additional propofol doses were needed for successful intubation. Total propofol dose (mg/kg) and time to intubation for the 4 patient groups are presented in Figure 3.

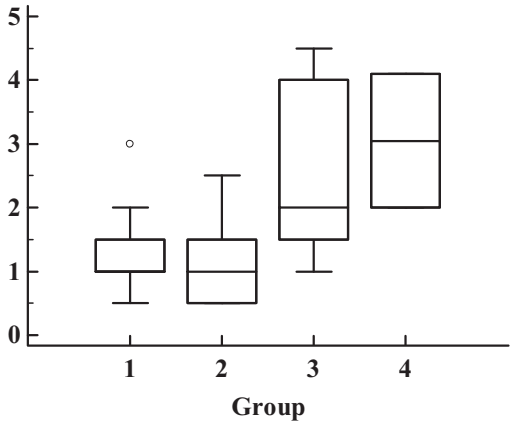

Figure 3a

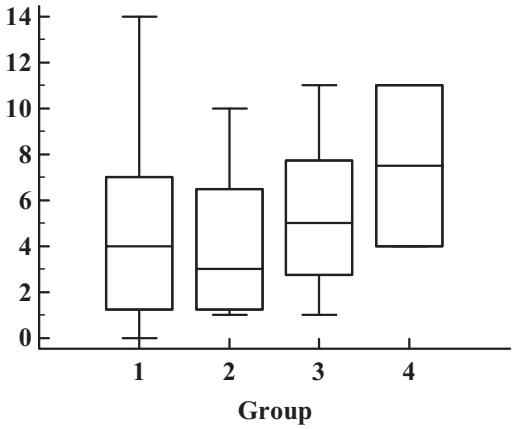

Figure 3b

Figure 3: (a) Total propofol dose (mg/kg) and (b) time to intubation (minutes) after propofol administration presented as boxplots for the 4 patient groups as defined in Table 8.

Table 11: Clinical characteristics of the 35 included patients. Data are provided for the 4 patient groups, as presented in Table 8. To explore continuous and dichotomous covariates between the patient groups, the Kruskal-Wallis test and Fisher exact test was used, respectively. A p-value <0.05 was considered statistically significant. HR: heart rate, MABP: mean arterial blood pressure, SaO_2: peripheral oxygen saturation, cFTOE: cerebral fractional tissue oxygen extraction, $rScO_2$: regional cerebral oxygen saturation, RR: respiration rate, PI: perfusion index.

Clinical characteristics	Group 1 (n=11)	Group 2 (n=15)	Group 3 (n=7)	Group 4 (n=2)	p-value
Gender (male / female)	8/3	7/8	3/4	1/1	0.594
Insure / non-insure indication	9/2	14/1	6/1	1/1	0.360
Birth weight (gram)	970 (540-1200)	1440 (780-1900)	2520 (1600-2775)	2740 (2190-3290)	<0.000
Current weight (gram)	970 (540-1200)	1440 (780-1900)	2435 (1600-2775)	2990 (2690-3290)	<0.000
GA (weeks)	26.86 (25.00-27.57)	30.14 (27.00-31.86)	33.71 (32.00-36.57)	36.36 (35.57-37.14)	<0.000
PMA (weeks)	27 (25.00-27.71)	30.14 (28.00-31.86)	33.71 (32.00-36.86)	37.78 (37.28-38.28)	<0.000
PNA (days)	1 (1-4)	1 (1-19)	1 (1-3)	10.5 (2-19)	0.077
PNA (hours:minutes)	5:49 (1:10-85:28)	3:59 (1:35-454:46)	14:16 (6:35-67:42)	236:39 (24.34-448.44)	0.055
Apgar score 1 minute	8 (4-9)	7 (4-9)	8 (6-9)	7 (6-8)	0.642
Apgar score 5 minutes	9 (6-10)	9 (6-9)	9 (7-10)	8 (8-8)	0.441
Baseline HR (bpm)	152 (128-167) (n=10)	157 (123-185)	147 (136-205)	158 (153-163)	0.553
Baseline MABP (mmHg)	36 (25-49) (n=9)	41 (31-54) (n=11)	53 (40-57) (n=4)	58.5 (42-75)	0.036
Baseline SaO_2 (%)	91 (87-98) (n=10)	91 (80-100)	94 (79-99)	96 (94-98)	0.154
Baseline cFTOE	0.234 (0.140-0.423) (n=9)	0.236 (0.000-0.411)	0.172 (0.000-0.362)	0.102 (0.000-0.204)	0.249
Baseline $rScO_2$ (%)	70 (53-77.5) (n=9)	70 (47.5-92)	78 (50-85)	86 (77-95)	0.062
Baseline RR (breaths/minute)	40 (31-61) (n=10)	42 (23-56)	49 (38-58)	60 (57-63)	0.564
Baseline PI (%)	0.763 (0.480-1.200) (n=10)	0.990 (0.290-1.800)	0.910 (0.470-1.500)	0.720 (0.520-0.920)	0.672

Using the up-and-down dose finding approach, successful outcome (i.e. successful intubation with satisfactory sedation and relaxation as defined by the treating physician without the need for additional propofol, and in case of INSURE procedure, with subsequent successful extubation within 60 minutes) was achieved in 51.4% of cases (54.5% in group 1, 60% in group 2, 42.9% in group 3 and 0% in group 4). Of the 30 INSURE cases, 17 had a successful outcome, 13 failed. Of these 13 failed cases, 3 neonates were (still) intubated 60 minutes after propofol administration (2 were not yet extubated: 1 due to pneumothorax, 1 due to repeated bradycardia and desaturation events, additionally 1 patient needed reintubation 24 minutes after extubation). In 11 failed cases, additional propofol was needed to achieve sufficient sedation prior to intubation.

Since most included neonates received propofol for INSURE indication, information concerning time to intubation, surfactant administration and extubation (all expressed as minutes after propofol administration) is presented in Table 12.

Table 12: Time to intubation, surfactant administration and extubation (expressed as minutes after propofol administration) for patients receiving propofol for INSURE indication. Data are presented as median and range. Mann Whitney U test was used to compare cases with outcome success (i.e. successful intubation with satisfactory sedation and relaxation as defined by the treating physician without the need for additional propofol, and in case of INSURE procedure, also successful extubation within 60 minutes) versus failure.

INSURE				
Parameter	**All patients (n=30)**	**Outcome success (n=17)**	**Outcome failure (n=13)**	**p-value**
Time to intubation (min)	4 (0-14)	2 (0-7)	7 (2-14)	0.0002
Time to surfactant (min)	6 (1-17)	4 (1-8)	9 (3-17)	0.0006
Time to extubation (min)	10 (4-2687)	8 (4-14)	15 (7-2687)	0.0011

Propofol whole blood concentrations

Median (range) propofol concentration at 3 h after propofol administration, based on 28 collected samples, was 0.200 (0.034-1.110) µg/mL. At 12 h after propofol administration, 19 samples were available and median (range) concentration was 0.093 (0.035-0.467) µg/mL.

Taking all samples into account, total propofol dose (mg/kg) was significantly associated with concentrations achieved at 3 h (p=0.0003) and at 12 h (p=0.0001). Neonates with a successful outcome, displayed significantly lower median propofol concentrations at 3 h and 12 h compared to those with a failed outcome (at 3h 0.101 µg/mL versus 0.274 µg/mL, p=0.010; at 12 h 0.068 µg/mL versus 0.163 µg/mL, p=0.041). This can be explained by the fact that most cases with a failed outcome were due to the need of insufficient sedation after the initial propofol dose (n=11/13), requiring additional propofol administration. In a multiple regression analysis, only total propofol dose remained significant to explain variability in propofol concentrations at 3 h and 12 h after administration.

When exploring covariates of propofol concentrations in the subgroup receiving a total propofol dose <2 mg/kg, propofol concentrations at 3 h were significantly associated with weight (BW and CW), age (GA and PMA) and total propofol dose (mg/kg). Since both BW and CW, and GA and PMA are highly correlated, only PMA and total dose were included in a multiple regression analysis. Both remained significantly associated with concentrations at 3 h. Concentrations at 12 h were significantly associated with weight (either BW or CW).

Propofol pharmacodynamics

Relaxation, sedation and intubation condition scores

Evolution of relaxation and sedation scores achieved in the 35 patients are presented in Figure 4 and 5 respectively. Median (range) intubation condition score was 7 (5-13), 6 (5-10), 9 (6-10) and 8 (7-9) for group 1, 2, 3 and 4 respectively and did not differ significantly across the 4 groups (p=0.231).

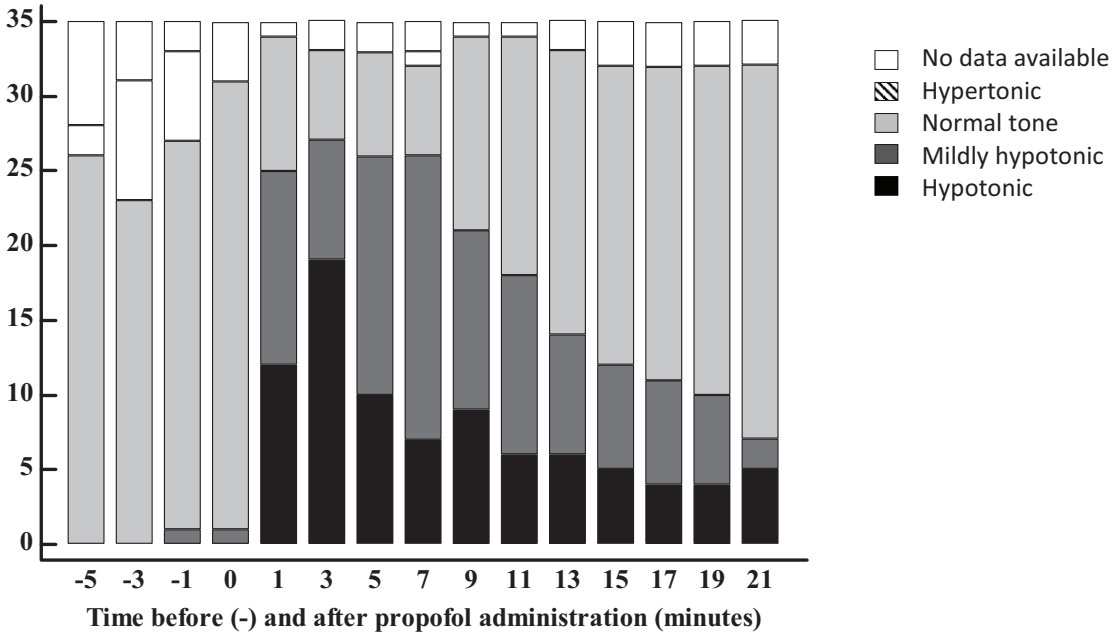

Figure 4: Relaxation scores, adapted from Naulaers et al [54,55], collected in the 35 patients from 5 minutes before up to 21 minutes after propofol administration (time=0). Y-axis: number of patients.

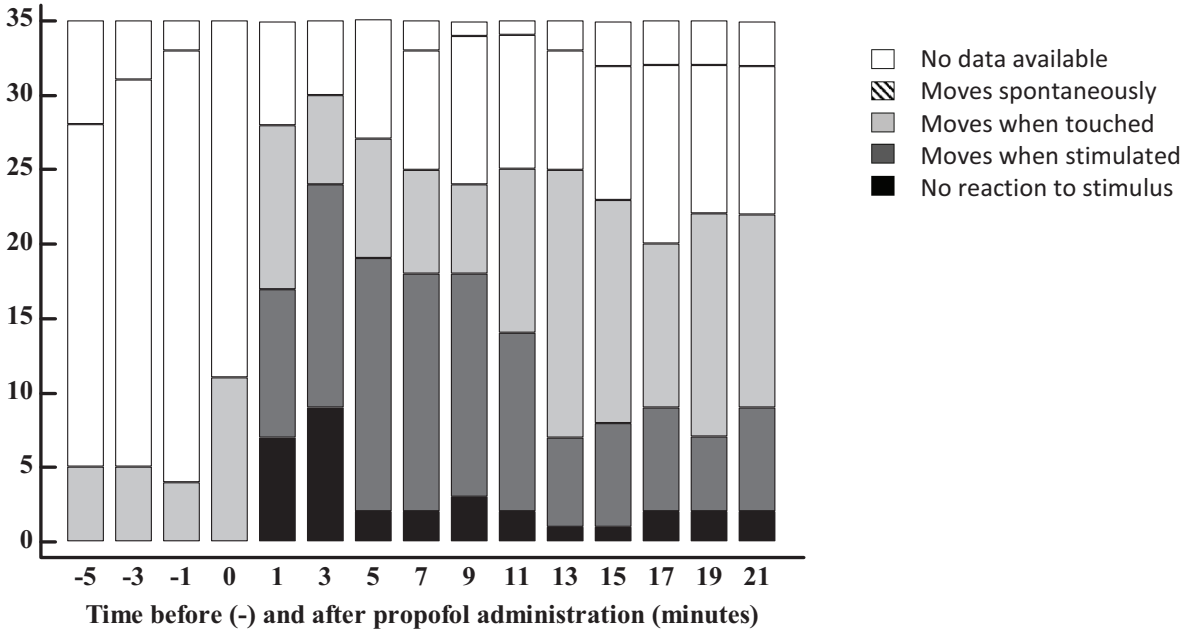

Figure 5: Sedation scores, as reported previously [54,55], collected in the 35 patients from 5 minutes before up to 21 minutes after propofol administration (time=0). Y-axis: number of patients.

Vital signs and cerebral oxygenation

In Table 13, a summary of the analyses for MABP, HR, SaO_2, $rScO_2$, FTOE and PI is presented. When exploring the age-corrected MABP values (cMABP), median (range) of the lowest values was -3 (-5, 1), -3 (-6,7.5), 2 (-9, 5) and -5.5 (-7, -4) for group 1, 2, 3 and 4 respectively. During the first 24 h after propofol administration, 5 patients received a fluid bolus (normal saline or colloids), with initiation between 8.5 h and 21.5 h after propofol administration.

For MABP, HR, SaO_2, $rScO_2$, FTOE and PI a median trend line with interquartile range (p25-p75) up to 720 minutes after propofol administration for the 4 patients groups (as defined in Table 8) is presented in Figures 6a, 7a, 8a, 9a, 10a and 11a respectively. To provide more details concerning the first hours after propofol administration, individual trend lines were plotted up to 240 minutes after propofol administration (Figure 6b, 7b, 8b, 9b, 10b and 11b).

Optimal interpretation of the vital signs requires review of the values provided in Table 13, since they cover the full 12 h period, as well as visual inspection of the graphs. The latter are of add-on value to unveil short-lasting changes immediately after propofol administration.

Univariate regression revealed no significant association between either AUC cMABP, AUC SaO_2 or AUC $rScO_2$ and clinical covariates (age, weight, total propofol dose) or propofol concentrations at 3 h or 12 h. Furthermore, AUC cMABP, AUC SaO_2 or AUC $rScO_2$ was not significantly associated with gender or outcome (success/failure).

Propofol ED_{50} calculation

Stratum 1, 3 and 5, as defined in Table 8, contained an effective sample size sufficiently high to calculate the propofol ED_{50} value. The initial propofol dose (mg/kg) sequentially administered to the neonates included in these strata are visually presented in Figure 12. The ED_{50} (95% confidence interval) value was 0.749 (0.331-1.167) mg/kg, 0.480 (0.110-0.851) mg/kg and 1.287 (0.721-1.852) mg/kg for stratum 1, 3 and 5, respectively.

Table 13: Vital signs presented for the 4 patients groups. The median (range) of the minimum values is presented as % decrease from baseline. AUC is calculated as the AUC below the indicated reference value. If not otherwise stated, data of all 34 patients of whom continuous registration of vital signs are available, were included. Ranges are reported as (minimum, maximum). NA: not applicable.

Parameter		Group 1 (n=10)	Group 2 (n=15)	Group 3 (n=7)	Group 4 (n=2)	p-value
MABP	Decrease from baseline (%)	-29.41 (-51.02, -20.00)°	-31.82 (-50.00, -17.07)°°	-33.59 (-55.56, -7.50)$^{+}$	-39.09 (-58.67,-19.51)	0.984
	Minimum cMABP <0 mmHg (y/n)	7/2°	7/4°°	1/3^{+}	2/0	0.293
	AUC cMABP (<0 mmHg)	19.20 (0, 3765.10)°	49.30 (0, 2627.10)°°	0 (0, 149.90)$^{+}$	438.45 (156.20, 720.70)	0.232
HR	Decrease from baseline (%)	-22.70 (-43.18, -9.766)	-25.16 (-40.00, -10.40)	-32.90 (-54.26, -20.59)	-42.03 (-53.99, -30.06)	0.075
	Minimum HR <100 bpm (y/n)	1/9	0/15	3/4	1/1	0.016*
	AUC HR (<100 bpm)	0 (0, 2.91)	0 (0, 0)	0 (0, 11.27)	186.70 (0, 373.40)	0.025*
SaO2	Decrease from baseline (%)	-39.06 (-92.36, -20.04)	-40.05 (-97.38, -13.74)	-22.49 (-61.22, -16.70)	-37.60 (-49.89, -25.31)	0.258
	Minimum SaO_2 <85%	all	all	all	all	NA
	AUC SaO_2(< 85%)	146.41 (50.26, 469.33)	110.16 (3.322, 507.63)	99.67 (0.36, 246.72)	55.51 (9.10, 101.91)	0.310
rScO2	Decrease from baseline (%)	-50.70 (-80.58, -24.29)°	-38.04 (-57.69, -18.06)	-34.12 (-50.00, -13.75)	-35.27 (-38.96, -31.58)	0.127
	Minimum $rScO_2$ <65% (y/n)	9/0°	15/0	6/1	1/1	0.028*
	AUC $rScO_2$(<65%)	268.30 (18.40, 3569.30)°	106.10 (4.80, 5589.10)	55.10 (0, 293.00)	169.45 (0, 338.90)	0.3038
PI	Decrease from baseline (%)	-77.73 (-90.70, -50.50)	-75.38 (-88.08,-31.03)	-83.67 (-92.17,-43.01)	-74.52 (-75.00,-74.04)	0.384
	Minimum PI < 0.44 %	all	all	all	all	NA
	AUC PI (<0.44%)	11.29 (0.4, 53.14)	7.42 (0.01,51.78)	11.08 (0.43,82.04)	34.89 (0.43,82.04)	0.701
cFTOE	Min	0.01 (0, 0.21)°	0 (0, 0.25)	0 (0, 0.12)	0 (0, 0)	0.541
	Max	0.49 (0.33, 0.65)°	0.41 (0.26, 0.55)	0.35 (0.31, 0.49)	0.37 (0.25, 0.49)	0.105
	AUC cFTOE (<baseline)	9.41 (1.40, 85.64)°	4.69 (0, 83.05)	4.90 (0, 79.15)	1.09 (0, 2.18)	0.254
	AAC cFTOE (>baseline)	13.85 (0.24, 38.31)°	16. 47 (0.16, 90.59)	13.94 (0.09, 99.64)	24.23 (3.92, 44.55)	0.928

Mean arterial blood pressure (MABP), heart rate (HR), oxygen saturation (SaO_2), regional cerebral oxygen saturation ($rScO_2$), cerebral fractional tissue oxygen extraction (cFTEO), perfusion index (PI), AUC: Area under the curve, AAC: Area above the curve, min: minimum, max: maximum, °: based on n=9 patients, °°: based on n=11 patients, $^{+}$: based on n=4 patients.

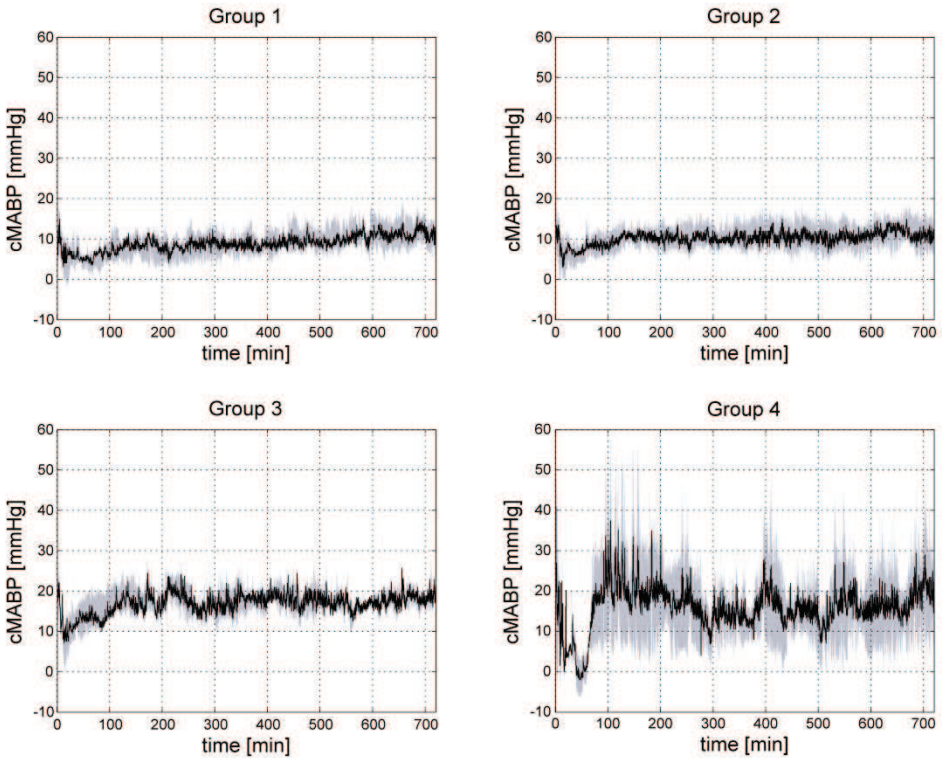

Figure 6a

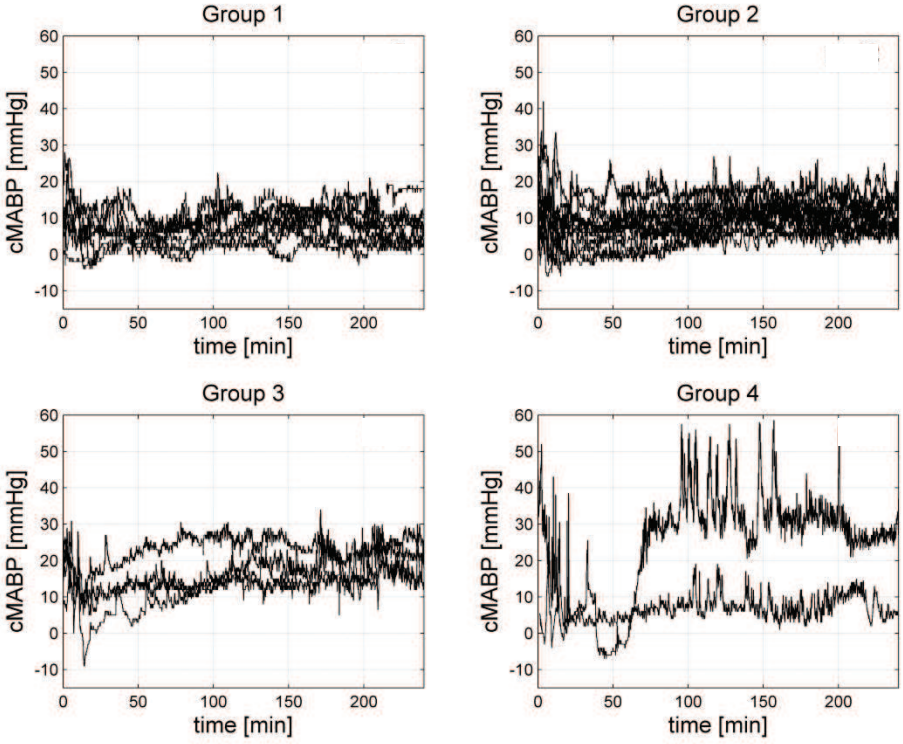

Figure 6b

Figure 6: Mean arterial blood pressure corrected for postmenstrual age (cMABP) for the 4 groups. Patients with invasive monitoring (n=26) were included. (a) Median trend line and interquartile range (grey zone) up to 720 minutes and (b) individual trend lines up to first 240 minutes after propofol.

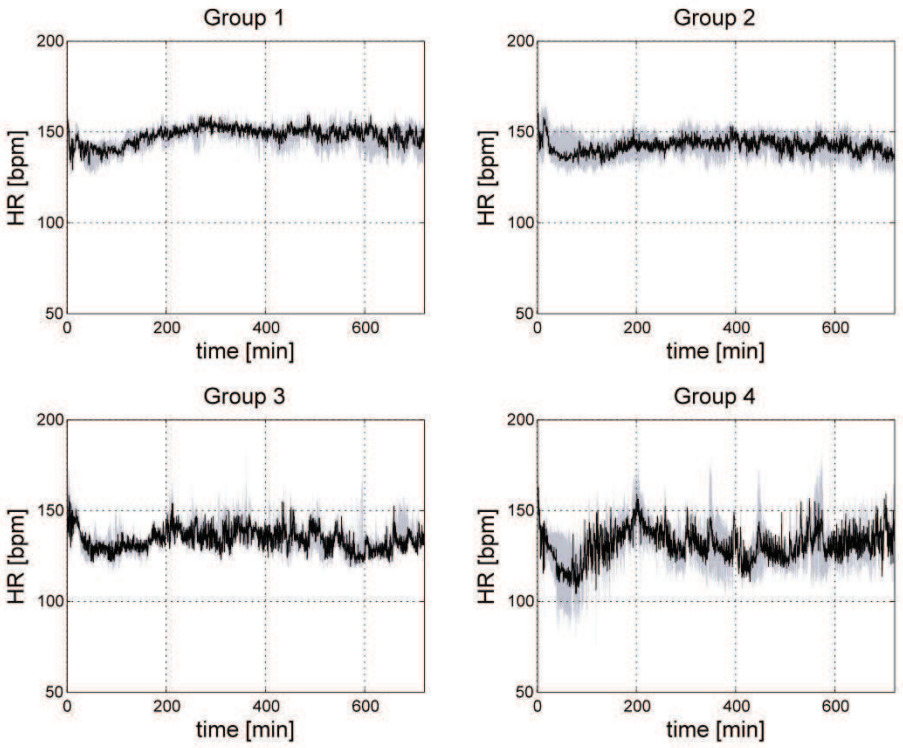

Figure 7a

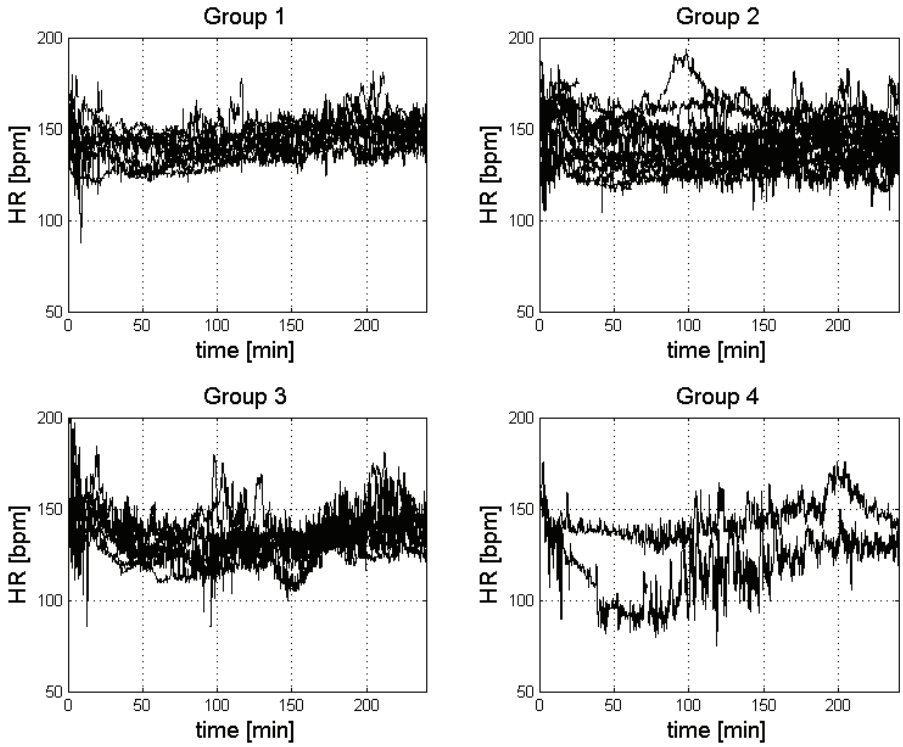

Figure 7b

Figure 7: Heart rate (beats per minute, bpm) for the 4 patient groups. Data of 34 patients were included. (a) Median trend line with interquartile range (grey zone) up to 720 minutes and (b) individual trend lines up to first 240 minutes after propofol.

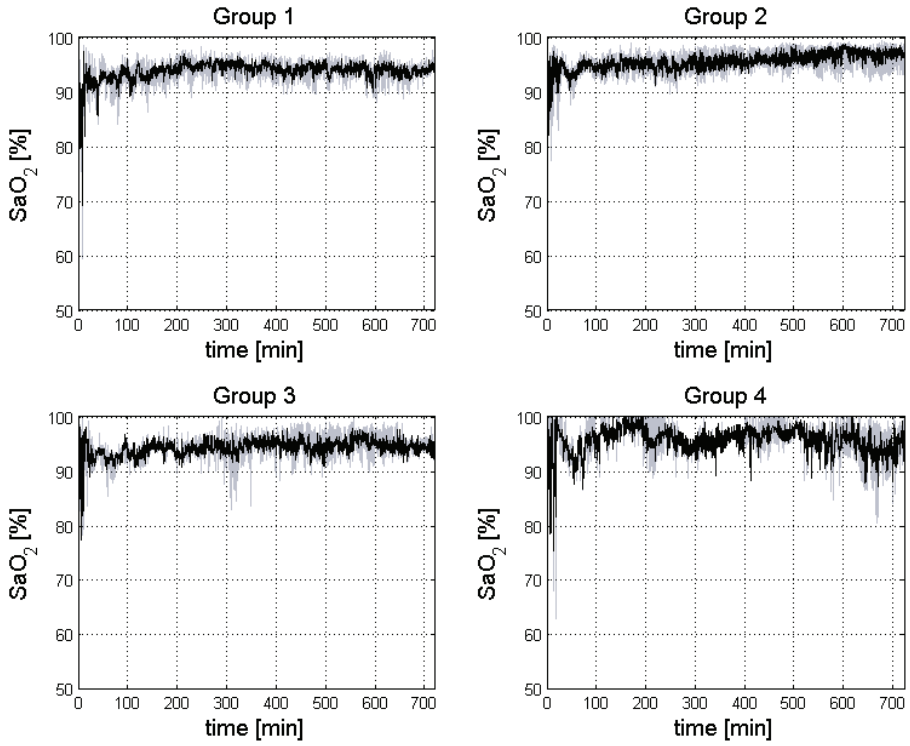

Figure 8a

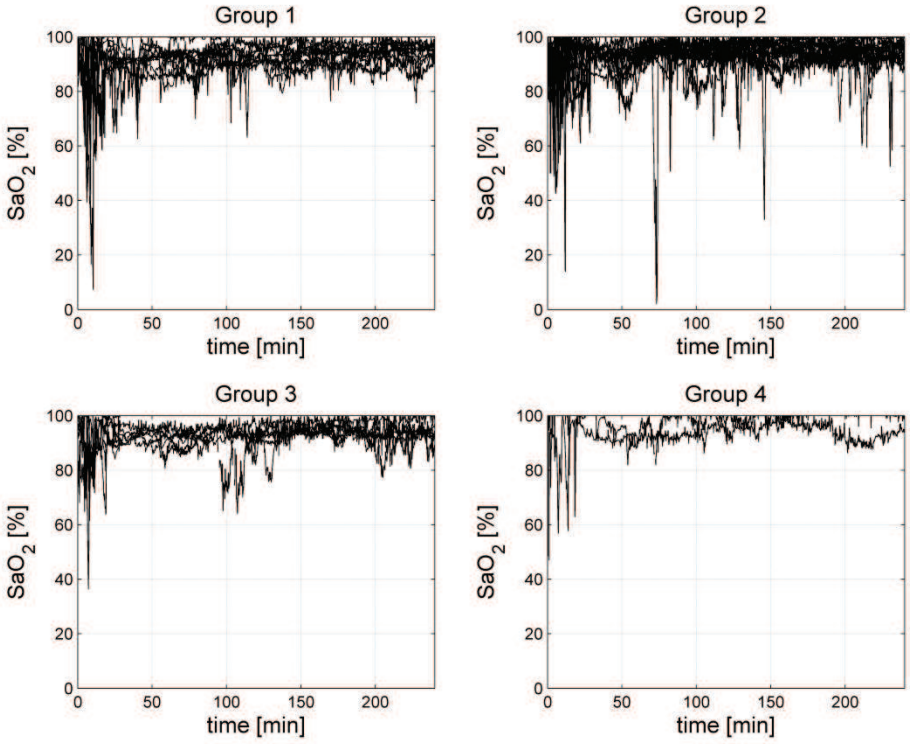

Figure 8b

Figure 8: Oxygen saturation (SaO_2,%) for the 4 patient groups. Data of 34 patients were included. (a) Median trend line with interquartile range (grey zone) up to 720 minutes and (b) individual trend lines up to first 240 minutes after propofol.

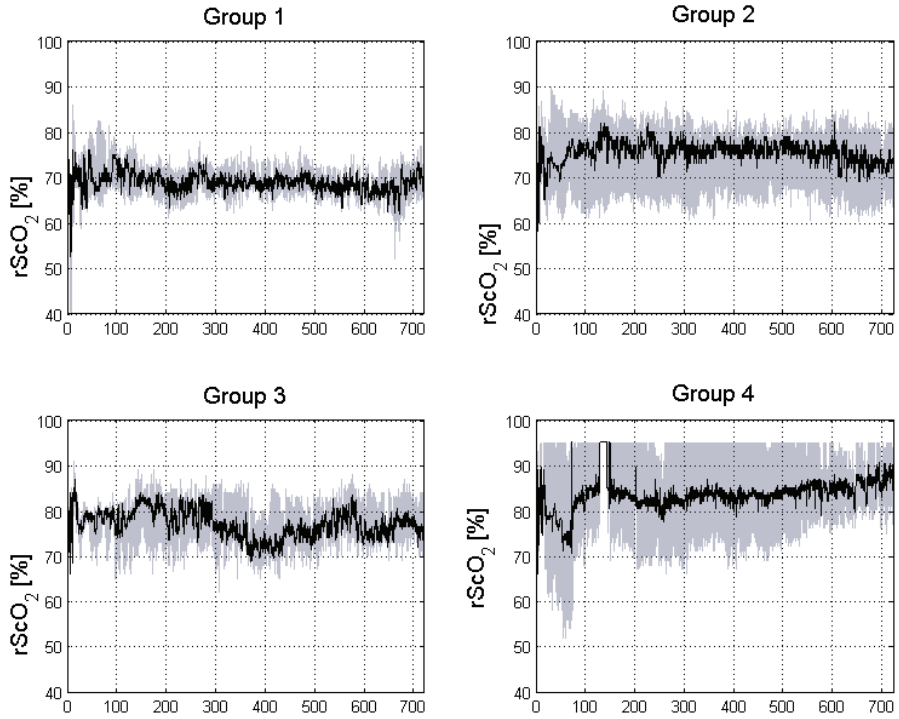

Figure 9a

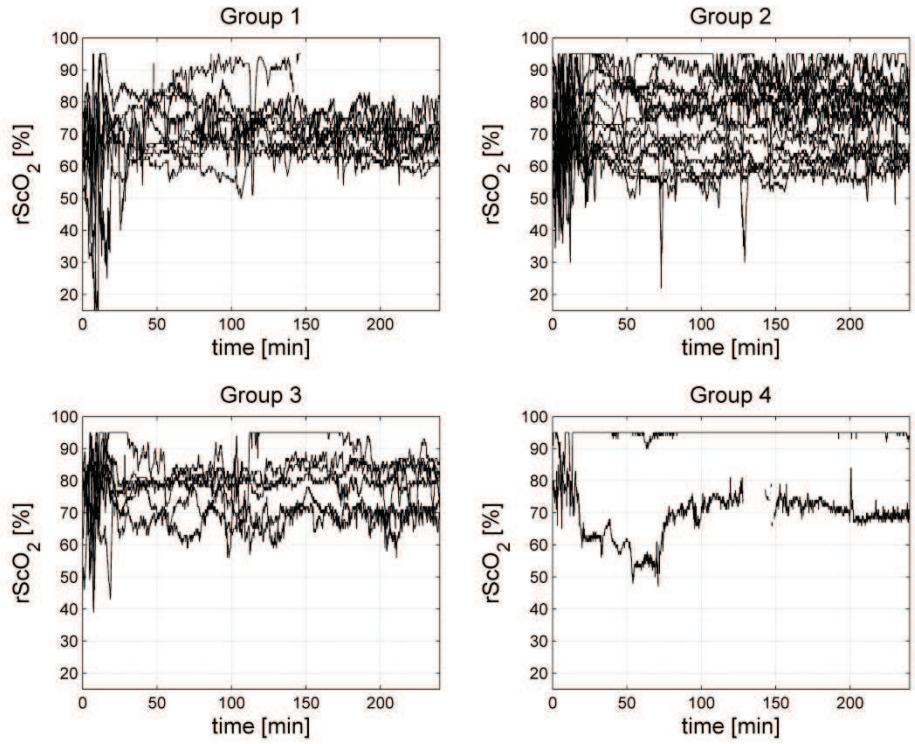

Figure 9b

Figure 9: Regional cerebral oxygen saturation ($rScO_2$,%) for the 4 patient groups. Data of 34 patients were included. (a) Median trend line with interquartile range (grey zone) up to 720 minutes and (b) individual trend lines up to first 240 minutes after propofol.

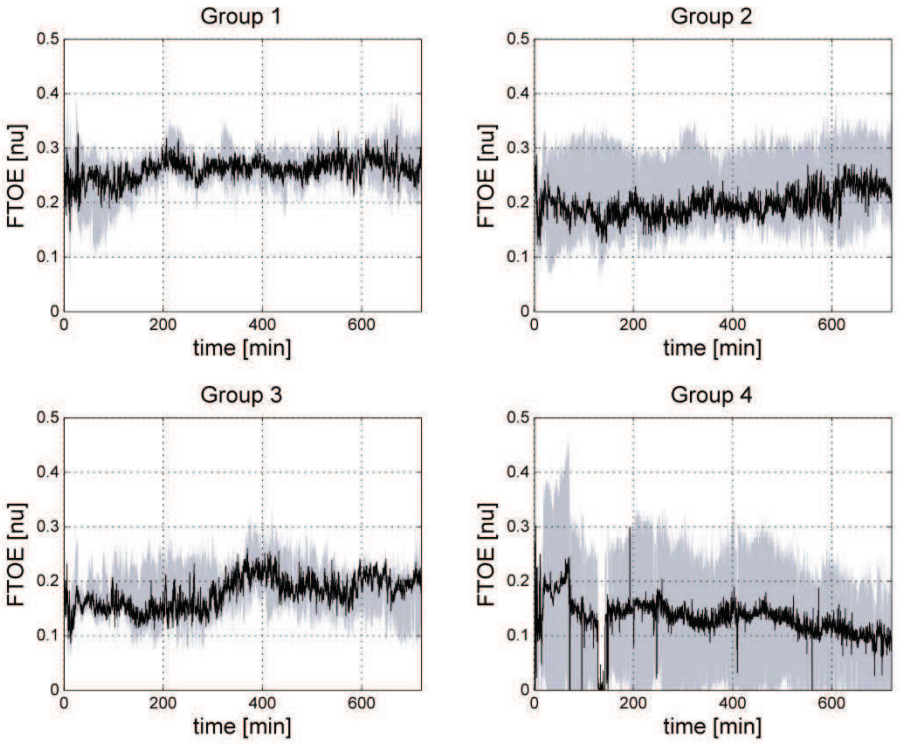

Figure 10a

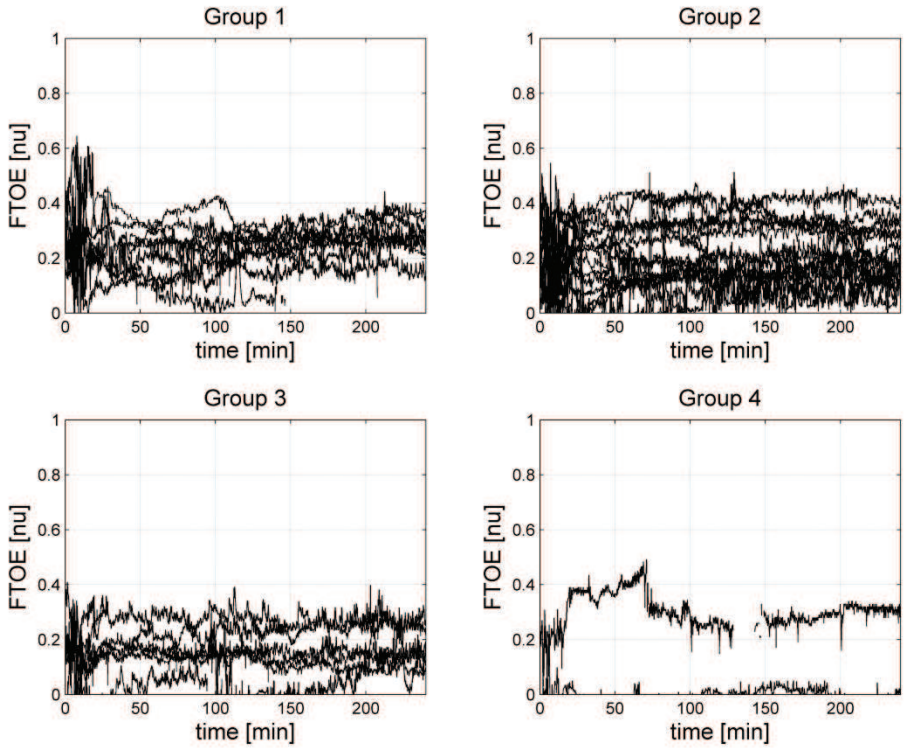

Figure 10b

Figure 10: Cerebral fractional tissue oxygen extraction (cFTOE, normalized units) for the 4 patient groups. Data of 34 patients were included. (a) Median trend line with interquartile range (grey zone) up to 720 minutes and (b) individual trend lines up to first 240 minutes after propofol.

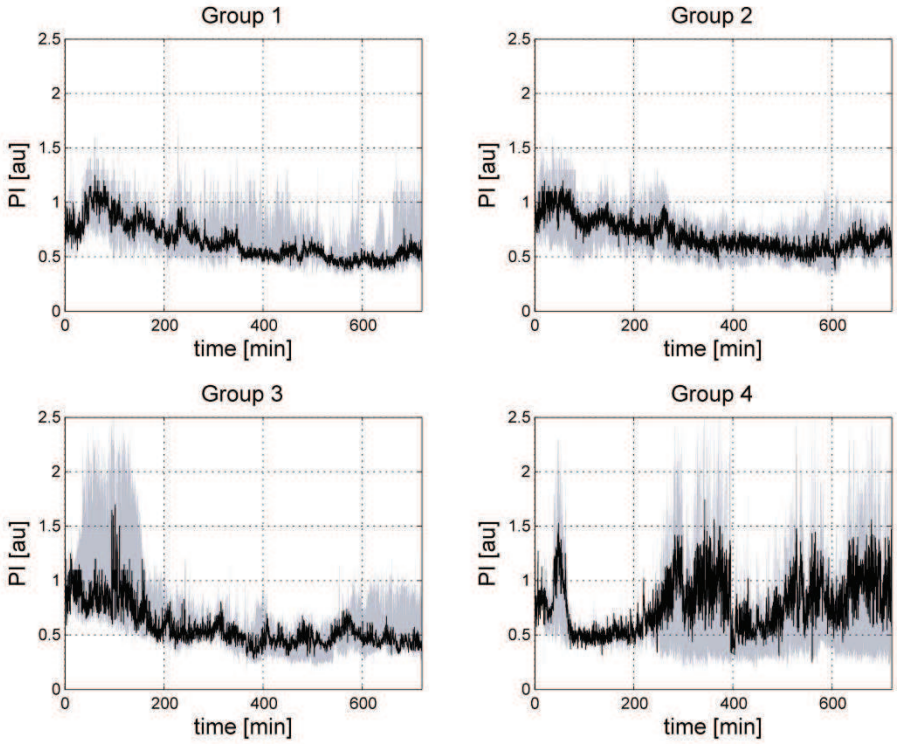

Figure 11a

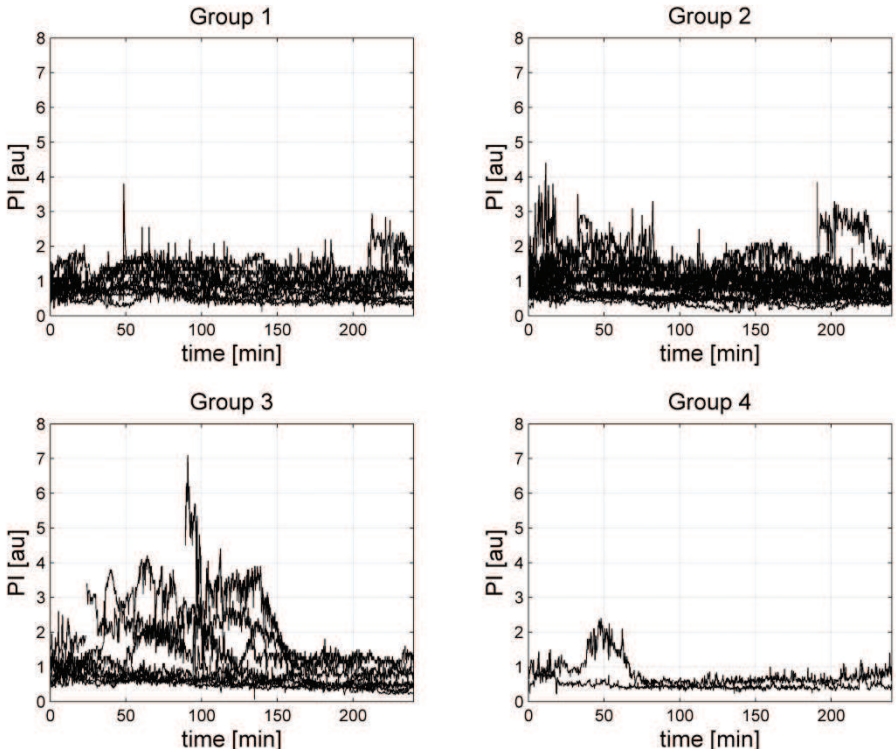

Figure 11b

Figure 11: Perfusion index (PI, arbitrary units) for the 4 patient groups. Data of 34 patients were included. (a) Median trend line with interquartile range (grey zone) up to 720 minutes and (b) individual trend lines up to first 240 minutes after propofol.

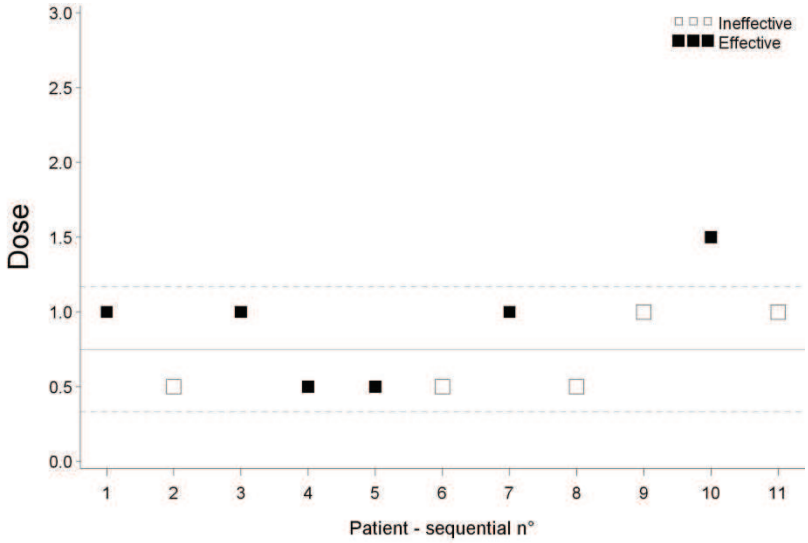

Figure 12a: Stratum 1

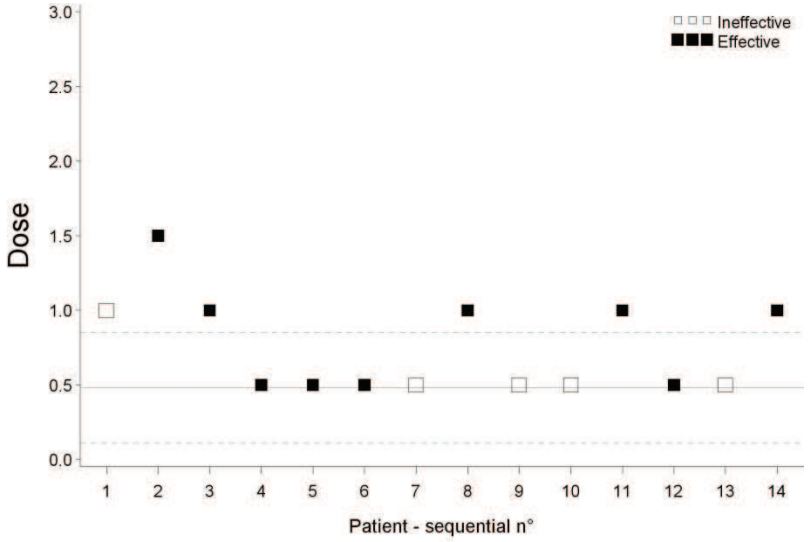

Figure 12b: Stratum 3

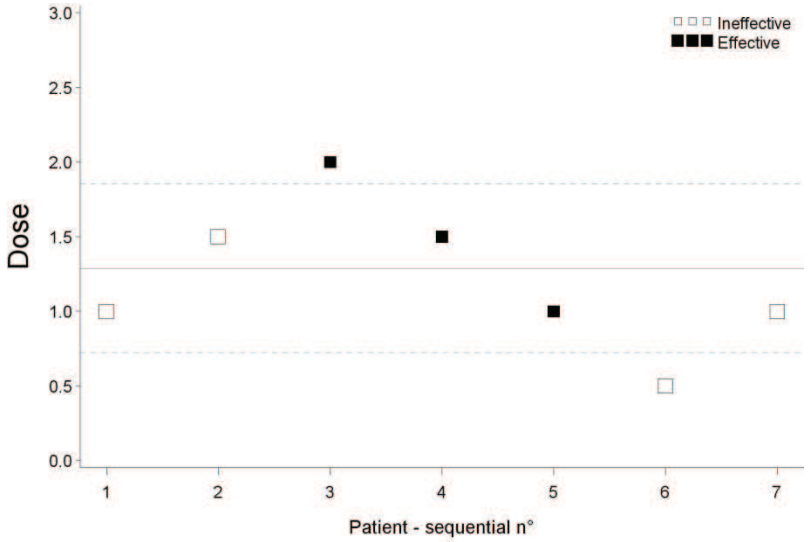

Figure 12c: Stratum 5

Figure 12: Initial propofol dose (mg/kg) sequentially administered in a) stratum 1, b) stratum 3 and c) stratum 5, for which ED_{50} calculation was possible (i.e. effective sample size at least equal to 6).

Discussion

The interest to use propofol for pre-intubation sedation in neonates is growing, but evidence concerning optimal dosing and safety is limited. We therefore performed an exploratory propofol dose-finding study in neonates needing pre-medication for endotracheal intubation. Besides data concerning intubation efficacy, we also explored propofol pharmacodynamics (sedation, relaxation and intubation condition scores, detailed and continuous vital sign measurements) as well as propofol blood concentrations, and finally provided propofol ED_{50} doses.

Efficacy

Propofol is a short acting drug and administered to create optimal intubation conditions and fast recovery of awake status afterwards. Achievement of adequate sedation is needed for patient comfort and to avoid trauma, but it also facilitates the intubation procedure for the clinician. Throughout the present study, initial and total propofol bolus dose ranges of 0.5-2 mg/kg and 0.5-4.5 mg/kg, respectively, were used. Simons *et al* [16], reported that a starting dose of 2 mg/kg for endotracheal intubation was only sufficient in 37% of cases (but 77% on the first day of life) and that success rate of the first intubation attempt was 49% [6]. Compared to his observations, our initial propofol dose (median = 1 mg/kg) was lower but still had a comparable success rate in terms of predefined outcome as well as successful first intubation attempts. Importantly, the median PNA of the patients described by Simons *et al* was 5 days and no INSURE cases were included. The percentage of patients intubated at first attempt in our study is also in line with other reports (69% current study versus 60% by Welzing *et al* [65]).

Propofol whole blood concentrations

As mentioned in the introduction of this chapter, propofol clearance in neonates increases with PMA. Due to immature glucuronidation capacity in early life, a PNA below 10 days additionally impairs metabolic elimination of this compound [7,8]. The propofol concentrations collected in this study mainly cover the late distribution phase (3 h and 12 h sampling). In patients receiving <2 mg/kg total propofol dose, covariates weight and age in part explained variability in propofol concentrations, especially at 3 h. This regression analysis hereby confirms the relevance of maturational covariates earlier described in this chapter (section 4.1

and 4.2). As a future perspective, the currently collected data can be used to further validate the reported propofol PK models in neonates.

Propofol pharmacodynamics

Relaxation, sedation and intubation condition score

Following the study of Ghanta *et al* [11] - one of the first reports of propofol (and its PD aspects) as induction agent for (semi)elective intubations in neonates - the drug became introduced in many neonatal intensive care units. One of the claimed advantages attributed to propofol is the continuation of spontaneous breathing and fast recovery of the sedative effect. However, we want to attenuate this clinical perception for use in neonates, since the suggested 'fast' recovery strongly depends on the outcome measures assessed. At least in our hands, both sedation and relaxation scores were not yet returned to pre-sedation condition at 21 minutes after propofol administration, as presented in Figures 4 and 5. In contrast, Ghanta *et al* [11] described a median time to recovery of 840 seconds (recalculated as time to sleep or muscle relaxation of 60 seconds plus time from sleep to return of spontaneous muscle movement of 780 seconds) (= 14 minutes) after administration of a 2.5 mg/kg propofol dose. However, it is questionable if spontaneous, voluntary muscle movement sufficiently covers the term 're'-covery, and in addition, to what extent this parameter reflects propofol effects in other body compartments (e.g. central nervous system). While it is not yet fully clear how propofol disrupts neural transmission, activation of the $GABA_A$ receptor is one of the mechanisms involved [66]. Since $GABA_A$ in early life primarily displays excitatory function and only later in life changes into inhibitory signaling [67,68], understanding or estimating the sedative drug effects in early infancy (developmental PK/PD) becomes even more complex. The same holds true for other GABA-related phenomena like dissociative (the presence of peripheral but no central inhibition) effects in early life [16].

One of the strengths of the current analysis is the continuous registration of vital signs over a relevant period (12 h) after propofol administration. When compared to baseline, a median MABP decrease between -29.41% and -39.09% was documented. This is in line with the mean blood pressure trend published by *Simons et al* [16]. Based on the median values of mean arterial blood pressure prior to and after propofol administration, provided by Welzing *et al* [11], a MABP decrease of -36.8% (38 to 24 mmHg) after propofol bolus administration and -24% (37 to 28) mmHg after propofol administration over 60 seconds, was recalculated. Although the study of Welzing was stopped, the % decrease of MABP is comparable with our findings.

As commented by Lerman *et al* [69], it is open for discussion whether the early termination of the Welzing study was needed since systolic blood pressure within 10 minutes after intubation decreased, on average, only 20% from baseline and values >30% were only found in 23% of neonates. Similar to the assessment of sedation and relaxation, this reflects different opinions on how these trends in vital signs should be interpreted. To put these trends into perspective, we also determined of age-corrected blood pressure values and its AUC. Based on these age-corrected blood pressure values (cMABP), a relevant proportion of patients indeed displayed hypotension (defined as cMABP <0), but mainly in the area considered as 'permissive hypotension' (defined as cMABP <0, without clinical signs of shock and no need for treatment) [70,71] (Table 13). Only a limited number of neonates (n=2) received a fluid bolus during the 24 h after propofol administration, with initiation at least 8 h after propofol, making the possibility of a causal link with this drug very unlikely. There was no need for inotropes.

Based on the observations of Vanderhaeghen *et al* [12], persistence of a decrease in blood pressure beyond the first 60 minutes after propofol administration was anticipated. Visual inspection of groups 1, 2 and 3 on Figure 6a, suggests that the duration in blood pressure decrease is present up to 120-200 minutes after propofol administration. This transient decrease in blood pressure after propofol has been described in neonates, but also in older children and adults. It is hypothesized to be the result of a propofol-induced vasodilatation, which seems more relevant when the drug is administered in the first hours of life. At present there is evidence that preterm neonates, in general, can maintain cerebral blood flow (CBF) despite changes in hemodynamic parameters e.g. blood pressure [72,73].

Irrespective of how clinicians assess the trends in blood pressure, this is only an indirect marker of blood flow and cerebral oxygenation [58]. This is the reason that we simultaneously collected data on both vital signs and cerebral oxygenation and extraction. It is known that the sedative effect of propofol results in a decreased cerebral oxygen consumption by inhibition of the N-methyl D-aspartate receptors and activation of the $GABA_A$ receptors [12]. Despite this reduced consumption, a mild and short-lasting decrease in $rScO_2$ was seen in the first 20-30 minutes after propofol administration in the current study (Figure 9). This decrease in $rScO_2$ can be caused by a decreased SaO_2 (hypoxic hypoxia) or by a decrease in CBF secondary to a decreased MABP (ischemic hypoxia) when autoregulation mechanisms fail. Due to the brisk decreases in SaO_2 with stable HR around the intubation procedure, we hypothesize the

decrease in $rScO_2$ can be explained by hypoxic hypoxia since in most cases cFTOE remains stable (Figure 10a) [74]. However, in some infants in group 1 and 2 an increase in cFTOE up to 0.6 (Figure 10b) can be seen. We attribute this to decrease in SaO_2 and MABP which will lead to higher oxygen extraction in order to provide sufficient oxygen delivery to the brain. This effect is short lasting (less than 30 minutes) and can be considered as safe [75]. After recovery, the $rScO_2$ value remains quite stable.

A lower but more stable trend in cFTOE can be seen up to the first 200 minutes (Figure 10a, groups 1, 2 and 3) . This might indicate less oxygen consumption with stable supply or equal oxygen consumption with increased supply. Taking into account our study setting, we hypothesize this mainly indicates a decrease in cerebral oxygen consumption due to propofol, with intact neurovascular coupling. An exception can be seen in group 4 were a child with renal failure and hypertension displays a drop in cMABP with sustained increase of cFTOE. In our opinion, autoregulation in this patient has failed with an increased oxygen consumption due to impaired CBF during +/- 70 minutes. Although based on one observation, caution is warranted when administering propofol to neonates with hypertension. Interestingly, to the other patient in group 4 high oxygenation was applied, reflected by SaO_2 and $rScO_2$ values at the upper limit. The coincidental cFTOE hereby approximates 0. This illustrates the limitations of 'calculated' parameters like cFTOE (Figure 10b) or relates to the fact that the $rScO_2$ parameter was measured by the neonatal OxyAlert NIRSensor, which gives $rScO_2$ values on average 10% higher compared to the small adult SomaSensor [63].

After propofol administration, the PI displays an initial increase with subsequent decrease to 0.5 % (Figure 10). This might be related to the propofol induced vasodilatation. PI is considered to be a valuable tool in clinical practice, providing information on e.g. illness severity and heamodynamic stability, but it needs to be combined with other parameters and, importantly, further validation in neonates is necessary [57]. Therefore, we only describe the PI trend documented in this specific population.

Although Welzing *et al* [13] described that both HR and SaO_2 remained stable throughout an INSURE procedure with propofol (1 mg/kg) as premedication, a short-lasting and minor decrease in HR and SaO_2 after propofol (3 mg/kg) for chest tube removal in neonates was previously reported [11,12]. In our cohort, a modest decrease in HR persisted up to 200 minutes is documented (Figure 7a). Although the administration of propofol resulted in hypotension, no accompanying tachycardia was seen. This is probably due to the inhibitory effect of

propofol on the baroreceptor reflex [12]. Since AUC for HR <100 bpm is equal to 0 in groups 1, 2 and 3, no clinically significant bradycardia was noticed for these patient groups. Due to the older age of the patients in group 4 (n=2), lower HR values (up to 80 bpm) are clinically tolerated and considered as normal. This in part explains the higher AUC HR value for group 4.

The decrease in SaO_2 after propofol administration was most pronounced during the first minutes after propofol administration, related to the intubation procedure, with a fast return to baseline (Figure 8).

Within the propofol dose range applied in this study, variability in AUC cMABP, AUC SaO_2 and AUC $rScO_2$, could not be explained by covariates representing ontogeny (weight, age), nor by propofol blood concentrations at 3 h and 12 h after administration. This is of relevance since clinicians still might be reluctant to use procedural sedation in especially the smallest and youngest neonates [65,76].

Propofol ED_{50} calculation

Besides reporting efficacy and propofol PD (including clinical scores, vital signs for safety evaluation and concentration-effect relationships) an important aim was to provide propofol ED_{50} values. We succeeded to determine these concentrations, effective in 50% of patients, for preterm neonates with PNA <10 days (i.e. strata 1, 3 and 5). Our results indicate that overall low propofol doses can be used to achieve successful outcome. We hereby want to stress that the majority of our patients (86%) were INSURE cases. The combined outcome of successful intubation and successful extubation hereby needed a balanced approach, i.e. tailored sedation. The fact that our patients, to a certain extent, still react during the intubation procedure, is also reflected in the ICS scores (overall range 5-13).

We are aware that an ED_{50} value cannot be considered as a robust dosing recommendation, but at least provides a start to develop future dose-finding and validation studies. This is important, since simple extrapolation of dosing regimens from older children is likely incorrect since on can assume that besides propofol metabolism itself (PK), also transporters and/or receptors involved could be immature in neonates (PD) [16]. In addition, the impact and maturational expression of polymorphisms of propofol metabolizing enzymes and receptors on its PK and PD in early life is unknown. In adults, polymorphisms studies are not conclusive and certainly cannot explain large variations in propofol effects [66].

Clinical reflection

In contrast to some other reports [13,16], we used clinical scores already published previously [1,54,55] and succeeded to collect them systematically. As mentioned above, an additional strength of this study is the continuous registration of different vital signs. We are aware that these data include every minor as well as major fluctuation in vital signs, but this reflects the clinical setting of the intubation procedure with possible artefacts. Importantly, we hereby illustrated that this type of study is feasible within an intensive care patient setting. With exception of determination of the initial propofol dose, there was no interference with routine clinical practice during the intubation procedure itself.

Although this study provides relevant data for safety evaluation of propofol use in neonates, there are some limitations. First of all, knowledge on neonatal vital sign trends, definitions, threshold values, validated reference values and the role of these vital parameters as biomarkers reflecting neonatal drug effects is very poor. Additionally, caution is needed when interpreting propofol effects of different reports. Besides variability in parameter criteria (e.g. hypotension) also blood pressure measurement frequency and techniques applied, as well as clinical characteristics of included patients will contribute to variability in the collected data.

After providing the reader with an overview of our observations, including its limitations, we leave it to the individual clinician to decide whether or not the results are sufficiently safe to use a single propofol bolus in neonates. Based on both the clinical scores and safety PD analysis, we feel it appropriate to use this compound in the dose-ranges studied for neonatal intubation, especially INSURE conditions. At induction, the respective ED_{50} doses can be applied. Subsequently, since questions concerning the mechanism of action and covariates of neonatal propofol pharmacokinetics still remain, we strongly advise to only increase the propofol bolus dose by up-titration based on perceived clinical need instead of routinely use of higher initial propofol doses in neonates. Strict follow-up of vital signs in the first hours after propofol administration is compulsory.

We conclude that a dose-finding study with intravenous propofol bolus as premedication for intubation resulted in ED_{50} values for preterm neonates below 10 days PNA. Simultaneous propofol PD data indicate that clinical recovery is not yet achieved at 21 minutes after propofol administration. Although a moderate hypotensive effect, considered as permissive hypotension, was confirmed, available PD data allow the safe use of propofol in neonates

within a total dose range of 0.5-4.5 mg/kg. As a future perspective, further propofol dosing exploration, based on the 50 patients included in this research project will be performed and subsequently need to be validated.

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PART II: Drug exposure prediction

Population pharmacokinetic analysis as a tool for covariate exploration and internal PK/PD model validation

CHAPTER 5

Disposition of intravenous cefazolin in neonates

This chapter is based on

Cefazolin plasma protein binding and its covariates in neonates. *Eur J Clin Microbiol Infect Dis* 2012, 31:3359-3365

Cefazolin plasma protein binding in different human populations: More than cefazolin-albumin interaction. *Int J Antimicrob Agents* 2014, 43:195-200

Population pharmacokinetic modelling of total and unbound cefazolin plasma concentrations as a guide for dosing in preterm and term neonates. *J Antimicrob Chemother* 2014, 69:1330-1338

Abstract

Introduction Pharmacokinetic data of cefazolin in neonates are limited and based on total drug concentrations. However, only the unbound drug is pharmacologically active. The aims of the study were to explore cefazolin protein binding and its covariates in neonates, to compare cefazolin protein binding between different populations and finally, to describe neonatal cefazolin pharmacokinetics (PK) based on both total and unbound concentrations.

Methods In neonates (n=40) receiving intravenous cefazolin (50 mg/kg) for surgical prophylaxis, total and unbound cefazolin plasma concentrations were determined. Linear and multiple regression analyses were used to document covariates of unbound cefazolin fraction. To explore cefazolin protein binding across different populations, the neonatal data were pooled with observations of 3 other published cohorts (i.e. pregnant women, surgical adults and non-surgical adults). Kruskal-Wallis tests and multiple regression were applied. Finally, a population pharmacokinetic analysis (using non-linear mixed-effect modelling) with subsequent Monte Carlo simulations was performed using the neonatal dataset.

Results In neonates, between- and within patient saturability of cefazolin protein binding were documented: 49.6% of neonatal unbound cefazolin fraction was explained by albuminaemia, total cefazolin concentration, indirect bilirubinaemia and postmenstrual age (PMA). Median unbound cefazolin fraction differed significantly across the observed populations. 76.8% of variability in unbound fraction was explained by total cefazolin concentration, unbound cefazolin concentration, albuminaemia but also patient subgroup. For the neonatal data, a one-compartment PK model was developed. Current bodyweight was identified as covariate for volume of distribution (Vd), birth weight and postnatal age for clearance (Cl) and albumin for maximal protein binding (B_{max}), explaining 50%, 58% and 41% of inter-individual variability in Vd, Cl and B_{max} respectively.

Conclusions The unbound cefazolin fraction in neonates is higher compared to adults. Besides alterations in albuminaemia, also subpopulation-specific characteristics determine variability in unbound cefazolin fraction across different populations. The concept of drug protein binding needs to be integrated in PK analyses to optimize drug dosing regimens. To illustrate this, a neonatal PK model taking into account total and unbound cefazolin concentrations was identified and a model-based cefazolin dosing regimen based on current bodyweight and postnatal age, documented as most important PK covariates, was proposed.

What is already known on this topic

- Cefazolin is highly and saturably bound to human serum albumin in adults.
- Only the unbound drug can have a pharmacological effect and is available for elimination.
- Neonatal cefazolin pharmacokinetic (PK) data are rare and based on total drug concentrations. Available neonatal cefazolin dosing regimens cover a 3-fold mg/kg range.

What this study adds

- Median unbound cefazolin fraction in neonates is 0.39, which is higher than in adults.
- A one-compartment PK model taking into account both total and unbound cefazolin concentrations in neonates was developed.
- A neonatal model-based cefazolin dosing approach reaching a predefined exposure, but with a total daily dose reduction up to 67%, was presented.

Introduction

Cefazolin, a first-generation cephalosporin, interferes with bacterial cell wall synthesis and covers especially gram-positive bacteria. Cefazolin is administered parenterally. Based on a European survey, 15% of antimicrobial use for surgical prophylaxis in children is accounted for by first generation cephalosporins [1]. In a US point prevalence survey of patients in paediatric intensive care units and neonatal intensive care units, cefazolin was used in 17.6% and 1.2% of patients on the day of the survey, respectively [2]. Indications for cefazolin administration in neonates are mainly prophylactic (72%), and to a lesser extent therapeutic (17%) (e.g. coagulase-negative staphylococcal sepsis) [3] or empirical (11%) [2]. While the pharmacokinetics of cefazolin have been described in adults, information on cefazolin pharmacokinetics in early life is limited [4-6].

In plasma, cefazolin is bound to human serum albumin (HSA) [7-9]. Protein binding influences drug disposition but also drug action since only the unbound drug is pharmacologically active [10]. Furthermore, unbound drug concentrations in plasma are assumed to reflect drug concentrations at the effect site, and are available for elimination. In vitro, cefazolin protein binding ranges from 73-92%, depending on variations in albumin concentrations in plasma as well as differences in methodology used to measure protein binding. In vivo, cefazolin protein binding has been documented separately in pregnant woman [11] and adult (surgical) patients [12,13]. Within the adult population, important intra- and interindividual variability as well as concentration-dependency (saturability) of cefazolin protein binding has been documented [11,13].

As highlighted in the general introduction of this thesis, drug disposition in neonates differs from adults based on their physiological characteristics, including protein binding (e.g. hypoalbuminaemia) as well as specific pathological processes. Furthermore, ontogeny (reflected by age and weight) needs to be considered. Since protein binding is of relevance both in the pharmacokinetic (PK) and pharmacodynamic (PD) evaluation of cefazolin, the aim of the present study is first to describe cefazolin protein binding and its covariates in neonates.

At present, there is little data defining whether cefazolin protein binding varies significantly across different human patient groups. Therefore, as a side step, the current neonatal data

were pooled with cefazolin protein binding observations of published cohorts of pregnant women and (surgical) adults to explore cefazolin protein binding across different human patient populations.

The collected data on cefazolin protein binding in neonates were subsequently integrated to improve cefazolin pharmacokinetic estimates.

Up to now, neonatal clearance (Cl) values for cefazolin are mainly based on total cefazolin concentrations, necessitating a neonatal cefazolin pharmacokinetic analysis integrating both total and unbound drug concentrations. Therefore, as a final aim, the neonatal cefazolin dataset was used to describe pharmacokinetics of cefazolin in (pre)term neonates based on both total and unbound cefazolin concentrations.

Finally, these pharmacokinetic estimates taking into account protein binding, were integrated in a population PK approach with subsequent exposure evaluation.

When an antimicrobial drug is administered to a patient, this is done with the intention to achieve optimal efficacy. For cefazolin, efficacy relates to the time unbound cefazolin concentrations exceed the minimal inhibitory concentration (MIC) for a given pathogen ($T_{>MIC}$) [14]. In neonates, often regarded as vulnerable and even immunocompromised patients, effective cefazolin therapy requires at least 60% of $T_{>MIC}$ [15]. However, currently available cefazolin dosing regimens for neonates are variable (Table 1) [16-22], hereby inducing uncertainty of efficacy attainment. To illustrate exposure to cefazolin using our current dosing approach and to propose a model-based dosing regimen for (pre)term neonates resulting in unbound concentrations during >60% of $T_{>MIC}$, Monte Carlo simulations were performed.

Table 1: 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. PNA: postnatal age, PMA: postmenstrual age, g:grams, h:hours

Reference	Age		Weight	Cefazolin dose and interval
NICU UZ Leuven				50 mg/kg/dose, q8h
Dutch Children's Formulary [16]	< 1 week PNA		< 2000 g > 2000 g	25 mg/kg/dose, q12h 50 mg/kg/dose, q12h
	1-4 weeks PNA			50 mg/kg/dose, q8h
Neonatal and pediatric pharmacology, Yaffe and Aranda 2011 [17]	0-4 weeks PNA		< 1200 g	20 mg/kg/dose, q12h
	< 1 week PNA		1200-2000 g > 2000 g	20 mg/kg/dose, q12h 20 mg/kg/dose, q12h
	≥ 1 week PNA		1200-2000 g > 2000 g	20 mg/kg/dose, q12h 20 mg/kg/dose, q8h
The Harriet Lane Handbook 2012 [19]	≤ 1 week PNA			20 mg/kg/dose, q12h
	> 1 week PNA		≤ 2000 g > 2000 g	20 mg/kg/dose, q12h 20 mg/kg/dose, q8h
Neofax 2011 [20]	≤ 29 weeks PMA	0-4 weeks PNA > 4 weeks PNA		25 mg/kg/dose, q12h 25 mg/kg/dose, q8 h
	30-36 weeks PMA	0-2 weeks PNA > 2 weeks PNA		25 mg/kg/dose, q12h 25 mg/kg/dose, q8 h
	37-44 weeks PMA	0-1 week PNA > 1 week PNA		25 mg/kg/dose, q12h 25 mg/kg/dose, q8h
	≥ 45 weeks PMA	all		25 mg/kg/dose, q6h
Nelson's Textbook of Pediatrics 2007 [18]	< 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 [21]	≤ 29 weeks PMA	0-4 weeks PNA > 4 weeks PNA		50 mg/kg/dose, q12h 50 mg/kg/dose, q8h
	30-36 weeks PMA	0-2 weeks PNA > 2 weeks PNA		50 mg/kg/dose, q12h 50 mg/kg/dose, q8h
	37-44 weeks PMA	0-1 week PNA > 1 week PNA		50 mg/kg/dose, q12h 50 mg/kg/dose, q8h
	≥ 45 weeks PMA	all		50 mg/kg/dose, q6h
Redbook 2012 [22]	≤ 1 week PNA		≤ 2000 g > 2000 g	25 mg/kg/dose, q12h 25 mg/kg/dose, q12h
	> 1–4 weeks PNA		≤ 2000 g > 2000 g	25 mg/kg/dose, q12h 25 mg/kg/dose, q8h

5.1. Cefazolin plasma protein binding and its covariates in neonates

Methods

Ethics, drug dosing and clinical characteristics

All patients were admitted to the Neonatal Intensive Care Unit of the University Hospitals Leuven, Belgium. The study was registered at ClinicalTrials.gov (NCT01295606) and approved by the ethical board of our hospital. After informed written consent of the parents, neonates were considered for inclusion if cefazolin (Cefazolin Sandoz®, Sandoz, Vilvoorde, Belgium) was intravenously administered over 30 min as routine prophylactic drug prior to a surgical procedure. At induction of surgery, a cefazolin dose of 50 mg/kg was administered. According to the local standard of care (depending on foreign body implantation or contamination risk of the procedure), additional cefazolin dose(s) of 50 mg/kg could be administered every 8 hour up to a maximum of 48 hours. Clinical characteristics recorded at inclusion were gestational age at birth (GA), postmenstrual age (PMA), postnatal age (PNA), birth weight, current body weight, cardiopathy (yes/no), gender and indication for cefazolin prophylaxis. Albuminaemia (g/L), indirect serum bilirubin concentrations (mg/dL) and serum creatinine (mg/dL) in a time interval of 24 h before or after the first cefazolin administration were extracted from clinical files. Threshold values for hyperbilirubinaemia are based on earlier reported cut-off values according to PNA, to adapt for the normal postnatal transient increase with subsequent decrease in neonatal life [23]. Plasma free fatty acids concentrations (FFA, mmol/l) were additionally determined on study samples at the end of the study.

Plasma 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.

Drug assay

The initial High Performance Liquid Chromatography (HPLC) method was developed in our laboratory and reported in literature [11]. The method was adapted for measurement of cefazolin in small volume plasma samples of neonates.

To determine total cefazolin plasma concentration, to 0.1 mL plasma were added: 10 µL of standard cefazolin dilutions in water (final concentration range 0.1-100 mg/L), 10 µL of cefoxitin dilution (100 mg/L) in water and 0.1 mL 0.1 M ammoniumacetate buffer pH 4.0. After vortexing and centrifuging for 5 min at 1800x*g* these samples were ready for application to the solid-phase extraction columns (Oasis HLB 30 mg, 1 ml).

The solid-phase extraction was performed with a Vac Elut SPS24 vacuum extraction system. The columns were activated with 2 times 1 mL methanol and 2 times 1 mL water, applying slight vacuum to the columns. The prepared standards, controls or samples were subsequently passed through the columns over a time period of 2 to 3 min. Then 1 mL water was applied and vacuum maintained for 2 min, followed by 1 mL methanol/water (80/20, *v/v*) and again vacuum maintained for more 2 min. Elution of the columns was performed with 2 times 0.5 ml of methanol (+0.2 % triethylamine). The eluates were evaporated with an airstream in a water bath at 45° C and the dried residues dissolved in 200 µL mobile phase. Injection volumes varied between 20 and 50 µL.

To determine the unbound cefazolin, to 0.1 mL of plasma was added 10 µL of 1M HEPES buffer pH 6.0. The samples were vortexed and incubated for 1 h in a water bath at 37 °C. Ultrafiltration was performed with Centrifree micropartition devices in a fixed rotor centrifuge for 30 min at 1100x*g* at room temperature (final temperature in centrifuge was 32 °C). The volume of the ultrafiltrate was adjusted to 0.1 mL with 0.5 % BSA in 0.9 % NaCl. After addition of 10 µL of cefoxitin (100 mg/L) and 0.1 mL of 5 % trichloroacetic acid, vortexing for 15 s, and waiting for 10 min, the samples were centrifuged for 8 min at 12000x*g*. Standard curves were prepared in 0.5 % BSA in 0.9 % NaCl, instead of plasma. A hundred µL of the supernatant was transferred to injection vials and injected onto the analytical column. 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. In this way, HPLC conditions remained the same and method performance showed the same recovery and reproducibility as earlier reported [11] and was in line with FDA analytical recommendations [24,25].

Biochemical assays

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

Statistical analysis

Statistical analysis was performed using SPSS (IBM statistical software, version 19) and Medcalc® (Mariakerke, Belgium). Clinical characteristics were reported by median and range or incidence. Two separate treatment episodes with cefazolin, documented in the same patient at different postnatal ages (i.e. PNA 25 days and PNA 53 days) were considered as 2 distinct observations. Cefazolin plasma concentrations (mg/L) were also reported by median and range. Unbound cefazolin fraction was calculated as the ratio of unbound to total drug concentrations. The impact of continuous and dichotomous covariates on unbound cefazolin fraction was determined using linear regression and Mann Whitney U tests respectively. Significant results of monovariate linear regression were entered in a multiple forward regression analysis. Within patient comparison of unbound cefazolin fraction at peak *versus* trough cefazolin concentration was assessed using Wilcoxon signed rank test. For this paired analysis, only neonates with cefazolin concentrations on both peak (0-2 h after first cefazolin administration) and trough (>6 h after first cefazolin administration but before next cefazolin administration, if scheduled) times were included. For different clinical conditions (i.e. albuminaemia <35 versus ≥ 35 g/l, albuminaemia <30 versus ≥30 g/l, PMA <37 versus ≥37 weeks, PNA <10 versus ≥10 days and indirect hyperbilirubinaemia versus no indirect hyperbilirubinaemia), relationship between unbound and total cefazolin concentration was expressed using regression equation.

Results

Forty neonates (male/female ratio 25/15) were included. Clinical characteristics and indications for cefazolin administration are provided in Table 2. Based on 131 samples, median (range) total and unbound cefazolin plasma concentrations were 100.63 (9.65-404.22) mg/L and 40 (2-261.38) mg/L, respectively. Median unbound cefazolin fraction was 0.39 (0.10-0.73).

Table 2: Clinical characteristics of all included study patients (n=40), reported by median and range or number of cases. Indirect bilirubinaemia and plasma free fatty acids (FFA) values were available in 39/40 patients.

Clinical characteristics	Median (range) or number of cases
Postmenstrual age (PMA, weeks)	39 (25-45)
Postnatal age (PNA, days)	9 (1-108)
Body weight at inclusion (g)	2767 (830-4200)
Albuminaemia (g/l)	34.70 (28.2-43.70)
Indirect bilirubinaemia (mg/dl)	2.80 (0.10-11.13)
Free fatty acids (mmol/l)	0.10 (0.00-0.84)
Creatinaemia (mg/dl)	0.44 (0.21-1.03)
Cefazolin dose (first administration) (mg/kg)	50.13 (30.70-55.25)
Cardiopathy	23
Indication for cefazolin prophylaxis	
Percutaneous cardiovascular procedures	21
Cardiac surgery	6
Thoracic surgery	6
Gastrointestinal surgery	4
Neurosurgery	2
Prophylaxis uropathy	1

Linear regression of unbound cefazolin fraction as a function of unbound cefazolin plasma concentration resulted in an R^2 value of 0.39 ($p<0.001$) and a slope significantly different from zero ($p<0.001$) (Figure 1). Linear regression analysis was significant between unbound cefazolin fraction and total cefazolin concentration ($R^2=0.10$, $p<0.001$), PMA ($R^2=0.29$, $p<0.001$), PNA ($R^2=0.17$, $p<0.001$), GA ($R^2=0.04$, $p=0.018$), albuminaemia ($R^2=0.12$, $p<0.001$) and indirect bilirubinaemia ($R^2=0.10$, $p<0.001$), but not between unbound cefazolin fraction and plasma FFA concentrations ($R^2=0.001$, $p=0.657$). Unbound cefazolin fraction was not significantly influenced by cardiopathy (MWU-test, $p=0.08$), nor by gender (MWU-test, $p=0.31$). In a multiple forward regression analysis, based on all (n=131) samples, 4 independent covariates of unbound cefazolin fraction (PMA, albuminaemia, total cefazolin concentration and indirect bilirubinaemia) resulted in an R^2 value of 0.496 ($p<0.001$). Linear regression graphs of the 4 individual covariates retained, are shown in Figure 2 a, b, c and d.

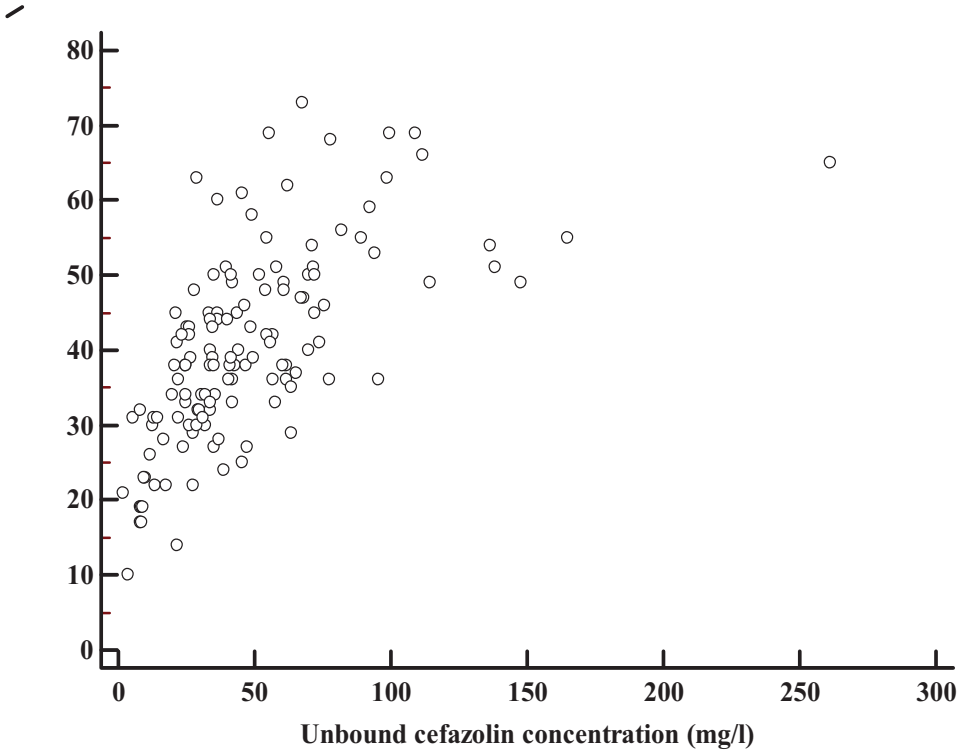


Figure 1: Linear regression of unbound cefazolin fraction as a function of unbound cefazolin plasma concentration. Regression equation was expressed as y= 29.6 + 0.22x

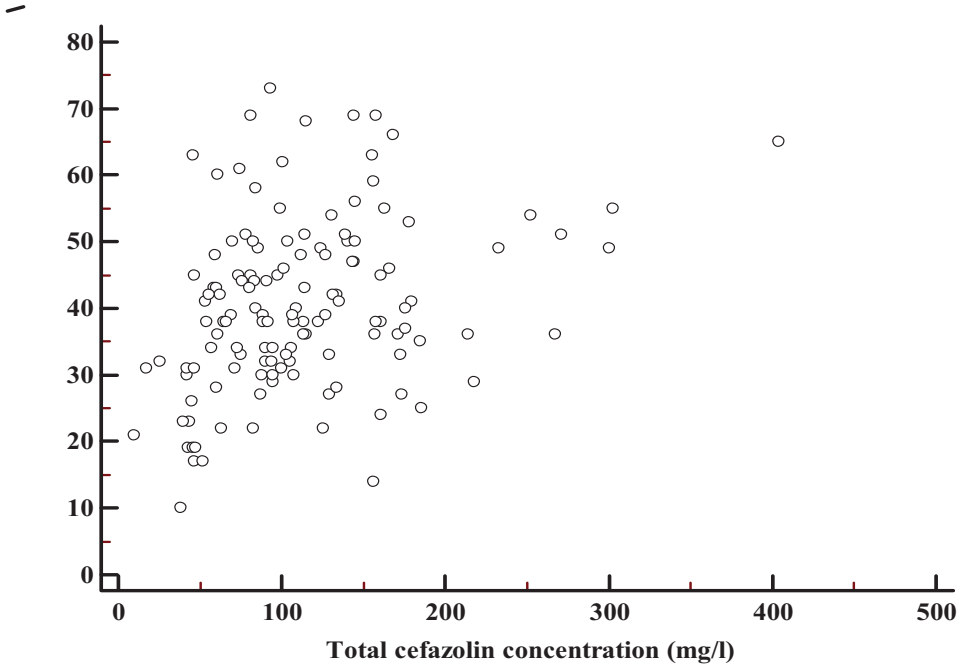


Figure 2a

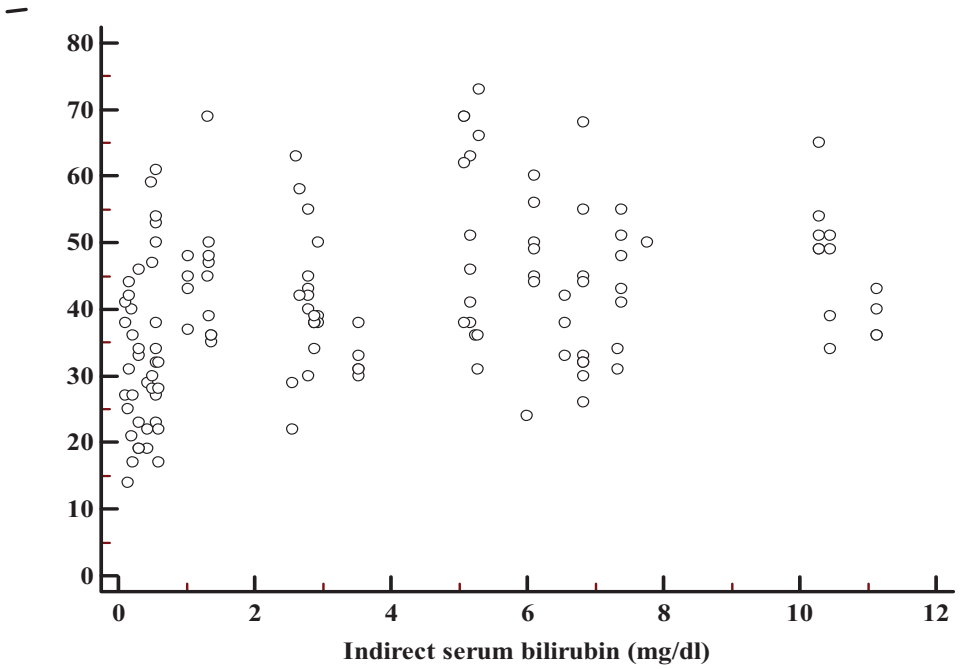


Figure 2b

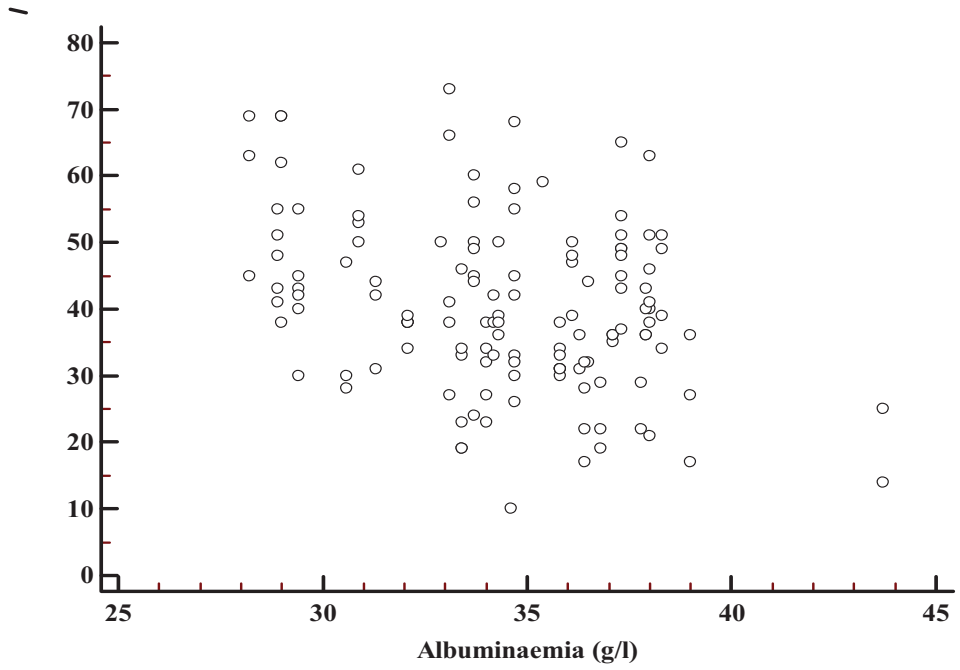


Figure 2c

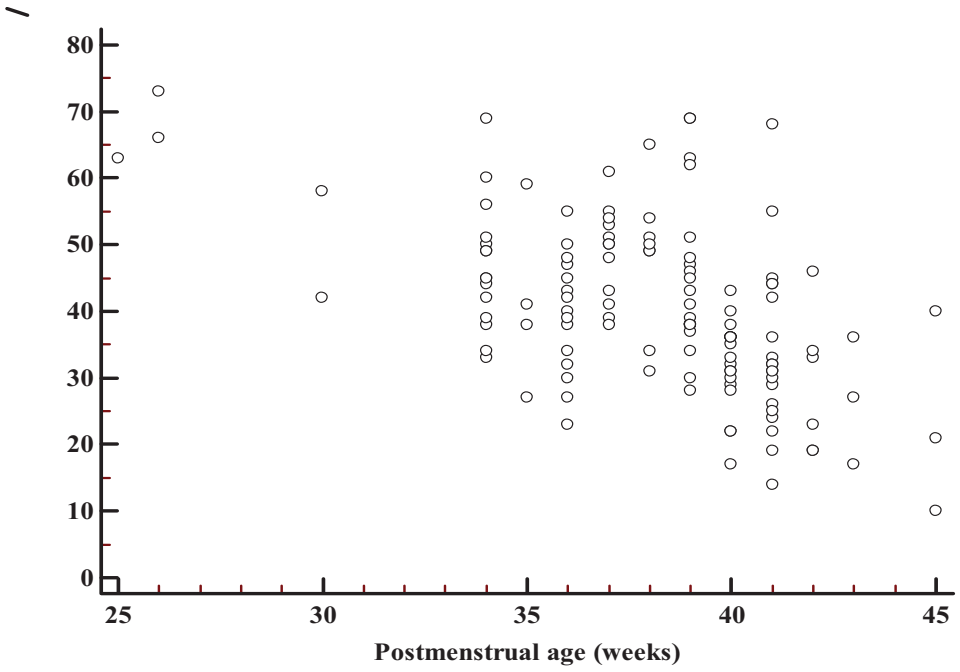


Figure 2d

Figure 2: Linear regression of unbound cefazolin fraction as a function of (a) total cefazolin plasma concentration (R^2=0.10), (b) indirect serum bilirubin concentrations (R^2=0.10), (c) albuminaemia (R^2=0.12) and (d) postmenstrual age (R^2=0.29).

Paired plasma samples were available in 29 neonates. Median peak and trough sampling times and corresponding cefazolin concentrations are reported (Table 3). Median (range) unbound cefazolin fraction at peak level (0.46, 0.28-0.69) was significantly higher compared to trough level (0.36, 0.17-0.73) ($p<0.001$).

Table 3: Paired unbound cefazolin (CFZ) plasma concentration, total cefazolin plasma concentration and unbound cefazolin fraction in 29 patients at peak (i.e. 0-2 h after first cefazolin administration) and trough (i.e. >6 h after first cefazolin administration but before next cefazolin administration, if scheduled) times. Data are represented by median and range. ***** Statistical significant difference.

Parameter	Peak	Trough
Sampling time (h)	1.00 (0.28-1.93)	8.00 (6.47-8.75)
Unbound CFZ concentration (mg/L)	68.10 (35.47-165.04)	21.92 (2-67.74)
Total CFZ concentration (mg/L)	155.60 (84.23-302.45)	60.42 (9.65-156.91)
Unbound CFZ fraction *	0.46 (0.28-0.69)	0.36 (0.17-0.73)

In Table 4 we estimated total cefazolin concentrations needed when an unbound cefazolin concentration of 8 mg/L is used as minimal inhibitory concentration (MIC) for susceptible pathogens e.g. CONS (Clinical and Laboratory Standards Institute MIC value) [26]. Estimates were provided for different clinical conditions. Considering the total study population, unbound cefazolin concentration was ≥ 8 mg/L in 128 of 131 samples (97.7%).

Table 4: Estimates of total cefazolin (CFZ) concentration (mg/L) needed for a time above minimal inhibitory concentration ($T_{>MIC}$) of 100% if an unbound cefazolin concentration of 8 mg/L is used as MIC value for susceptible pathogens e.g. coagulase-negative staphylococci [26]. Estimates are based on the regression equations of the unbound *versus* total cefazolin concentrations collected in our study population for different clinical conditions. Threshold values for hyperbilirubinaemia are >115 µmol/L if PNA<2 days, >155 µmol/L if PNA 2-5 days, >120 µmol/L if PNA 6-12 days, >80 µmol/l if PNA 13-19 days, >45 µmol/L if PNA 20-26 days and >10 µmol/L if PNA ≥27 days, as reported in literature, to adapt for the normal postnatal increase and subsequent decrease of bilirubin in neonatal life [23]. PMA: postmenstrual age, PNA: postnatal age.

Clinical inclusion criteria	Total CFZ concentration (mg/L)	Clinical inclusion criteria	Total CFZ concentration (mg/L)
Albuminaemia <35 (g/l)	30.38 *(y = -8.10 + 0.53x)*	Albuminaemia ≥35 (g/l)	48.74 *(y = -18.32 + 0.54x)*
Albuminaemia <30 (g/l)	24.76 *(y = -6.61 + 0.59x)*	Albuminaemia ≥30 (g/l)	38.90 *(y = -12.23 + 0.52x)*
PMA <37 weeks	29.89 *(y = -8.14 + 0.54x)*	PMA ≥37 weeks	39.29 *(y = -12.43 + 0.52x)*
PNA <10 days	35.76 *(y = -12.74 + 0.58x)*	PNA ≥10 days	30.76 *(y = -3.38 + 0.37x)*
Indirect hyperbilirubinaemia	42.57 *(y = -17.97 + 0.61x)*	No indirect hyperbilirubinaemia	25.87 *(y = -3.64 + 0.45x)*

Discussion

Effective treatment with cefazolin requires integration of protein binding, which will affect disposition since only the unbound drug is responsible for antimicrobial action. We documented a median (range) unbound cefazolin fraction of 0.39 (0.10-0.73) in neonates and established between-patient as well as within-patient saturability of cefazolin protein binding. Neonatal unbound cefazolin fraction in our study is higher than reported values in adults (0.25, 0.14-0.41 in pregnant woman and 0.19, 0.10-0.51 in non-pregnant adults) [11,13]. We hereby confirm the findings of Deguchi *et al.* who reported a mean unbound cefazolin fraction of 0.51 (± 0.17) in neonates and also referred to a significant difference in unbound cefazolin fraction between newborns and children (0.22±0.03, n=6) *versus* adults (0.11±0.02, n=12) [4].

In addition to the paper of Deguchi *et al*., we explored covariates of the inter-individual variability in neonatal unbound cefazolin fraction. Albuminaemia, total cefazolin plasma concentration, indirect bilirubinaemia and PMA explained 49.6% of this variability. Covariates ‘albuminaemia’ and ‘total cefazolin plasma concentration’ are in line with findings in adults [11]. Since neonates frequently display a lower albuminaemia compared to children or adults, an elevated unbound drug fraction can be expected. Within the neonatal population, albuminaemia increases with age reaching adult levels before the age of 5 months [27]. The variation of unbound cefazolin fraction with total cefazolin concentration confirms that the

concept of cefazolin protein binding saturability is also applicable to neonates, but saturation is reached sooner compared to other populations because of a lower binding capacity [13].

In addition to findings in adults, covariates 'indirect bilirubinaemia' and 'PMA' were specific for neonatal cefazolin protein binding. In early life, indirect hyperbilirubinaemia is common due to an increased production and decreased removal (by glucuronidation) of indirect bilirubin. Bilirubin displays an association constant 100 to 1000 times greater than most drugs for albumin [27]. It can displace cefazolin from plasma albumin, resulting in an increased unbound drug fraction in case of an increased indirect bilirubin level. Additionally, the binding of bilirubin to albumin can induce an allosteric effect on albumin resulting in a decreased cefazolin binding percentage [4,8]. However, cefazolin itself can also displace bilirubin from albumin binding sites [28], suggesting that the competition between bilirubin and cefazolin for albumin binding sites is a mutual interaction [29]. Our observation is an illustration of this competitive binding concept. Structural changes of albumin in pathological conditions or the possible influence of unknown endogenous inhibitors on albumin binding need further research.

PMA showed the strongest relation with unbound cefazolin fraction (R^2=0.29). Changes in neonatal plasma profile and body composition can partly be attributed to PMA and can influence drug protein binding [27]. The importance of age on neonatal protein binding was also documented for other compounds. Pullen *et al*. reported a significant correlation of neonatal flucloxacillin protein binding with age (gestational age, postconceptional age) [30].

The impact of protein binding on cefazolin PK and PD needs special attention. Cefazolin elimination occurs by renal route (glomerular filtration and active tubular secretion). In general, higher protein binding is associated with lower drug elimination by tubular secretion and correlates negatively with glomerular filtration, because only the unbound drug is filtered [31-33]. Since a higher unbound cefazolin fraction is documented in neonates, it is reasonable to postulate that clearance is proportionally higher compared to adults. However, the combination of an overall low glomerular filtration rate (GFR) and less effective renal tubular functions in neonates makes it difficult to predict the effect of protein binding on neonatal cefazolin clearance and needs further study.

Protein binding also affects volume of distribution, because only the unbound drug can reach the extravascular space [34]. Deguchi *et al.* documented that inter-individual changes in cefazolin volume of distribution per body weight in neonates are mainly due to individual differences in the unbound cefazolin plasma fraction [4].

The efficacy of cefazolin is related to the drug concentration at the receptor site (i.e. bacterial cell wall), but also to the time drug concentration exceeds the MIC for a given pathogen [31,33,35]. Measurement of total cefazolin concentration without considering aspects of unbound cefazolin fraction is, therefore, not reliable for estimating PD effect. For prophylactic use, we aim for a $T_{>MIC}$ of 100%. In our study, 97.7% of samples had an unbound cefazolin concentration ≥8 mg/L, reflecting good prophylaxis for susceptible pathogens e.g. CONS [26]. For different patient subgroups, we provided total cefazolin concentration needed to exceed an unbound concentration of 8 mg/L (Table 4). These estimates are based on the regression equation of the unbound versus total cefazolin concentrations collected in our study population and illustrate variability in different clinical conditions. To introduce these results in clinical practice, further population modeling is needed. Since the unbound cefazolin fraction is higher in neonates, lower doses (mg/kg) might theoretically be used to provide the same drug effect as in adults although final dosing guidelines should also consider cefazolin distribution and clearance. We did not focus on cefazolin PD but rather want to stress that the influence of protein binding on drug PD in neonates is of relevance beyond compound specific observations. In general, a minor decrease in protein binding -for very highly (e.g. >90%) protein bound drugs- will have a major effect on the free drug proportion. For drugs with a high toxicity profile, this can result in altered (side)effects and administration of lower drug doses have to be considered. For drugs with minor toxicity, like cefazolin, we have to be aware that dose adaptations may result in ineffectivity (drug dose too low) or increased free bilirubin (drug dose too high). Overall, data concerning protein binding, population specific PK/PD as well as drug characteristics have to be taken into account in future initiatives to optimize dosage regimens.

Our study has its limitations, but also some strengths. Due to restrictions in blood volume available for sampling in neonates, we could not collect all predefined samples in each patient. FFA can influence drug-albumin interaction [8,36], however FFA were no significant determinant of cefazolin protein binding in our study and concentrations were lower than earlier reported values in neonates and infants [27,37]. This can be due to interferences of co-medication and parenteral nutrition and sample handling [38]. Finally, review of individual patient data showed that the highest values of total (404.22 mg/L) and unbound (261.38 mg/L) cefazolin concentration belong to the same patient. Chart review indicated this patient unintentionally received an additional cefazolin dose 1.8 h after the induction dose. These limitations reflect the difficult balance between research aims and clinical care in neonatal

intensive care setting. We suggest that the strengths of our study are the relevant study size (40 neonates) and the repeated, focused sampling instead of opportunistic sampling strategies (e.g. Pullen *et al*, remnants), enabling paired analysis.

We conclude that albuminaemia, total cefazolin concentration, indirect bilirubinaemia and PMA are the main covariates explaining 49.6% of inter-individual variability of neonatal cefazolin unbound fraction. Between- and within-patient saturability of cefazolin protein binding were also documented in neonates, but compared to adults a higher unbound cefazolin fraction was found in neonates. The integration of cefazolin protein binding aspects in PK/PD research is warranted to optimize neonatal cefazolin dosing regimens. Since neonatal PK studies often assume a fixed degree of protein binding, we recommend the measurement of protein binding, especially for highly protein bound and clinically relevant drugs. This need to be studied in sufficient numbers of patients to give a reliable point estimate for the neonatal population as well as a distribution for the extent of protein binding. How our data can be used to perform population pharmacokinetic modeling, will be illustrated in section 5.3.

5.2. Cefazolin plasma protein binding in different human populations

Methods

Study population and cefazolin plasma protein binding data

Cefazolin plasma protein binding data of 4 published patient cohorts were collated. In total, the analysis consisted of total cefazolin concentrations, unbound concentrations and unbound fractions of 352 plasma concentrations-time points, collected in 124 patients.

In a cohort of pregnant women (30 surgical interventions) [11] and a cohort of 40 neonates [39], both receiving cefazolin for surgical prophylaxis, respectively 130 and 131 blood samples were collected. In a cohort of 12 surgical adults undergoing (semi-)elective abdominal aortic aneurysm open repair with cefazolin as prophyaxis, cefazolin protein binding was determined in 36/104 plasma concentration-time points [12]. Finally, data of a non-surgical adult cohort consisting of 31 adults treated with cefazolin either within a home antibiotic program or as in-patients and of 12 other non-surgical hospitalized adults treated with cefazolin were included [13]. In Table 5, general clinical characteristics, cefazolin dosing regimen and indication of the 4 cohorts are presented. For further details we refer to the individual references [11-13,39].

Table 5: Characteristics of included patient groups. n= number of patients

Parameter	Pregnant women [11] (n=30)	Neonates [39] (n=40)	Surgical adults [12] (n=12)	Non-surgical adults [13] (n=43)
Median (range) age	Childbearing age	9 (1-108) days	70 (62-81) years	19-91 years
CFZ indication	Prophylaxis	Prophylaxis	Prophylaxis	Therapy
CFZ Dosing	2 gr/8h	50 mg/kg/8h	single 2 g bolus	2-6g/day, q8h or ct.
Plasma samples	130	131	36	55

Drug assay

Cefazolin was quantified using a validated liquid chromatography-tandem mass spectrometry (adult surgical cohort) or high performance liquid chromatography (other patients). Ultrafiltration was performed on all samples to determine unbound cefazolin fraction.

Statistical analysis

To explore single continuous variables between the datasets, the Kruskal-Wallis test was used. For further data exploration 3 patient subgroups were defined i.e. pregnant women, neonates and (non-pregnant) adults. Each data point was considered as a unique observation of cefazolin protein binding. Observations with data on unbound cefazolin concentration, total cefazolin concentration and albuminaemia (n=237) were included in a multiple regression analysis to define covariates of unbound cefazolin fraction. A p-value <0.05 was considered statistical significant. Statistical analysis was performed using SPSS (SPSS, version 20.0, Armonk, New York, IBM Corp.) and Medcalc (Mariakerke, Belgium).

Results

Median total cefazolin concentrations, unbound concentrations and unbound fractions differed significantly between the 4 original populations (Kruskal-Wallis test, $p<0.0001$). For the 237 observations retained, median (range) total [55.34 (1.96-148.26) mg/L, 100.63 (9.65-404.22) mg/L, 122 (2.30-547) mg/L] and unbound cefazolin concentrations [15.59 (0.31-46.69) mg/L, 40 (2-261.38) mg/L, 18.98 (0.40-192.60) mg/L] for respectively pregnant women, neonates and adults as well as unbound fraction (Figure 3a) and albuminaemia (Figure 3b) differed significantly between the 3 subgroups (Kruskal-Wallis test, $p<0.05$). Albumin concentration in neonates hereby differed significantly from pregnant women, but not from non-pregnant adults probably due to the large range of albuminaemia in the non-pregnant adults. Seventy-seven percent ($R^2=0.768$) of the variability in unbound cefazolin fraction was explained by covariates total cefazolin concentration, unbound concentration, albuminaemia and patient subgroup (represented by 2 dummy variables).

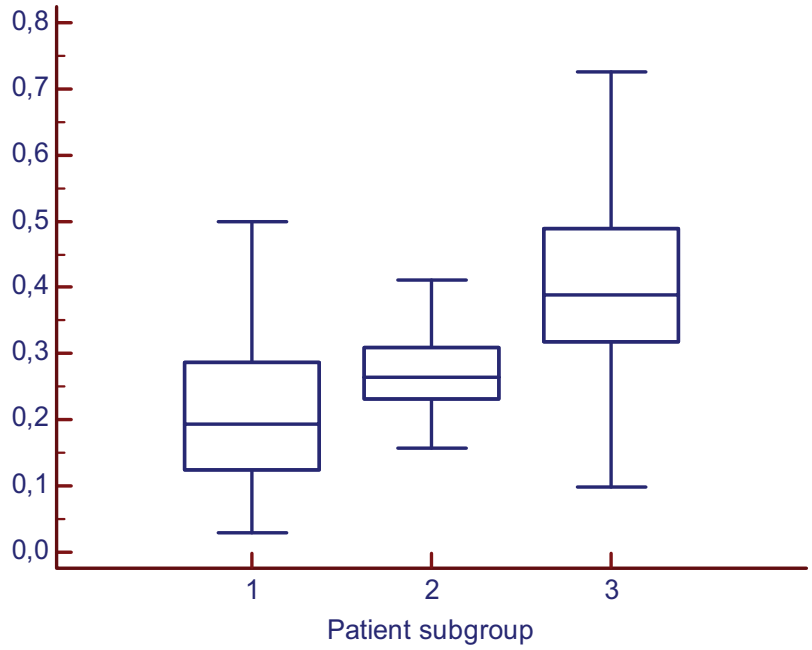


Figure 3a

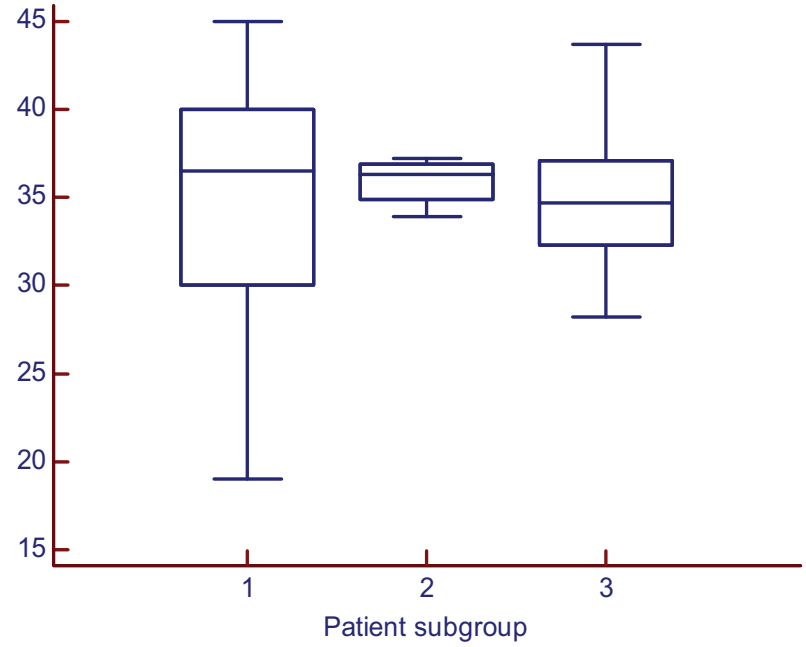


Figure 3b

Figure 3: (a) Unbound cefazolin (CFZ) fraction and (b) albuminaemia for different patient subgroups (1= adults, 2= pregnant women and 3= neonates). Only observations (n=237) with available total cefazolin concentrations, unbound cefazolin concentrations and plasma albumin concentrations were included.

Discussion

Significantly higher median unbound cefazolin fractions were documented in pregnant women and neonates compared with adults. Since only the unbound drug can have a pharmacological effect, integration of the present observations in pharmacokinetic/pharmacodynamics (PK/PD) analyses may be useful to develop optimized, population-specific cefazolin dosage regimens. To explore whether this binding pattern also applies for other highly albumin-bound compounds across these populations, similar investigations are needed in the future.

The impact of albuminaemia on unbound cefazolin fraction was anticipated. Both pregnancy and neonatal life display hypoalbuminaemia. During pregnancy, median albuminaemia decreases with increasing gestational age. In neonates, albumin concentration increases rapidly up to the age of 5 months. As illustrated in Figure 3, the differences in unbound cefazolin fraction between patient subgroups can, however, not solely be attributed to alterations in albumin concentrations ('quantitative' aspect), but also depend on the covariate 'patient subgroup'. This may in part be explained by an altered affinity constant for the drug-albumin interaction ('qualitative' aspect) owing to endogenous agents (e.g. bilirubin, free fatty acids, urea) [8]. Interference of these agents with drug-albumin binding is based on competition for albumin binding sites or by inducing structural albumin modifications. The variation of unbound cefazolin fraction with total cefazolin concentration confirms protein binding saturability, which was documented previously [11,13,39].

As a highly protein bound, renally eliminated drug, of which dosing is not titrated to effect, alterations in protein binding of cefazolin may be clinically relevant, even if limited, since they can influence PK parameters and achievement of PD targets. To the best of our knowledge, this is the first pooled analysis of cefazolin protein binding data.

In conclusion, cefazolin protein binding varies between different patient groups. This can not only be attributed to alterations in plasma albumin concentrations. Subpopulation specific characteristics also matter. These data can be considered in the optimization of cefazolin dosing regimens. Further exploration of whether the protein binding pattern described applies beyond compound specific observations would be of interest.

5.3. Population pharmacokinetic modeling of total and unbound cefazolin plasma concentrations as a guide for dosing in preterm and term neonates

Methods

Ethics, study population and drug dosing

The patients included in this analysis are based on the cohort of 40 neonates and young infants described in section 5.1. Details on study approval, cefazolin dosing regimen, collection of clinical characteristics and laboratory data can be found in section 5.1. As in the present analysis only neonates with postnatal age (PNA) 1-30 days were included, patients with PNA 48, 51, 53 and 108 days) were excluded from the original dataset. Clinical characteristics of the study population are presented in Table 6.

Blood sampling and drug assay

Blood sampling procedure and quantification of total and unbound cefazolin plasma concentrations are extensively described in section 5.1.

Table 6: Clinical characteristics of the neonates included in the pharmacokinetic analysis.

Patient characteristics	Median (range) or number of cases
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

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 used to visualize and evaluate the model were 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).

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 [40]. A decrease in objective function value (OFV) of ≥3.9 points was considered statistically significant ($p<0.05$ based on χ^2 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 [41] and shrinkage [42] were assessed.

Structural and statistical sub-model

A one and two compartment pharmacokinetic models 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 [43]:

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

(Equation 1)

In this equation $C_{unbound}$ represents the unbound cefazolin concentration, C_{total} the total cefazolin concentration, 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 [bBW (g), bodyweight on the day of birth], current bodyweight [cBW (g), bodyweight on the day of blood sampling], postnatal age [PNA (days)], gestational age [GA (weeks)], postmenstrual age [PMA (weeks), combination of GA and PNA in weeks], albuminaemia (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):

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

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 OFV decreased by ≥7.8 points (p-value <0.005). The covariate causing the largest reduction in OFV 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 used. To perform this analysis the dataset was simulated 1000 times after which each observed concentration was compared with the simulated concentrations using the NPDE package in R [44,45].

Monte Carlo simulations

To evaluate $T_{>MIC}$, the Clinical and Laboratory Standards Institute (CLSI) 2012 [26] 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 7) [26]. As effective cefazolin therapy is reported to require at least 60% of $T_{>MIC}$, the probability of attaining unbound cefazolin concentrations during 60% of the dosing interval [15] 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 [16]. 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 and cBW ≤ 2000g; group 2: PNA ≤ 7 days and cBW > 2000g; group 3: PNA > 7 days and cBW ≤ 2000g; and group 4, PNA > 7 days, cBW > 2000g) were created. Based on these results, a new model-based dosing regimen was proposed.

Table 7: 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	≤ 8	16	≥ 32
2) *S. hominis*	9.49	12.04	≤ 8	16	≥ 32
3) *S. aureus*	6.57	8.33	≤ 8	16	≥ 32
4) *S. capitis*	6.57	8.33	≤ 8	16	≥ 32
5) *E. coli*	4.38	5.56	≤ 2	4	≥ 8

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

Results

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 101.09 (range 17.44-404.22) mg/L and 41.15 (range 5.34-261.38) mg/L, respectively. The median unbound fraction was 0.40 (range 0.14-0.73). Clinical characteristics are presented in Table 6.

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 cefazolin concentrations, was parameterized in terms of clearance (Cl), volume of distribution (Vd), maximum protein binding B_{max} and the dissociation constant K_D (Figure 4).

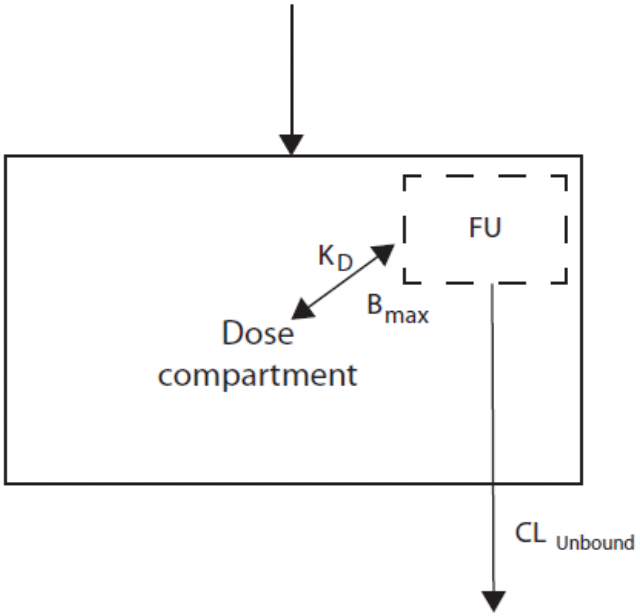


Figure 4: Schematic representation of the pharmacokinetic model using both total and unbound concentrations of cefazolin. K_D = Dissociation constant, B_{max} = Maximum protein binding, FU = unbound fraction of cefazolin, $CL_{unbound}$ = Clearance of unbound cefazolin

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.

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 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, both analyses resulted in a comparable improvement of the model (i.e. same reduction in OFV of 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 8). The model was further improved (reduction in OFV of 12 points, $P< 0.005$) by introducing albumin on B_{max} using a linear function (Table 8).

The parameter estimates of the simple and the final pharmacokinetic model and the values obtained from the bootstrap analysis are provided in Table 8.

Table 8: Model-based population pharmacokinetic parameter estimates and the values obtained after the bootstrap analysis.

Parameter	Simple model without covariates Value (CV%)	Final pharmacokinetic covariate model Value (CV%)	Bootstrap final pharmacokinetic model 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

In Figure 5, the observed *versus* predicted concentrations are plotted for the total and unbound concentrations showing that the model adequately describes the data. In Figure 6, the inter-individual variabilities in clearance, Vd and B_{max} are plotted against the relevant covariates for the simple and the final pharmacokinetic model. A significant part of the interindividual variability is explained (Figure 6). This is also reflected in the decrease in the estimates of the interindividual variability when comparing the simple and the final pharmacokinetic model, which resulted in a decrease of 50% in the interindividual variability in Vd, 58% in clearance and 41% in B_{max} (Table 8). In Figure 7 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 can be explained by differences in current bodyweight, birth bodyweight and PNA of the subjects (Figure 7).

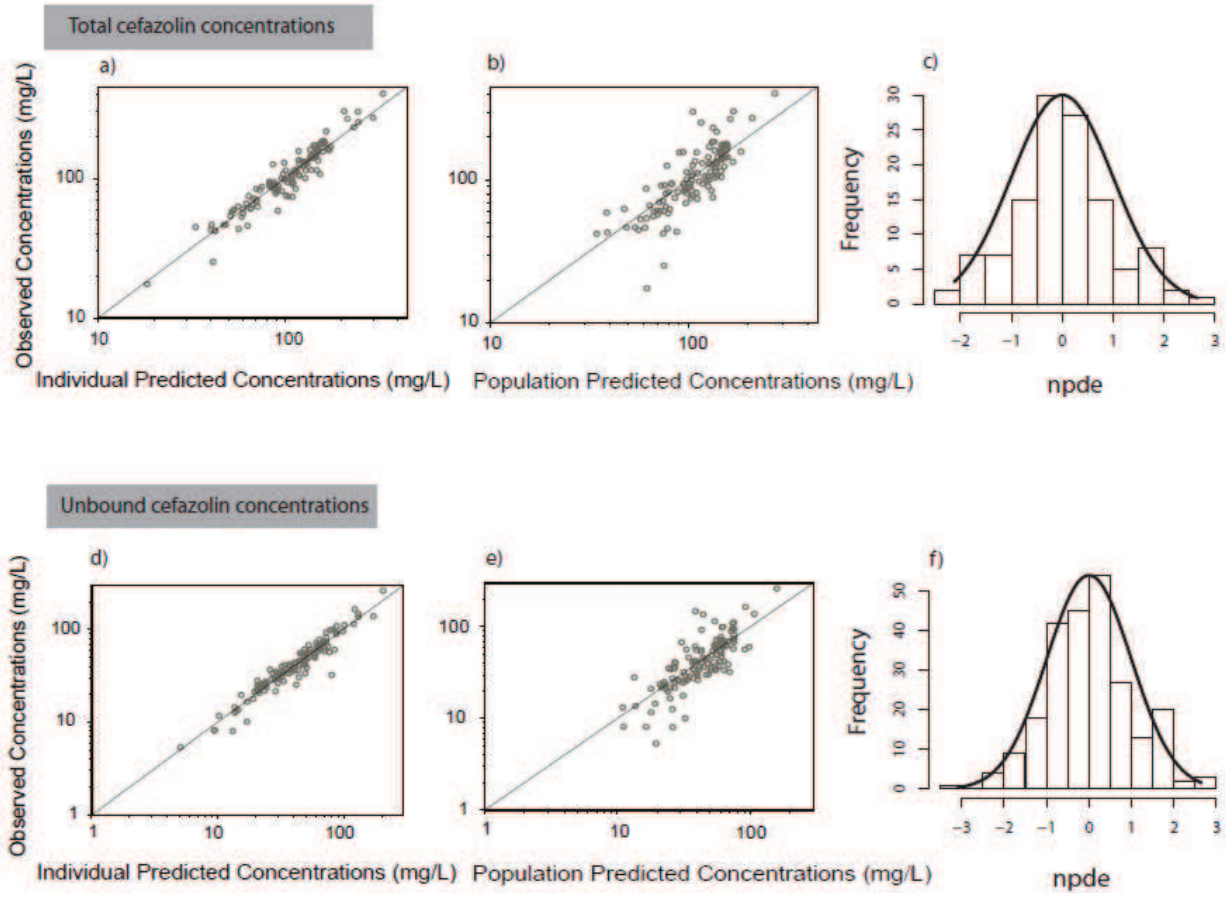
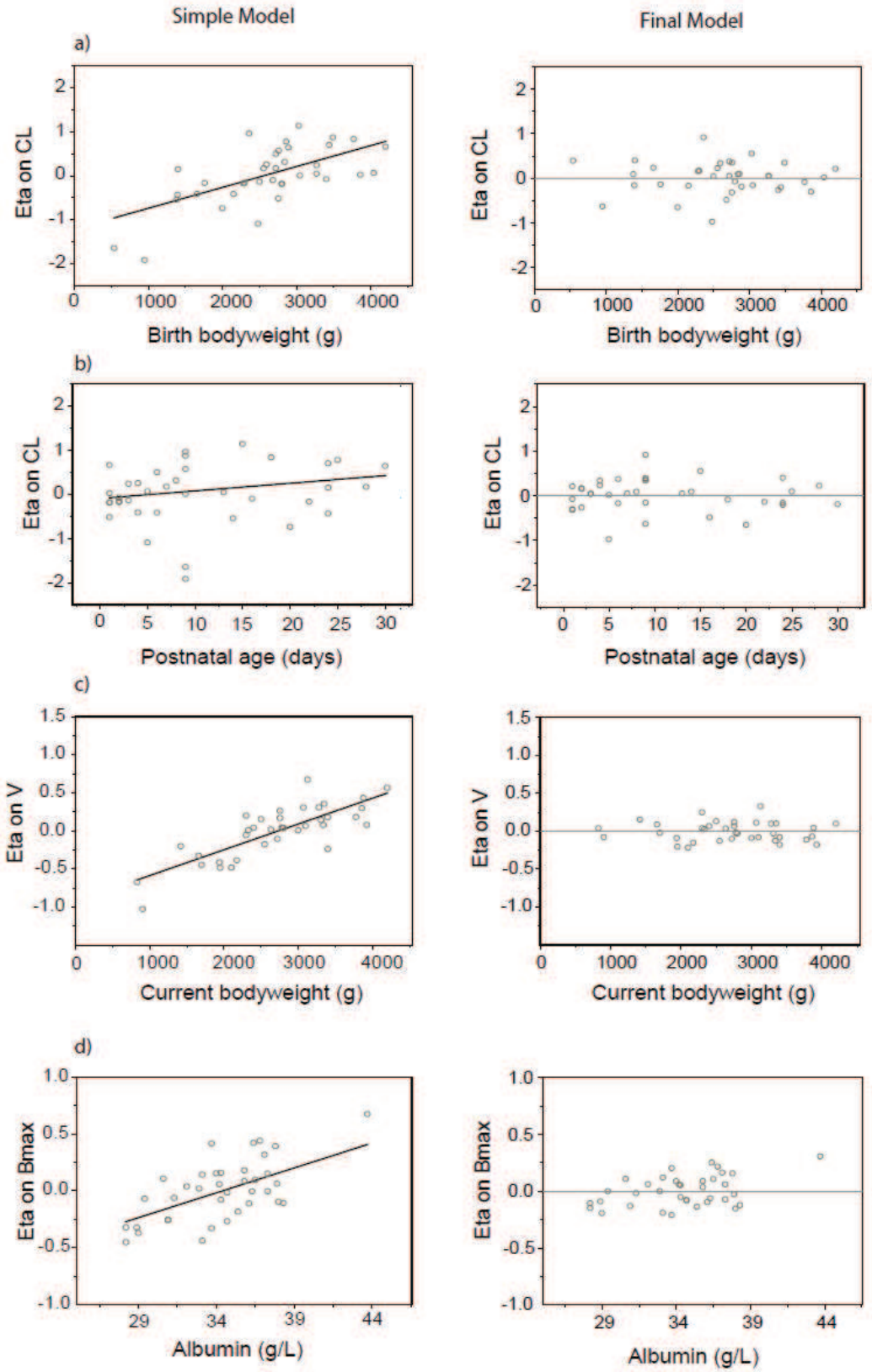


Figure 5: Observed *versus* individual predicted concentrations (a,d) and population predicted concentrations (b,e) for total (upper panels) and unbound (lower panels) cefazolin concentrations. The histograms show the distribution of the normalized prediction distribution error (NPDE) methods for total (c) and unbound (f) cefazolin concentrations.



Figur 6: 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).

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 was calculated to be 0.6.

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

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

Model Validation

The results of the bootstrap analysis (Table 8) show that the median estimated values based on the resampled dataset were within 10% of the values obtained in the final model. The NPDE histograms follow the normal distribution, indicating the accuracy of the final pharmacokinetic model (Figure 5). Furthermore, no trend was seen in the NPDE *versus* time or *versus* predicted concentrations. The number of ill-conditioning (74.6) was far below

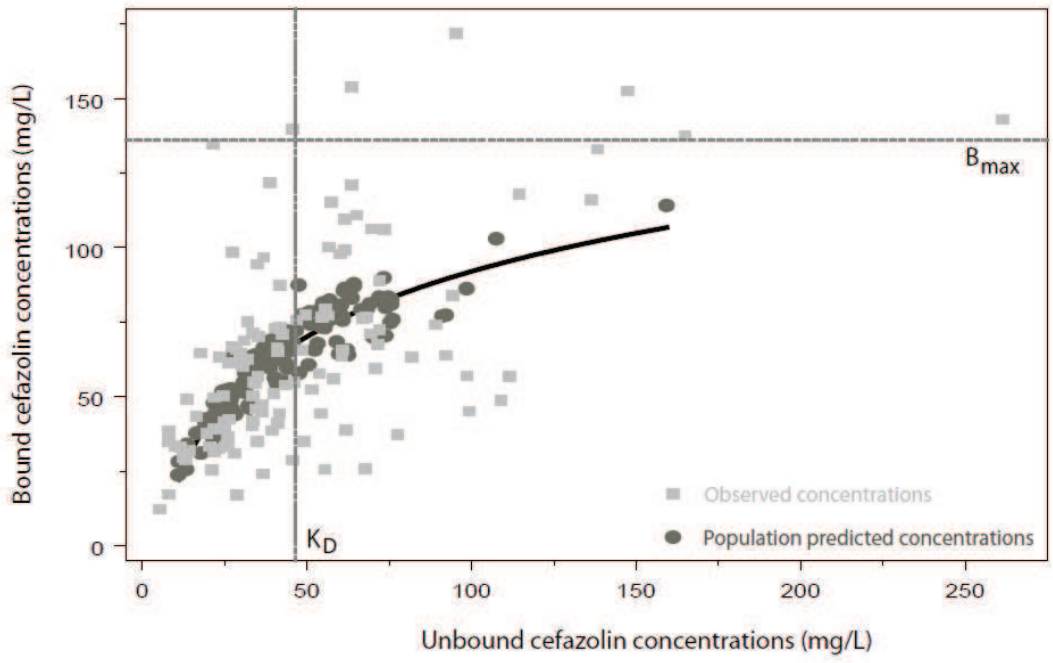


Figure 7: The relationship between the observed (*square*) and model-based predicted (*circle*) bound and unbound cefazolin concentrations (mg/L) in 36 (pre)term neonates. B_{max} (protein binding defined as the maximum estimated concentration bound to albumin) and K_D (dissociation constant defined as the unbound concentration which corresponds to 50% of the maximum binding capacity) are illustrated.

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}.

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 9) were predicted based on Monte Carlo simulations using the final pharmacokinetic model (Figure 4). In Figure 9, box plots illustrate the median and interquartile ranges (5% to 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 8, 9). 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 9). 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 9B).

Table 9: 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 8. PNA = postnatal age, cBW = current bodyweight

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
	8-28 days		50	8
Proposed dosing regimen	≤ 7 days	≤ 2000g	25	12
	≤ 7 days	> 2000g	50	12
	8-28 days	≤ 2000g	25	8
	8-28 days	> 2000g	50	8

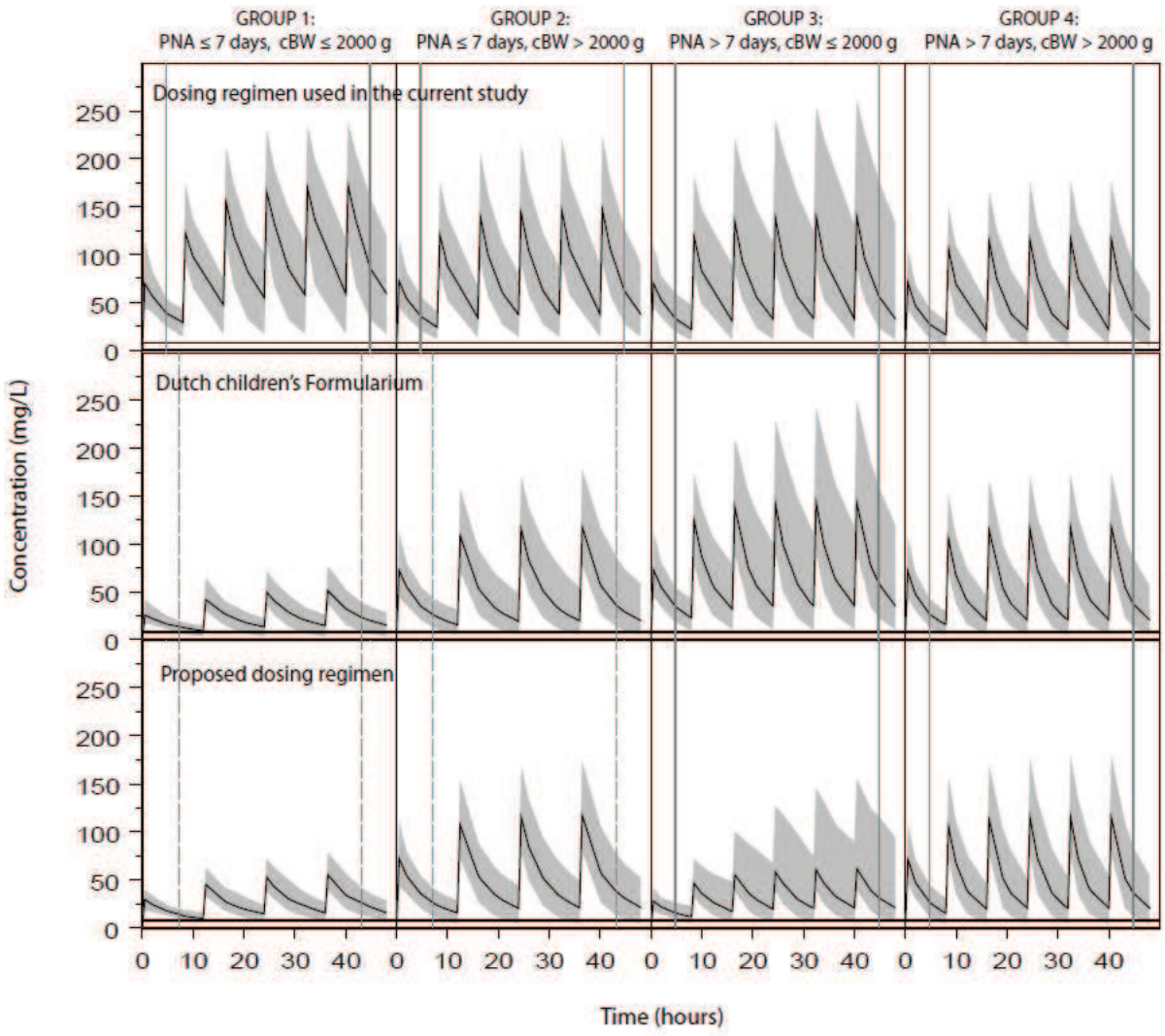


Figure 8: Concentration-time profiles based on 1000 Monte Carlo simulations using the final pharmacokinetic model following the dosing regimen used in this study (upper row), the dosing regimen proposed by the Dutch Children's Formulary (middle row) and the new model-based proposed dosing regimen (bottom 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 full 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 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.

Discussion

Neonatal cefazolin PK data and cefazolin dosing regimens (Table 1) 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 7), 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.

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 reflect the prenatal and postnatal maturation, respectively [46]. Furthermore, age and bodyweight were earlier documented as clearance predictors of other beta-lactams in neonates [47-50]. 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 [47,51]. 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 [30,48]. Therefore, results of amoxicillin and flucloxacillin may not be directly applied to cefazolin.

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) [8,52,53].

We documented relatively high cefazolin plasma concentrations based on a 50 mg/kg/8h cefazolin dosing regimen, administered to all study patients. This is probably 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 8 and 9, 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 9). 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 1. As a consequence of cefazolin dose reduction, albumin binding places become available 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 multidrug therapies, this is a relevant and population specific advantage. Recent pharmacokinetic 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 [47] and an increase of initial dose with subsequent dose reduction depending on the microbiological isolate, for flucloxacillin [51] were suggested in neonates. This emphasizes the need for population specific pharmacokinetic studies in neonates. Since study methodologies can differ, a correct definition of the aimed pharmacokinetic target is required to achieve reliable dosing evaluations in this specific population [15,54]. In general, we have to be aware that total daily dose reduction of an antimicrobial may lead to increased bacterial resistance and ineffectiveness [55]. 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

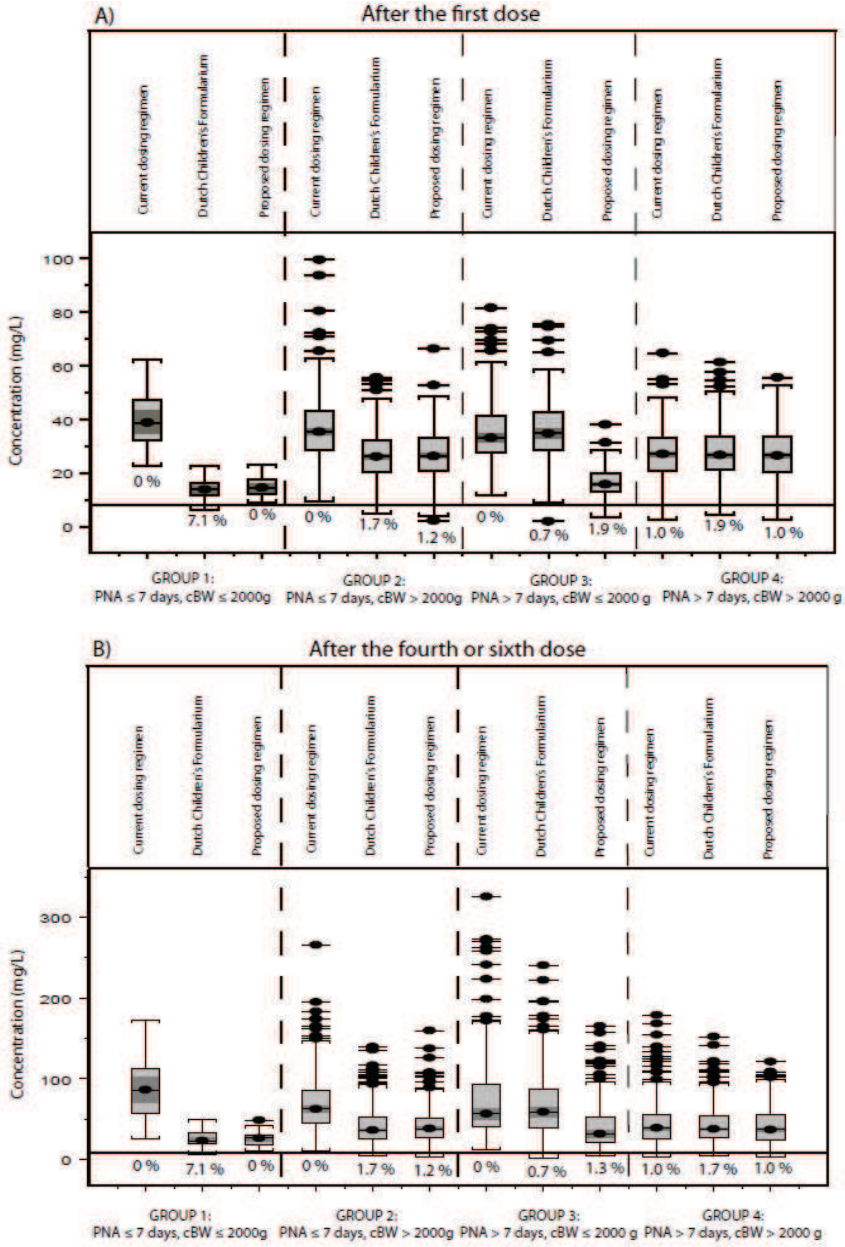


Figure 9: Individual predicted concentrations based on Monte Carlo simulations in 1000 individuals *versus* 4 different groups based on current bodyweight (cBW) and postnatal age (PNA). Plot A represents the individual predicted concentrations at 60% of the dosing interval after the first dose which 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.

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 correct, well-timed drug administration and subsequent tissue distribution. Direct measurement of drug concentrations in the surgical site tissues [56,57] may provide additional information to include in pharmacokinetic models, but is very challenging in this population [58].

We conclude that total and unbound cefazolin concentrations in neonates could be described by a one compartment pharmacokinetic 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.

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PART III: Prospective dosing validation

Development of individualized dosing regimens in neonates

CHAPTER 6

Prospective validation of a model-based amikacin dosing regimen in neonates

This chapter is based on

Prospective validation of a model-based dosing regimen for amikacin in preterm and term neonates

(ready for submission)

Abstract

Introduction A novel neonatal amikacin dosing regimen was previously developed based on a population pharmacokinetic model. The aim of the current study was to prospectively validate this model-derived dosing regimen in clinical practice.

Methods First, early therapeutic drug monitoring (TDM) observations were evaluated. Secondly, all observed TDM concentrations were compared with concentrations predicted by the model, whereby the results of an NPDE (normalized prediction distribution error) were considered, after which Monte Carlo simulations were performed. Finally, remaining causes limiting amikacin predictability (prescription errors and disease characteristics of outliers) were explored.

Results In 579 (pre)term neonates [median birth bodyweight 2285 (range 420-4850)g, postnatal age (PNA) 2 (1-30) days, gestational age 34 (24-41) weeks], 90.5% of the observed peak levels reached 24 mg/L and 60.2 % of the early trough levels was <3 mg/L (93.4% ≤5 mg/L). All prospective observations were accurately predicted by the model without bias, which was confirmed by the NPDE. Monte Carlo simulations showed that peak concentrations >24 mg/L were reached in almost all patients. Trough values <3 mg/L were documented in 78-100% and 45-96% of simulated cases, respectively when ibuprofen was co-administered or not, with largest percentages of trough levels >3 mg/L in patient subgroups with postnatal age <14 days and current weight >2000g.

Conclusions Prospective validation of a model-based neonatal amikacin dosing algorithm resulted in optimized peak and trough concentrations in almost all patient groups. Slightly adapted dosing regimens for patient subgroups with suboptimal trough levels (PNA<14 days in combination with current weight >2000g) were proposed. This model-based approach substantially contributes to drug dosing individualization in neonates.

What is already known on this topic

- Extended interval dosing of amikacin contributes to achieve higher peak ('more efficacy') and lower trough ('less toxicity') amikacin concentrations in neonates.
- Neonatal amikacin clearance can be predicted by birth bodyweight (reflecting prenatal renal maturation), postnatal age (reflecting postnatal maturation) and co-administration of ibuprofen.
- A model-based amikacin dosing regimen for neonates was recently developed, and underwent both internal and external, but not yet prospective, validation.

What this study adds

- Prospective validation of this model-based dosing regimen resulted in optimal peak and trough concentrations for a relevant proportion of neonates.
- In neonates with specific clinical conditions influencing amikacin pharmacokinetics, therapeutic drug monitoring remains of utmost importance.
- Prospective validation resulted in a further improvement of the current amikacin dosing regimen, for neonates with postnatal age <14 days in combination with a current weight >2000g. This illustrates the importance of validation studies.

Introduction

Despite profound differences between neonates and adults, neonatal dosing regimens are usually derived from adult regimens using extrapolations based on bodyweight. However, this may lead to under- or overdosing [1,2] with subsequently therapeutic failure or occurrence of adverse drug effects. In order to establish rational and evidence-based dosing regimens for neonates, detailed information on population specific pharmacokinetics (PK) and pharmacodynamics (PD) is needed [3]. Population PK and/or PD analysis using non-linear mixed effect modeling (NONMEM) is a useful tool to generate such knowledge and to improve neonatal dosing regimens. A crucial, but often missing part in the development of new model-derived dosing regimens is a thorough evaluation and validation of these dosing regimens in a large prospective clinical study [3].

Amikacin is commonly recommended to treat (suspected) neonatal sepsis. In our department, co-treatment with amoxicillin (early-onset cases) or vancomycin (late-onset cases) is usually prescribed. Despite the frequent use of amikacin, currently used dosing regimens may lack efficacy or systematically reach the toxic range [4]. Therefore, a model-based dosing regimen was recently developed in which neonatal amikacin dosing is based on current bodyweight [a covariate found for volume of distribution (Vd)], birth bodyweight, postnatal age and co-administration of ibuprofen [covariates for clearance (Cl)] [4]. This model-based dosing regimen was derived from a neonatal pharmacokinetic model that was based on amikacin data from preterm and term neonates (n=874) varying in age between 1 and 30 days, and was both internally and externally (n=239) validated [4]. In July 2011, a simplified version of this dosing regimen (Table 1) was introduced in the neonatal intensive care unit of UZ Leuven, Belgium.

The aim of the current study was to prospectively evaluate this model-based dosing regimen for amikacin in neonates in the same clinical setting. As such, we first defined the proportion of early therapeutic drug monitoring (TDM) results (i.e. first trough and peak samples) achieving target concentrations (trough <3 mg/L or ≤ 5mg/L, peak >24 mg/L). Secondly, using all TDM results, the predictive performance of the previously published model was evaluated based on a comparison of the observed *versus* the model-based predicted concentrations and an NPDE analysis after which Monte Carlo simulations were performed. Finally, we hypothesize that observations outside the target values can also be due to errors on

the application of the dosing regimen during clinical practice or due to covariates not covered by the current dosing approach [4]. We consequently also reviewed prescription errors as well as outliers defined by visual inspection of the plots.

Table 1: Original (1) and simplified (2) model-based dosing regimen of amikacin in neonates used in the current study [4]. The dosing regimen proposed after the prospective validation (3) is also presented. The differences between dosing regimens 1 and 2 are highlighted in bold and italic, between 2 and 3 in bold, italic and grey.

	1. Original model-based dosing regimen		**2. Simplified model-based dosing regimen**		**3. Final dosing regimen after prospective validation**	
CW (g)	PNA <14 days	PNA ≥14 days	PNA <14 days	PNA ≥14 days	PNA <14 days	PNA ≥14 days
0-800	16 mg/kg/48h (group 1)	20 mg/kg/42h (group 2)	16 mg/kg/48h	20 mg/kg/42h	16 mg/kg/48h	20 mg/kg/42h
800-1200	16 mg/kg/42h (group 3)	20 mg/kg/36h (group 4)	16 mg/kg/42h	20 mg/kg/36h	16 mg/kg/42h	20 mg/kg/36h
1200-2000	15 mg/kg/36h (group 5)	19 mg/kg/30h (group 6)	15 mg/kg/36h	***18 mg/kg/30h***	15 mg/kg/36h	18 mg/kg/30h
2000-2800	13 mg/kg/30h (group 7)	18 mg/kg/24h (group 8)	***15 mg/kg/30h***	18 mg/kg/24h	***15 mg/kg/36h***	18 mg/kg/24h
≥2800	12 mg/kg/24h (group 9)	17 mg/kg/20h (group 10)	***15 mg/kg/24h***	***18 mg/kg/20h***	***15 mg/kg/30h***	18 mg/kg/20h
Dosing interval was prolonged 10 hours when ibuprofen was co-administered or when asphyxia was considered by the treating physician. Duration of the iv infusion was 20 minutes, PNA=postnatal age, CW=current bodyweight.						

Methods

Patients

All neonates admitted to the Neonatal Intensive Care Unit of the University Hospitals Leuven in whom routine amikacin TDM samples were available between July 2011 and December 2012 were considered for inclusion in this study. The study was approved by the ethical board of our hospital. A simplified version of the previously developed model-based dosing regimen [4] was applied, and based on current weight and postnatal age 10 different patient groups were considered (Table 1). Patients were excluded from this analysis if initiation of amikacin administration was based on a previously used dosing regimen [5], when data (prescription or

clinical characteristics) were missing or if patients had a postnatal age above 30 days. Clinical characteristics at birth [gestational age (GA, weeks), birth bodyweight (grams), Apgar score at 1, 5 and 10 minutes after birth, asphyxia (yes/no/initially suspected but not retained as final diagnosis at discharge)], as well as characteristics at the moment of amikacin TDM [postmenstrual age (PMA, weeks), postnatal age (PNA, days), current bodyweight (grams), concurrent ibuprofen (yes/no) or inotropic drugs (yes/no), respiratory support (i.e. continuous positive airway pressure or mechanical ventilation, yes/no), mechanical ventilation (conventional or high frequency, yes/no)] were retrospectively extracted from the patient files. Additionally, blood culture results at start of amikacin therapy were collected from the individual laboratory reports. The daily nursing progress reports were used to collect amikacin prescription (dose and interval) data.

Drug administration and TDM sampling

Amikacin (Amukin, Bristol Myers Squibb, Braine-L'Alleud, Belgium) was administered as an intravenous infusion over 20 minutes. As part of routine clinical care, blood samples for early amikacin TDM were collected just before (trough) and 1 hour after administration of the second dose (peak). In case of unexpected results or dosing adaptation, additional TDM samples were collected, based on the decision of the attending physician.

Amikacin assay

Up to May 31th 2012, amikacin concentrations were measured with fluorescence polarization immunoassay (Abbott TDx kit, Abbott Laboratories, Diagnostics Division, Abbott Park, IL 60064 USA). The lower limit of quantification (LLOQ) was 0.8 mg/L. According to the insert, the coefficient of variation was <5% (assessed at 5, 15 and 30 mg/L). From May 31th 2012, amikacin quantification was based on a kinetic interaction of microparticles in solution (KIMS) immunoassay (Roche/Hitachi Cobas c systems, Roche Diagnostics GmbH, Mannheim, Germany). Also in this assay, the LLOQ was 0.8 mg/L. According to the insert, the coefficient of variation was <4%. To avoid censoring of data below the LLOQ, these concentrations were replaced by LLOQ/2 (i.e. 0.4 mg/L) as suggested in literature [6,7].

Prospective validation

1. Early amikacin TDM observations

To evaluate early amikacin exposure, trough and peak concentrations obtained just before and 1 hour after the second dose of each amikacin treatment episode were considered. The

percentage of samples achieving target trough <3 mg/L and peak concentrations >24 mg/Lwere defined. These targets were chosen since dosing regimen adaptation during clinical practice in our unit is considered if early trough concentrations exceed 3 mg/L and/or peak levels are below 24 mg/L. In addition, trough levels ≤5 mg/L were evaluated since this target is associated with toxicity. Descriptive statistics were performed using MedCalc®12 (Mariakerke, Belgium).

2. Pharmacokinetic analysis and Monte Carlo simulations

2.1. Pharmacokinetic analysis

To evaluate the predictive performance of the recently developed pharmacokinetic model [4], a pharmacokinetic analysis was performed using NONMEM VI in which model-based individual and population predicted concentrations were simulated for each observation in the prospectively collected dataset. These model-predicted concentrations were obtained by use of the recently developed pharmacokinetic model [4] in which all parameters were fixed to the final values with MAXEVAL = 0 and without covariance step. Subsequently, both the individual and population predicted concentrations were visually compared to the observed concentrations. Additionally, to evaluate accuracy, the recently developed pharmacokinetic model [4] was used to compute a NPDE (normalized prediction distribution error) [8,9] for each of the observations of the prospective dataset. A histogram of the NPDE distribution and scatterplots showing the NPDE *versus* time and *versus* predicted concentration were used as evaluation tools [8,9]. Finally the parameters of the recently developed pharmacokinetic model [4] were re-estimated based on the data of the prospective dataset.

2.2. Monte Carlo simulations

Monte Carlo simulations were performed to evaluate whether target peak (>24 mg/L) [10] and trough concentrations (<3.0 mg/L) [11] of amikacin were reached following the simplified and the original model-based dosing regimen (Table 1) [4]. For these Monte Carlo simulations, 5 consecutive doses of amikacin were administered over 20 minutes. For the peak values, concentrations <24 mg/L, between 24 – 35 mg/L [10] and >35 mg/L were evaluated. For trough values, concentrations between 1.5 - 3.0 mg/L were evaluated because this was the primary target of the model-based dosing regimen [4]. In addition, the percentage of trough concentrations <1.5, between 3.0 – 5.0 and >5.0 mg/L was evaluated. Results of the Monte Carlo simulations were compared among different neonatal dosing groups as defined in Table 1. For these simulations, the covariates identified in the recently developed final

pharmacokinetic model [4] - birth bodyweight and PNA (covariates found on clearance) and current bodyweight (covariate found on volume of distribution) - were sampled from the prospective dataset taking into account their correlation. The Monte Carlo simulations were performed twice in 5000 individuals following the model-based dosing regimen (either or not with ibuprofen co-administration) [4].

3. Remaining causes limiting amikacin exposure predictability

To assess the performance of the dosing regimen implementation in clinical practice, prescription errors were evaluated based on chart review. Four categories of prescription errors were distinguished i.e. a) incorrect dose (>10% deviation of intended dose [12]), b) incorrect interval, c) incorrect dose and interval, and d) incorrect interval adaptation in case of asphyxia or ibuprofen.

In addition, based on visual inspection of the plots of the pharmacokinetic analysis and Monte Carlo simulations, outliers were identified and clinical characteristics were reviewed to search for remaining clinical causes impairing amikacin exposure predictability in neonates.

Results

Patients

From July 2011 until December 2012, 701 neonates were evaluable for prospective evaluation of the model-based dosing regimen. In total, 122 patients were excluded from the analysis due to application of a previous dosing regimen (n=32), missing data (n=76) or PNA above 30 days (n=14). A summary of the clinical characteristics of the patients included in the final prospective dataset (n=579) is presented in Table 2, as well as the clinical characteristics of the patients used for initial development of the pharmacokinetic model [4]. In total, 579 included patients underwent 701 amikacin treatment episodes, resulting in 1195 amikacin TDM observations. In 93/701 episodes, a bacterial species was isolated from at least one blood culture. Additional data on pathogens isolated and related MIC values are provided in Table 3 [10,13,14].

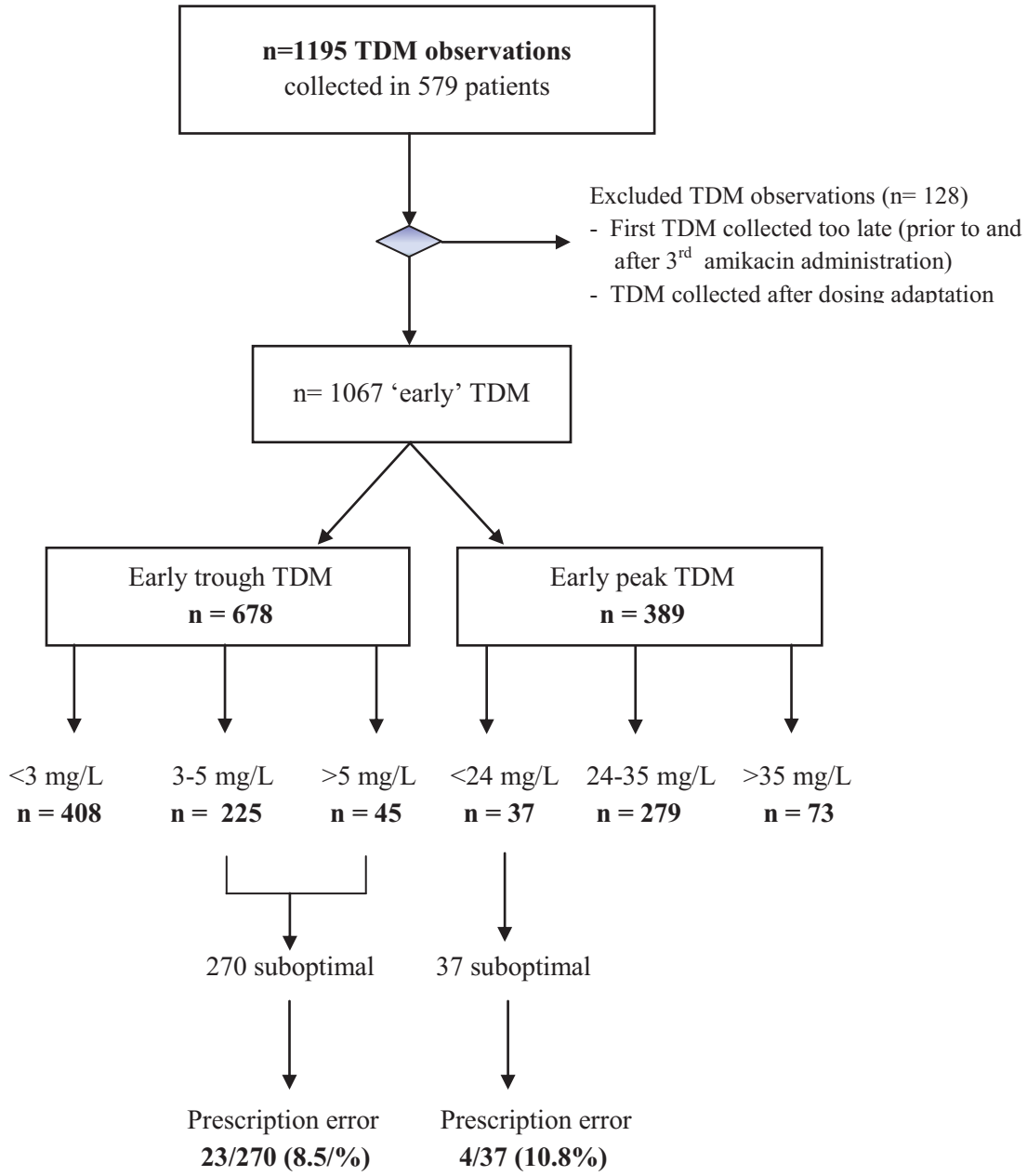

Figure 1: Flowchart of included early trough and peak amikacin therapeutic drug monitoring (TDM) observations. The number of trough observations <3 mg/L, between 3-5 mg/L and >5 mg/L and peak observations <24 mg/L, between 24-35 mg/L and >35 mg/L are presented. The percentage of prescription errors documented in suboptimal trough and peak observations are calculated. n= number of TDM observations.

Table 2: Clinical characteristics of neonates included in the recently published analysis on amikacin [4] and in the current prospective analysis (median and range, or absolute number and incidence).

Characteristics	Recently developed amikacin model [4] n= 874	Current prospective amikacin analysis n= 579
Gestational age (weeks)	32 (24-43)	34 (24-41)
Postmenstrual age (weeks)	33 (24-43)	34 (24-45)
Postnatal age (days)	2 (1-30)	2 (1-30)
Birth bodyweight (g)	1750 (385-4650)	2285 (420-4850)
Current bodyweight (g)	1760 (385-4760)	2100 (420-5040)
Co-administration of ibuprofen (n (%))	118 (13.5)	29 (5)

Table 3: Pathogens isolated from blood cultures collected at start of the amikacin treatment episodes included in the prospective analysis. Isolates are ranked based on frequency.

Blood culture result	Number	Percentage	MIC reference values*
Negative	601	85.7%	
Positive	93	13.3%	
1) Staphylococcus epidermidis	*32*	*34.4%*	1.5-12 [10]
2) Escherichia coli	*23*	*24.7%*	≤ 2 [13], MIC90=1 [14]
3) Staphylococcus aureus	*6*	*6.5%*	0.75-3 [10]
4) Streptococcus mitis	*5*	*5.4%*	NA
5) Others	*27*	*29%*	NA
Unknown / Not available	7	1%	
Total	701	100%	

* MIC: Minimal inhibitory concentration. NA: not applicable.

Prospective validation

1. Early amikacin TDM observations

Of the 1195 TDM observations, 678 early trough and 389 early peak samples were identified (Figure 1). Overall, 60.2%, 33.2% and 6.6% of first amikacin trough levels were <3 mg/L, between 3-5 mg/L and >5 mg/L respectively. Taking into account all first peak levels, 90.5%

reached 24 mg/L (71.7% between 24-35 mg/L, 18.8% exceeded 35 mg/L) (Figure 1). The percentages of first trough and peak TDM observations for the 10 different groups are presented in Table 4.

Table 4: Early therapeutic drug monitoring (TDM) observations included in the prospective dataset (n=678 early trough and n=389 early peak observations). For the subgroups 1-10 as defined in Table 1, the percentages of trough samples <3 mg/L, between 3-5 mg/L and >5 mg/L and peak samples <24, between 24-35 and >35 mg/L are provided. Conc= concentration.

Conc (mg/L)	group 1	group 2	group 3	group 4	group 5	group 6	group 7	group 8	group 9	group 10
Simplified model-based dosing regimen										
<3	74%	100%	66%	92%	72%	84%	44%	92%	48%	88%
3-5	26%	0%	25%	8%	25%	16%	49%	0%	41%	12%
>5	0%	0%	9%	0%	3%	0%	7%	8%	12%	0%
<24	23%	0%	23%	0%	14%	8%	4%	0%	5%	0%
24-35	71%	100%	68%	70%	76%	46%	71%	33%	74%	78%
>35	6%	0%	9%	30%	10%	46%	25%	67%	21%	22%

2. Pharmacokinetic analysis and Monte Carlo simulations

2.1. Pharmacokinetic analysis

Figure 2 shows the individual and population predicted concentrations *versus* concentrations observed in this prospective study, for the different dosing groups based on current bodyweight and postnatal age (Table 1). Both panels indicate absence of bias and an adequate prediction of the observed concentrations across the different groups. Moreover, the distribution of the data points around the line of unity of the observed *versus* predicted plot indicates that the interindividual variability is both acceptable and similar across the entire neonatal population. This is also reflected in Figure 3 in which the interindividual variability on clearance is plotted against birth bodyweight, postnatal age and co-administration of ibuprofen. Figure 3 illustrates that the pharmacokinetic model of amikacin is able to describe the prospective dataset accurately across the different covariates as no trend is seen in the interindividual variability on clearance *versus* these covariates. This result was obtained despite the fact that there were small differences in clinical characteristics between the dataset that was used to build the model [4] and the prospective dataset (Table 2). Table 5 gives an overview of the parameter estimates of the recently published final pharmacokinetic model [4]

together with the parameter estimates obtained for the current prospective dataset in 579 individuals. Based on the values in Table 5, fairly similar parameter estimates are obtained when comparing the prospective dataset with the previously obtained parameters, hereby indicating the stability of the model.

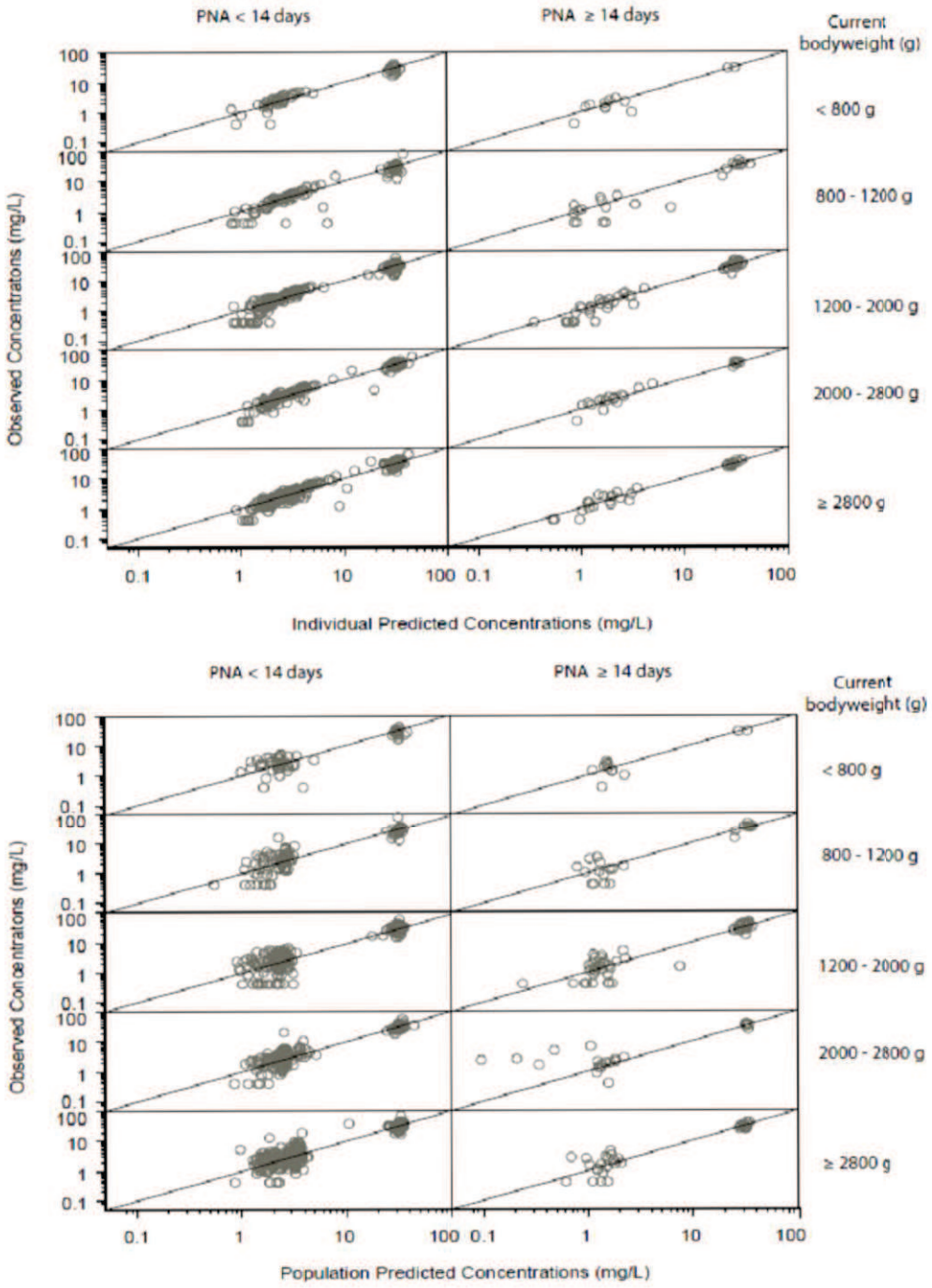

Figure 2: Observed *versus* model-based individual and population predicted concentrations (logarithmic scale) for the 10 different dosing groups based on current bodyweight and postnatal age (Table 1) for the current prospective dataset.

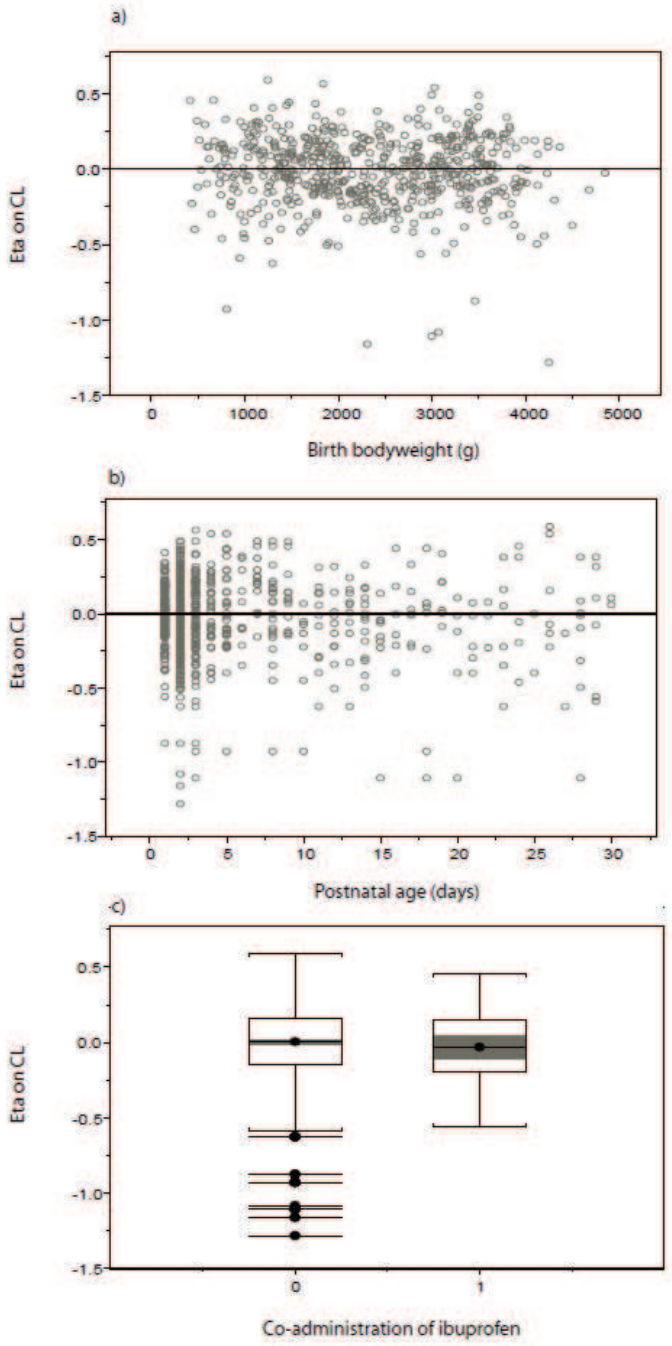

Figure 3: Interindividual variability (eta) on clearance (CL) versus birth bodyweight (a), postnatal age (b) en co-administration of ibuprofen (c) for the current prospective dataset using the recently developed pharmacokinetic model [4].

Figure 4 shows the results of the NPDE analysis. The histogram follows the normal distribution indicated by the black solid line. Additionally, no trend is seen in NPDE *versus* time and NPDE *versus* predicted concentrations indicating model accuracy.

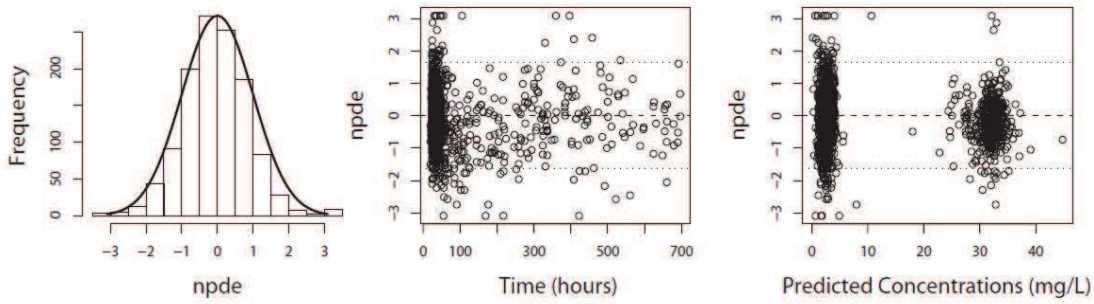

Figure 4: Results of the NPDE analysis performed for the prospective dataset using the recently developed pharmacokinetic model [4]. Left panel: Histograms of the NPDE distribution with the solid line representing a normal distribution as a reference, Middle panel: NPDE *versus* time (hours); Right panel: NPDE *versus* observed concentrations (mg/L).

2.2. Monte Carlo simulations

Monte Carlo simulations were performed to illustrate the exposure to amikacin in two times 5000 neonates (with and without ibuprofen administration) following the simplified model-based and the original model-based dosing regimen [4] after 5 consecutive doses. In Table 6a the percentages of the individuals with simulated trough concentrations after 5 doses <1.5 mg/L, between 1.5-3 mg/L, between 3-5 mg/L and >5 mg/L and peak concentrations after 5 doses <24 mg/L, between 24-35 mg/L and >35 mg/L are given when ibuprofen is not co-administered. In Table 6b, these simulated percentages are shown when ibuprofen is co-administered. Both tables are graphically presented in Figures 5a and 5b. In both figures the upper panels (A) represent the trough concentrations while the lower panels (B) represent the peak concentrations. Based on the Tables 6a and 6b and Figures 5a and 5b, it can be seen that for the model-based dosing regimens (with and without ibuprofen) the percentages for trough concentrations between 1.5 and 3.0 mg/L are relatively constant across the 10 different age and weight groups. This confirms the predictions performed in the previously published analysis [4]. Overall, these Figures and Tables show that trough concentrations <3 mg/L [11] are reached in most individuals of the different dosing groups upon the simplified (78-100% and 45-96%) and original model-based dosing regimen (86-100% and 62-92%), when ibuprofen is co-administered or not, respectively. Trough concentrations >5 mg/L which are associated with oto- and nephrotoxicity, were observed in 0-20% and 0-9% of the individuals of the different dosing groups as defined in Table 1, for the simplified dosing regimen and the original dosing regimen [4] respectively (Tables 6a and 6b). Considering the peak concentrations, simulations using the simplified model-based dosing regimen result in peak

concentrations >24 mg/L in almost all individuals. For the original model-based dosing regimen [4], target peak concentrations are not reached in all individuals of one or more dosing subgroups.

Table 5: Final parameter estimates and coefficients of variation (CV%) of the pharmacokinetic model that was recently developed on the basis of the original dataset (n=874) [4] and on the basis of the current prospective dataset (n=579). Clinical characteristics of the datasets are provided in Table 2.

Parameter	**Recently developed amikacin model [4] n=874 patients** Value (CV%)	**Current prospective amikacin analysis n=579 patients** Value (CV%)
Fixed effects		
CLp in CL = CLp x (bBW/median)^{m} x (1+ n x (PNA/median)) x o (ibuprofen)	0.049 (2.21)	0.066 (3.2)
m	1.34 (2.04)	1.30 (2.95)
n	0.213 (9.81)	0.302 (9.34)
o	0.838 (3.88)	0.846 (6.55)
Vp in V1 = Vp x (cBW/median)^{p}	0.833 (1.34)	1.03 (1.47)
p	0.919 (2.46)	0.863 (4.03)
Q = r x CL	0.415 (12.3)	0.480 (13.5)
V2=V1	V2=V1	V2=V1
Interindividual Variability		
ω^2 (CL)	0.0899 (14.9)	0.0921 (19.3)
Residual Variability		
σ^2 (proportional)	0.0614 (8.2)	0.0448 (22.3)
σ^2 (additive)	0.267 (27.2)	0.315 (16.3)

CLp = population value for clearance (L/h), Vp = population value for volume of distribution of the central compartment (L), bBW = bodyweight at birth (g), cBW = current bodyweight (g), PNA = postnatal age (days), Q = intercompartmental clearance (L/h), V2 = Volume of distribution of the peripheral compartment (L), median values for the recently developed model for amikacin: bBW = 1750g, PNA = 2 days, cBW=1760g; median values for the current prospective study: bBW = 2285g, PNA = 2 days, cBW = 2100g

3. Remaining causes limiting amikacin exposure predictability

Prescription errors using the simplified model-based dosing regimen were found in 64/701 (9.1%) of the amikacin treatment episodes. Of these errors, an incorrect dose was prescribed in 17/64 (26.6%) episodes, an incorrect interval was found in 20/64 (31.2%) errors. Dose and interval were both incorrect in 11/64 (17.2%) cases. Interval prolongation in case of asphyxia or ibuprofen use was not correctly implemented in 16/64 (25%) of the error cases. When suboptimal trough (>3 mg/L) or peak levels (<24 mg/L) were obtained, a prescription error was documented in respectively 8.5% and 10.8% of cases (Figure 1).

Although no trend was seen in Figure 3, a subgroup of 7 patients presented an interindividual variability (eta) on clearance <-0.6. When exploring the individual characteristics, 2 cases displayed perinatal asphyxia, 1 course was suggestive for asphyxia, 2 neonates presented with hydrops foetalis at birth, 1 case suffered a fulminant septic shock and 1 patient was born at 35 weeks and had toxic trough and peak samples without specific underlying disease.

Discussion

Recently, a population pharmacokinetic model and a model-based dosing regimen for amikacin in neonates aged between 1-30 days were developed [4]. The main aim of this dosing regimen was to obtain trough concentrations of 1.5-3 mg/L and peak concentrations >24 mg/L across the entire neonatal population [4]. As a final step to evaluate whether this model-based dosing guideline indeed results in the target amikacin concentrations aimed for in the clinical setting, a prospective clinical trial was conducted to validate this dosing regimen.

For most patient groups, adequate amikacin peak and trough levels were achieved. The observed values (60.2% of trough values <3 mg/L, 93.4% ≤5 mg/L, and 90.5% of peak values >24 mg/L) hereby represent the early treatment phase, in which steady-state amikacin concentrations are not yet presumed. The results obtained by the Monte Carlo simulations (trough values <3 mg/L in 78-100% and 45-96% respectively when ibuprofen was co-administered or not, and peak values >24 mg/L in almost all patients) more likely reflect steady-state condition, since simulations were performed up to 5 administrations. Overall, the prospective validation of the model-based dosing regimen resulted in better amikacin exposure when compared to evaluations of dosing regimens found in reference books as reported by De Cock et al [4]. To further illustrate this, we performed Monte Carlo simulations

Table 6: The percentage of individuals of subgroups 1-10 as defined in Table 1 with trough concentrations after 5 amikacin doses <1.5 mg/L, between 1.5-3 mg/L, between 3-5 mg/L and >5 mg/L and peak concentrations (grey background) after 5 doses <24 mg/L, between 24-35 mg/L and >35 mg/L following Monte Carlo simulations in 5000 individuals according to the simplified model-based dosing regimen and the original model-based dosing regimen [4], when ibuprofen was not co-administered (Table 6a) and when ibuprofen was co-administered (Table 6b).

Table 6a

Conc (mg/L)	group 1	group 2	group 3	group 4	group 5	group 6	group 7	group 8	group 9	group 10
Simplified model-based dosing regimen										
< 1.5	34%	55%	30%	65%	27%	58%	17%	53%	8%	58%
1.5-3	45%	30%	45%	30%	45%	38%	45%	36%	37%	36%
3-5	16%	15%	21%	5%	23%	4%	27%	11%	35%	6%
> 5	5%	0%	4%	0%	5%	0%	11%	0%	20%	0%
< 24	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%
24-35	95%	50%	94%	71%	97%	83%	90%	97%	81%	94%
> 35	5%	50%	6%	29%	3%	17%	10%	0%	19%	6%
Original model-based dosing regimen [4]										
< 1.5	33%	55%	29%	43%	25%	56%	25%	60%	16%	61%
1.5-3	41%	30%	48%	36%	47%	36%	47%	32%	46%	25%
3-5	21%	15%	18%	14%	22%	8%	23%	5%	29%	11%
> 5	5%	0%	5%	7%	6%	0%	5%	3%	9%	3%
< 24	0%	0%	0%	0%	0%	0%	9%	3%	24%	9%
24-35	96%	50%	93%	64%	97%	70%	91%	89%	76%	84%
> 35	4%	50%	7%	36%	3%	30%	0%	8%	0%	7%

Table 6b

Conc (mg/L)	group 1	group 2	group 3	group 4	group 5	group 6	group 7	group 8	group 9	group 10
Simplified model-based dosing regimen										
< 1.5	34%	55%	30%	65%	27%	58%	17%	53%	8%	58%
1.5-3	45%	30%	45%	30%	45%	38%	45%	36%	37%	36%
3-5	16%	15%	21%	5%	23%	4%	27%	11%	35%	6%
> 5	5%	0%	4%	0%	5%	0%	11%	0%	20%	0%
< 24	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%
24-35	95%	50%	94%	71%	97%	83%	90%	97%	81%	94%
> 35	5%	50%	6%	29%	3%	17%	10%	0%	19%	6%
Original model-based dosing regimen [4]										
< 1.5	33%	55%	29%	43%	25%	56%	25%	60%	16%	61%
1.5-3	41%	30%	48%	36%	47%	36%	47%	32%	46%	25%
3-5	21%	15%	18%	14%	22%	8%	23%	5%	29%	11%
> 5	5%	0%	5%	7%	6%	0%	5%	3%	9%	3%
< 24	0%	0%	0%	0%	0%	0%	9%	3%	24%	9%
24-35	96%	50%	93%	64%	97%	70%	91%	89%	76%	84%
> 35	4%	50%	7%	36%	3%	30%	0%	8%	0%	7%

using the Neofax [15] and BNFc [16] dosing regimen, to compare them with the simulations of the model-based dosing regimens as can be seen in Figure 5a and 5b.

A ratio of peak concentration divided by the minimal inhibitory concentration of a given pathogen (peak/MIC) of at least 8-10 is recommended for effective amikacin therapy [17]. While the amikacin peak concentration depends on the dose administered and the distribution volume of the individual patient, the peak/MIC also depends on the sensitivity of the specific pathogens targeted for. Taking the non-specified *Staphylococcus species* MIC values (0.75-3 mg/L) as published by Sherwin et al [10] and the *Escherichia coli* MIC values (our department ≤2 mg/L, Pacifici 2009 MIC^{90}=1 mg/L) into account [14], a peak/MIC ratio ≥8 is obtained if peak concentrations reach 24 mg/L (Table 3). Consequently, the current extended interval dosing approach reaches sufficient high (≥8) peak/MIC values to treat pathogens causing neonatal infections in most patients, as documented in both early TDM, as well as in the Monte Carlo simulations (Table 6a and 6b).

Besides the overall positive evaluation of the model-based dosing regimen, we anticipated that the exposure for some patient groups can be further improved. Considering observed trough levels, group 7 (current weight 2000-2800g, PNA<14 days) and 9 (current weight >2800g, PNA<14 days), had a relevant proportion of early trough observations (respectively 50% and 41%) between 3-5 mg/L, and this elevated incidence was also confirmed in the Monte Carlo simulations (Table 6a and 6b) after 4-5 administrations. In the setting of peak levels on target, this indicates that an additional interval prolongation (+6h) for the subgroups 7 and 9 may be of additional benefit to avoid toxicity. Based on these observations, we suggest a further adapted dosing regimen for these two subgroups. Since optimal peak concentrations are achieved using the simplified model-based dosing regimen, the 15 mg/kg dose can remain the same, but an interval of 36h instead of 30h, and 30h instead of 24h, for respectively group 7 and 9 can be considered, as presented in Table 1. At least, this illustrates the importance of prospective validation studies. Consequently, a new prospective validation is needed in these specific subgroups to evaluate whether lower trough concentrations could be obtained when the dosing interval is prolonged as suggested.

In addition to dosing regimen validation, the predictive performance of the pharmacokinetic model was assessed. Based on the current results, it can be concluded that the final pharmacokinetic model is indeed able to predict the observed concentrations in the current

study without bias across the entire neonatal range (Figure 2). Moreover, the covariates birth bodyweight, postnatal age and co-administration of ibuprofen are correctly implemented on clearance as no trend is seen when plotting the interindividual variability on clearance versus the different covariates (Figure 3). When evaluating Figure 2 and Figure 3, we concluded that only random variability remained in the model while variability allocated to the explored covariates is explained.

Finally, we also explored remaining causes of impaired amikacin exposure predictability. First of all, prescription errors were evaluated. In the current study, the extent of suboptimal early trough and peak amikacin levels associated with prescription errors (8.5% and 10.8% respectively) is in line with the extent of overall prescription errors (9.1%) in the prospective dataset, suggesting that this is not a major cause of unexplained variability per se. Overall, drug prescription errors are common and multifactorial. Efforts to reduce these errors, like computerized physician orders with electronically provided dosing regimens [18] as well as the development of care bundles (i.e. a combination of evidence-based practices to improve a care process) are gradually being introduced in neonatal departments [19] and improve patient safety. We therefore encourage that when new neonatal dosing regimens are introduced, a simultaneous evaluation on prescription and administration feasibility is performed. Secondly, we documented that amikacin exposure predictability was impaired in individual neonates with hydrops foetalis, perinatal asphyxia or severe septic shock. Although the impact of sepsis on the clearance [20] and volume of distribution of gentamicin in neonates are described, the influence of other clinical conditions on aminoglycoside disposition in neonates is not yet confirmed [20,21]. Furthermore, robust parameters to define conditions like (suspected) asphyxia are lacking. Therefore we want to stress that TDM monitoring remains indicated to guide safe and effective amikacin therapy, at least in these specific cases.

Following dosing regimen development, a prospective study was performed and confirmed the accuracy of the model. In addition, the covariate model itself was also validated, and this may be of relevance beyond the amikacin specific observations. This is because it was shown by two different groups that the pharmacokinetic covariate model of amikacin contains system-specific information on the developmental changes in glomerular filtration rate (GFR) throughout neonatal life. [21,22]. Consequently, the covariate model on amikacin clearance with birth bodyweight, postnatal age and co-administration of ibuprofen as most important covariates could be used to predict the dosage regimens of other drugs excreted by GFR.

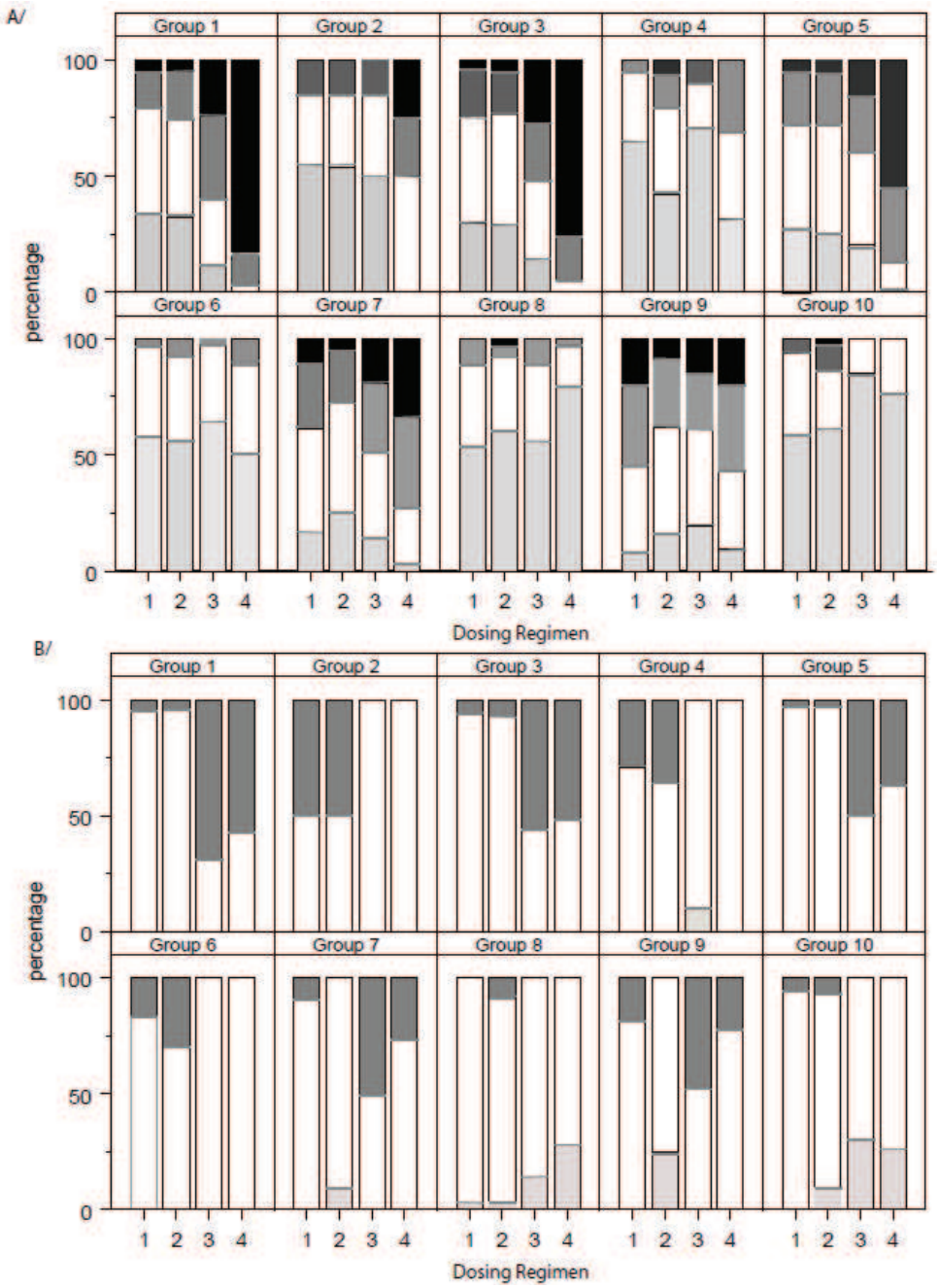

Figure 5a: Bar graphs to illustrate the percentages of individuals of the different dosing groups as defined in Table 1 with A/ trough concentrations after 5 doses <1.5 mg/L (light grey), between 1.5-3 mg/L (white), between 3-5 mg/L (dark grey) and >5 mg/L (black) and B/ peak concentrations after 4 doses <24 mg/L (light grey), between 24-35 mg/L (white) and >35 mg/L (dark grey) following the Monte Carlo simulations in 5000 individuals according to the different dosing regimens (1= simplified model-based dosing regimen, 2= original model-based dosing regimen [4], 3= Neofax [15], 4= BNFc [16]) when ibuprofen was not co-administered.

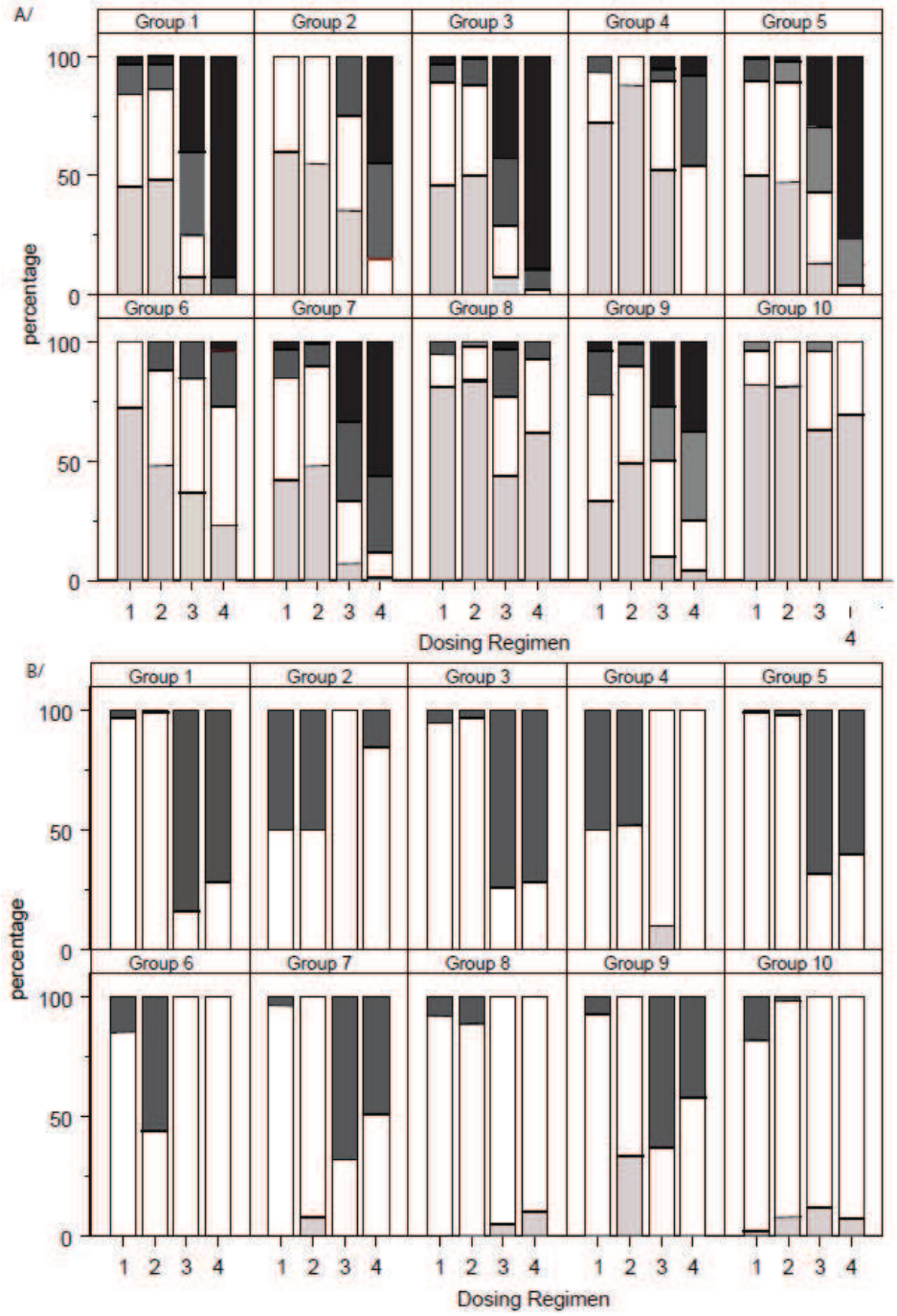

Figure 5b: Bar graphs to illustrate the percentages of individuals of the different dosing groups as defined in Table 1 with A/ trough concentrations after 5 doses <1.5 mg/L (light grey), between 1.5-3 mg/L (white), between 3-5 mg/L (dark grey) and >5 mg/L (black) and B/ peak concentrations after 4 doses <24 mg/L (light grey), between 24-35 mg/L (white) and >35 mg/L (dark grey) following Monte Carlo simulations in 5000 individuals according to the different dosing regimens (1= simplified model-based dosing regimen, 2= original model-based dosing regimen [4], 3= Neofax [15], 4= BNFc [16]) when ibuprofen was co-administered.

(netilmicin, tobramycin, gentamicin and vancomycin) in neonates [21,22]. This semi-physiological approach may be used to optimize sparse data analysis and may facilitate development of pharmacokinetic models and evidence-based dosing regimens following prospective validation [23].

In conclusion, we documented that a large percentage of neonatal amikacin TDM observations achieved using a model-based dosing regimen reached predefined targets. Furthermore, we illustrated that population pharmacokinetic modeling facilitates the development of drug dosing regimens in neonates. Finally, we raised awareness on other causes possibly impeding amikcin exposure predictability in neonates.

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Part 4: General discussion

CHAPTER 7

General discussion and future perspectives

General discussion

The neonatal period is characterized by large and intriguing inter-and intra-individual variability in drug disposition and drug action. In this doctoral thesis, we aimed to improve pharmacotherapy in neonates based on pharmacokinetic (PK) and/or pharmacodynamic (PD) studies of frequently used -but still insufficiently understood- drugs in this population. The drugs selected for the studies represent a larger group of compounds with similar physicochemical characteristics and/or with similar metabolic or renal excretion pathways. Therefore, the drugs studied can be seen as 'probe' drugs, of which PK/PD results are of relevance beyond the compound specific observations.

Research sequence and individual studies performed

Throughout this doctoral thesis, a 3-step research sequence was used, illustrating how a pharmacology-related problem or question, arising during clinical care, can ultimately result in improved pharmacotherapy through validated drug dosing. Population pharmacokinetic modelling hereby needs to be considered as an advanced methodological tool turning neonatal drug therapy from explorative to confirmatory [1]. *First*, to gain insight into the behavior of a drug in neonates, **exploration of covariates** contributing to variability in drug exposure and/or effect is warranted. *Secondly*, integration of these covariates in dosing regimens can improve **prediction of drug exposure** for the individual patient. *Finally*, new model-derived dosing regimens need **prospective validation**. For each research step, observations were collected using different probe drugs.

Part 1: Problem identification and covariate exploration

Although vancomycin is an old drug used in the treatment of neonates, suboptimal vancomycin dosing was presumed since low trough levels were often measured during clinical care. In **chapter 2** we retrospectively assessed that indeed up to 70% of vancomcyin serum trough levels in our NICU patients were below the target of 10 mg/L. For this evaluation we used routine therapeutic drug monitoring (TDM) results achieved using 2 published neonatal vancomycin dosing regimens. Especially the small [low birth weight (BW), current weight (CW)] and immature [low gestational age (GA), postmenstrual age (PMA)] neonates showed suboptimal trough concentrations, indicating ineffective or at least off target therapy. Besides confirming the impact of covariates reflecting ontogeny on

vancomycin exposure (which is in line with observations in literature [2]), we hereby also illustrated the need for prospective validation of dosing regimens, preferably before publication [3].

Measurement of drug exposure usually occurs in the blood compartment. However, the ability to reach therapeutic concentrations at the effect site is necessary for drug efficacy. In **chapter 3** we explored this PK aspect for amikacin by describing neonatal amikacin concentrations in a deep body compartment, i.c. the bronchial epithelial lining fluid (ELF). Quantification was feasible and required urea (introduced by Renard *et al* [4]) to correct for bronchoalveolar lavage (BAL)-related dilution [5]. The specific chromatographic and electrophoretic methods were developed and subsequently applied to neonatal samples [5]. Median (range) amikacin ELF concentration was 6.5 (1.5-23) mg/L [6]. Only 1 additional published observation on amikacin disposition in a deep body compartment (cerebrospinal fluid, CSF) in neonates could be found [7]. To compare both (CSF, ELF) deep body compartment studies, we provide a summary of the clinical and biochemical characteristics in Table 1.

Although we are aware that there are differences in sampling times, the ELF compartment attained significantly higher median amikacin concentrations (6.01 mg/L) than the CSF, where a median value of 1.08 mg/L in neonates was reported previously [7]. In that study, amikacin CSF concentrations in neonates without meningitis were analysed. The lower amikacin detection in CSF illustrates that passing the blood-CSF barrier is more difficult for amikacin than passing the blood-ELF barrier, a finding similar to many other water soluble drugs [8].

We are aware that primary respiratory tract infections are a rare indication for amikacin use in neonates. However, we encourage the investigation of drug penetration in ELF as well as other deep compartments in neonates since such data can be integrated in physiology-based pharmacokinetic models (PBPK) or by pooling of different data, in a population pharmacokinetic modelling analysis with Monte Carlo simulations. This allows to evaluate plasma and ELF concentrations with regard to the achievement of predefined PD targets. This is of relevance since -at present-, the relation between clinical outcomes (including efficacy, but also the potential to induce microbiological resistance) and deep compartment concentrations, including ELF concentrations, is under-studied [9]. Unfortunately, such efforts

are laborious and demanding, resulting in overall small cohorts of patients as reflected in Table 1 of chapter 3.

Table 1: Comparison of amikacin quantification in cerebrospinal fluid (CSF group [7]) and epithelial lining fluid in neonates (ELF group [6]). CSF: cerebrospinal fluid; ELF: epithelial lining fluid. Data are presented as median and range if not otherwise stated. *: Mean and standard deviation. °: Hours after amikacin administration.

Clinical characteristics and fluid analysis	**CSF group [7] (n=43)**	**ELF group [6] (n=17)**
Clinical characteristics		
Weight (kg)	2.430 (0.865-3860)	1.715 (0.550-3.540)
Postmenstrual age (weeks)	36 (26-41)	31.9 (25.1-41)
Postnatal age (days)	3 (1-29)	3.5 (2-37)
Creatinaemia (mg/L)	0.86 (0.48-1.26)	0.62 (0.35-0.98)
Fluid analysis		
Serum amikacin trough (mg/L)	3.8 +/- 2.5 *	2.1 (1-7.1)
Serum amikacin peak (mg/L)	35.7 +/- 5.9 *	39.1 (24.1-73.2)
CSF or ELF sampling (h)°	25 (2.5-93.7)	13.5 (1.5-23.5)
Amikacin concentration (mg/L)	CSF: 1.08 (0.34-2.65)	ELF: 6.01 (0.26-23.03)

Comparable with many other drugs, the prescription of propofol in neonates is off-label. Available propofol PD data are conflicting and dosing recommendations in neonates are absent. In **chapter 4**, we first explored PK covariates of a single propofol bolus. Based on 24 h urine collections, the median total urinary recovery of propofol equivalents was 40.95 (2.01-129.81)% and PG/QG (propofol glucuronide/quinol glucuronides) ratio was 0.44 (0.01-5.93). In this analysis, postnatal age (PNA) was a significant covariate of propofol metabolic profile. A significant correlation between %PG and PNA as well as between early versus late PNA (10 days as cut-off point) and PG/QG ratio was documented [10].

Secondly, we introduced bilirubin in a published propofol population PK model for neonates to explore if hyperbilirubinaemia is a valuable covariate explaining neonatal propofol clearance. It turned out that the model with PMA and PNA as covariates remained the most optimal. Although both UGT1A1 and UGT1A9 mature simultaneously, hyperbilirubinaemia can be the final result of either increased production or decreased elimination. This indicates that UGT activity is only one of the contributing factors to the newborn with jaundice [11]. As

main conclusion of both covariate explorations on propofol metabolism, the clinician administering propofol to a neonate, has to be aware of the age (PNA + PMA) to estimate propofol clearance.

Especially in the first days after birth, propofol induced vasodilatation and decrease in blood pressure might occur more often due to impaired clearance besides potential pharmacodynamic differences (more pronounced hemodynamic instability) [12]. At this age, respiratory distress syndrome typically occurs, sometimes requiring endotracheal intubation or an INSURE procedure. Therefore, in a third study, we explored the propofol ED_{50} dose and simultaneously recorded PD aspects in neonates needing propofol for pre-intubation sedation. The initial and total propofol dose ranges administered were 0.5-2 and 0.5-4.5 mg/kg, respectively. Within this dose ranges a moderate decrease in blood pressure, considered permissive, and a short-lasting decrease in peripheral and cerebral oxygen saturation was seen. Since 86% of patients received propofol prior to an INSURE procedure, both successful in-and extubation are requested to consider the procedure as successful. This resulted in the use of overall low propofol doses and patients who still remained active to a certain level. Notwithstanding this tailored sedation approach, it was interesting to see that clinical recovery (sedation, relaxation state) takes time. At the end of our scoring period (i.e. 21 minutes after propofol administration) baseline scores were not yet reached. The ED_{50} propofol dose for neonates in stratum 1, 3 and 5 (as defined in Table 1 chapter 4) was 0.749, 0.480 and 1.287 mg/kg respectively. We recommend to start with the respective ED_{50} doses and subsequently to increase the propofol bolus dose by up-titration based on perceived clinical need, combined with strict follow-up of vital signs, until validated dosing regimens become available.

Besides propofol, also remifentanil, a short-acting opioid, has been suggested as suitable candidate for premedication prior to endotracheal intubation in neonates. Its metabolism by nonspecific tissue and plasma esterases theoretically results in a fast termination of its clinical effect. However, also for this compound, the impact on intubation conditions and vital signs vary across different reports [13-16]. Since the time to extubation after remifentanil could be rather long, the appropriateness of its use for INSURE conditions is debatable [17]. It should be stressed that whatever compound is used (propofol, remifentanil) in a specific NICU, one has to remain attentive for side effects. In our opinion, it is only once validated ED_{50} values for both drugs are available and their safety is assessed, that a randomized controlled trial can be performed to further compare both compounds in the search for the most optimal pre-intubation medication.

Part 2: Drug exposure prediction

After a drug has been administered to a patient, we may not simply assume that the total drug concentration measured in the blood (or another body compartment e.g. CSF, BAL fluid), is responsible for the drug effects. In essence, only the unbound drug can have an effect (PD), can diffuse to deep compartments or is available for elimination. Protein binding is one of the interfering covariates of this unbound drug concentration. Cefazolin is highly bound to albumin in plasma. However, available PK analyses in neonates are old and are based on total cefazolin concentrations. Therefore, in **chapter 5**, we first explored cefazolin protein binding in 40 neonates and subsequently developed a neonatal population PK model based on both total and unbound cefazolin concentrations. We documented a median unbound cefazolin fraction in neonates of 0.39 (0.10-0.73), which was higher compared to pregnant women and adults. Overall, 49.6% of variability in unbound cefazolin fraction could be explained by covariates PMA, albuminaemia, total cefazolin concentration and indirect bilirubinaemia [18]. As a side step we compared cefazolin protein binding in neonates with published cohorts of other specific populations. Despite the limited number of clinical characteristics available for evaluation and despite the (minor) differences in analytical techniques used, this was -to the best of our knowledge- the first pooled analysis of CFZ protein binding data [19]. Besides total and unbound cefazolin concentrations, variability in unbound cefazolin fraction across the populations also seemed to depend on albuminaemia and characteristics of the patient subgroup itself [19].

According to literature, both pregnancy and neonatal life are typically characterized by hypoalbuminaemia. The decrease in albuminaemia during gestation is assumed to result from haemodilution, and is only in part compensated by an increase in the amount of circulating albumin [20]. In the pregnant cohort included in the pooled analysis, median albuminaemia decreased with gestational age (GA), but did not appear to differ significantly from non-pregnant adult values. This could be explained by the extensive range of albuminaemia, reflecting different pathophysiological conditions in the non-pregnant adults.

As shortly announced in section 5.2, not only the number of albumin binding places (i.e. ‘quantitative’ aspect, depending on the number of albumin molecules and on the presence of other compounds in competition for the same binding places), but also the drug-albumin binding affinity (i.e. ‘qualitative’ aspect) matters. Alterations in affinity can be due to the presence of endogenous substances [e.g. bilirubin, free fatty acids (FFA), urea] which by binding to albumin induce conformational changes of the albumin molecule with subsequent alterations in binding affinity [21,22]. Elevated FFA are reported during pregnancy and the first

days of life, although robust reference values of the latter are lacking. Decreased protein binding of cefazolin, phenytoin and valproic acid has been shown in the presence of increased FFA concentrations [21,23]. In uremic conditions, decreased drug binding (e.g. diazepam, salicylic acid) but also increased drug binding (e.g. doxycycline, metoclopramide) is reported [24,25]. For cefazolin, binding in uremic conditions is yet to be described. As a highly protein bound, renally eliminated drug, where dosing is not titrated to effect [26], alterations in protein binding of cefazolin may be of -perhaps limited- clinical relevance. Because the concept of protein binding certainly matters, our neonatal data were subsequently, integrated in a population PK analysis using non-linear mixed effect modelling, to describe cefazolin disposition and to define covariates improving exposure predictability. A one-compartment PK model was developed. Current weight was identified as covariate for volume of distribution (Vd), birth weight and postnatal age for clearance (Cl) and albumin for maximal protein binding (B_{max}), explaining 50%, 58% and 41% of inter-individual variability in Vd, Cl and B_{max} respectively [27]. Based on Monte Carlo simulations, an advanced statistical tool to evaluate and predict drug exposure using different dosing regimens, a model-derived neonatal cefazolin dosing regimen based on current weight and postnatal age as most important PK covariates, was proposed. The attainment of unbound concentrations for at least 60% of the dosing interval above 8 mg/L [i.e. the CLSI Minimal Inhibitory Concentration (MIC) for staphylococcal species [28]] for the different weight and age groups defined, was the a priori target used in the development of the model-based dosing regimen [27]. We hereby would like to stress that the distribution of bacterial species and their MIC values usually differ between units, hospitals, regions and countries. This illustrates why not only the choice of an antibiotic agent, but also the dosing regimen used, the concentrations achieved in the subcutaneous tissues and the susceptibility pattern of the bacterial species all contribute to the success of surgical prophylaxis. At least, our dataset can also be used to develop dosing regimens with other a priori target concentrations. However, when referring to the research sequence presented in this doctoral thesis, every new model-derived dosing regimen needs prospective validation (*'can the final PK model adequately describe a dataset prospectively collected using the model-derived dosing regimen?'*), before integration in clinical care is possible.

Part 3: Prospective dosing validation

In our unit, amikacin is used for both (suspected) early and late onset sepsis. In **chapter 6**, we performed a prospective validation of a population PK model-based amikacin dosing regimen for neonates. This dosing regimen, based on postnatal age (covariate for clearance) and

current weight (covariate for volume of distribution) was implemented in our neonatal department in 2011, but was not yet prospectively validated. In total 1195 routine amikacin TDM results collected in 579 neonates were included. During clinical practice we aim for early trough levels <3 mg/L and peak >24 mg/L. 90.5% of the observed peak levels reached 24 mg/L and 60.2 % of the early observed trough levels were <3 mg/L (93.4% ≤5 mg/L). The observed concentrations were accurately predicted by the model. Monte Carlo simulations showed that peak predicted concentrations >24 mg/L were reached in almost all patients. Trough values <3 mg/L were documented in 78-100% and 45-96% of simulated cases, respectively when ibuprofen was co-administered or not, with largest percentages of trough levels >3 mg/L in subgroups with PNA <14 days and current weight >2000g. For these subgroups, a slightly adapted dosing regimen was proposed. Except for these subgroups, the model-based dosing regimen overall resulted in optimized trough and peak concentrations.

With this study, we illustrated the relevance of prospective validation. Although routine sampling of amikacin TDM peak values was in the meanwhile abandoned in our unit, monitoring of trough values remains important, especially in specific clinical conditions impairing exposure predictability. Secondly, although TDM helps the clinician to adapt dosing regimens, -even for amikacin- different neonatal target values can be found in literature. Again, this illustrates the need for validated targets in neonatal PK/PD research. Finally, the introduction of the model-based dosing regimen in 2011 [29] resulted in an almost similar total daily dose range compared to the dosing regimen administered previously in our unit [30], but dosing is now refined on a weight-and age-based approach instead of only considering age. Appropriate dosing for neonates with PNA< versus ≥14 days is an add-on value in the current approach compared to the regimen of Langhendries *et al* [30], which mainly focused on the first days of life.

Future perspectives: Closing the gaps between developmental PK, PD and clinical care

Throughout this doctoral thesis, some concerns, limitations and challenges, of relevance beyond the individual studies performed appeared. First, it would be time consuming to conduct the same research sequence for every individual drug. Therefore, the methods described in the drug-specific studies should be used and extrapolated to further explore PK/PD patterns of other compounds or in other patient populations. Secondly, optimal pharmacotherapy is more than only providing adequate dosing regimens. Selection of the

appropriated treatment indication and duration needs to be studied. Third, bioanalytical assay-related variability as well as the lack of clinical definitions are challenges to perform covariate analyses in neonates. Finally, the translation of PK/PD data into clinical guidelines will be a future challenge.

Beyond compound specific observations

We performed studies with selected 'probe' drugs to explore compound-specific PK/PD questions. However, these observations are of relevance for drugs with 'similar' characteristics. For example, besides vancomycin, teicoplanin is also a glycopeptide. As an aminoglycoside, amikacin is considered as a probe for gentamycin, netilmicin and tobramycin [31]. To a certain extent, amikacin shares PK characteristics with other water soluble drugs mainly excreted by GFR like other aminoglycosides or glycopeptides [31,32]. Compared to aminoglycosides, the renal excretion of cefazolin -at least in adults- is a combined result of GFR and tubular secretion. Furthermore, cefazolin is a probe drug illustrating the importance of protein binding determination. Future efforts to compare amikacin versus cefazolin PK aspects, will presumably improve knowledge on the role of renal tubular functions in neonates [33]. Propofol represents the lipophilic drugs which immediately after intravenous administration distribute to the central nervous system and fat tissue. In contrast to the aforementioned probes, propofol undergoes hepatic metabolisation by glucuronidation. Similar to the GFR maturational pattern, a glucuronidation covariate model has also been extrapolated from morphine glucuronidation to zidovudine glucuronidation [34]. Such initiatives, in which drug-specific data are used to improve knowledge of other compounds, or to explore patterns in other populations, should be further encouraged.

'Less is More?'

For some of the probe drugs investigated (propofol, cefazolin), a trend towards lower drug doses was found. This can be of add-on value to avoid side-effects [including toxicity or disturbance of vital signs (propofol)] or to increase the available albumin binding places (cefazolin study) for endogenous (bilirubin) or other exogenous compounds. In general, the fact that 'less may very well be more' is not only applicable to 'dosing'. Also about the selection of indications and treatment durations in neonatal pharmacotherapy, improvements can be made. Based on point prevalence surveys, to which we in part contributed [35], it was documented that antimicrobial drugs in NICUs are mainly administered to neonates with PNA <7 days [35] and on empirical basis [36]. In essence, we only need to treat those patients who need

it and we have to reduce the need to treat [37]. These two tenets are for example of relevance in case of suspected early onset sepsis [37,38].

Challenges in neonatal covariate analysis

Difficulties in the correct interpretation of biomarkers, covariates or TDM results, may rely on bioanalytical techniques (serum creatinine, free fatty acids, vancomycin), definitions of clinical covariates (weight, hypotension, perinatal asphyxia) or overall evolutions in neonatal care (trends in practices throughout time in a given unit). It is important to raise awareness about these factors, since they can influence study results and hereby limit the transferability of PK models to other settings, or at least limit the suggested PK models in time. Each of these factors will be discussed to stress their possible impact as well as to put the results of this thesis into a broader perspective.

Bioanalytical techniques

Serum creatinine: Although serum creatinine (Scr) is often integrated in neonatal population PK analyses as a potential covariate of drug disposition, it is usually not retained as a major factor explaining drug exposure variability. However, Scr reflects -to a certain extent- GFR in neonates and supports the clinician to adapt drug doses, fluid administration and electrolyte support in neonates. Besides variability related to (patho)physiology, differences and related inaccuracy of bio-analytical techniques may also in part explain this variability.

As reported for extreme low birth weight (ELBW, < 1000 g) neonates [39,40], postnatal Scr observations depend on the quantification assay used, e.g. the Jaffe (based on a colorimetric assessment) or the enzymatic technique. In an attempt to further assess the impact of the Scr measurement technique we retrospectively extended the dataset with Scr observations of neonates with higher birth weight. Figure 1 presents these Jaffe Scr data of 1139 neonates compared to enzymatic Scr data of 1110 neonates of our UZ Leuven NICU. All Scr data during the first 42 days were collected [41]. The normal postnatal pattern of initial Scr increase (highest and last in the smallest neonates) followed by a decrease (most delayed in the smallest neonates) was confirmed. Jaffe hereby always resulted in higher Scr values compared to the enzymatic assay, without fixed conversion factor to correct for the difference between both techniques. This figure also nicely documents postnatal trends for cohorts <1 kg, 1-2 kg, 2-3 kg and >3 kg for both techniques. To reduce between assay Scr differences, Jaffe and enzymatic Scr methods were calibrated to isotope dilution mass spectrometry (IDMS), in a

Scr standardization program. Even after introduction of IDMS traceability and standardization, there are still limited differences between neonatal Scr values determined by Jaffe versus enzymatic assay [42]. Irrespective of IDMS traceability we still need a more creative approach to turn Scr into a useful covariate of clearance. We suggest to consider age- or weight-adapted reference values with centiles or Z scores. Using such an approach, PNA-dependent Scr centiles (<p25, p25-75, >p75) were a relevant covariate of amikacin clearance in ELBW neonates. Higher amikacin clearance was observed in cases with lower creatinine [43].

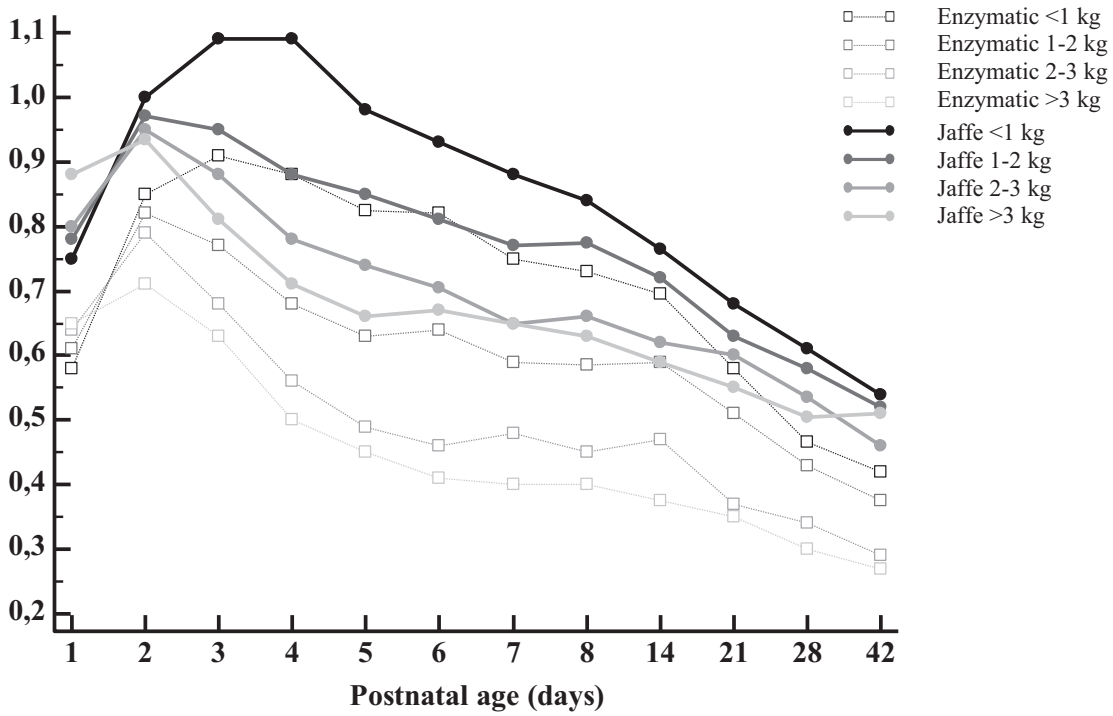

Figure 1: Median postnatal trends of serum creatinine (Scr, mg/dL) for consecutive birth weight categories (<1kg, 1-2 kg, 2-3 kg, >3 kg). Scr values were determined by either Jaffe (full lines, year 2001-2006) or enzymatic (dotted lines, year 2007-2011) method. Creatinine conversion: 1 mg/dL is equal to 88.4 µmol/L [41].

One can assume that instead of Scr, other biomarkers can be used to assess renal function, e.g. cystatin C (CysC). However, CysC observations in neonates are limited, cover a 4-5 fold range and age-specific CysC reference values for neonates are lacking. Furthermore, and similar to creatinine, the impact of different measurement techniques on CysC values need to be considered [44,45]. In essence, the remarks mentioned for Scr and CysC are applicable for many other biomarkers. For infants and older children, a research project defining age- and

gender- appropriate reference intervals for a wide range of blood tests [e.g. the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER)] is still ongoing and tries to close some gaps in paediatric reference intervals [46,47]. Similar projects, specifically focusing on the preterm neonatal population, are a future challenge, but urgently needed.

Free Fatty Acids: One of the covariates suggested in literature to influence the disposition of albumin-bound drugs (like cefazolin) in neonates are free fatty acids (FFA). Literature search strongly suggests that neonates display higher FFA values compared to adults [20], and that FFA values depend on age [48], disease state, co-medication (e.g. heparin) and nutritional state. Reports on neonatal FFA concentrations are rare, differ methodologically and often do not mention feeding regimen. As mentioned in the discussion of section 5.1 we documented FFA concentrations in remnant CFZ plasma samples. Surprisingly, our median (range) FFA concentration was 0.10 (0-0.84) mmol/L, which is lower than intervals reported in literature. We hypothesized that this was probably due to heparin interference (samples collected by arterial line or use of heparinized tubes) and sample handling (freezing and thawing) [18]. As a proof of concept, we therefore prospectively collected FFA values in neonates (n=48), using standardized sampling methods and an enzymatic quantification assay (DiaSys, Diagnostic systems, Holzheim Germany). However, it turned out that median (range) FFA (C16-C18) concentration was 0.19 (0.06-0.79) mmol/L. Despite this 10-fold difference, neither weight, age nor feeding regimen (parenteral, formula feeding, mothers milk) were covariates of this variability.

Vancomycin TDM measurement: Measurement-related problems are not only present on the covariate level, also methods used for drug TDM differ. An external evaluation of neonatal vancomycin PK models documented that differences in predictive performance of the models were in part attributed to both vancomycin and creatinine assays used. This illustrates that besides patient-related characteristics, also measurement techniques should be considered in dosing individualization of vancomycin in neonates [49,50]. Since between-assay differences in vancomycin quantification may influence clinical decisions, a novel LC-MS/MS method to measure vancomycin (unbound and total) in human plasma was recently developed at the laboratory department of our hospital. Data collection for a paired analysis with the currently applied immunoassay and the novel LC-MS/MS of neonatal blood samples is ongoing. Furthermore, the extent of vancomcyin protein binding within the neonatal population will be explored (EudraCT nummer 2014-001124-29; ClinicalTrials.Gov).

Definitions of clinical covariates

Besides bioanalytical factors, also clinical definitions can be a challenge to perform covariate analyses in neonates. A challenging 'clinical' covariate is perinatal asphyxia. Although guidelines to start hypothermia therapy in severely asphyxiated term neonates are straightforward, the clinical definition of perinatal asphyxia remains unclear. Not all cases qualify for hypothermia (e.g. preterm neonates, limited clinical signs), which makes the retrospective but also prospective handling of this covariate complex in neonatal PK/PD research. The combined report of potential indicators of asphyxia e.g. Apgar score, lactate levels and Tompson score [29] in medical files should be encouraged. The same holds true for other straightforward covariates, like weight. Neonatal dosing regimens often contain weight as clinical covariate of drug exposure, without further specification of the weight referred to (i.e. birth weight, or current weight, and how to manage this when the current weight is below the birth weight).

To illustrate the clinical relevance of both covariates, we refer to Figure 3 in chapter 6. Especially patients with clinical conditions influencing clearance (asphyxia) and/or distribution volume (sepsis [51], hydrops foetalis) can display an impaired predictability of amikacin exposure, requiring TDM.

Evolutions in neonatal care

Not only patients are dynamic biological systems, also PK/PD covariates and endpoints can change over time, due to evolutions in clinical practice.

With the introduction of extended dosing intervals for aminoglycosides in neonates, target trough and peak values also shifted over time. Although we earlier mentioned the lack of integrated and validated amikacin targets, -at least in our unit- an evolution towards lower trough and higher peak target values was seen (from trough <5 to trough <3 mg/L, from peak >20 to 24-35 mg/L). The final aim of new PK/PD knowledge is improved exposure predictability for the individual patient. In 2006, the consecutive steps to optimize amikacin dosing and administration, with the resulting impact on exposure predictability in a specific subgroup of neonates (i.e. PMA <31 weeks, PNA <3 days and on respiratory support) in our unit was published [52]. The subsequent introduction of a PMA-based amikacin dosing regimen (2002, Langhendries *et al* [30]), the introduction of a pediatric amikacin vial (2004, 50 mg/mL instead of 250 mg/mL) reduced the inter-individual variability in amikacin clearance. This was obvious by the increase in attaining the predefined TDM targets. In an effort to explore

the impact of the step taken after 2006 (i.e. the implementation of the age-and weight based model-derived dosing regimen [29]), we extracted the results of neonates with PMA <31 weeks, PNA <3 days and on respiratory support from the dataset used for the prospective amikacin validation as described in chapter 6 (Table 2).

During this 14-year period (1999-2012), also the diagnostic and treatment modalities in the neonatal landscape have changed dramatically. As derived from Table 2, the use of prenatal indomethacin, postnatal non-steroidal anti-inflammatory drugs (e.g. ibuprofen) and dopamine decreased in the past decade. This in part reflects an altered neonatal 'population' and/or outcome variables applied.

Table 2: Clinical characteristics and amikacin plasma concentration measurements of 4 cohorts of neonates studied, indicating the increase in the number of neonates attaining predefined targets. The table is adapted and extended from Allegaert *et al* [52]. ° Mean (± SD), * Target zones used up to 2011.

	1999-2002 [52]	**2002-2004** [52]	**2004-4006** [52]	**2011-2012**
Number of neonates	129	75	69	79
PMA (weeks)	28 (24-30)	28 (24-30)	28 (24-30)	29 (25-30)
Birth weight (g)	1047 ± 346	1130 ± 332	1080 ± 314	1229 ± 308
Prenatal indomethacin	10%	3%	4%	NA
Prenatal betametasone	79%	76%	82%	NA
Aspirin/ibuprofen	89%	52%	23%	7.6%
Dopamine	54%	39%	41%	11.39%
Peak amikacin (mg/L)°	45.7 ± 17.8	38.3 ± 13.1	40.9 ± 9.1	29.1 ± 6
Trough amikacin (mg/L)°	8.2 ± 4.4	4.8 ± 2.6	4.3 ± 1.8	3.1 ± 1.4
Peak >20 mg/L*	**95%**	**95%**	**98%**	**98% (90% 24-35 mg/L)**
Trough <5 mg/L*	**24%**	**63%**	**73%**	**90% (53% <3 mg/L)**

Challenges in translating neonatal PK/PD data into clinical guidelines

Throughout this doctoral thesis, it became obvious that dosing regimens available in reference textbooks for neonates highly vary, as illustrated for vancomycin (chapter 2, Table 1) and cefazolin (chapter 5, Table 1). Efforts to improve neonatal dosing regimens of frequently used, but off-label drugs are increasing. It will ultimately be a challenge to further explore the transferability of PK/PD results obtained in one NICU center or in a specific clinical setting to

other centers and finally to translate PK/PD data into useful bedside tools and guidelines. The joined forces of multicenter and/or international initiatives will certainly be helpful to achieve these goals.

Conclusion

At the end of this PhD thesis, we conclude that the 3-step research sequence suggested in the introduction, may not be simply considered as a straight line trajectory with one final endpoint but rather as a continuous process. Every result hereby announces the beginning of a new exploration, towards improved predictability (Figure 2).

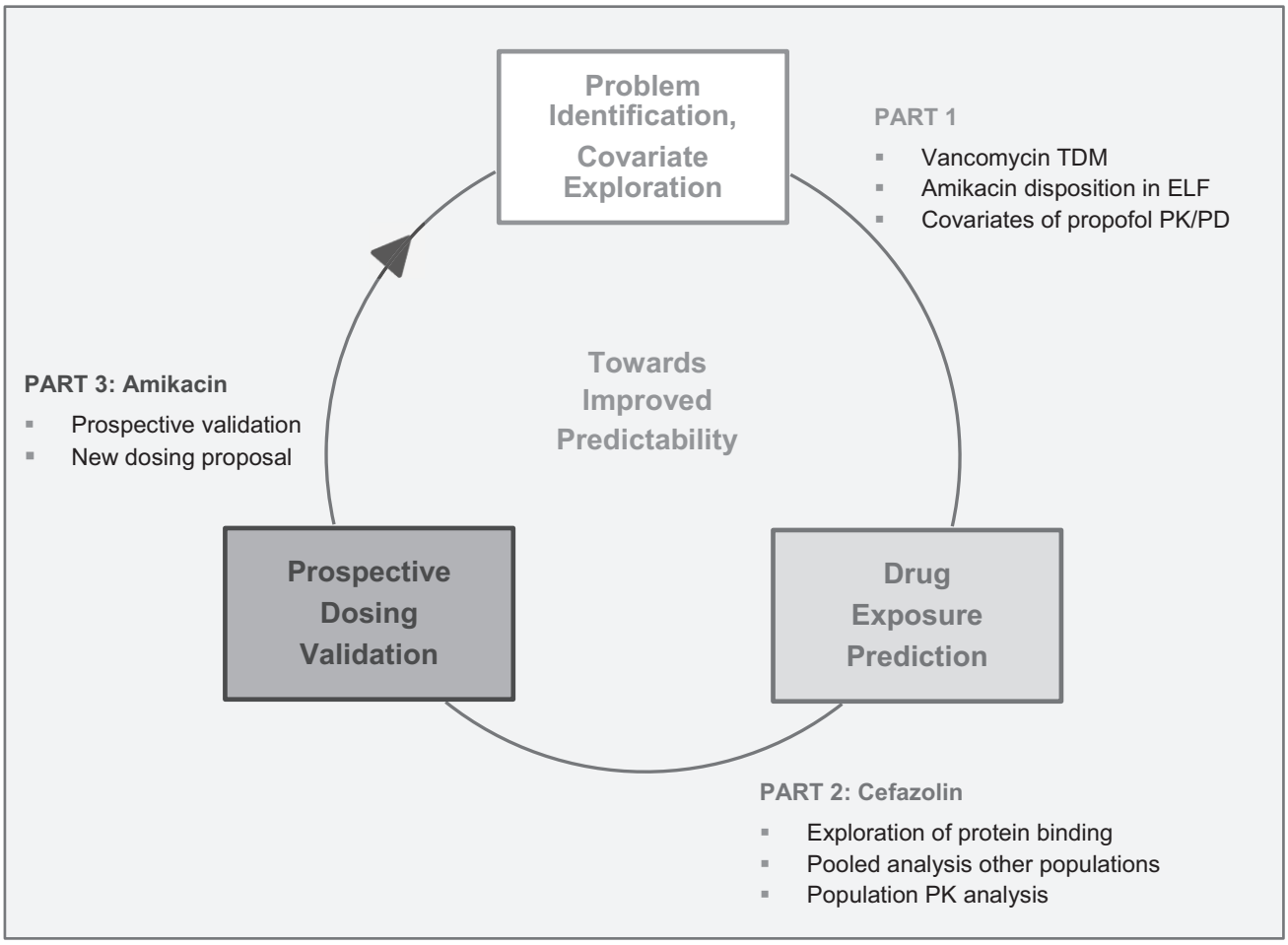

Figure 2: Research process towards improved predictability and more individualized pharmacotherapy. The studies conducted in this PhD thesis are mentioned in the corresponding part of the figure.

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Summary

Summary

Clinical pharmacology has the intention to predict drug effects based on pharmacokinetics (PK) and pharmacodynamics (PD). The general principles hereby used, also apply to neonates. However, fast developmental changes during the neonatal period result in extensive inter- and intra-individual PK/PD variability, making both clinical care and research in this specific subpopulation more challenging.

In this doctoral thesis we performed an **exploration of covariates** explaining this extensive PK/PD variability, for commonly used -but still insufficiently understood- drugs in neonates. We reported up to 70% of vancomycin routine trough levels below the target level (10 mg/L), despite the use of published dosing regimens. We hereby illustrated the extent of unanticipated problems in neonatal drug exposure. Small and immature neonates, and with higher postnatal age (PNA) seemed to be most prone to display subtherapeutic vancomycin exposure (chapter 2). Similar, disposition of drugs at an effect site or deep body compartment in neonates is under-studied. In ventilated neonates, amikacin concentrations were quantified in epithelial lining fluid (chapter 3). The highest concentration measured was reached between 6-14.5 h after administration, which is delayed compared to adults. In chapter 4 covariates of propofol disposition were further explored. Using 24 h- urinary propofol metabolite profiles, postnatal age turned out to be the main driver of propofol metabolism. The limited contribution of glucuronidation to propofol metabolism was in line with other reports of drug glucuronidation in early life. Based on the link between phenotypic glucuronidation and propofol clearance, and on the fact that bilirubin also needs glucuronidation, we explored if indirect hyperbilirubinaemia could be a predictive biomarker to anticipate further reduced propofol clearance in neonates. Since both iso-enzymes involved (UGT1A1 for biliruin and UGT1A9 for propofol) display a similar maturational pattern, this hypothesis seemed reasonable. However, it turned out that postmenstrual age (PMA) and PNA as covariates were most optimal, irrespective of the presence of hyperbilirubinaemia. This can be explained by the fact that neonatal jaundice is the final result of either increased production or decreased elimination. Elevated bilirubin, respiratory distress syndrome, hemodynamic instability are all characteristics most often seen in the first days of life. Since we use propofol for (semi)elective endotracheal intubation in these neonates, without appropriate propofol dosing regimens, a prospective dose-finding study with simultaneous assessment of propofol PD

(sedation and relaxation state, vital signs) was conducted. A trend towards lower doses compared to previous reports was documented, while clinical recovery after the propofol bolus takes time. Based on continuous vital sign data, safety was assessed with only a moderate decrease in blood pressure and a short decrease in peripheral and cerebral oxygen saturation. For preterm neonates <10 days PNA, propofol ED_{50} values were provided. Until validated dosing guidelines appear, these ED_{50} values can be used at induction with subsequent up-titration based on clinical need. At least for INSURE indications this targeted policy is requested.

Integration of covariates, explaining variability in neonatal PK and/or PD processes, in drug dosing regimens, can improve **drug exposure prediction**. This was illustrated with cefazolin in chapter 5. Based on a population PK model, covariates of neonatal cefazolin disposition were determined, taking into account saturable protein binding. Since the unbound fraction of a drug is responsible for drug (side)effects, we encourage both the determination and implementation of protein binding data in PK/PD models. The unbound cefazolin fraction in neonates was in part explained by covariates PMA, albuminaemia, total cefazolin concentration and unbound cefazolin concentration. Pooling of our cefazolin protein binding data with published adult cohorts revealed that besides anticipated covariates (albuminaemia, total cefazolin concentration, unbound cefazolin concentrations), also the patient subgroup contributes to variability in unbound fraction across different populations.
Monte Carlo simulations, using the neonatal cefazolin data, indicated that lower cefazolin doses could be used while still reaching unbound concentrations above a target of 8 mg/L, during >60% of the time. A weight-and age-based model-derived cefazolin dosing regimen for neonates was proposed. Although this dosing regimen should theoretically result in improved cefazolin exposure in NICU patients, prospective validation by a clinical trial is needed.

In chapter 6, amikacin was used to document how such a **prospective dosing validation** can lead *Towards Improved Predictability* of drug exposure in neonates. An amikacin population PK-based dosing regimen was validated using 1195 routine therapeutic drug monitoring (TDM) results. Overall, target peak and trough values were attained in most patient groups. Specific subgroups who would benefit from a limited additional dosing adaptation were identified. Consequently, this prospective validation effort ended with the proposal of a new, improved dosing regimen, which in turn needs adequate evaluation and subsequent validation.

This at least illustrates that optimizing pharmacotherapy needs to be considered as a continuous research process, in which every result announces the beginning of a new exploration.

Throughout this scientific journey, it became obvious that many gaps in neonatal clinical pharmacology still have to be resolved. Nevertheless, the results obtained in this doctoral thesis yield a small contribution to bridge some gaps. Besides improvements in compound-specific pharmacotherapy for neonates, our observations provide a basis to further explore PK/PD patterns of other compounds or in other patient populations.

Nederlandse samenvatting

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Klinische farmacologie tracht de effecten van een geneesmiddel te voorspellen op basis van de farmacokinetiek (PK) en farmacodynamiek (PD). De algemene principes die hierbij van toepassing zijn, gelden evenzeer voor pasgeborenen. Veranderingen ten gevolge van de snelle ontwikkeling tijdens de neonatale fase zorgen echter voor een grote inter- en intra-individuele variabiliteit. Dit brengt extra uitdagingen met zich mee, zowel in de klinische zorg als bij het uitvoeren van het wetenschappelijk onderzoek in deze specifieke populatie.

In deze PhD thesis hebben we gezocht naar covariabelen, die de grote variabiliteit in PK/PD (mede) kunnen verklaren. We hebben hiervoor geneesmiddelen bestudeerd die frequent toegediend worden aan pasgeborenen, maar nog onvoldoende begrepen zijn.
Tot 70% van de vancomycine dalspiegels, bekomen na gebruik van gepubliceerde doseerschema's, zijn lager dan de vooropgestelde grens voor effecitiviteit (10 mg/L). Dit illustreert de uitgebreidheid van onverklaarde, en soms onverwachte, problemen in geneesmiddelen blootstelling bij pasgeborenen. Immature neonaten met een laag gewicht, en met hogere postnatale leeftijd (PNL), blijken het meest vatbaar om subtherapeutisch vancomycine dalspiegels te behalen (hoofdstuk 2). De mate waarin een geneesmiddel zijn plaats van effect of een diep lichaamscompartiment bereikt, is onvoldoende bestudeerd bij pasgeborenen. In kunstmatig beademde neonaten hebben we amikacine concentraties bepaald in het bronchiaal epitheliaal vocht (hoofdstuk 3). De hoogst gemeten concentratie werd bereikt tussen 6-14.5 uren na de amikacine toediening. Dit is later, in vergelijking met volwassenen. In hoofdstuk 4 werd een verdere exploratie verricht naar variabelen van propofol dispositie. Op basis van de propofol metabolieten bepaald in 24 uur urine collecties bij pasgeborenen, bleek postnatale leeftijd de belangrijkste drijfveer te zijn van propofol metabolisme. De beperkte bijdrage van glucuronidatie tot het metabolisme van propofol is vergelijkbaar met andere studies betreffende geneesmiddelen glucuronidatie in het jonge leven. Omwille van de link tussen fenotypische glucuronidatie en propofol klaring, en omwille van het feit dat ook bilirubine glucuronidatie ondergaat, werd nagegaan of indirecte hyperbilirubinemie een voorspellende biomarker zou kunnen zijn van lagere propofol klaring bij pasgeborenen. Gezien beide betrokken iso-enzymen (UGT1A1 voor bilirubine en UGT1A9 voor propofol) een vergelijkbaar maturatie patroon vertonen, was dit een aannemelijke hypothese. Echter, postmenstruele leeftijd (PML) en PNL waren de meest

optimale covariabelen, ongeacht de aanwezigheid van hyperbilirubinemie. Deze bevinding kan verklaard worden door het feit dat icterus bij een pasgeborene het gecombineerd resultaat kan zijn van een toegenomen productie en/of een verminderde eliminatie van bilirubine. Gestegen bilirubinemie, het respiratoire distress syndroom en hemodynamische instabiliteit zijn kenmerken die vaak gezien kunnen worden tijdens de eerste levensdagen. Omdat propofol gebruikt wordt als premedicatie voor (semi)electieve endotracheale intubatie op deze leeftijd en in afwezigheid van aangepaste propofol doseerschema's, werd een prospectieve dose-finding studie uitgevoerd met gelijktijdige evaluatie van farmacodynamische aspecten van propofol (sedatie en relaxatie status, vitale tekens). In vergelijking met vroegere bevindingen, kunnen lagere propofol dosissen gebruikt worden, terwijl het klinische herstel na toediening van de lagere propofol bolus toch nog tijd vraagt. Op basis van continue metingen van vitale tekens werd de veiligheid van de propofol toediening geëvalueerd. Enkel een matige bloeddrukdaling en een kortdurende daling in perifere en cerebrale zuurstofsaturatie werd gedocumenteerd. Voor preterme neonaten met een PNL <10 dagen konden ED_{50} dosissen voor propofol bepaald worden. In afwachting van gevalideerde propofol doseerschema's, kunnen deze ED_{50} dosissen gebruikt worden als startdosis, met individuele titratie van de bolus dosis indien klinisch nodig. Zeker voor INSURE indicaties is deze geleidelijke aanpak vereist.

Integratie van covariabelen, die variabiliteit in neonatale PK/PD verklaren, in doseerschema's kan leiden tot een verbeterde voorspelling van geneesmiddelen expositie. Dit werd aangetoond voor cefazoline in hoofdstuk 5. Op basis van een populatie PK model werden covariabelen van cefazoline dispositie bij pasgeborenen gedefinieerd. Hierbij werd rekening gehouden met concentratie-afhankelijke eiwit binding. Gezien de vrije fractie van een geneesmiddel verantwoordelijk is voor de (neven)effecten van het geneesmiddel, is het zinvol de eiwitbinding te bepalen en vervolgens te implementeren in PK/PD modellen. De vrije cefazoline fractie bij pasgeborenen werd gedeeltelijk verklaard door de covariabelen PML, albuminemie, totale cefazoline concentratie en vrije cefazoline concentratie. De neonatale data over cefazoline eiwitbinding werden samengevoegd met gepubliceerde observaties bij volwassenen. Naast verwachte covariabelen (albuminemie, totale cefazoline concentratie, vrije cefazoline concentratie), was ook de patiënt subgroep een verklarende factor voor de verschillen in vrije cefazoline fractie tussen de verschillende populaties.
Monte Carlo simulaties op basis van deze neonatale cefazoline data, toonden dat lagere cefazoline dosissen gebruikt kunnen worden terwijl nog steeds vrije concentraties boven 8

mg/L bereikt worden gedurende >60% van de tijd. De populatie PK analyse resulteerde dan ook in een voorstel tot aangepast cefazoline doseerschema voor pasgeborenen, op basis van gewicht en leeftijd. Dit nieuwe doseerschema zou tot betere cefazoline blootstelling moeten leiden bij pasgeborenen, maar hiervoor is eerst nog prospectieve validatie noodzakelijk.

In hoofdstuk 6 werd met behulp van amikacine getoond hoe prospectieve validatie van een doseerschema tot een betere voorspelbaarheid van geneesmiddel blootstelling kan leiden bij pasgeborenen. In totaal werden 1195 routine amikacine therapeutische drug monitoring (TDM) resultaten gebruikt om een doseerschema, afkomstig van een populatie PK analyse, te valideren. Vooropgestelde piek- en dalwaarden werden bereikt in de meeste patiënten. Specifieke subgroepen voor wie een bijkomende aanpassing van het doseerschema zinvol zou zijn, werden geïndentifieerd. Hiermee werd duidelijk dat prospectieve validatie tot een nieuw doseervoorstel kan leiden, dat op zijn beurt een adequate evaluatie en validatie moet ondergaan.

Het verbeteren van farmacotherapie moet beschouwd worden als een continu proces, waarbij elk resultaat het begin van een nieuwe exploratie aankondigt.

Doorheen dit onderzoekswerk werd vastgesteld dat vele facetten binnen het domein van de neonatale klinische farmacologie nog bestudeerd moeten worden. De resultaten uit deze PhD thesis kunnen beschouwd worden als een kleine bijdrage. Naast het verbeteren van geneesmiddel-specifieke farmacotherapie voor pasgeborenen, vormen de resultaten een basis voor verdere exploratie van PK/PD patronen voor andere geneesmiddelen of binnen andere populaties.

Curriculum vitae

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