# Towards Precision Dosing in Pediatric Oncology

## Challenges and Recommendations

Sebastiaan D.T. Sassen

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# Towards Precision Dosing in Pediatric Oncology

**Challenges and Recommendations**

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# Contents

# Chapter 1

## General introduction

## General introduction

Acute lymphoblastic leukemia (ALL) is the most common form of childhood malignancy. The overall incidence in children from 0-19 years old is two to four cases per 100,000 person-years with a peak between the ages of one to four years old.[1,2] In the last decades, major advances have been made in the treatment, which improved 5-year overall survival from around 10-30% in the seventies to over 90% in recent years.[3–5] The treatment has become more individualized and is based on risk adaptation, for example by stratification on early response (minimal residual disease) and genetic aberrations.[2,6] The current treatment of ALL consist predominantly of rotating combinations of chemotherapeutic agents. Adequate exposure to chemotherapy is of utmost importance to avoid adverse drug related toxicities and subtherapeutic treatment. However, studies evaluating the variability in drug exposure (for example between patients or treatment phases) and its relation to clinical outcome parameters remain scarce. These studies might be especially important in a pediatric population, where the differences in exposure are more pronounced compared to adults, as children undergo different phases in their growth and development.[7–9]

Population pharmacokinetics (popPK) is the study of the relationship between dose and concentration, including the variability between and within patients and the identification of the source of this variability. PK comprises the effects of the body on the drugs e.g., absorption, distribution, metabolism and excretion (ADME). However, PK by itself might not be of much use if the relation between concentration and effect is not considered. This relationship between concentration and effect is studied in pharmacodynamic (PD) studies in which the effect of the drug on the body is assessed. The combination of PK and PD analyses provides a powerful tool to improve and individualize dosing. For example, Evans *et al.* compared individualized PK based dosing of high-dose methotrexate in pediatric B-lineage ALL patients to standard dosing and observed a significantly higher percentage of patients to remain in continuous complete remission compared to patients with non-individualized standard dosing.[10] The latter study is just one example of how PK/PD research can improve treatment in pediatric cancer patients. Chapter two of this thesis describes in detail how PK/PD research is currently applied in clinical practice and the benefits of this approach from a clinical perspective.

Pediatric oncology is a good candidate for PK based treatment optimization considering the severity and complexity of the treatment in a pediatric population. The scarcity of PK data in pedi-

atric oncology might therefore be surprising. However, the limited number of available PK studies is likely due to several factors which complicate PK research in this group of vulnerable patients. First, classic PK studies require dense blood sampling which is often seen in phase one studies in a controlled setting. This is typically not feasible in small children due to the limited volume of blood that can be withdrawn and it is often impractical with respect to their treatment.[11] Second, in most cases the population of children with specific forms of cancer and specific chemotherapeutic agents is small and it is unethical to administer therapeutic dosages of chemotherapeutic agents to healthy children. However, the use of sensitive high-end analytical quantification methods and the flexibility provided by the use of nonlinear effects modeling (NONMEM®) may allow PK studies in oncologic children. By application of high-end liquid chromatography coupled to mass spectrometric detection the volume of blood withdraw can be reduced as the sensitivity to detect the drug in plasma of this technique is high. The application of NONMEM allows the popPK to be assessed in data sets in which patients have undergone limited blood sampling. Furthermore, this mathematical approach allows the combination of heterogeneous data sets.[12–14] For the scope of this thesis several drugs currently used in the treatment of pediatric ALL are studied in order to gather more insight in the PK and its relation with PD to determine whether individualized treatment can be beneficial.

In chapter three *Erwinia* asparaginase was studied, which is used as an alternative to *Escherichia coli* [*E. coli*] derived asparaginase after allergic reactions or silent inactivation. Different forms of asparaginase exhibit different PK profiles.[15–18] Native *E. coli* has a half-life of 17-19 hours and 1.3 days for respectively intramuscular and intravenous administration.[15–17] PEGylated asparaginase has a much longer half-life of 2.4-11.8 days and exhibits time dependent clearance.[18] *Erwinia* asparaginase has the shortest half-life, especially after intravenous administration (about 6.4-7.6 hours); however, information concerning its PK is limited.[19,20] Currently, therapeutic drug monitoring (TDM) is performed to achieve trough concentrations above the target concentration of 100 IU/L, which leads to complete asparagine depletion.[21–26] Chapter three describes the popPK of *Erwinia* asparaginase and its association with patient characteristics to improve target attainment in individual patients especially at the start of the treatment of *Erwinia* asparaginase prior to TDM. With TDM the dose is adjusted based on individual trough concentrations. However, no concentrations are available at the start of the treatment and model-based predictions based on patient characteristics may be used to determine an individualized starting dose.

Another drug commonly administered in the treatment of pediatric ALL is ciprofloxacin, an antibiotic used as prophylactic treatment in order to prevent infections in this immunosuppressed population. Studies have shown the effectiveness of antimicrobial prophylaxis for gram negative pathogens in pediatric acute leukemia and the superiority of quinolones over other antibiotics.[27–29] However, there is no guideline concerning prophylactic dosing of antibiotics; therefore, therapeutic doses are used. The duration of prophylactic ciprofloxacin use is much longer compared to the treatment of infection, where the former can last over a year. The prolonged use of ciprofloxacin raises questions concerning long term side effects such as possible joint and cartilage toxicities and the emergence of resistance to fluoroquinolones.[30,31] A ratio of ciprofloxacin exposure (area under the concentration time curve; AUC) over minimal inhibitory concentration (MIC) of >125 is considered the PK/PD target.[32–34] Although, it is not known whether this target is valid for prophylactic dosing and whether this target is achieved throughout this population. Routine surveillance cultures are taken prior to the start and during the treatment of ALL, which allowed the evaluation of bacteremia and emergence of resistance during ciprofloxacin prophylaxis. In chapter four the popPK of ciprofloxacin was studied in order to determine whether the target ratio of $AUC_{24}$/MIC is achieved in the total population and the correlation between exposure versus the incidence of infections and emergence of resistance was evaluated.

In chapter five the popPK and PD of prednisolone was studied. Prednisolone is a glucocorticoid like dexamethasone, which form the backbone of pediatric ALL treatment. The response to glucocorticoids is an important prognostic indicator in pediatric ALL.[35,36] Many studies have been performed on steroid resistance; however, very little information is available with respect to the PK in pediatric patients.[37–40] For both prednisolone and dexamethasone, weight-normalized clearance is higher in younger children.[41,42] This raises the question whether younger children might benefit from higher dosages of glucocorticoids in leukemia treatment. To our knowledge no studies have been performed regarding the correlation between *in vivo* prednisolone exposure and clinical treatment response in ALL, nor is it known whether steroids need age-based dosing. In addition, studies from Yang *et al.* and Kawedia *et al.* observed higher dexamethasone clearance in patients with anti-asparaginase antibodies.[41,43] This might result in lower exposure in patients with such antibodies compared to other patients. Whether this is also the case for prednisolone is unknown. Although the molecular structure of prednisolone and dexamethasone are very similar, the PK/PD profiles differ. Dexamethasone has higher potency and longer biological half-

life compared to prednisolone. Dexamethasone exhibits linear plasma protein binding whereas the plasma protein binding of prednisolone is concentration dependent, and changes from 95% bound to plasma proteins at low prednisolone concentrations to 60% at high prednisolone concentrations.[42,44,45] Only unbound prednisolone is active, therefore the change in free prednisolone in plasma could affect treatment outcome.[42] In chapter five of this thesis the PK/PD of prednisolone was studied to assess whether exposure is age-related, especially whether younger children may need higher dosages to obtain similar AUCs as in older children. Additionally, the effect of asparaginase on prednisolone PK was evaluated. Lastly, the relationship between prednisolone exposure and anti-leukemic response was studied. Altogether this might clarify whether patients (or subset of patients) might benefit from individualized dosing and whether PK based dosing is recommended.

The overall focus of this thesis was to improve the treatment of pediatric ALL patients through optimized and individualized dosing of chemotherapeutic agents and supportive care agents. Precision dosing was studied by gathering insight in the PK of these drugs, quantification of inter- and intraindividual variability, and identification of the cause of these variabilities by application of population PK modeling techniques. The developed PK models were used to clarify drug specific questions regarding their concentration-effect relation. Is there a correlation between (unbound) prednisolone exposure and treatment outcome? Do patients achieve the target $AUC_{24}$/MIC ratio of ciprofloxacin and does the exposure correlate with the incidence of gram-negative bacteremia or the emergence of resistance? Is the starting dose of *Erwinia* asparaginase sufficient to reach the target trough concentration in all patients and whether (subsets) of patients require dose adjustments? All these questions converge to one overarching question: can PK based precision medicine improve the treatment via individualized dose adjustments in all or subsets of patients?

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# Chapter 2

## Pharmacokinetics and population pharmacokinetics in pediatric oncology

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## Abstract

Pharmacokinetic research has become increasingly important in pediatric oncology as it can have direct clinical implications and is a crucial component in individualized medicine. Population pharmacokinetics has become a popular method especially in children, due to the potential for sparse sampling, flexible sampling times, computing of heterogeneous data and identification of variability sources. However, population pharmacokinetic reports can be complex and difficult to interpret. The aim of this chapter is to provide a basic explanation of population pharmacokinetics, using clinical examples from the field of pediatric oncology, to facilitate the translation of pharmacokinetic research into the daily clinic.

## Introduction

Despite cure rates approximating 80%-90%, cancer is the leading cause of death due to disease in children. [1,2] The treatment of pediatric cancer consists predominantly of combinations of chemotherapeutic agents. The level of exposure to these agents is an important determinant for the therapeutic efficacy and the toxicity. Whether a drug is safe and effective depends on the drug exposure in the body. Drug exposure should be within the therapeutic window, which means sufficiently high to produce the intended effect (the minimal effective concentration (MEC)) and below the level resulting in (unacceptable) toxicity or unwanted side effects (the minimum toxic concentration (MTC) or maximum tolerated dose (MTD)). Oncolytic drugs often have a narrow therapeutic window, and combined with a large variability between drug plasma concentrations observed in pediatric oncology patients, this can result in suboptimal therapy or increased toxicity.

Pharmacokinetics (PK) studies the relationship between dose and concentration within the body, where pharmacodynamics (PD) studies the effects of the drug. Knowledge of the relationships between dose-concentration and concentration-effect are fundamental in establishing the right dose and dose adjustments. Unfortunately, pediatric PK and PD data of anti-cancer drugs is often lacking, and dose regimens have been established empirically. In general, most drugs in pediatric oncology are dosed based on body weight or body surface area (BSA) if no specific pediatric dosing information is available. This is done by extrapolating the adult dose per kg body weight or $m^2$ BSA to the pediatric situation. However, this extrapolation is only valid on two conditions; first, the processes of distribution, metabolism and elimination of the drug are proportional over the weight or BSA range. Second, the relationship between concentration and effect of the drug is similar between adults and children. In many cases drug dosing for adults and children is correlated and extrapolation is possible. [3] However, due to developmental changes in children, especially in infants and children below two years of age, these conditions might not be met. [4,5]

The lack of pediatric PK/PD data is due to the fact that pediatric oncology patients generally form a small population, and it is not ethical to administer therapeutic dosages of chemotherapeutic agents to healthy children. Traditional PK sampling is invasive requiring multiple blood samples (e.g. n ≥10) at fixed time intervals. Additionally, the amount of blood which can be withdrawn from infants is limited. [6] Population PK/PD modeling has many advantages; it allows time flexible and limited sampling (for example 2-3 samples), quantify variability in concentrations

and identify variability sources, as well as extrapolation based on statistical models and simulations with virtual patients to expand population scenarios and further individualize dosing.[7,8]

In conclusion, knowledge of pharmacokinetics and its variability in the pediatric oncologic population is important in the optimization of (individualized) drug dosing in this population, with the final aim to improve prognosis. However, population pharmacokinetic studies can be complex and difficult to interpret due to their technical nature. The aim of this chapter is to provide a basic explanation of PK and population PK, through the use of examples from the field of pediatric oncology, in order to facilitate the implementation of pharmacokinetic research into the daily clinic.

## Part I: What is pharmacokinetics?

Pharmacokinetics is the study of the kinetics of pharmacological substances within the body and describes the processes of drug absorption, distribution, metabolism and excretion. It focuses predominantly on the relation between the administered dose and the concentration-time profile of the drug in a body compartment (e.g. plasma). The pharmacodynamics describes the dose response relationship, for example tumor response or side-effects.[9,10] Pharmacokinetics and –dynamics are often studied together in PK/PD models in order to determine the relationship between dose, concentration and effect. PK studies are performed in different areas, like preclinical drug development, Phase I, II and III trials, to provide and establish dosing guidelines for registration.[11–13] Pharmacokinetic analyses may also be performed clinically in individual patients for individualized dosing, i.e. therapeutic drug monitoring (TDM).[14–16]

> *Example 1, clinical relevance of PK:* Individualizing the dose and treatment with the use of PK parameters can improve outcome and avoid unnecessary toxicities. Evans *et al.* showed that individualized based dosing of high-dose methotrexate in pediatric B-lineage ALL patients, based on their clearance and AUC, resulted in a significantly higher percentage of patients remaining in continuous complete remission compared to conventional fixed dosing based on BSA.[17] PK modeling can help predict the individual clearance and AUC, hence improve outcome.

## Linear versus nonlinear PK

The concept of linearity in drug elimination is of great clinical importance (assuming a strong correlation between concentration and effect), as it determines how the drug concentration in the body changes in relation to dose adjustments. A drug is considered to have linear PK if there is a linear relation between the administered dose and the exposure (AUC). Thus, increasing the dose by a factor two results in a factor two increase in exposure. Most drugs follow linear PK within the clinically applied concentration range. A drug is considered to have nonlinear PK when increasing the dose produces a non-proportional increase in exposure (figure 2.1). nonlinearity often occurs when certain PK processes become saturated, e.g. (re)absorption pathways (limiting the uptake

and availability of the drug from the gut) or from saturation of metabolic and excretion pathways (limiting elimination and producing accumulation of the drug). Drugs with linear PK are generally preferable in the clinic due to their predictable dose-concentration relation. nonlinearity is clinically not ideal, as increasing doses might result in disproportional shifts in the concentration. With nonlinear elimination TDM is recommended, especially if the drug has a small therapeutic window.

*Example 2, nonlinearity:* Asparaginase concentration should remain above a threshold for complete asparagine depletion, hence TDM is important to ensure sufficient levels throughout treatment, and to detect immune mediated inactivation.[18,19] Several forms of asparaginase are available, e.g. native *E. coli* derived asparaginase, PEGylated asparaginase and *Erwinia* asparaginase, which have different PK profiles. Native *E. coli* asparaginase and *Erwinia* asparaginase exhibit linear pharmacokinetics, whereas PEGylated asparaginase has a time-dependent elimination.[20–23] PEGylated asparaginase is conjugated with polyethylene glycol (PEG), to reduce the clearance therefore increasing the dose interval from every two or three days to every two weeks. However, popPK studies by Hempel *et al.* and Würthwein *et al.* show that the PEGylation results time-dependent elimination, where clearance increases with time after dose. This can result in lower than expected (subtherapeutic) trough levels and should be accounted for.[21,24] PK models can help predict these trough levels. Asparaginase concentrations might also affect the clearance of other drugs like dexamethasone, which could lead to a lower exposure of both asparaginase and dexamethasone. This could result in a worse outcome concerning an increased risk of relapse.[21,24,25]

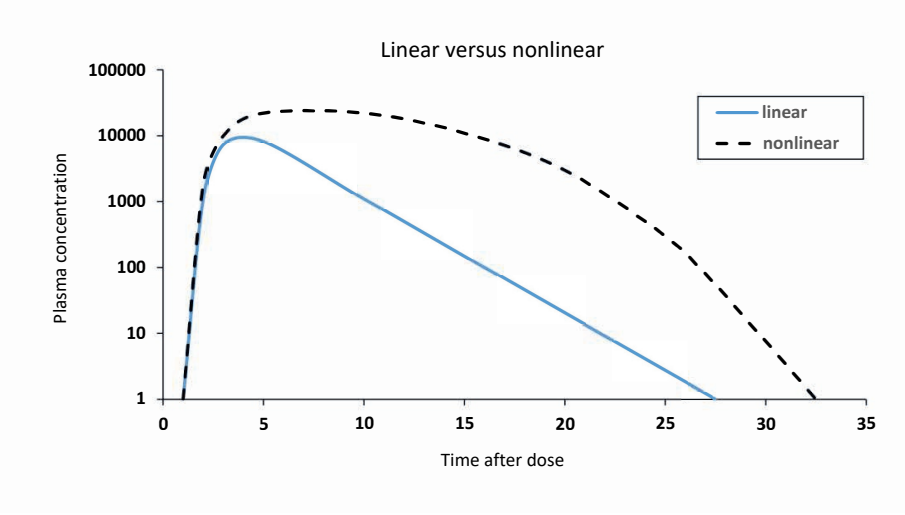


**Figure 2.1:** Linearity versus nonlinearity. Concentration versus time curve. Solid line: linear PK clearance independent of concentration ($t_{1/2}$ constant); Dashed line: nonlinear kinetics, drug clearance depends on concentration, slow clearance (longer $t_{1/2}$) at high concentrations (e.g. due to saturation metabolic pathway) subsequent faster clearance (shorter $t_{1/2}$) when concentration decreases (pathway becomes less saturated) assuming unaltered distribution.

## ADME

PK can be divided into four main processes: drug absorption, distribution, metabolism and excretion (ADME). Knowledge of the processes that determine the PK is important, as it can have major clinical implications. This is especially true for chemotherapeutic drugs, which often have a small therapeutic window. The ADME processes can differ between patients, within patients and affected by external factors like comedication. In children these differences are even more pronounced. The human body does not develop isometrically but allometrically, meaning different organs and processes develop at different rates in terms of growth and maturation. Therefore, in pediatric PK modeling, allometric scaling is often used to adjust for growth and maturation. Especially in the first two years of life, using a linear approach or even a corrected approach (like allometric scaling) to describe PK parameters might not suffice.[26–28] Several changes during the development of a child complicate dosing linearity, and affect how drugs are absorbed, distributed and eliminated from the body.[4,29–33]

## Absorption

The absorption phase describes the uptake of the drug into the bloodstream, for example from the gastrointestinal-tract. In PK the rate of absorption is referred to by the first-order rate constant '$ka$'. Most drugs are not completely absorbed when administered orally. The percentage of absorbed drug available to the body is referred to as the bioavailability ($F$). The absorption phase can be preceded by a dissolution phase in the gut (e.g. dissolution of a tablet). Different pharmaceutical formulations can result in different absorption profiles. In children the absorption rate and bioavailability can differ from adults due to anatomical and developmental differences. These factors, predominantly in the first two years of life include, reduced gut transit time, increased intestinal permeability and altered passive and active drug transporters.[33–35] A drug can also bind to food or other medication in the stomach, hence inactivating or inhibit absorption of the drug, like ciprofloxacin and milk or tube-feeding.[36]

> *Example 3, absorption:* Topotecan is used for pediatric brain tumors. Topotecan can be administered both intravenously and orally. However, topotecan shows extensive variability between and within patients, especially in very young children.[37,38] Roberts *et al.* studied the popPK of oral topotecan in infants and young children focusing on the effects of age and drug efflux transporters on absorption. Polymorphism of the efflux transporter ABCG2 showed to have a significant effect on absorption, resulting in an almost two-fold difference in maximum concentration.[39] PK analysis provide a useful tool in studying these effects and make better predictions.

## Distribution

After absorption the drug is distributed throughout the body based on its physicochemical properties.[40] Distribution is expressed as an apparent volume ($V_d$). This is a proportional factor defined as the volume into which the drug appears to be distributed with a concentration equal to the plasma concentration, as if the body was *'one well-stirred compartment'*. For some drugs, plasma concentrations decrease rapidly after administration due to distribution to body tissues. In this case a one-compartment does not suffice to describe the concentration-time profile of distribution and multiple compartments may be required to adequately describe the profile. On a

more advanced level the intracellular distribution can be taken into account.[41,42] Distribution in children is affected by altering body composition, for example changes in water/fat ratio, muscle ratio and extracellular water.[32,43,44] Plasma protein concentration and binding can differ as well, which is important for highly protein bound drugs. If 98% of a drug is bound to proteins, a decrease to 96% result in twice the concentration of the available drug.[45]

*Example 4, plasma protein binding:* The corticosteroid prednisolone used in acute lymphoblastic leukemia has both linear and nonlinear binding to plasma proteins.[46–48] The binding to albumin is linear in contrast to the saturable binding to Corticosteroid Binding Globulin (CBG). The concentration of free unbound prednisolone depends on CBG, albumin and free prednisolone. With higher dosages, relatively higher concentrations of free prednisolone will be present and could potentially increase the risk of negative side-effects.[46–48] Ionita *et al.* and Petersen *et al.* showed how population PK models are used to include the linear and nonlinear binding in order to improve the prediction of free concentrations which can establish the drug effects.[46,47]

*Example 5, body composition:* As most chemotherapeutics, doxorubicin has severe side effects. Krischke *et al.* showed in a popPK model, that children <3 years of age had a significantly lower clearance compared to older children even after correction for BSA, resulting in higher exposure in younger children.[49] Additionally, Thompson *et al.* showed that doxorubicinol, an active metabolite of doxorubicin, was dependent on body composition: children with >30% body fat showed a significantly lower clearance of doxorubicinol, with a mean of 37 l/h/m$^2$ compared to 64 l/h/m$^2$ for <30% body fat, however the groups were small. The metabolite doxorubicinol may contribute to the cardiotoxicity after doxorubicin administration.[49–52]

## Metabolism

Metabolism describes the processes involving conversion of the drug into metabolites. Most anti-cancer drugs are excreted from the body after being metabolized into active (pro-drug), less active or inactive metabolites. Metabolization occurs predominantly in the liver and gut through the cytochrome P450 enzyme system. Differences or changes in the enzyme system can result in increased or decreased clearance of the drug, therefore decreasing or increasing the exposure. This can occur due to inherited genetic differences in the metabolic pathway, e.g. polymorphism of CYP450 subfamilies (isoenzymes). Another common cause of altered metabolization is due to drug-drug interactions, where a drug inhibits or induces the metabolic pathway of other drugs or itself. In adults, metabolism is often the cause of large PK variability. However, developmental changes in children superimposes on this variability, due to relative high liver mass, increased hepatic blood flow, liver enzyme synthesis and concentration, and differences in gut wall enzymes including bacterial enzymes. [53–56]

*Example 6: polymorphism:* Pharmacogenetic variation can affect the treatment. Polymorphisms of thiopurine methyltransferase (TMPT) can result in lower activity of the enzyme which competes with 6-thioguanine nucleotides. This increases the effect of 6-thioguanines like 6-mercaptopurine and 6-thioguanine, affecting the relapse risk in pediatric ALL. [57,58] Hawwa *et al.* used popPK to examine the effects of genetic polymorphism and developed a PK model to improve dosing of 6-mercaptopurine, which showed a large effect of TMPT on the metabolism. [59]

*Example 7: drug-drug interactions:* Azole antifungals are used as antifungal prophylaxis in pediatric cancer, especially during high-risk periods. Azoles are strong inhibitors of a number of CYP450 subfamilies and P-glycoprotein (transporter protein; P-gp). Toxicities of have been reported of azoles with concomitant Vinca alkaloids and calcineurin inhibitors due to increasing their exposure. TDM is recommended. [60–63] The PK of azoles, like voriconazole, is complex. Many PK models and simulations have been performed to determine variability, dosing schedule and to study the drug interactions. [64–69]

*Example 8, drug interaction and genetic polymorphism:* Etoposide is a substrate of P-gp, CYP3A4 and CYP3A5. Glucocorticoids, like dexamethasone and prednisolone, can inhibit or induce CYP3A and P-gp. Kishi *et al.* showed in a PK study an almost two-fold increase in etoposide clearance with concomitant treatment of prednisolone in pediatric acute lymphoblastic leukemia (ALL) patients, resulting in a lower etoposide AUC. Additionally, the effect of genetic polymorphism showed that the *MDR1* exon 26 CC genotype predicted higher etoposide clearance.[70] Both concomitant glucocorticoids and *MDR1* exon 26 CC genotype result in higher clearance and therefore less etoposide exposure.

## Excretion and elimination

Excretion and elimination describe the removal of the drug from the body. Drug and metabolites can be excreted by the kidneys in the urine and/or by the liver through biliary excretion into feces based on their physicochemical properties. Other routes of excretion, like sweat or breath, are in most cases negligible. Metabolism and excretion are quantitatively described by the clearance ($CL$), which is the overall ability to eliminate a compound from the body. It is expressed as the volume that is cleared of the compound per unit of time (e.g. L/h).

Elimination can be linear (first-order kinetics) or nonlinear (zero-order, Michaelis-Menten and nonlinear elimination kinetics). In first-order PK the amount of drug eliminated per time period is proportional to the concentration in blood. The time to clear the body of 50% of the drug (half-life; $t_{1/2}$) remains constant and is independent of the concentration. $CL$ and $t_{1/2}$ are inversely correlated, assuming unaltered distribution. In "zero-order pharmacokinetics" a constant amount of drug is removed from the bloodstream per unit of time regardless of the concentration. Therefore, high concentrations have a relatively low clearance and long elimination half-life compared to relatively high clearance and short half-life at low concentrations. The nonlinear Michaelis-Menten (enzyme) kinetics is due to saturation of the elimination pathway. When saturation occurs, the body cannot increase the clearance with increasing concentrations, as it lacks the capacity, and will therefore continue to eliminate the drug at the maximum (fixed) rate. Other types of nonlinearity are for example time-dependent kinetics. Decreased clearance and prolonged half-life can result in unwanted accumulation of the drug. Clearance in children can differ from

adults due to altered renal excretion rate (relatively large kidney size), active and passive tubular transporter mechanisms and urinary pH.[28,29,71]

> *Example 9, Michaelis-Menten kinetics:* Voriconazole is an antifungal used in immunocompromised children such as hematological malignancies. Karlsson *et al.* showed with a popPK model that voriconazole follows nonlinear Michaelis-Menten kinetics. They also showed important PK differences between children and adults. The concentration at which half of the maximum enzyme activity is achieved (Michaelis-Menten constant) is higher in children. Therefore, the nonlinearity is less pronounced and occurs at higher concentrations and doses compared to adults. Additionally, with covariate analysis CYP2C19 genotype and alanine aminotransferase levels were identified as significant factors affecting the clearance.[72] Gastine *et al.* also used popPK and simulations to evaluate target voriconazole dose and interval using a nonlinear model with allometric scaling.[65]

In summary, alterations in eliminating organs, genetic polymorphisms and growth and development cause significant PK variability. Consequently, when this variability is not accounted for, variability in drug exposure will affect treatment outcome in case of a concentration and effect correlation. Hence studying the PK of drugs and providing insight in the characteristics of the drugs and possible dependency of demographic and pathophysiological factors is important for optimal treatment and enables tailoring the treatment to the individual patient.

## Part II: Pharmacokinetic assessment

There are several ways to study pharmacokinetics. The traditional 'two-stage' approach requires full individual concentration-time profiles which are obtained through serial sampling of 10 to 20 blood samples per patient. PK parameters are calculated for each individual patient and summarized to obtain population PK values (mean ± standard deviation). This method is useful in case of a limited number of patients of a homogeneous population with a rich data set such as collected in a phase I study in adults. However, this method is impractical and inconvenient in children as blood sampling in pediatric patients, especially infants, is limited by the total blood volume that can be withdrawn to remain within safe limits, and strict sampling times might hamper the treatment schedule or interfere with daily activities.[6,73] Chemotherapeutic agents cannot ethically be tested on healthy volunteers, therefore limiting the available population. Fortunately, these problems can be easily circumvented through the use of population pharmacokinetic modeling. Due to their flexibility and possibilities, PK/PD modeling is useful throughout different phases of drug development. For example in the translation of preclinical trials to clinical trials, simulating exposure effect relationship and assessment of variability.[74–76] Two common PK-modeling approaches are non-compartmental and compartmental analysis. The former does not rely on the assumptions of body composition where the latter assumes *'well-stirred'* interconnected compartments. Non-compartmental analyses are useful for example within a single study with a homogeneous population. However, with heterogeneous data (e.g. across trials) and high variability (e.g. due to patient characteristics and different occasions) compartmental analysis are useful. Comparison of different methods are described by Ette and Williams 2014 and Kiang *et al.* 2012.[14,77,78]

## Compartmental PK analysis

A *'top down'* approach (population PK; popPK) starts with clinical data like blood samples and patient demographics. Subsequently mathematical and statistical models are developed and fitted to the data in order to determine which model best describes the data. For practical reasons the body is often considered as *'one well-stirred compartment'*. However, drugs do not distribute evenly among tissues outside the bloodstream, therefore additional compartments can be used to describe the data. These compartments do not necessarily reflect a physiological volume, but are empirically derived on the basis of mathematical equations. On the contrary, physiologically-based

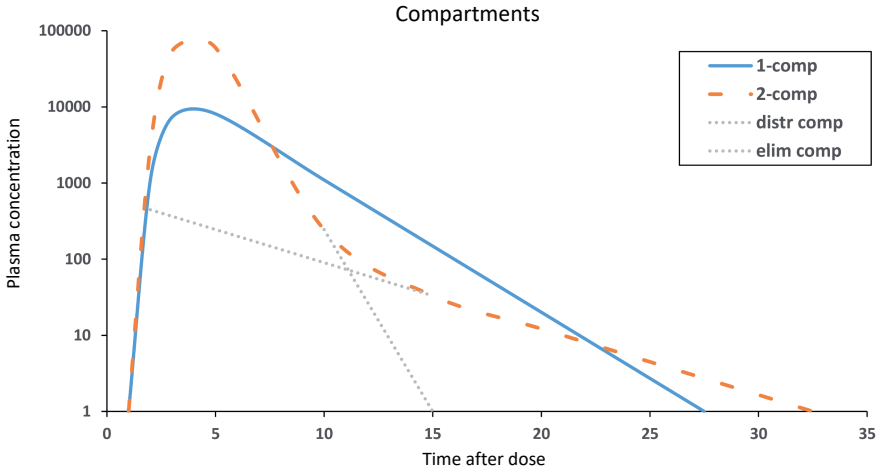


**Figure 2.2:** Compartments: concentration versus time curve. Solid line: linear one-compartment, clearance independent of concentration ($t_{1/2}$ constant); Dashed/dotted line: linear two-compartment, clearance independent of concentration but consists of a distribution phase (first part) and an elimination phase (second part).

PK (PB/PK) models do reflect physiologic compartments, which are connected through vascular transport systems. These systems are built on mechanistic insights from *in vitro* and *ex vivo* experiments.[79] This is a *'bottom-up'* approach which starts with mathematical models/systems and is validated and optimized using clinical data. Ideally, it would also encompass the PK in tumor tissue, e.g. the cellular uptake and excretion from malignant cells. The disadvantage can be the limited available models/systems.[80–84] More information on PB/PK can be found for example in articles of Jones and Rowland, and Khalil and Läer.[85,86]

Most popPK models contain one or two compartments, although they may comprise more compartments. A one-compartment model uses one central compartment (e.g. plasma). A two-compartment model generally has a central compartment ($V_c$) reflecting blood and highly perfused tissues with rapid distribution, and a peripheral compartment ($V_p$) with poorly perfused tissues like adipose tissue. After administration the drug is quickly transported from the central to the peripheral compartment until an equilibrium situation occurs (steady-state). This distribution is commonly referred to as the distribution phase. The second phase, where the drug is eliminated

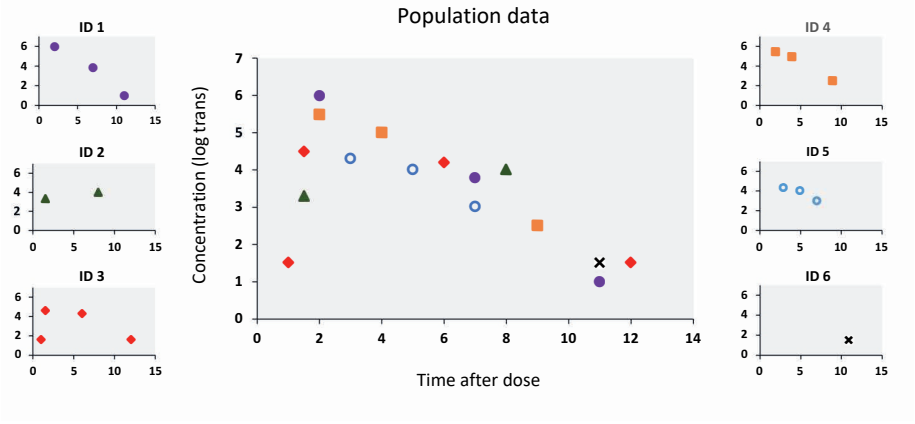


**Figure 2.3:** Population Data Pooling: Population data; the sparse individual data might not be sufficient for individual PK analyses; however, the pooled population data is. The calculated PK parameters from the pooled data (e.g. $CL$ and $V_d$) are population means. Individual information is retained as individual variability from the mean.

from the central compartment and slowly redistributed from the peripheral, is referred to as the elimination phase. The movement between the compartments is described by intercompartmental clearance ($Q$). A two-compartment model has two $t_{1/2}$ values, one for the distribution and one for the elimination phase. If only one value is presented, it is usually the $t_{1/2}$ of the elimination phase or a combined (hybrid) value calculated from the distribution and elimination phase (figure 2.2).[87,88]

## Population PK approach

In the seventies the population PK based approach has been developed, which facilitated the derivation of PK parameters from only a few samples per patient (sparse sampling). Additionally, the approach provided more flexibility both in number of samples and time at which samples were taken, as long as the exact collection times were registered. The population approach enables the use of heterogeneous data; e.g. using data from different trials, across different populations, a combination of dense and sparse data and flexible sampling times. These advantages are particularly useful in pediatric oncology as the population is small, for example with rare forms of cancer. Therefore, the ability to combine data from different trials, countries and groups allows increas-

ing the population. Sparse sampling is particularly useful to the decreased burden due to invasive sampling, for example in phase I trials.[89] Figure 2.3 shows how limited individual data generates a useful population concentration time curve.

For the population approach several mathematical methods can be used, of which nonlinear mixed-effects (NLME) modeling is the most frequently used method.[90,91] The use of different models is described by Mould and Upton.[80,90] The *'mixed-effects'* refer to the estimation of both fixed and random effects. The fixed effects reflect the average population parameters (e.g. population $CL$, $V_d$). The random effects refer to the variability in these parameters. Variability can be estimated at different levels: interpatient which is between subjects (interindividual variability; IIV), intrapatient which is within a patient (e.g. inter-occasion variability; IOV) and residual variability, the remaining (unexplained) variability , due to model misspecification, time errors, errors in chemical analysis, etc.[80,92] The different types of variability commonly estimated during a population PK analysis are depicted in figure 2.4, which shows a visual representation of variability based on concentration time curves of two subjects. Patient one, with two drug administrations (solid line and dots) at different time points; and patient two, with one administration (dashed line). It shows the variability between patients, between different occasions and the unaccounted variability as residual variability.

## Covariate analysis

Estimation of variability is important. For instance, if large interpatient variability is present in $CL$, unexpected high or low drug concentrations can be found within individual patients, which might result in subtherapeutic or toxic levels. Hence, quantification of the variability is important to determine the expected range between patients.[14,90,93,94] After population analysis the derived model may be used to perform TDM of the drug. By Bayesian combination of popPK parameters with the individual concentrations estimates the individual PK parameters can be obtained. These estimates may be used to adjust the dose (Bayesian forecasting). The statistical model quantifies variability; however, it does not provide the source of variability. The identification of variability sources can be achieved through the evaluation of covariates. Covariates include patient or group characteristics like age, weight, kidney function, disease state, comedication or any other variable that could reasonably account for the variability in patients. A covariate can be correlated to one or more PK parameters. The identification of covariates can be used in children to identify vari-

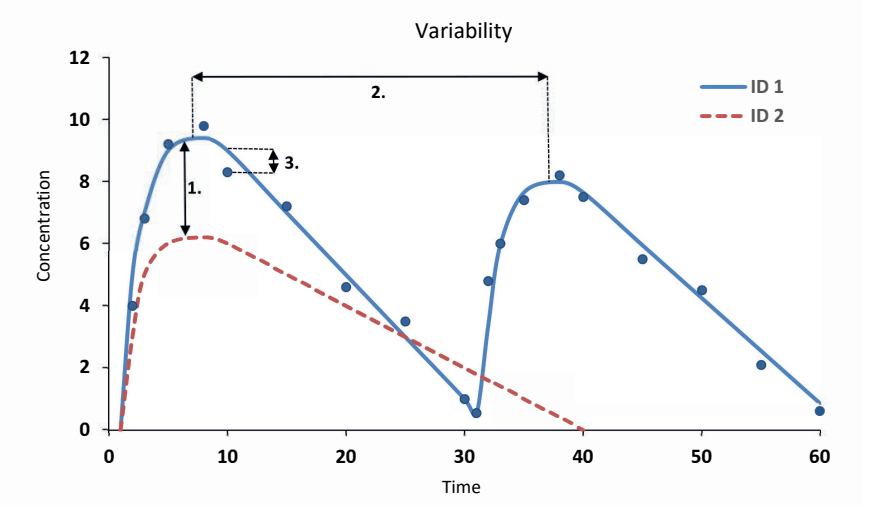


**Figure 2.4:** Types of variability: Concentration time curves depicting the 3 forms of variability. ID1 has two concentration time curves from two subsequent dose administrations (Solid line); ID2 has a single concentration time cure after a single administration. 1. Interindividual variability (IIV), differences (e.g. in peak concentration) between the two patients; 2. Interoccasion variability (IOV), Difference between dose administration time points; 3: Residual variability, due to model misspecification.

ability related to developmental changes. Additionally, the covariate analysis can also study the PK differences with different comedication, disease groups, treatment centers, etc. The implementation of a significant covariate effect in the model decreases the variability and the estimated covariate can be used for dose recommendations.[14,90,95] More information on covariate analysis is described by Joerger *et al.* and Hutmacher and Kowalski.[96,97]

## Allometric scaling

In pediatrics a wide array of body sizes is found. Body size is considered the most important predictor of $CL$ and $V_d$.[4,30,98,99] $V_d$ generally increases linearly with body weight, whereas clearance generally increases nonlinear (figure 2.5). This explains why children in the age range two to four years have higher clearances and dose on basis of kg body weight than older children or adults. To better describe this nonlinearity of $CL$ in children allometric scaling is used. The ¾ power model is the most common approach, where $CL$ is multiplied by normalized weight (indi-

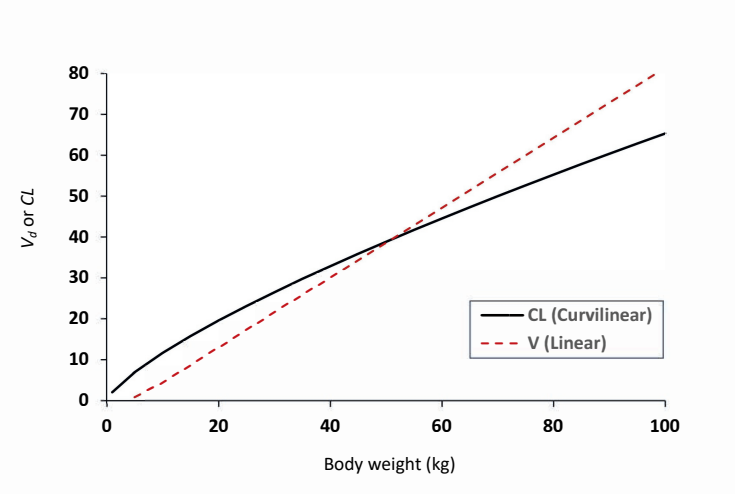


**Figure 2.5:** Correlation Clearance and Distribution versus body weight:The curvilinear relation between clearance $CL$ (L/h) and body weight (solid line) and linear relation volume of distribution $V_d$ (L) and body weight (dashed line). The clearance is normalized using the exponent for allometric scaling (0.75).

vidual weight / standard weight) raised to the power of 0.75. $CL$ and $V_d$ are often normalized for a body weight of 70 kg, which facilitates comparison between different studies.[29,30,73,100] Details and comparison of allometric approaches can be found in e.g. Wang *et al.* 2012, and Anderson & Holford 2012.[30,73]

*Example 10 development and maturation:* Busulfan exhibits substantial interpatient variability, especially in children.[101–105] High concentrations result in severe toxicities, whereas subtherapeutic levels put the patient at risk of graft failure. Therefore TDM adjusted individualized dosing is indicated.[106] McCune *et al.* showed low and fluctuating clearances of busulfan below the age of 3 where it peaked and plateaued until the age of 17.[107] Paci *et al.* showed a higher increase in clearance per body weight for infants <9 kg (2.4 fold) compared to children >9 kg (1.7 fold). PK studies with busulfan showed a vast improvement with the implementation of allometrically scaled body weight to describe this nonlinear correlation between body weight and clearance. In some studies, it was superimposed with age to better describe the changes with age. Paci *et al.* showed a reduction of 63% to 27% in interpatient vari-

ability in busulfan concentrations after implementation of allometric scaling in their PK model.[102,103,105,108] Bartelink *et al.* developed a PK model that could be used with good precision as basis of individualized busulfan dosing, including the nonlinear body weight clearance relation.[102,109]

## Model testing and validation

Throughout the development process of PK modeling, different mathematical models are fitted to the data to determine which model best describes the data. Visual tests (comparison of predicted and observed drug concentrations) and numerical tests (statistical tests, precision) are performed. For diagnostic purposes several goodness-of-fit plots may be evaluated showing model predicted versus observed concentration (figure 2.7). Two common diagnostic tools are the visual predictive check (VPC; simulation) and the bootstrap procedure (resampling). A VPC is used to assess how well the predictions made by the PK model describe the actual observed data, including the variability.[110] The observed and simulated data are presented on top of each other. VPCs are created by simulating a large number of replicates of the original dataset (Monte Carlo simulations). The VPC shows the dependent variable (e.g. concentration) versus independent (e.g. time) for both the simulated and observed data (figure 2.6). The concentrations predicted by the model should be in line with the observed data. Detailed information on VPCs is described by Bertrand *et al.*[110] Other simulation-based methods are available, and include for example the posterior predictive check (PPC), numerical predictive check (NPC) and normalized predictive distribution error (NPDE), which are explained in more detail by Sherwin *et al.* and Karlsson and Savic.[93,111–113]

Bootstrapping is a computer-based resampling technique (creating new datasets utilizing the original dataset) which can be used to assess the accuracy and stability of the model results.[111,114–118] Bootstrap samples are generated by repeated independent random sampling with replacement of samples from the original data set.[117,118] The results of the estimated PK parameters using the resampled datasets should be in line with the results of the original dataset. If not, this might indicate that the model describes the dataset rather than the population and might not be extrapolated outside of the studied population.

Due to technological progression, especially computational power, increasingly complex (mathematical) models can be developed. However, a major concern with these complicated models is overparameterization, where too many parameters are included in the model resulting in unreliable or unrealistic estimates. The model and parameter estimates describe the dataset rather than the population. To reduce overparameterization the number of parameter estimates can be reduced or fixated. Hence, validation of developed models is an important aspect of PK modeling. Different validation methods are described by Sherwin *et al.*[77] Validation can be internal (within the dataset) or external (using a different dataset). The best technique depends on the available data and intended use.[93] Validation using an external (new independent) dataset is most stringent.[118] However, an external dataset might not always be available. Splitting the data is possible for large datasets, where one part is used for model development and the other for validation. Often available data is limited, hence simulation and resampling techniques are used.[117] Other resampling technique is cross-validation, using repeated data splitting.[93,115,118]

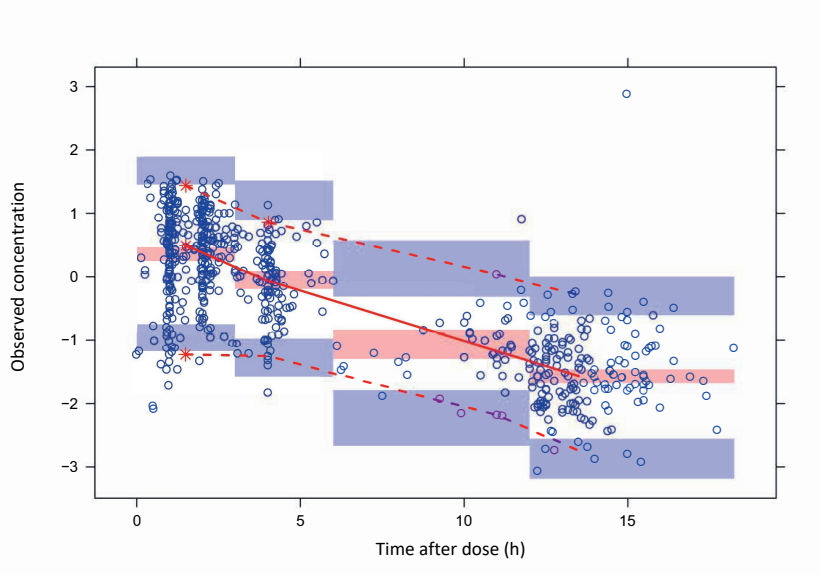


**Figure 2.6:** Visual Predictive Check (VPC): Concentration versus time after bolus dose to 100 patients at t=0 and subsequent sampling around t=1, t=2, t=4 and trough samples prior to next administration. Percentiles (e.g. $2.5^{th}$ and $97.5^{th}$) are calculated for individual values or bins (e.g. different time frames). The lines present the observed data (solid = mean; dashed = percentiles) and the semi-transparent fields present the simulated data based on the population PK model (red = mean; blue = percentiles). In this example the predicted concentration medians and 95% intervals fit the observed concentration well, which shows a good predictive level of the PK model.

## Conclusion

Although the population PK approach has been around for a while, the clinical implications of this approach are not always obvious and translation into the clinic can be difficult. This might be due to the mathematical complexity of the models and the analysis. This chapter describes the implications of PK in the clinic and how it is involved in the work of clinicians. As shown throughout the chapter the pharmacokinetics of an individual patients can be affected by many factors including: the size (body weight, body surface area and age), pharmacogenetics (e.g. enzyme polymorphism), disease state, drug-drug interactions, external factors (e.g. food; drug formulations), drug administration route and development/maturation (e.g of kidneys, liver, metabolism pathways). The nonlinearity of processes, like growth development and saturated metabolic pathways, com-

plicating the extrapolation from one group to the other. Pediatric oncology patients need specific attention due to the aspects of growth and development, the severity of illness, the different combinations of medication (including supportive care), and the small therapeutic window of most chemotherapeutics. Toxicities are a main concern, however subtherapeutic treatment can also be fatal, due to disease progression, and should be avoided. Hence, pediatric oncology can greatly benefit from pharmacokinetic studies for individualized and optimized dosing. Population PK is the ideal method to study PK in children due to sparse and time flexible blood sampling, the use of heterogeneous data, the quantification and identification of variability and simulations. Hopefully an increasing number of PK studies will be performed in order to individualize dosing based on specific patient (and disease) characteristics to optimize treatment and limit side effects.

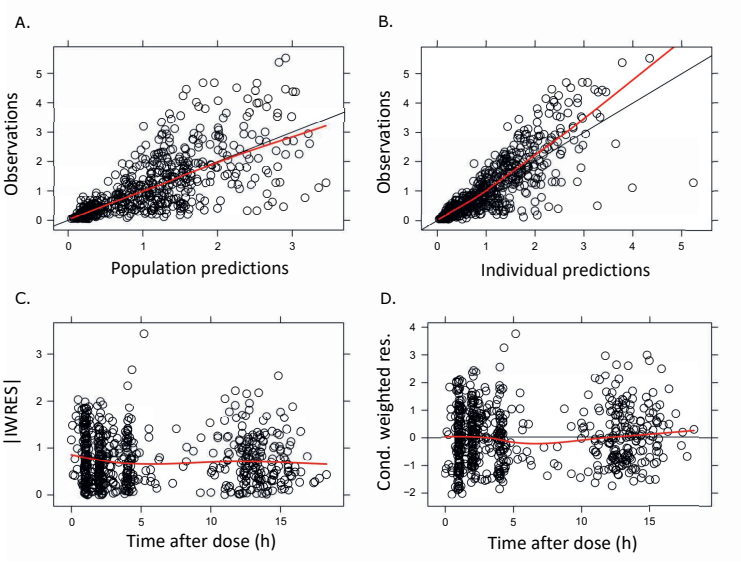


**Figure 2.7:** Goodness-of-Fit plots; Plot A shows the predicted by the PK model based on the population estimates (x-axis) versus the actual measured concentrations (y-axis); Plot B shows the individual prediction versus population prediction. It is similar to plot A, however in this plot predictions are corrected for the individual deviations; Plot C; shows the absolute individual weighted residuals (|IWRES|). It calculates the differences between the observed and predicted values using the standard deviation of the residual variabil-ity; Plot D shows the conditional weighted Residuals (CWRES), it is similar to WRES however a more advanced method of linearization is used. It is conditioned around each individual estimate of between-subject variability. Both plots for WRES and CWRES should be evenly distributed around zero.

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# Chapter 3

## Population pharmacokinetics of intravenous *Erwinia* asparaginase in pediatric acute lymphoblastic leukemia patients

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## Abstract

*Erwinia* asparaginase is an important component in the treatment of pediatric acute lymphoblastic leukemia. A large variability in serum concentrations has been observed after intravenous *Erwinia* asparaginase. Currently in the Dutch Childhood Oncology Group protocols dose alterations are based on trough concentrations to ensure adequate asparaginase activity (≥100 IU/L). The aim of this study was to describe the population pharmacokinetics of intravenous *Erwinia* asparaginase to quantify and gather insight in the interindividual and inter-occasion variability. The starting dose was evaluated based on the derived population pharmacokinetic parameters. In a multicenter prospective observational study, a total of 714 blood samples were collected from 51 children (1–17 years) with acute lymphoblastic leukemia. The starting dose was 20,000 IU/m$^2$ thrice weekly and adjusted according to trough levels from week three onwards. A population pharmacokinetic model was developed using NONMEM®. A two-compartment linear model with allometric scaling best described the data. interindividual and inter-occasion variability of clearance were 33% and 13%, respectively. Clearance in the first month of treatment was 14% higher ($p<0.01$). Monte Carlo Simulations with our pharmacokinetic model demonstrated that patients with a low weight might require higher doses to achieve similar concentrations compared to patients with high weight. The current starting dose of 20,000 IU/m$^2$ might result in inadequate concentrations especially for smaller patients, hence dose adjustments based on individual clearance is recommended.

## Introduction

Asparaginase is an enzyme that catalyzes the hydrolysis of asparagine to aspartic acid and ammonia. Leukemic cells rely on exogenous supplies of asparagine for their protein synthesis. Hence the depletion of asparagine results in cell death.[1] Asparaginase has become an important component in the treatment of pediatric acute lymphoblastic leukemia (ALL), and therefore every effort should be made to expose the patient to the protocol-prescribed cumulative dose.[2–6] *Erwinia* asparaginase is derived from *Erwinia* chrysanthemi, whereas the other forms of asparaginase (native *Escherichia coli* [*E. coli*] and PEG-asparaginase) are derived from *E. coli*. Currently the *E. coli* derivatives are the first choice in treatment naive patients, and the use of *Erwinia* asparaginase is indicated in patients who develop hypersensitivity to the *E. coli* derived asparaginase or in case of silent inactivation of *E. coli* asparaginase.[4,7,8]

Little is known about the pharmacokinetics (PK) of *Erwinia* asparaginase, especially after intravenous administration. Currently, in the Dutch Childhood Oncology Group (DCOG) ALL-11 protocol (and the preceding ALL-10), the interval and/or dose alterations of *Erwinia* asparaginase are based on therapeutic drug monitoring (TDM), with the aim to keep the trough asparaginase activity above the 100 IU/L threshold, which leads to complete asparagine depletion.[7,9–13] A concentration of 100 IU/L is considered safe concerning asparagine depletion, however complete depletion has been observed at lower concentrations.[14–16] Currently the consensus for the target threshold is 100 IU/L.[17] No evidence based guidelines for increasing or decreasing the dose are available and dose-adaptations are based on empirical knowledge.

The PK data of different asparaginase forms are not transferrable. Intramuscular native *E. coli* asparaginase has an elimination half-life of 1.3 days[18], and intravenous recombinant and native *E. coli* asparaginase 17.3-19.0 hours (h)[19,20], and follow linear elimination, whereas *E. coli* PEG-asparaginase has time dependent pharmacokinetics and a half-life with an observed range of 2.4 to 11.8 days.[9,18,21,22] *Erwinia* asparaginase has the shortest half-life with means of 12.6-22.1 h after intramuscular administration[7,9,23,24], and 6.4-7.6 h after intravenous administration.[23,25]

The aim of the present study was to describe the population PK of intravenously administered *Erwinia* asparaginase in pediatric ALL patients, to quantify the random parameters interindividual (IIV), inter-occasion (IOV) and residual variability, and to determine the association between

patient characteristics and the PK parameters. Quantification of random parameters is important for proper therapeutic drug monitoring, as IIV can be compensated by TDM, whereas IOV cannot. Additionally the current starting dose was evaluated taking the requirement of having a trough concentration above 100 IU/L into account.

## Methods

*Patients and treatment*

The study was designed as a prospective multicenter study in seven pediatric oncology centers in the Netherlands. Children aged 1-18 years with acute lymphoblastic leukemia (ALL) were eligible for the study when treated according to the DCOG ALL-10 (1-Nov-2004 – 1-Apr-2012) or ALL-11 (1-Apr-2012 – ongoing) protocol with *Erwinia* asparaginase (Erwinase®; EUSA Pharma [Europe]) after the development of an allergy to *E. coli* derived asparaginase or silent inactivation of *E. coli* derived asparaginase. The starting dose of *Erwinia* asparaginase was 20,000 IU/m$^2$ intravenously (iv) over one hour thrice weekly (Mon/Wed/Fri). The dose was fixed for the first two weeks. Subsequently, if the 72 h concentration was >100 IU/L, the dose interval was adjusted to twice weekly. Additionally in ALL-11, the dose was increased based on clinical expertise if the 72 h concentration was <100 IU/L. When insufficient, the dose interval was set to every other day. The TDM samples were collected between 1-May-2009 and 5-Feb-2015. These are trough samples prior to their next dose, predominantly 48 or 72 h after last *Erwinia* asparaginase administration. For the purpose of this study additional peak concentrations were collected between 1 and 4 h in a subset of patients. *Erwinia* asparaginase activity concentrations in serum were analyzed as previously described by us, with a lower limit of quantification (LLoQ) of <5 IU/L.[26] The protocols were IRB approved.

*Pharmacokinetic analysis*

Time profiles of *Erwinia* asparaginase concentrations were analyzed using the nonlinear mixed effects modeling approach implemented in NONMEM® (version 7.2; Icon Development Solutions, Ellicott City, Maryland, USA). Additionally Pirana (version 2.7.1, for the model environment Pirana, Pirana Software & Consulting BV, The Netherlands), Xpose4 (version 4.4.1, Nicholas Jonsson and Mats Karlsson, Uppsala, Sweden) and Perl speaks NONMEM (PsN) (ver-

sion 4.2.0, Uppsala, Sweden) were used.

All *Erwinia* asparaginase concentrations were log transformed prior to analysis. First order conditional estimates with interaction (FOCE+I) was used as method of analysis throughout the model building procedure. The data was initially fitted to a one-compartment linear model without an absorption compartment as the drug was administered intravenously. More complex models were evaluated; improvement of the fit of the model was evaluated by the precision of the estimated PK parameters, the change in the objective function values (OFV), goodness-of-fit plots (GOF) and visual predictive checks (VPC). A 3.84 point decrease in OFV for one degree of freedom was considered a significant improvement with a p-value of <0.05.

The data was obtained in a pediatric population, hence PK parameters were allometrically scaled to adequately describe the parameters across a wide range of body weights. For allometric scaling standard fixed exponent values of 0.75 for the flow dependent physiologic process parameters clearance ($CL$) and intercompartmental clearance ($Q$), and 1 for the volume related parameters apparent volume of distribution of the central compartment ($V_c$) and peripheral compartment ($V_p$) were used.[27–29] Inter-patient and inter-occasion variability in clearances and volumes of distribution were characterized with exponential models. An occasion was defined as one month of treatment due to the limited number of samples per occasion. For example, clearance in the $i^{th}$ individual at the $j^{th}$ occasion was estimated using equation:

$$CL_i = CL_{pop} * \left(\frac{WT}{70}\right)^{0.75} * exp^{\eta_i + \kappa_j} \tag{3.1}$$

Where $CL_{pop}$ (= $\Theta_{CL}$) is the typical population value for clearance in a patient with a standardized body weight of 70 kg and $\eta_i$ and $\kappa_j$ represents the random effect accounting for interindividual deviation from the typical population value (IIV) and typical individual values (IOV) respectively. $\eta_i$ and $\kappa_j$ are assumed to be symmetrically distributed with a mean of 0 and estimated variance of $\omega^2$ and $\pi^2$, respectively. An additive error model was used to describe the residual error in plasma concentrations.

After the finalization of the structural model, covariate models were built by a stepwise forward inclusion procedure. The covariate with the greatest reduction in OFV was added to the base model. This was iterated over all the covariates until no statistically significant decrease in OFV occurred. For the internal validation of the model non-parametric bootstrap procedures (n=1000) were performed and VPCs were obtained. The final model including covariates was used to perform Monte Carlo simulations (n=5000) for different doses and patient weight.

$$CL_i = CL_{pop} * \left(\frac{COV}{median\ COV}\right)^{\theta_{COV}} * \left(\frac{WT}{70}\right)^{0.75} * exp^{\eta_i + \kappa_j} \tag{3.2}$$

$$CL_i = CL_{pop} * {\theta_{Cat}}^{FLAG} * \left(\frac{WT}{70}\right)^{0.75} * exp^{\eta_i + \kappa_j} \tag{3.3}$$

Where COV is the continuous covariate, $\theta_{cov}$ is the estimated exponent parameter of the continuous covariate. $\theta_{cat}$ is the estimated fraction parameter of the categorical covariate. FLAG is either 1 (covariate present) or 0 (not present). Other parameters are described in equation 3.1.

Covariates were included one at the time. The covariate with the greatest reduction in OFV was added to the base model. This was iterated over all the covariates until no statistically significant decrease in OFV occurred. The available covariates were: weight, age, height, body surface area (BSA), sex, treatment protocol (ALL-10 and ALL-11) and treatment center. Dose interval was evaluated as covariate for patient on thrice weekly or every other day *Erwinia* asparaginase versus patients who switched to twice weekly *Erwinia* asparaginase.

For the internal validation of the model a non-parametric bootstrap procedures (n=1000) was performed and prediction corrected visual predictive checks (VPC) were obtained. The final model including covariates was used to perform Monte Carlo simulations (n=5000) for doses ranging from 100 to 2000 IU/kg (per 100 IU/kg steps) for patients weighing 10 to 100 kg (per 10 kg steps).

## Results

*Patients and samples*

During the study period 53 pediatric patients were switched from *E. coli* derived asparaginase to *Erwinia* asparaginase due to allergic reactions or silent activation. Data from 51 of these 53 patients were included in the PK analysis. Two patients were excluded due to *Erwinia* asparaginase concentrations below the LLoQ. One of these patients had anti-*Erwinia* asparaginase antibodies neutralizing the drug and prohibiting sufficient exposure, for the other patient the reason of unmeasurable concentrations is unknown. Both patients discontinued treatment with *Erwinia* asparaginase.

**Table 3.1:** Patient characteristics

| Item | |
|---|---|
| **Total patients (n)** | 51 |
| **Age (y)** | |
| Median | 6 |
| Range | 1.9 – 17.7 |
| **Gender (n; %)** | |
| Male | 32 (62.7%) |
| Female | 19 (37.3%) |
| **Weight (kg)** | |
| Median | 24.5 |
| Range | 11.7 – 99.0 |
| **BSA ($m^2$)** | |
| Median | 0.92 |
| Range | 0.53 – 2.22 |

*y= years, kg = kilograms, BSA = body surface area*

A summary of the patient characteristics can be found in table 3.1. A total of 741 samples were available, with a median of 11 samples per patient (2-43 samples). 27 samples (3.6%) were excluded from the analysis, due to missing sampling data (n=20), no measurable asparaginase (n=4) or unrealistic high concentrations (n=3) due to sampling artefacts. The four unmeasurable trough concentrations were all from the same patient, however this patient did have measurable *Erwinia* asparaginase concentrations within 24 hours after administration.

Samples were collected for 2 weeks up to 12 months after the start of *Erwinia* asparaginase treatment. Samples were predominantly trough concentrations taken around 48 (52.2%) and 72 (36.8%) hours after *Erwinia* asparaginase administration. Figure 3.1 shows the combined *Erwinia* asparaginase concentrations versus the time after dose for all patients throughout therapy, demonstrating a large variability. The concentrations can be stratified for the first two weeks of treatment (no dose adjustments), and after two weeks with potential adjustments (TDM) in *Erwinia* asparaginase dose frequency (both ALL-10 and ALL-11 protocol) and dose (ALL-11 protocol only). The median asparaginase trough concentrations two days after administration (42 – 50 hours), for re-

spectively the first 2 weeks and during TDM, were 166.2 (IQR: 103.4 – 270.1) IU/L and 191.0 (IQR: 115.0 – 296.5) IU/L; 75.4% and 82.6% of the patients had asparaginase trough concentrations ≥100 IU/L; 90.16% and 91.30% had trough concentrations ≥50 IU/L. Three days after administration (65 – 80 h) the median trough concentrations, for respectively the first 2 weeks and during TDM, were 48.4 (IQR: 29.9 – 104.7) IU/L and 83.7 (IQR: 49.5 – 98.7) IU/L; 26.4% and 39.5% of the patients had trough concentrations ≥100 IU/L; 50% and 74.3% had trough concentrations ≥50 IU/L.

**Table 3.2:** Samples

| Samples | n (%) |
|---|---|
| **Total samples** | 741 |
| Evaluable samples | 714 (96.4%) |
| Not evaluable | 27 (3.6%) |
| Pre TDM | 225 (31.5%) |
| During TDM | 489 (68.5%) |
| **Samples per patient** | |
| Median | 11 |
| Range | 2 - 43 |
| **Sample time*** | |
| Within 5 h | 17 (2.4%) |
| Between 5 – 40 h | 9 (1.3%) |
| After 48 h | 373 (52.2%) |
| After 72 h | 263 (36.8%) |
| Between 80 – 120 h | 52 (7.3%) |

**Sample time is the time after last Erwinia asparaginase administration in hours*

The summary of the number of samples and time points can be found in table 3.2. A total of 311 samples (43.6%) were collected in the first month of *Erwinia* asparaginase treatment. The number of samples during the following months ranged from 86 (in month 2) to 1 (in month 12). Especially trough concentrations taken at 72 h frequently dropped below the desired therapeutic target threshold of 100 IU/L. Eleven patients (21.6%) were switched from thrice to twice weekly interval after asparaginase 72 h trough concentrations of >100 IU/L during their treatment. A total of 117 samples (15.5%) were drawn during a twice weekly interval.

*Pharmacokinetic model*

Both one and two-compartment models were evaluated. The estimated PK parameters were normalized to a weight of 70 kg using the ¾ allometric model. The two-compartment model provided a better fit to the data than a one-compartment model based on the OFV and the goodness-of-fit plots. The OFV significantly decreased 127.2 points from 366.5 to 239.3 ($p<0.001$). A slight decrease in additive error was observed from 0.70 IU/L (one-compartment model) to 0.64 IU/L (two-compartment model). Addition of a third compartment did not improve the model. Michaelis-Menten elimination model did not improve the model either.

The estimated IIV on $CL$ was 36%, whereas this parameter could not be estimated for $V_c$, $Q$ and $V_p$. Complete removal of the *a priori* incorporated allometric scaling from the model, based on body weight (standardized for 70 kg) and fixed exponents, resulted in a worse model with a 8.3 points increase in OFV and an increase of the IIV for $CL$ from 33% to 40%. Samples were collected throughout therapy on different occasions. Addition of IOV resulted in an improvement of the population model with an estimated value of 14%. By incorporation of the IOV, the OFV decreased with 44.9 points, additive error decreased from 0.64 to 0.57 and the IIV for $CL$ decreased from 36% to 33%. The shrinkage was 6% for IIV on $CL$, 32% for IOV on $CL$ and 9% for residual variability. This was considered the structural model and was used for the stepwise forward inclusion of covariates.

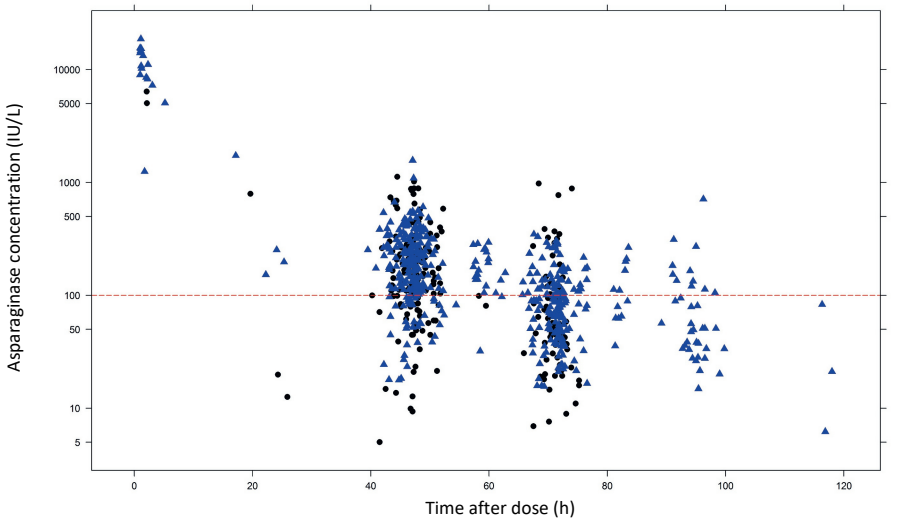


**Figure 3.1:** Asparaginase concentration (IU/L) versus time after dose in hours (h) for all patients and all occasions on a semi-log plot. This shows the large inter-patient variability in plasma concentrations as collected throughout treatment. Triangles (black) show concentrations prior to possible dose adjustments (first 2 weeks) and circles (blue) show observed concentrations with possible dose adjustments [therapeutic drug monitoring (TDM) after week two].

All covariates were tested one at the time for improvement of the structural model. The clearance in the first month was 14% higher in comparison to the subsequent months with a decrease of 17.0 points in OFV ($p<0.001$). Dose as a covariate on $CL$ also improved the model ($p<0.05$). However, during TDM, the dose is adjusted according to the patients' asparaginase concentrations and therefore indirectly for their clearance, which explains the correlation between dose and clearance. Similarly, an association between dose interval and clearance was detected. With dose interval as covariate, the OFV significantly decreased 6.11 points ($p<0.05$) and patients with twice weekly dosing (n=11) were associated with a 24% lower $CL$ in comparison with patients on thrice weekly dosing (n=40). Due to TDM, dose and dose interval were not incorporated in the model. The other covariates age, weight, height, BSA, sex, treatment protocol (ALL-10 or ALL-11) and treatment center did not result in a significant improvement of the base model.

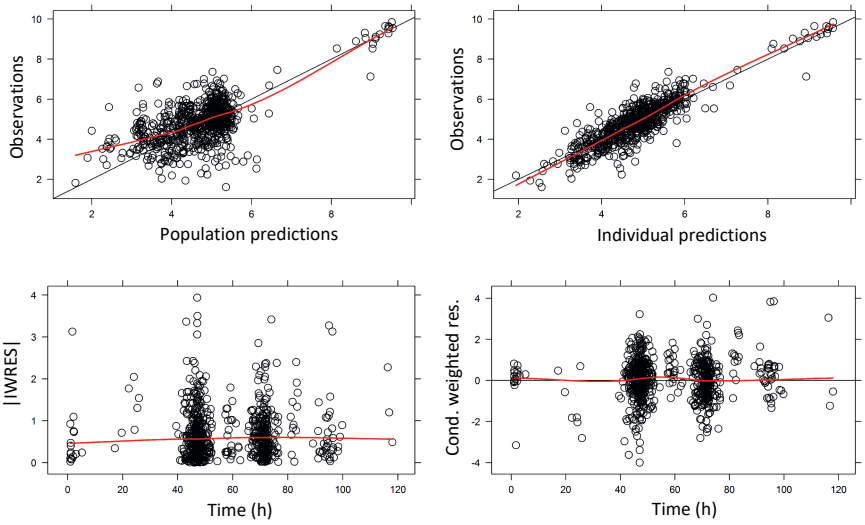


**Figure 3.2:** Goodness-of-fit plots final model. (Upper left) Predicted population concentrations versus observed concentrations of the final model. (Upper right) Predicted individual concentrations versus observed concentrations of the final model. (Lower left) Individual weighted residuals versus individual predictions, (lower right) conditional weighted residuals versus time after dose. h: time in hours; IWRES: individual weighted residual predictions.

The final model was a two-compartment model with fixed exponents allometric scaling, a correction factor for increased clearance in the first month, and interindividual and inter-occasion variability on clearance. The parameter estimates of the final model were: $CL$ 0.44 L/h/70kg, $V_c$ (central compartment) 3.2 L/70kg, $Q$ (intercompartmental clearance) 0.15 L/h/ 70kg and $V_p$ (peripheral compartment) 2.9 L/70kg. The calculated half-lives ($t_{1/2}$) for the two-compartment model are $t_{1/2,\alpha}$ 3.5 h and $t_{1/2,\beta}$ 19.6 h, which represent respectively the distribution phase and elimination phase. The interindividual variability of clearance was 33%. There was an inter-occasion variability of 13% based on monthly intervals (table 3.3). The basic goodness-of-fit plots (fig 3.2) show good model performance. Individual predictions versus observation are well distributed around the unity line. The weighted residuals are within a good range (2, -2) and evenly distributed. The population predictions show a deviation from the unity line for the lower concentrations where limited samples were available.

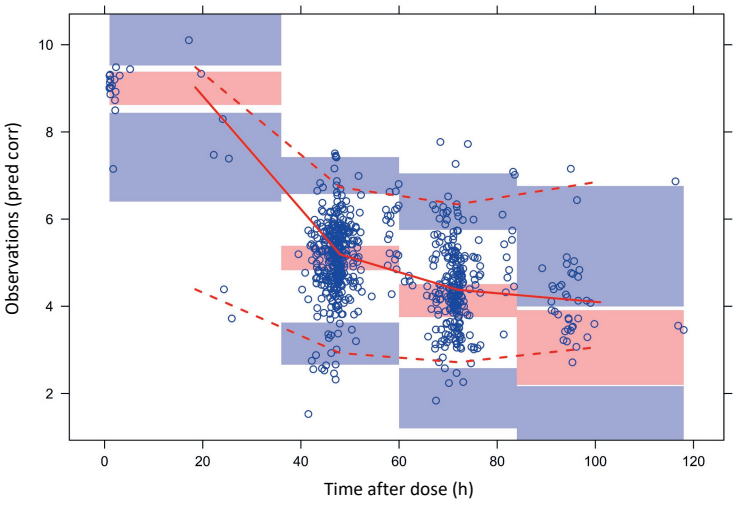


**Figure 3.3:** Visual predictive check. Prediction corrected (Pred Corr) visual prediction plot of observed log asparaginase concentrations versus time after last *Erwinia* asparaginase dose in hours (h) of the final model. Red solid line shows the median observed concentrations and the surrounding opaque red area the simulation based 95% interval for the median. Red dashed lines indicate the observed 5% and 95% percentiles; surrounding opaque blue areas show the simulated 95% confidence intervals for the corresponding predicted percentiles.

*Model validation*

The nonparametric bootstrap procedure was performed to test the robustness of the model. A total 998 of the 1000 runs were successful. The results are shown in table 3.3. The estimates of the final model are in accordance with the results from the 1000 bootstrap replicates. The plot of the prediction corrected visual predictive check (pcVPC) shows the median and 90% interval of the observed asparaginase concentrations (fig 3). The model adequately predicts the time course of the asparaginase plasma concentration during the first three time frames (0-36h, 36-60h and 60-84h). However an under-prediction of median concentrations and 5th and 95th percentiles was observed in the 84-118 hour timeframe.

*Evaluation of the Starting dose*

To investigate the appropriateness of the starting dose, Monte Carlo simulations with varying weights and doses, were performed utilizing the developed population PK model. Based on the simulations, patients with a lower body weight appeared to require higher weight normalized

starting doses to achieve sufficient *Erwinia* asparaginase concentrations after 48 hours. Patients weighing 100 kg require 500 IU/kg compared to doses exceeding 1000 IU/kg for patients with a body weight below 20 kg in order to have *Erwinia* asparaginase concentrations of ≥100 IU/L in 75% of the patients at 48 hours after administration (figure 3.4 and figure 3.5). With the current starting dose of 20,000 IU/m$^2$, circa 75% of the patients with a body weight >50 kg would have concentrations ≥100 IU/L after 48 hours. For patients between 30-50 kg the suggested starting dose would be 25,000 IU/m$^2$ and for patients 10-30 kg doses of 25,000 – 37,000 IU/m$^2$ to achieve *Erwinia* asparaginase concentrations ≥100 IU/L after 48 hours in at least 75% of the patients after the first dose. To achieve this in 90% of the patients, starting dose for all patients would above 33,000 IU/m$^2$. The dose in IU/kg was converted to IU/m$^2$ utilizing corresponding BSA to weight for pediatric oncology patients.[30] Figures 3.5 and 3.6 show required starting dose (in IU/kg and IU/m$^2$) versus weight to achieve 48 h trough concentration of ≥100 IU/L (fig 3.5) and ≥50 IU/L (fig 3.6) in a percentage of patients ranging from 10% to 90%. The simulations with the final PK model are in accordance with the observations in the patient data. Starting doses of 36 patients were evaluable (*Erwinia* asparaginase dose of 20,000 IU/m$^2$ and an available 48 h sample after administration of the first dose). With respect to weight, 12 of 31 patients (39%) with weight <50 kg and 1 of 5 (20%) of 5 patients weighing ≥50 kg had 48 h trough concentrations below 100 IU/L. Although these numbers are small, it shows the same trend in weight-concentration relationship as the Monte Carlo simulations.

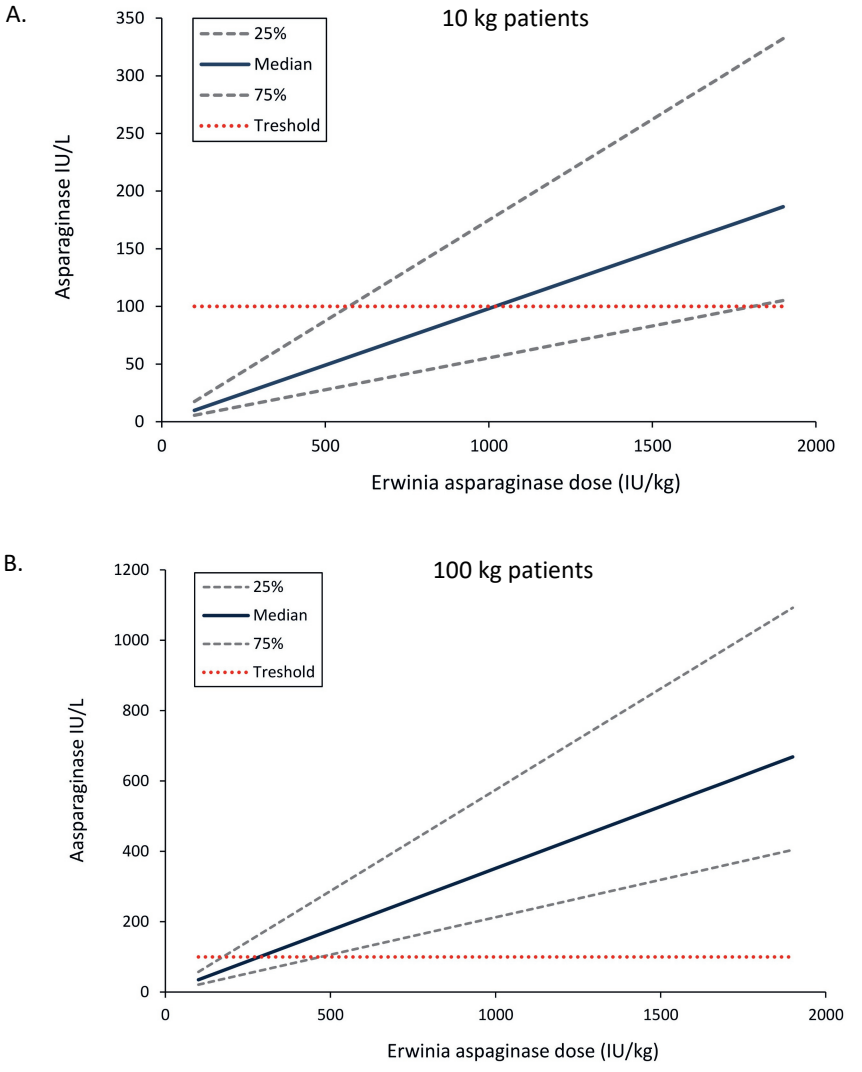


**Figure 3.4:** Simulated *Erwinia* asparaginase concentrations for a 10 kg and a 100 kg patient. Median and the 25% and 75% percentiles of the patients who achieve asparaginase concentrations (y-axis) after 48 hours for different *Erwinia* asparaginase doses (x-axis). (A) Concentrations for a 10 kg patient and (B) a 100 kg patient. Red dashed line is the target trough concentration of 100 IU/L.

**Table 3.3:** Population parameter estimates and nonparametric bootstrap

| | | NONMEM | | | | Bootstrap | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Estimate | RSE (%) | 95% CI (lower) | 95% CI (upper) | Shrink. (%) | Median | 95% CI (lower) | 95% CI (upper) |
| CL (L/h/70kg) | 0.44 | 11 | 0.35 | 0.53 | - | 0.42 | 0.32 | 0.54 |
| $V_c$ (L/70kg) | 3.2 | 22 | 1.8 | 4.6 | - | 3.3 | 2.2 | 6.9 |
| Q (L/h/70kg) | 0.15 | 44 | 0.02 | 0.28 | - | 0.13 | 0.01 | 0.33 |
| $V_p$ (L/70kg) | 2.9 | 35 | 0.9 | 5.0 | - | 2.7 | 0.7 | 5.3 |
| $CL_{\text{month 1 diff}}$ | 1.1 | 3 | 1.06 | 1.22 | - | 1.12 | 1.05 | 1.20 |
| IIV CL (%) | 33 | 20 | 20 | 45.2 | 5.5 | 30 | 19 | 42 |
| IOV (%) | 13 | 18 | 8 | 17.2 | 31.9 | 11 | 6 | 17 |
| Residual error | 0.57 | 6.4 | 0.50 | 0.65 | 8.5 | 0.57 | 0.50 | 0.63 |

$CL$ population estimate for clearance, $V_c$ population estimate of apparent volume of distribution in central compartment, $Q$ population estimate for intercompartmental clearance, $V_p$ population estimate of apparent volume of distribution in peripheral compartment, $CL_{month1diff}$ population estimate difference in clearance of first month. IIV is the interindividual variability and IOV the inter-occasion variability.

## Discussion

The PK of *Erwinia* asparaginase was best described with a two-compartment model with linear elimination and therefore more similar to native asparaginase than PEGylated asparaginase which has time dependent elimination, probably due to the polyethylene glycol.[21] There appears to be a negative correlation between weight and weight-normalized clearance, where the patients with a lower weight require higher weight-normalized doses based on Monte Carlo simulations with the final PK model. The same trend was observed in the actual patient data, however the number of patients above 50 kg was small. Also the clearance in the first month was significantly higher.

Asparaginase is an important component in the treatment of pediatric ALL, where it contributes 10-20% to the total treatment outcome.[6,11,12,19,31,32] However treatment with asparaginase at suboptimal dose schedules leads to an inferior outcome.[11–13,32] Asparaginase is available in different molecular forms (e.g. PEGylated or native). The PK properties of these different forms are however not similar, and can therefore not be used interchangeably.[9,18,33] *Erwinia* asparaginase has a shorter half-life in comparison with the other asparaginase forms, which results in lower concentrations and exposure when administered at equal dose schedules and, consequently, worse outcome.[11–13]

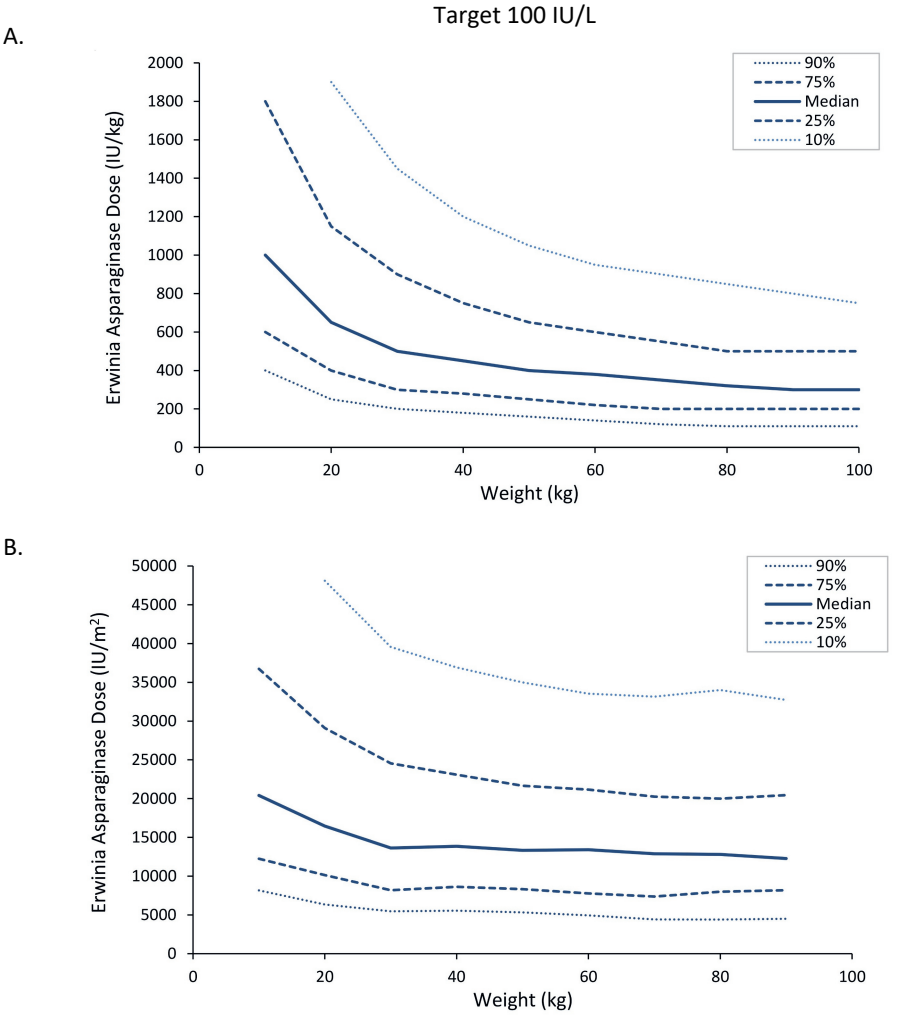


**Figure 3.5:** *Erwinia* asparaginase starting dose versus patient weight to achieve 100 IU/L or more after 48 hours (h). (A) Required starting dose in IU/kg and (B) IU/m$^2$ versus weight of patients in kilograms (kg) to achieve 100 IU/L or more after 48 h. Median (solid line), 25% and 75% percentiles (dashed line) and 10% and 90% percentiles (dotted line) of the patients with asparaginase concentrations of 100 IU/L or more with different weight (x-axis) and different starting doses (y-axis).

In North-America intramuscular injection of *Erwinia* asparaginase was the only FDA approved method of administration, but this has recently been extended with intravenous administration.[34] In Europe the intravenous administration has been the predominant method of *Erwinia* asparaginase administration. Several PK studies of intramuscular administration have been published.[8,9,23,24] This is important in studying the PK because, in addition to the asparaginase molecule or pharmaceutical form, the route of administration may also influence the PK. The PK of intramuscular administered *Erwinia* asparaginase differs from intravenous administration due to the presence of a rate limiting step in the absorption phase.[23,24,34] Bypassing the absorption from the muscle will result in faster elimination and probably more predictable concentrations, as variability in absorption rate is eliminated. The calculated terminal half-life was 19.6 h, which is similar to the terminal half-life of the iv administered native *E. coli* asparaginase of 19.0 h.[22] Previously published studies with iv *Erwinia* asparaginase showed a half-life of 6.4 and 7.5 h.[23,25] However, our study uses a two-compartment model which has a fast elimination half-life of 3.5 h during the distribution phase and a slower elimination half-life of 19.6 h for the terminal elimination phase. This presents itself in a concentration time curve with a steep decline in the first phase followed by a slower decline in the second (which can also be observed in fig 3.1 and 3.3).

During TDM, a large variability in *Erwinia* asparaginase concentrations was also observed after iv administered *Erwinia* asparaginase (fig 3.1). Hence, the population PK of *Erwinia* asparaginase was studied to evaluate the elimination of *Erwinia* asparaginase from the body in a quantitative manner and to explain and quantify the variability in order to improve individual dosing to achieve sufficient asparaginase concentrations prior to their next dose. PK based TDM dosing can compensate for interindividual variability (IIV), but not for inter-occasion variability (IOV). In this study the IIV was 33% and the IOV was 13%, which is favorable for PK based dosing.

The development of the PK model was successful despite the limited numbers of peak concentrations. The parameters were estimated with good precision, the shrinkage of IIV and the residual error was small. The bootstrap estimates were also in accordance with the model estimates. The VPC showed the model predictions to be in line with the observations, except for the last time frame (84-118h) which showed a slight under-prediction, as can be seen in figure 3.3. However samples in this timeframe were patients who were switched to twice weekly *Erwinia* asparaginase administration due to high asparaginase concentrations, hence presumably character-

ized by a lower clearance. Calculations based on the total population would therefore over-predict the clearance in this group resulting in the under-prediction of the *Erwinia* asparaginase concentrations. When the predictions are corrected for the dose interval, the under-prediction disappears and are in accordance with the observations (fig. S1).

Monte Carlo simulations of patients with different body weights and *Erwinia* asparaginase starting doses were performed aiming at trough concentrations of ≥100 IU/L. Based on the simulations, the current starting dose of 20,000 IU/m$^2$ seems rather low, especially for children <50 kg. If this starting dose is used, close monitoring of the patient is required to ensure sufficient *Erwinia* asparaginase concentrations. The PK model uses the standard allometric scaling based on weight, which is the golden standard for allometric scaling in population PK. Unlike weight based scaling it is unclear how scaling based on BSA should be implemented. The implementation of BSA in the PK model might depend on the chosen method of BSA calculation (e.g. Mosteller, Dubois & Dubois, Haycock), as these methods use different internal (exponential) correction factors.[35–37] However to our knowledge this has currently not been studied. Therefore weight based allometric scaling was used for the development of the PK model. Monte Carlo simulations were also expressed on a per weight basis. Additional simulations were performed with scaling based on BSA resulting in similar results. For clinical convenience the dose was converted to IU/m$^2$ using the corresponding BSA to weight in pediatric oncology patients for the representation of figures 3.5b and 3.6b.[30] With the registered dose of 25,000 IU/m$^2$ about 75% of the patients have *Erwinia* asparaginase concentrations above 100 IU/L 48 hours after the first dose and 90-100% of the patients above 50 IU/L.

When increasing the dose to achieve sufficient trough concentrations, one has to keep in mind that the peak concentrations ($C_{max}$) and exposure (AUC) increase as well. This might lead to side effects which include hypersensitivity or infusion reactions, pancreatitis, liver abnormalities, central neurotoxicities, glucose intolerance or coagulation abnormalities,[24,38,39] although we showed no significant correlation between asparaginase activity concentrations and pancreatitis, thrombosis or central neurotoxicities.[39] High concentrations of asparaginase were associated with high triglyceride and high cholesterol concentrations, and was more pronounced in children ≥10 years old. This might be explained by the lower weight normalized clearance in older children.[39] Information concerning specific toxic concentrations were not available, hence maximum concentrations ($C_{max}$) and exposure (area under the curve) were not evaluated. Additionally due to the

increased clearance in the first month, the plasma concentrations will be lower compared to the following months. Hence increasing the dose might not be necessary.

A potential limitation of this study was that samples were collected during the TDM procedure and predominantly withdrawn at 48 and 72 hours after administration. Additional peak samples were collected during the first hours after administration. Patients were at home in the period between the peak (first hours after dose) and trough concentrations (prior to the next dose), therefore *Erwinia* asparaginase concentrations within this timeframe were not available. However with *Erwinia* asparaginase dosing the aim is to achieve sufficient trough concentrations to assure complete asparagine depletion prior to the next dose (which is after 48 or 72 hours), and the dose will be adjusted according to those time points. Two patients were excluded from the analysis due to non-measurable asparaginase levels. One patient had antibodies which could explain the lack of asparaginase, the other patient might have had a very fast asparaginase clearance. Excluding these patient could result in lower variability and estimated clearance.

With the registered *Erwinia* asparaginase dose of 25,000 IU/m$^2$, about 25% of the patient will not have 48 h trough concentrations above 100 IU/L after their first dose. This will be more pronounced in the patients with a low body weight. However the PK analysis showed an increased clearance in the first month, therefore *Erwinia* asparaginase concentrations will increase after the first month. Monitoring the plasma concentrations and adjusting the dose for the individual patients presented with concentrations below target threshold is recommended. Asparagine is completely depleted with *Erwinia* asparaginase concentrations of ≥100 IU/L, although some studies show complete depletion at lower concentrations.[7,9,13,15,16,40] Increasing the dose for the group as a whole might lead to unnecessary high concentrations in the majority of the patients with concentrations already above 100 IU/L, hence resulting in possible (long term) side-effects and unnecessary costs. Therefore individual dose adjustments are recommended.

The optimal treatment would be dose adjustments based on the patients' individual PK parameters. With the PK model developed in this study, the individualized dose requirements can be calculated via *post-hoc* Bayesian analysis. This might reduce possible under-exposure potentially resulting in relapse, as well as reduce high concentrations. A prospective randomized controlled trial could compare conventional dosing versus individualized PK based *Erwinia* asparaginase dosing to evaluate whether individual *Erwinia* asparaginase concentrations would improve and whether

this affects treatment outcome. However, due to the limited number of *Erwinia* asparaginase treated patients in the Netherlands this is not possible and should be performed on an international level. Dosing should be done rationally, to ensure sufficient trough concentrations.

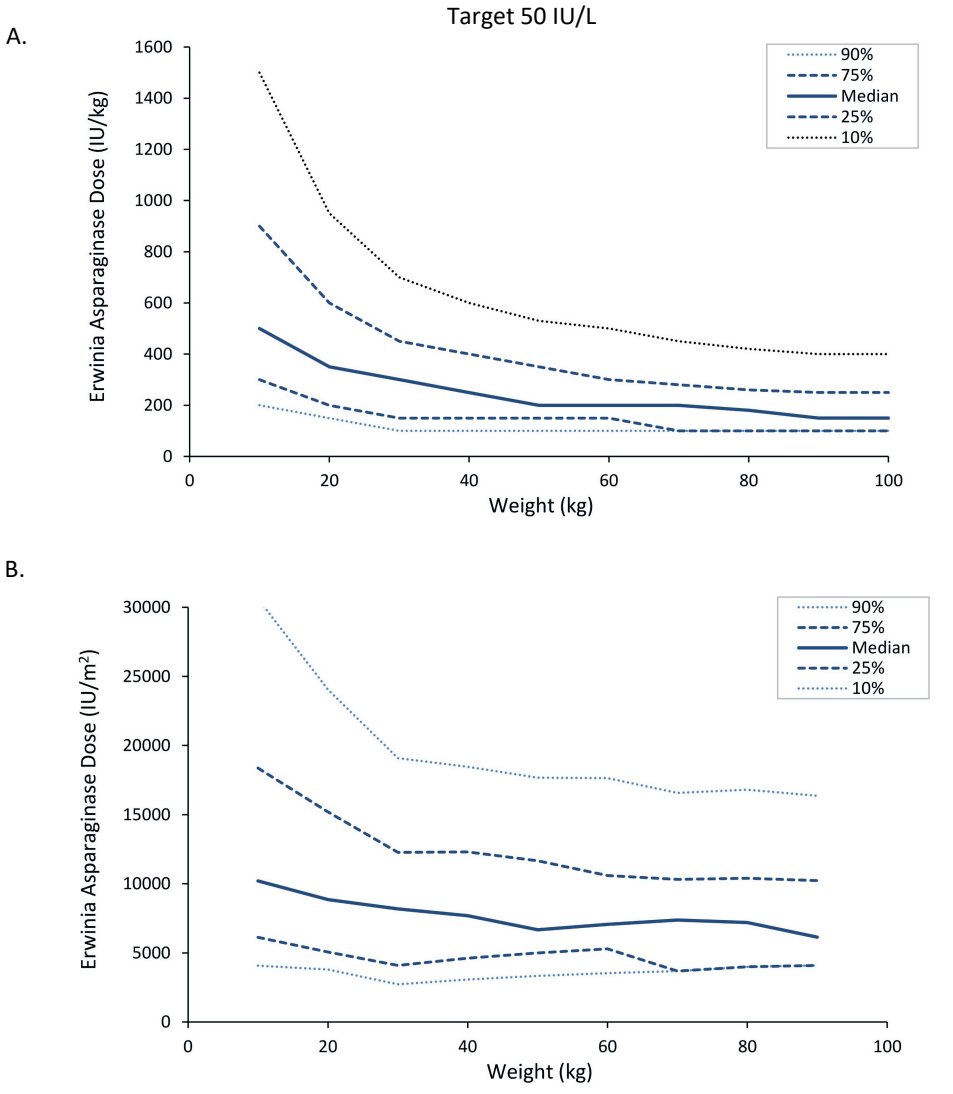


**Figure 3.6:** *Erwinia* asparaginase starting dose versus patient body surface area to achieve 50 IU/L or more after 48 hours (h). (A) Required starting dose in IU/kg and (B) IU/m$^2$ versus weight of patients in kilograms (kg) to achieve 50 IU/L or more after 48 h. The median (solid line), 25% and 75% percentiles (dashed line) and 10% and 90% percentiles (dotted line) of the patients with asparaginase concentrations of 50 IU/L or more with different weight (x-axis) and different starting doses (y-axis).

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# SUPPLEMENT

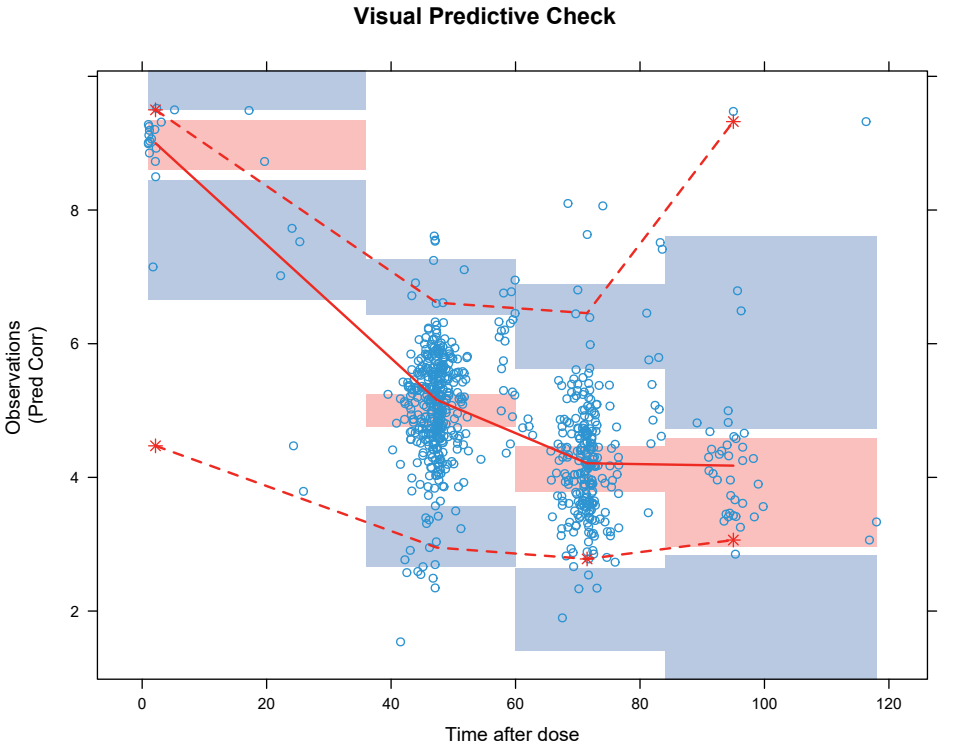


**Figure S1:** Prediction corrected Visual prediction plot of observed log asparaginase levels versus time after dose (hours) of the final model with covariate dose interval. The red solid line indicates the median observed levels and the surrounding opaque red area the simulation based 95% interval for the median. The red dashed lines indicates the observed 5% and 95% percentiles and the surrounding opaque blue areas show the simulated 95% confidence intervals for the corresponding predicted percentiles.

```
;; 1. Based on: run065
;; 2. Description: PK model Erwinase
;; x1. Author: SDTSassen

$PROBLEM PK model Erwinia Asparaginase pediatric ALL patients

$INPUT
      DROP
      AORTA=DROP
      ID
      AMT
      CNTR
      PROT
      DV1=DROP
      DV2=DROP
      DV                ; log transformed (ln)
      EVID
      MDV
      FLAG=DROP         ; per week
      FLAG              ; per month
      GNDR=DROP
      BIRTH=DROP
      AGE
      WT
      HT
      BSA
      DATE=DROP
      TIME1=DROP
      TIME
      TAD1=DROP
      TAD
      TAFD=DROP         ; Time after first dose
      DSE               ; Dose amt

$DATA ERW_NONMEM_COMP_020715V2.csv IGNORE=C

$SUBROUTINES ADVAN3 TRANS4

$PK
      FLAG0 = 0
      FLAG1 = 0
      FLAG2 = 0
```

```
FLAG3 = 0
FLAG4 = 0
FLAG5 = 0
FLAG6 = 0
FLAG7 = 0
FLAG8 = 0
FLAG9 = 0
FLAG10 = 0
FLAG11 = 0
FLAG12 = 0

IF(FLAG.EQ.0) FLAG0 = 1
IF(FLAG.EQ.1) FLAG1 = 1
IF(FLAG.EQ.2) FLAG2 = 1
IF(FLAG.EQ.3) FLAG3 = 1
IF(FLAG.EQ.4) FLAG4 = 1
IF(FLAG.EQ.5) FLAG5 = 1
IF(FLAG.EQ.6) FLAG6 = 1
IF(FLAG.EQ.7) FLAG7 = 1
IF(FLAG.EQ.8) FLAG8 = 1
IF(FLAG.EQ.9) FLAG9 = 1
IF(FLAG.EQ.10) FLAG10 = 1
IF(FLAG.EQ.11) FLAG11 = 1
IF(FLAG.EQ.12) FLAG12 = 1


IOVCLA = FLAG0*ETA(2)+FLAG1*ETA(3)+FLAG2*ETA(4)+FLAG3*ETA(5)
IOVCLB = FLAG4*ETA(6)+FLAG5*ETA(7)+FLAG6*ETA(8)+FLAG7*ETA(9)
IOVCLC = FLAG8*ETA(10)+FLAG9*ETA(11)+FLAG10*ETA(12)+
                                      FLAG11*ETA(13)+FLAG12*ETA(14)

TVCL = THETA(2)  * (WT/70)**(0.75) * (THETA(6)**FLAG0)
CL = TVCL *EXP(ETA(1)+ IOVCLA + IOVCLB + IOVCLC)

TVV1 = THETA(3) * (WT/70)**(1)
V1 = TVV1

TVQ = THETA(4) * (WT/70)**0.75
Q = TVQ

TVV2 = THETA(5) * (WT/70)**1
V2 = TVV2
```

```
    S1 = V1

$THETA
    (0, 0.649)  ;add err
    (0, 0.786)  ; CL
    (0, 5.17)   ; V1
    (0, 0.669)  ; Q
    (0, 8.92)   ; V2
    (0, 0.1)    ; CL M1

$OMEGA 0.127              ; IIV for CL
$OMEGA BLOCK(1) 0.01      ; IOV for CL
$OMEGA BLOCK(1) SAME      ;
$OMEGA BLOCK(1) SAME      ;
$OMEGA BLOCK(1) SAME      ;
$OMEGA BLOCK(1) SAME      ;
$OMEGA BLOCK(1) SAME      ;
$OMEGA BLOCK(1) SAME      ;
$OMEGA BLOCK(1) SAME      ;
$OMEGA BLOCK(1) SAME      ;
$OMEGA BLOCK(1) SAME      ;
$OMEGA BLOCK(1) SAME      ;
$OMEGA BLOCK(1) SAME      ;

$SIGMA
    1 FIX

$ERROR
    IPRED=LOG(0.0001)
    IF(F.GT.0)IPRED=LOG(F)
    IRES = DV-IPRED
    W=1
    IF(F.GT.0)W = SQRT(THETA(1)**2)
    IWRES = IRES/W
    Y = IPRED+W*EPS(1)

$EST METHOD=1 MAXEVAL=99999 SIG=3 PRINT=5 NOABORT POSTHOC
    INTERACTION

$COV PRINT=E UNCONDITIONAL

$TABLE ID TAD IPRED IWRES CWRES NOPRINT ONEHEADER FILE=SDTAB085
```

```
$TABLE ID CL V1 Q V2 ETA1 NOPRINT ONEHEADER FILE=PATAB085
$TABLE ID WT AGE FLAG NOPRINT ONEHEADER FILE=COTAB085
$TABLE ID NOPRINT ONEHEADER FILE=CATAB085
```

# Chapter 4

## Population pharmacokinetics and -dynamics of ciprofloxacin prophylaxis in pediatric ALL patients

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**Clin Infect Dis. 2020 Oct;71(8):e281-e288.**

## Abstract

**Background:** Ciprofloxacin is used as antimicrobial prophylaxis in pediatric acute lymphoblastic leukemia (ALL) to decrease infections with gram-negative bacteria. However, there are no clear guidelines concerning prophylactic dose.

**Aims:** To determine the pharmacokinetics and -dynamics of ciprofloxacin prophylaxis in a pediatric ALL population. The effect of patient characteristics and anti-leukemic treatment on ciprofloxacin exposure, the area under the concentration time curve over minimal inhibitory concentration ($AUC_{24}$/MIC) ratios, and emergence of resistance were studied.

**Methods:** A total of 615 samples from 129 children (0 – 18 years) with ALL were collected in a multicenter prospective study. A population pharmacokinetic model was developed. Microbiological cultures were collected prior to and during prophylaxis. An $AUC_{24}$/MIC of ≥125 was defined as target ratio.

**Results:** A one-compartment model with zero-order absorption and allometric scaling best described the data. No significant ($P<0.01$) covariates remained after backwards elimination and no effect of asparaginase or azoles were found. Ciprofloxacin $AUC_{24}$ was 16.9 mg*h/L in the prednisone prophase versus 29.3 mg*h/L with concomitant chemotherapy. Overall 100%, 81% and 18% of patients at respectively MIC of 0.063, 0.125 and 0.25 mg/L achieved $AUC_{24}$/MIC ≥125. In 13% of the patients, resistant bacteria were found during prophylactic treatment.

**Conclusion:** Ciprofloxacin exposure shows an almost two-fold change throughout the treatment of pediatric ALL. Depending on the appropriateness of 125 as target ratio, therapeutic drug monitoring or dose adjustments might be indicated for less susceptible bacteria starting from ≥ 0.125 mg/L to prevent the emergence of resistance and reach required targets for efficacy.

## Introduction

During the treatment of hematological malignancies, patients may receive antimicrobial prophylaxis to suppress gram-negative bacterial colonization and prevent infection in this immunosuppressed population.[1] Studies have shown the effectiveness of antimicrobial prophylaxis in pediatric acute leukemia and superiority of quinolones over other antibiotics.[2–4] However, there is no guideline concerning the prophylactic dose of antibiotics. Hence, in most situations therapeutic dose-levels are used.[5,6] In contrast to beta-lactam antibiotics, quinolones have a fast and concentration-dependent killing with a more sustained post-antibiotic effect against most gram-negative pathogens.[7,8] Therefore, the area under the concentration time curve over the minimal inhibitory concentration ($AUC_{24}$/MIC) is used as PK/PD target for quinolones.[6,7,9] Studies showed higher probabilities of clinical and microbiologic cure rates with $AUC_{24}$/MIC >125.[6,7] However, it also showed that $AUC_{24}$/MIC of >125 might not be achieved in all patients, especially with less susceptible bacteria.[7,9–11] Emergence of resistance is another area of concern, especially in our patients receiving antibiotics for an extended period. *De novo* resistance develops in a gradual, stepwise manner, usually from the accumulation of mutations.

In this study the pharmacokinetics (PK) and -dynamics (PD) of ciprofloxacin were evaluated in a large pediatric ALL population to determine the effects of patient characteristics and treatment on the ciprofloxacin plasma concentrations. The presence of gram-negative bacteria and susceptibility to ciprofloxacin was evaluated. Subsequently, Monte Carlo simulations were performed to evaluate dosing regimens and MIC values in relation to $AUC_{24}$/MIC ratios.

## Materials and methods

*Patients and treatment*

The study was designed as a prospective multicenter Dutch Childhood Oncology Group (DCOG) study performed in the seven pediatric oncology centers in the Netherlands. Children aged 0-18 years with ALL were eligible for the study when treated according to the DCOG ALL-11 protocol (April 1st 2012 – ongoing), or the Interfant-06 protocol (February 2006 – August 2016), receiving ciprofloxacin as antimicrobial prophylaxis in a dose of 15 mg/kg twice daily (maximum 1000 mg/day). Patients with Down syndrome were excluded from the study due to possible altered pharmacokinetics.[12–14] One infant was treated according to the Interfant-06 protocol with

samples in week one containing prednisolone. The DCOG ALL-11 and DCOG Interfant-06 protocols were institutional review board (IRB) approved (EudraCT: 2012-00006725 (ALL-11) & 2005-004599-19 (Interfant-06); Dutch Trial Registry nr. 3379).

*Sample collection and analysis*

For the PK analysis, ciprofloxacin steady state samples were collected between February 2012 and August 2016. Samples were collected >24h after first administration and following a single dose (trough, t=1, t=2 and t=4 hours). Samples were collected during three treatment phases; week 1 [Block A], 52 days after start treatment [Block B] and additional trough samples between block A and B and during risk-group (MRG) intensification phase [Block C]. In block A patients received prednisolone and during block B and C concomitant chemotherapy (fig. 4.1). Samples were analyzed with LC-MS/MS at the department of Hospital Pharmacy in the Academic University Medical Centers in Amsterdam, The Netherlands. LC: Shimadzu LC-30 Nexera [Nishinokyo-Kuwabaracho, Japan]; MS: AB Sciex 5500 QTrap® [Framingham, MA, USA]; HPLC column: Thermo ScientificTM Hypersil GoldTM 50 x 2.1 mm, 1.9 $\mu$m [Waltham, MA, USA]. Blood samples were collected in K2 EDTA tubes and centrifuged at room temperature within two hours after withdrawal. Supernatant (serum) was collected and stored at -80° Celsius prior to analysis.

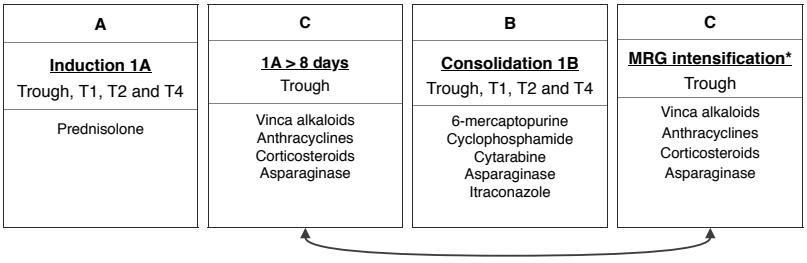


**Figure 4.1:** Treatment phases and sampling schedule. Overview of the treatment blocks and samples. t=1, t=2, and t=4 are, respectively, 1, 2, and 4 hours after last administration of ciprofloxacin. Comedication according to protocol are stated per block. *Only medium risk patients [MRG].

*Microbiology*

Routine surveillance cultures were taken according to DCOG supportive care guidelines prior to start prophylaxis and during treatment. Rectal and throat swabs were collected during periods of intense chemotherapy either weekly (when hospitalized) or every 2-3 weeks (outpatient clinic),

including additional patients treated according to the ALL-11 protocol (outside of PK-study). Ciprofloxacin susceptibility was tested with VITEK®-2 system [BioMérieux, Marcy-l'Étoile, France] at the department of Microbiology in the Erasmus MC, Rotterdam, The Netherlands. Results were presented as MIC ≤ 0.25, 0.5, 1 and > 2 mg/L (MIC >0.5 mg/L is considered resistant).[15] The incidence of febrile neutropenia during treatment was evaluated using reported episodes of febrile neutropenia to the DCOG. Febrile neutropenia was defined as neutrophil count $<1.0*10^9$ $L^{-1}$ with a single temperature of >38.3° Celsius or ≥38.0° Celsius an hour apart.

*Pharmacokinetic analysis*

The total concentration time profiles of ciprofloxacin were analyzed using the nonlinear effects modeling approach implemented in NONMEM® first-order conditional estimates (FOCE) with interaction (version 7.3, [Globomax LLC, Ellicott City, Maryland, USA]). The data was initially fitted to a one-compartment linear model with first-order absorption followed by more complex models. Improvement of the fit of the model was evaluated quantitatively by the precision of the estimated PK parameters and the change in the objective function values (OFV), and visually by goodness-of-fit plots (GoF) and visual predictive checks (VPC). *A priori* the parameters were normalized to a weight of 70 kilogram (kg) and allometrically scaled, with an exponent of 0.75 for $CL$ and 1 for $V_d$. A 3.84-point decrease in OFV for one degree of freedom was considered a significant improvement with a p-value of <0.05. The evaluation of covariates was done through stepwise regression with iterative forward selection ($p < 0.05$) and backward elimination ($p < 0.01$).[16] Continuous covariates were centered around the median. A proportional error model was used to describe the residual error in plasma concentrations. The robustness of the estimated model parameters was evaluated by a nonparametric bootstrap procedure (n=1000). A visual predictive check was performed for internal validation of the model. Monte Carlo simulations were performed with the final model (n=1000) for patients with body weights of 10kg - 100kg, and ciprofloxacin dose of 15 mg/kg with a maximum of 500 mg during treatment phases block A, B and C. The area under the curve (AUC) was calculated for the different patients and dosages.

*Statistical analysis*

The patient characteristics height, weight, age, albumin, creatinin, ASAT, ALAT, bilirubin and urea for different treatment phases were compared using two-sided Mann-Whitney U test with $\alpha = 0.05$. The Pearson's chi-squared test was used to compare gender, pharmaceutical formulation (tablet, capsule or oral liquid) and administration route (oral or via tube).

## Results

*Patients and samples*

A total of 134 patients were enrolled in the study between October 2012 and August 2016. Five patients were excluded due to missing data. A total of 129 patients were included for the PK analysis. 646 samples were available for analysis. 31 samples (4.8%) were excluded from the analysis, due to missing sampling data (n=2; 0.3%), technical issues (e.g. <250 $\mu$l plasma, n=7; 1.1%), <LLoQ of 0.02 mg/L (n=10; 1.5%), or unrealistic concentrations due to sampling artefacts (n=12; 2.0%). A total of 615 samples were used for the PK analysis. A detailed description of patient characteristics and samples is shown in table 4.1. Observed differences between patients in treatment phases (mean [interquartile range]) were, albumin (38 [34-40] vs 33 [26-37] g/L; p<0.001) and bilirubin (12 [6-14] vs 17 [9-18] $\mu$mol/L; p=0.02) for respectively block A vs B; urea (4.9 [3.8-6.2] vs 6.4 [4.5-7.3] mmol/L; p=0.02) for block A vs C; and ASAT (57 [31-54] vs 55 [24-43] U/L; p=0.03) and urea (4.8 [3.5-5.0] vs 6.4 [4.5-7.3] mmol/L; p<0.001) for block B vs C.

*Pharmacokinetic model*

Initially a one-compartment model with first-order absorption was evaluated. The samples were *a priori* stratified in three treatment periods, (block A, B and C) and associated with $CL$ and $V_d$. Compared to block A, $CL$ and $V_d$ were respectively 44% and 49% lower in block B and 31% and 33% lower in block C (decrease of 157 points in OFV (p <0.0001). The association between treatment blocks and PK parameters greatly influenced the stability of the model and was therefore included in the structural model. Addition of a peripheral compartment model decreased the OFV of 19.2 points (p <0.01). However, the parameters of the second compartment could not be estimated precisely and resulted in a less stable model.

Different absorption models were evaluated; first-order absorption, zero-order absorption, lag time and multi-compartment absorption models (up to twenty transit compartments). The absorption phase was best described with a zero-order absorption model. The final structural model was a one-compartment model with zero-order absorption with allometric scaling and the association between treatment phase and $CL$ and $V_d$. This model was used for the subsequent covariate analysis.

**Table 4.1:** Patient and sample characteristics per block

| | All patients median (range) | Block A median (range) | Block B median (range) | Block C median (range) |
|---|---|---|---|---|
| Patients (n) | 129 | 91* | 76* | 74* |
| Age (y) | 5.6 (0.3-17.7) | 5.6 (1.2 – 17.7) | 5.0 (0.3-17.0) | 6.2 (1.4-17.7) |
| Weight (kg) | 21 (9-86) | 20 (10-79) | 21.5 (9-72) | 25 (10-86) |
| Heigt (cm) | 120 (78-190) | 116 (81-188) | 120 (78-184) | 126 (79-190) |
| Male:Female | 39% vs 61% | 38% vs 62% | 39% vs 61% | 39% vs 61% |
| Creatinin ($\mu$mol/L) | 28 (8-67) | 28 (8-67) | 28 (10-63) | 28 (12-63) |
| GFR (ml/min/1.73m$^2$) | 179 (80-494) | 162 (94-494) | 181 (80-348) | 176 (80-340) |
| ASAT (U/L) | 37 (13-551) | 37 (13-551) | 37 (13-513) | 37 (10-513) |
| ALAT (U/L) | 62 (8-1321) | 62 (8-1321) | 62 (13-1321) | 62 (13-1321) |
| Bilirubin ($\mu$mol/L) | 9 (2-158) | 9 (2-64) | 10 (2-158) | 9 (2-64) |
| Ureum | 4.5 (0.8-33) | 4.5 (0.8-11.6) | 4.5 (0.8-33) | 4.9 (0.8-40) |
| Albumin (g/L) | 37 (13-100) | 37 (21-100) | 36 (13-47) | 37 (21-100) |
| Samples (n) | 615 | 323 | 204 | 88 |
| Samples per patient (n) | 4 (1-13) | 4 (1-13) | 3 (1-4) | 1 (1-3) |
| Dose ciprofloxacin (mg) | 300 (75-500) | 300 (75-500) | 300 (75-500) | 343 (80-500) |
| Azoles (%) | 51% | 3% | 63% | 38% |

*Patients with samples in A, B and C (n=13); A and B (n=18); A and C (n=19); A (n=23); B (n=14); C (n=11)

*Covariate analysis*

The covariates were tested one at the time for improvement of the structural model. The age adjusted GFR decreased the OFV with 5.3 points ($p < 0.05$), bilirubin resulted in a 4.1-point decrease in OFV ($p < 0.05$) and ASAT with 4.8 points ($p < 0.05$). Although IIV on absorption rate (D) could not be adequately assessed, the covariate age could, and decreased the OFV with 5.7 points ($p < 0.05$). Age showed an exponential correlation with absorption rate, with increasing age resulting in extended time in the gut. The other covariates height, body surface area (BSA), sex, ALAT, albumin and treatment center did not significantly improve the base model ($p > 0.05$). $CL$ and exposure of patients (n=14) who developed resistant micro-organisms during prophylaxis (MIC ≥ 0.5 mg/L), did not differ significantly from patients without (2.1-point decrease in OFV; $p > 0.05$). Neither pharmaceutical form nor administration route showed a significant effect. The covariates with a significant improvement were implemented in the PK model. However, none of the covariates were included in the final model after the more stringent backwards elimination ($p < 0.01$).

The concomitant use of azoles was different between the blocks, whereas 3% of the patients received azoles in block A, 63% did in block B, and 38% in block C. Azoles as covariate did not improve the model with a decrease in OFV of 0.12 points. Additionally, concomitant use of asparaginase was evaluated in a subset of patients (n=74) within block B and did not show a significant difference with a decrease of 0.53 points in OFV. The other chemotherapeutic drugs were received by all patients and could therefore not be compared within a single block. The final model was a one-compartment model with zero-order absorption with allometric scaling, an association between treatment phase and $CL$ and $V_d$. The parameter estimates of the final model for block A were: $CL$ 86 L/h/70kg, $V_d$ 695 L/70kg. $CL$ was reduced by 44% and 32% and $V_d$ was reduced by 49% and 34% in respectively block B and C compared to block A. The interindividual variability was 27% for $CL$ and 41% for $V_d$. For a detailed PK estimates refer to table 4.2. The ciprofloxacin protein binding showed a weak linear correlation over the concentration range with a coefficient of 0.16 ($p < 0.001$) (fig. S1). The median percentage of unbound ciprofloxacin was 63%. The $AUC_{24}$ and unbound $AUC_{24}$ ($fAUC_{24}$) are shown in table 4.3. A steep decline was observed in patients achieving $AUC_{24}$/MIC ratios of ≥125 for MIC values of ≥0.25 mg/L, which was especially low in block A and C with respectively 1% and 18% of the patients (table 4.3).

**Table 4.2:** Population parameter estimates and nonparametric bootstrap

| | NONMEM | | | | | Bootstrap | | |
|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Estimate** | **RSE (%)** | **95% CI (lower)** | **95% CI (upper)** | **Shrink. (%)** | **Median** | **95% CI (lower)** | **95% CI (upper)** |
| CL/F (L/h/70kg) | 86 | 5.5 | 76.6 | 95.0 | - | 88 | 79.1 | 97.4 |
| V/F (L/70kg) | 695 | 8.9 | 574 | 816 | - | 692 | 594 | 821 |
| $CL_{block\ B}$ | 0.56 | 6.5 | 0.49 | 0.63 | - | 0.58 | 0.50 | 0.66 |
| $V_{block\ B}$ | 0.51 | 9.1 | 0.42 | 0.60 | - | 0.54 | 0.44 | 0.65 |
| $CL_{block\ C}$ | 0.68 | 10.8 | 0.54 | 0.82 | - | 0.68 | 0.56 | 0.89 |
| $V_{block\ C}$ | 0.66 | 16.7 | 0.44 | 0.87 | - | 0.67 | 0.49 | 1.0 |
| $D_1$ | 0.62 | 25.0 | 0.32 | 0.92 | - | 0.65 | 0.41 | 1.0 |
| IIV CL (%) | 26.6 | 25 | 19 | 34 | 22 | 41 | 29 | 53 |
| IIV V (%) | 39.2 | 17 | 33 | 48 | 24 | 50 | 33 | 67 |
| Prop res error | 0.46 | 4 | 0.42 | 0.49 | 7 | 0.40 | 0.35 | 0.45 |

CI is confidence interval, IIV random effect parameter that represents interindividual variability for clearance (IIV *CL*) and distribution (IIV *V*), prop res error is the random effect parameter proportional residual error, *CL/F* population estimate for clearance including bioavailability, *V/F* population estimate of apparent volume of distribution including bioavailability, $CL_{block}$ population estimate for differences between block A and B or C on clearance, $V_{block}$ population estimate for differences between block A and B or C on distribution, $D_1$ is the population estimate for the absorption rate constant.

*Model validation*

The nonparametric bootstrap procedure was performed to test the robustness of the model. A total 916 of the 1000 runs were successful. The results are shown in table 4.2. The estimates of the final model were in accordance with the results from the 1000 bootstrap replicates. The plot of the prediction corrected visual predictive check (pcVPC) shows the median and 90% interval of the observed ciprofloxacin concentrations (fig. 4.2). The model adequately predicts the time course of the ciprofloxacin plasma concentration (fig. 4.3).

*PK/PD simulations*

Monte Carlo simulations were performed to show the percentage of patients achieving the target $AUC_{24}$/MIC of 125 with the current dose over a range of MIC values (fig. 4.4). For the MIC values of 0.063, 0.125, 0.25 and 0.5 mg/L respectively, 90%, 37%, 1% and 0% (Block A); 100%, 86%, 27% and 0% (Block B); and 99%, 74%, 13% and 0% (Block C) of patients achieved an $AUC_{24}$/MIC ratio of ≥125. The AUC was lower in patients with high body weight compared to low body weight. Note that the maximum dose is 500 mg/dose, hence patients with body weight >33.3kg received a relative lower dose on a weight basis.

**Table 4.3:** micro organisms

| Micro organism | No Pat |
|---|---|
| Escherichia coli | 14 |
| Pseudomonas aeruginosa | 3 |
| Klebsiella pneumoniae | 1 |
| Citrobacter freundii | 1 |
| Enterobacter cloacae complex | 1 |
| Acinetobacter baumannii complex | 1 |
| During prophylaxis | 13.22 % |

*Microbiology*

In sum, 251 rectal and throat surveillance cultures of 121 patients were collected and analyzed (including 67 patients from the PK analysis). MIC values were determined in case of positive bacteremia. Ciprofloxacin resistant gram-negative bacteria (MIC ≥ 0.5 mg/L) were identified in routine colonization rectal cultures in 26 out of 121 (21%) all with MIC > 2 mg/L. In 16 out of 121 (13%) patients, resistant gram-negative cultures emerged during ciprofloxacin prophylaxis, with a median of 34 days (range 5-279 days) after diagnosis, in four (3%) patients were colonized with resistant bacteria prior to prophylaxis. For six (5%) patients with resistant cultures no data was available prior to prophylaxis and remains inconclusive. Resistance occurred most frequently in *Escherichia coli* (67%) and *Pseudomonas Aeruginosa* (14%) (table 4.4). The AUC of patients in block A who developed gram-negative bacteremia during

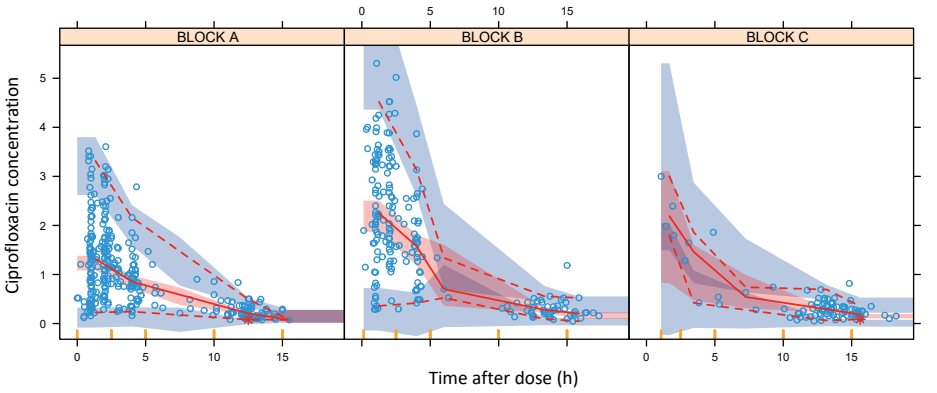


**Figure 4.2:** Visual predictive checks per block. The fit of the predicted ciprofloxacin concentrations versus the observed concentrations of the final model. The predictions are in line with the observed data. The red solid line indicates the median observed concentrations and the surrounding opaque red area the simulation based 95% confidence interval for the median. The red dashed lines indicates the observed 5% and 95% percentiles, and the surrounding opaque blue areas show the simulated 95% confidence intervals for the corresponding predicted percentiles

prophylaxis (n=12) was lower than patients without (n=54; p=0.025). However, no difference in AUC was observed in block B, C or overall (see fig. 4.5).

A total of 165 episodes of febrile neutropenia were reported to the DCOG in 85 of 108 patients during their ALL treatment. 74 (45%) of these episodes (64 patients) occurred in the first weeks of treatment. In 71 cases microbiological documentation was available, with 7 (10%) documented gram-negative blood or surveillance cultures (1 blood, 5 rectal, urinary tract or throat and 1 unknown). 38 of these 71 episodes occurred in the first weeks of treatment including 3 (8%) of the documented gram-negative bacteremia.

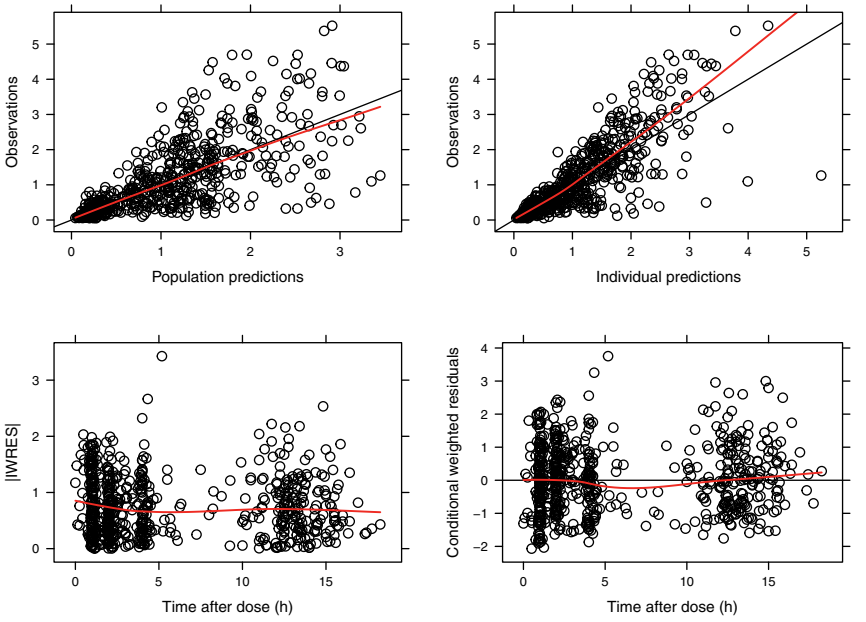


**Figure 4.3:** Goodness-of-fit plot. Goodness-of-fit plots final model. Predicted population concentrations versus observed concentrations of the final model (upper left);predicted individual concentrations versus observed concentrations of the final model (upper right). Individual weighted residuals versus individual predictions (lower left),conditional weighted residuals versus time after dose (h = time in hours) (lower right). Abbreviation: IWRES, individual weighted residuals.

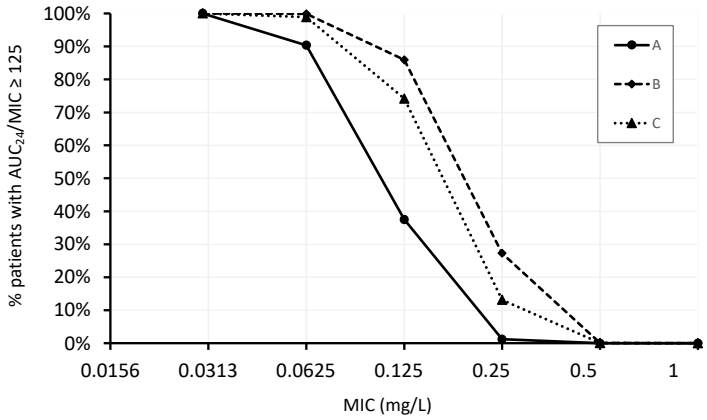


**Figure 4.4:** Patients with $AUC_{24}$/MIC ≥ 125 in block A, B, and C. The effect of treatment phase and patient weight on exposure and $AUC_{24}$/MIC ratio. Simulation (n = 1000) of patients with a weight of 10–100 kg and a dose of 15 mg/kg (max 500 mg) during different treatment phases (block A, B, and C). The x-axis shows different MIC values in mg/L, and the y-axis shows the percentage of patients exceeding the $AUC_{24}$/MIC threshold of 125. A steep decline is shown in patient exceeding the threshold ratio for MIC > 0.125 and >0.25. Abbreviation: $AUC_{24}$/MIC, 24-hour area under the curve/ minimal inhibitory concentration

**Table 4.4:** AUC and $AUC_{24}$/MIC ratios

| **Parameters** | **Overall** | **Block A** | **Block B** | **Block C** |
|---|---|---|---|---|
| Patients (n) | 129 | 91 | 76 | 74 |
| $AUC_{24}$ mg*h/L | 22.3 | 16.9 | 29.3 | 24.8 |
| (range) | (6.8-57.4) | (6.9-32.8) | (11.8-57.4) | (8.1-49.7) |
| $fAUC_{24}$ mg*h/L | 14.0 | 10.6 | 18.4 | 15.6 |
| (range) | (4.3-36.2) | (4.3-20.7) | (7.4-36.2) | (5.1-31.3) |
| $AUC_{24}$/MIC at MIC 0.125 mg/L | 178.4 (54.4-459.2) | 135.2 (55.2-262.4) | 234.4 (94.4-459.2) | 198.4 (64.8-397.6) |
| $AUC_{24}$/MIC at MIC 0.25 mg/L | 89.2 (27.2-229.6) | 67.6 (27.6-131.2) | 117.2 (47.2-229.6) | 99.2 (32.4-198.8) |
| **% of patients $AUC_{24}$/MIC ≥ 125** | **Overall** | **Block A** | **Block B** | **Block C** |
| MIC 0.063 | 100% | 97% | 100% | 99% |
| MIC 0.125 | 81% | 65% | 92% | 87% |
| MIC 0.25 | 18% | 1% | 40% | 18% |
| MIC 0.5 | 0% | 0% | 0% | 0% |

$AUC_{24}$, 24-hour area under the curve; MIC, minimal inhibitory concentration

## Discussion

Overall a total of 81% of the studied patients achieved an $AUC_{24}$/MIC ratio of ≥125 for ciprofloxacin susceptible bacteria with MIC of ≤0.125 mg/L and 100% with MIC of ≤0.063 mg/L However, the majority of patients did not achieve the target ratio for a MIC value of ≥0.25 mg/L (99% and 60% in respectively block A and B), which is still considered susceptible. Low rates above the $AUC_{24}$/MIC target of 125 were also found in other studies for MIC values >0.25 mg/L.[7,9–11,17] Although the susceptible MIC values were classified as ≤0.25 mg/L, the exact MIC values are likely much lower. The EUCAST reference database showed MIC predominantly ≤0.064 mg/L with mean of 0.015 mg/L for wildtype *Escherichia Coli*.[18] At these MIC levels the $AUC_{24}$/MIC target of 125 is reached in all patients. Patient did not reach target $AUC_{24}$/MIC for our MIC cutoff of >2 mg/L.

The observed rate of gram-negative bacteremia throughout the ALL treatment in the subset of patients was 16%, comparable to the study Alexander *et al.* (21%) in pediatric acute leukemia patients with levofloxacin.[19] Although higher cure rates have been shown above an $AUC_{24}$/MIC of ≥125, it is unclear how this translates to prophylactic treatment. In addition to treatment efficacy, the emergence of resistance and specific surface site colonization should be considered.[20] The $AUC_{24}$/MIC should be sufficient to prevent loss in susceptibility and emergence of resistance. In this study a correlation was found between exposure in week one (block A) and patients who developed resistant gram-negative bacteremia during ciprofloxacin prophylaxis. However, the $AUC_{24}$/MIC in these patients cannot be determined as the exact MIC value is not known.

The required $AUC_{24}$/MIC have been shown to differ between strains and fluorquinolones.[21,22] Felsenstein *et al.* observed a significant reduction in infections caused by gram-negative rods but a higher proportion of gram-positive bacterial and fungal infection with ciprofloxacin in pediatric AML patients.[23] Sung *et al.* used levofloxacin with higher gram-positive sensitivity.[2] This might be something to take into account with regard to prophylaxis or treatment concerning gram-positive bacteremia.

This study showed an almost two-fold change in ciprofloxacin clearance and exposure for different treatment phases. In the literature a wide range of $CL$ can be found from 15.9 L/h/70 kg to 102.5 L/h/70kg in a wide variety of pediatric patients (e.g. severe malnutrition, cystic fibrosis),

the $CL$ and AUC in this study falls in the upper range.[10,24–27] Ciprofloxacin is for 40-50% excreted in urine and 20-35% via biliary clearance or transintestinal elimination.[28–30] Most patients will have received hyperhydration and allopurinol/rasburicase (and sometimes diuretics) in week one to prevent tumor lysis syndrome, which could affect the estimation of $CL$. Other factors that might contribute to the difference in $CL$ and AUC include kidney and liver function, transporters (e.g. organic anion transporter (*OAT3*)), bioavailability and drug interactions.[28,31,32] A significant effect of GFR, bilirubin and ASAT ($p<0.05$) was observed, however it was not implemented in the final PK model after more stringent backwards elimination ($p<0.01$). All ciprofloxacin administrations were oral. Therefore, $CL$ is the ratio of clearance and bioavailability and changes in bioavailability (e.g. due to binding of ciprofloxacin to multivalent cations in milk or tube feeding) are reflected in the $CL$.

The PK of ciprofloxacin was best described with a one-compartment model with zero-order absorption. Other studies have established both one- and two-compartment models for ciprofloxacin.[23,26,32–34] A two-compartment model was not supported by the data in our analysis with twice daily ciprofloxacin. Several models were tested to fit the absorption phase; however, all absorption models showed an underestimation of the individual predicted maximum concentration ($C_{max}$). Therefore, the model predicts a slightly lower ciprofloxacin exposure (AUC). This might be due to the limited data available during the absorption phase.

In conclusion ciprofloxacin exposure shows a large difference throughout the treatment of pediatric ALL, with about twice the exposure during concomitant chemotherapy compared to the prednisone prophase. The current prophylactic treatment with ciprofloxacin seems to be adequate with limited emergence of resistance and few bacteremia. If the current $AUC_{24}$/MIC ratio of 125 is correct, the MIC cut-off of 0.25 mg/L might be too high. The target at an MIC of 0.25 mg/L is achieved in only 18% of the patients overall. However, if current prophylactic therapy suffices even with MIC levels of 0.25 mg/L, the target $AUC_{24}$/MIC is higher than necessary. Therapeutic drug monitoring might be recommended with increasing MIC levels in order to achieve sufficient $AUC_{24}$/MIC levels or using the mutant selection window (see Firsov *et al.* 2015 or Olofsson 2006)[20,21] to prevent the emergence of resistance and acquire efficacy targets.

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# SUPPLEMENT

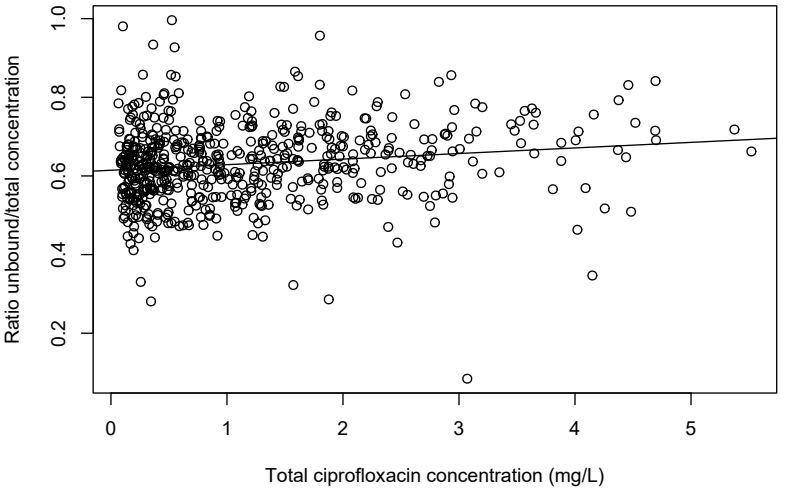


**Figure S1:** Plasma protein binding of ciprofloxacin shows a weak correlation over the concentration range of ciprofloxacin. The x-axis shows total ciprofloxacin concentration and the y-axis the ratio of unbound over total ciprofloxacin concentration.

```
;; 1. Based on: 010e
;; 2. Description: Ciprofloxacine
;; x1. Author: SDTSassen

$PROBLEM PK model

$INPUT
      CENSOR
      AORTA=DROP
      ID
      ID=DROP
      DATE=DROP
      TIME
      AMT
      RATE
      EVID
      MDV
      TAD
      DV                ;linear total conc
      DV=DROP           ;linear unbound conc
      DV=DROP           ;ln (total conc)
      DV=DROP           ;ln (unbound conc)
      BLOK
      HT
      WT
      AGE
      AGEM
      SEX
      VORM
      ROUTE
      ALB
      CREAT
      ALAT
      ASAT
      BILI
      UREUM
      HYP=DROP
      CNTR
      GFR
      AZOL

$DATA CIP_230718_rate.CSV IGNORE=C
```

```
$SUBROUTINES
     ADVAN1 TRANS2


$PK
     FLAG0 = 0
     FLAG1 = 0
     FLAG2 = 0

     IF(BLOK.EQ.1) FLAG0 = 1
     IF(BLOK.EQ.2) FLAG1 = 1
     IF(BLOK.EQ.3) FLAG2 = 1
     IF(BLOK.EQ.4) FLAG1 = 1

     SIZE = (WT/70)

     TVCL = THETA(3)*(SIZE**0.75)*(THETA(5)**FLAG2)*THETA(7)**FLAG1)
     CL = TVCL * EXP(ETA(1))

     TVV = THETA(4)*( SIZE**1)*(THETA(6)**FLAG2)*(THETA(8)**FLAG1)
     V = TVV * EXP(ETA(2))

     S1 = V

     TVD = THETA(9)
     D1 = TVD

$THETA
     (0, 0.4)      ;1 prop
     (0) FIX       ;2 add
     (0, 81.1)     ;3 CL
     (0, 696)      ;4 V1
     (0, 0.589)    ;5 BL-CLb
     (0, 0.533)    ;6 BL-Vb
     (0, 0.589)    ;7 BL-CLc
     (0, 0.533)    ;8 BL-Vc
     (-1, 1)       ;9 D1

$OMEGA BLOCK(2)
      0.09         ; IIV-CL
      0.01 0.09    ; IIV-V
```

```
$SIGMA
      1 FIX   ;residual variability

$ERROR
      DEL=0
      IF(F.EQ.0)DEL=0.0001
      IPRED = F
      IRES = DV-IPRED
      W = IPRED*THETA(1)+THETA(2)
      IF (W.EQ.0) W = 1
      IWRES = IRES/W
      Y= IPRED+W*ERR(1)

$EST METHOD=1 MAXEVAL=99999 SIG=3 PRINT=5 NOABORT POSTHOC
      INTERACTION

$COV PRINT=E UNCONDITIONAL

$TABLE ID TAD IPRED IWRES CWRES NOPRINT ONEHEADER FILE=SDTAB127
$TABLE ID CL V ETA1 ETA2 BLOK AGE WT D1 AMT EVID
      MDV NOPRINT ONEHEADER FILE=PATAB127
$TABLE ID WT BILI BLOK GFR CNTR ROUTE CREAT AGE
      ALAT ASAT AZOL ALB UREUM NOPRINT ONEHEADER FILE=COTAB127
$TABLE ID NOPRINT ONEHEADER FILE=CATAB127
```

# Chapter 5

## Pharmacokinetics of prednisolone in relation to early treatment response in pediatric ALL patients treated according to DCOG protocols

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**Br J Haematol. 2021 Jul;194(2):423-432.**

## Abstract

**Background:** Glucocorticoids form the backbone of pediatric acute lymphoblastic leukemia (ALL) treatment. Many studies have been performed on steroid resistance; however, few studies addressed the relationship between dose, concentration and clinical response.

**Aims:** The aim of this study was to evaluate the pharmacokinetics of prednisolone in the treatment of pediatric ALL and the correlation with clinical parameters.

**Methods:** 1028 bound and unbound prednisolone plasma concentrations were available from 124 children (0 – 18 years) with newly diagnosed ALL enrolled in DCOG studies. A population pharmacokinetic model was developed and post-hoc area under the curve (AUC) was tested against treatment outcome parameters.

**Results:** The PK of unbound prednisolone in plasma was best described with allometric scaling and saturable binding to proteins. Plasma protein binding decreased with age. The AUC of unbound prednisolone was not associated with any of the disease parameters or treatment outcomes.

**Conclusion:** : Unbound prednisolone plasma concentrations correlated with age. No effect of exposure on clinical treatment outcome parameters was observed and does not substantiate individualized dosing. Poor responders, high risk and relapsed patients showed a trend towards lower exposure compared to good responders. However, the group of poor responders was small and requires further research.

## Introduction

The overall long-term survival of children with acute lymphoblastic leukemia (ALL) has vastly improved over the last decades.[1–3] Glucocorticosteroids, like prednisolone and dexamethasone, cause apoptosis in malignant lymphoid cells and have significant anti-leukemic activity, and form the backbone of pediatric ALL treatment.[4] The Berlin-Frankfurt-Münster (BFM) based protocols have shown that the day 8 prednisone response is an important prognostic indicator and can be used in risk group stratification.[5,6]

Many studies have been performed on the pharmacodynamic aspects of steroid resistance and sensitivity, both *in vitro* and *in vivo*.[7–10] Differences in prednisolone sensitivity have been found between phenotypes and genetic subtypes.[6,11,12] Patients become more resistant to prednisolone with age (possibly due to higher frequency of T-ALL in older children), and throughout treatment.[12] Poor response to prednisolone is unfavorable and leads to a worse outcome, although this is treatment dependent as is the case for all prognostic factors.[5,6] The pharmacokinetic studies of glucocorticoids in pediatric ALL treatment is scarce.[13–15]

Dexamethasone is often used in pediatric ALL due to its higher potency and prolonged biological half-life compared to prednisolone. A wide range of equivalent concentrations can be found in literature ranging from 5-fold to 16-fold.[16,17] However, a higher incidence of induction-related treatment deaths has been reported in the 10 mg/m$^2$ dexamethasone versus 60 mg/m$^2$ prednisolone.[18] Prior studies have shown that dexamethasone pharmacokinetics in pediatric ALL patients is highly variable with younger patients exhibited higher clearances compared to older patients. Additionally, a possible effect of asparaginase on dexamethasone PK was observed.[15,19] It is not known whether this also applies to prednisolone, as studies on the *in vivo* pharmacokinetic exposure to prednisolone in ALL are limited.[13,20]

Prednisolone is highly bound to plasma proteins and shows both linear binding to albumin and nonlinear binding to corticosteroid binding globulin (CBG).[13,21–23] The binding to plasma proteins, and therefore the exposure to the active unbound prednisolone, might be affected by the disease and concomitant chemotherapy. However, no studies have been performed linking unbound prednisolone plasma concentrations to the clinical response in ALL. If a correlation is found between unbound prednisolone and clinical outcome parameters, patients might benefit

from individualized dosing.

The aim of this study was to assess the pharmacokinetics of unbound prednisolone and its relation to early treatment response in pediatric ALL patients. The relationship between prednisolone exposure and effect was evaluated using the day 8 prednisone response, the minimal residual disease (MRD) levels, and relapse risk in the total population as well as in well-defined genetic subgroups of sufficient size. [5,6,24,25]

## Materials and methods

*Patients and treatment*

The study was designed as a prospective multicenter Dutch Childhood Oncology Group (DCOG) study, performed in seven pediatric oncology centers within the Netherlands. Patients with ALL in the age of 0 to 18 years and treated according to the DCOG ALL-11 (April 2012 – July 2020) protocol or Interfant-06 (February 2006 – August 2016) protocol were eligible for enrollment. Both protocols were institutional review board (IRB) approved (EudraCT: 2012-00006725 (ALL-11); Dutch Trial Registry nr. 3379). Patients with Down syndrome were excluded from this PK-study due to potential altered pharmacokinetics. [26,27] Patients received 60 mg/m$^2$/day prednisolone either intravenously (iv) or orally (po) divided into three single doses per day. During the first week of treatment, patients received prednisolone and one intrathecal methotrexate (MTX) injection at the start of treatment, and patients often switched from iv to oral prednisolone during the 1st week. Induction treatment subsequently consisted of oral prednisolone with weekly vincristine and daunorubicin, PEG-asparaginase at day 12 and 26, and intrathecal injections (single MTX for prophylaxis, or triple MTX, cytarabine and prednisolone in case of CNS-involvement), for a total duration of four weeks.

Patients were stratified to patients with prednisone good response (PGR defined as <1000 leukemic blasts/$\mu$l blood on day eight after seven days of consecutive prednisolone treatment), and patients with >= 1000 leukemic blasts/$\mu$l, which were considered poor responders. Risk group classification was done according to the DCOG ALL-11 protocol criteria (supplement 2).

*Sample collection and analysis*

Blood samples were collected in the first week of treatment (prior to concomitant chemotherapy), and during week 2-4 of treatment (with concomitant chemotherapy). All samples were collected prior to administration (trough) of prednisolone around $C_{max}/T_{max}$ at 1, 2 and 4 hours after administration during steady-state (>24 hours after the start of prednisolone treatment).[28–30] Blood samples were collected in K2 EDTA tubes and centrifuged at room temperature within two hours after withdrawal. Supernatant (serum) was collected and stored at -80° Celsius prior to analysis. Samples were analyzed with LC-MS/MS (LC: Shimadzu LC-30 Nexera [Nishinokyo-Kuwabaracho, Japan] and MS: AB Sciex 5500 QTrap® [Framingham, MA, USA]) at the department of Hospital Pharmacy in the Academic University Medical Center in Amsterdam, The Netherlands. Details are specified in the supplement (S5).

*Pharmacokinetic analysis*

The concentration time profiles of prednisone and prednisolone were analyzed using nonlinear effects modeling approach in NONMEM® first-order conditional estimates (FOCE) with interaction [version 7.3, ICON, Development Solutions, MD, USA]. Pirana software version 2.9.5b [Certara, NJ, USA] was used as a modeling environment, and data were further handled in R version 3.6.1 [R Foundation for Statistical Computing, Vienna, Austria]. One- and multicompartment linear models with first-order absorption for oral administration, were fitted to the unbound prednisolone concentrations. Allometric scaling was implemented *a priori* to normalize the PK parameters over a wide range of body weights, using fixed exponent values of 0.75 for flow dependent process parameters and 1 for volume related parameters.[31–33] Parameters were normalized to a weight of 70kg. The fit of the model was evaluated both numerically by the precision of the estimated PK parameters and the change in the objective function values (OFV) and visually by goodness-of-fit plots (GoF) and visual predictive checks (VPC). A 3.84-point decrease in OFV for one degree of freedom was considered a significant improvement with a p-value of <0.05. Proportional and constant error models were tested to describe the residual error in plasma concentrations.

Prednisolone exhibits a nonlinear (saturable) binding to corticosteroid binding globulin (CBG) and a linear binding to albumin.[13,22] Prednisolone plasma protein binding was modeled using the formula reported by Ionita *et al.* (supplement 1).[22] For missing albumin concentrations, the population median value was used. A schematic overview of the final model is shown in figure

5.1. After finalization of the structural model, a covariate analysis was performed. Covariates included gender, age, BSA, treatment period, albumin, ALAT, ASAT, bilirubin and urea, treatment block, pharmaceutical formulation (tablet, suspension, intravenous), administration routes (oral, iv, tube), creatinin and glomerular filtration rate (GFR). Continuous covariates were centered around the median. Missing covariates were replaced by the covariate median. The evaluation of covariates was performed using stepwise regression with iterative forward selection.[34] A decrease of ≥ 3.84 points in OFV for one degree of freedom was used for forward selection (p-value of <0.05). The robustness of the parameter estimates was evaluated using a nonparametric bootstrap procedure (n=1000). A visual predictive check was performed for internal validation of the model.

*Pharmacodynamic analysis*

Individual AUC values were calculated on basis of post-hoc values for clearance. Correlation was evaluated of exposure and white blood cell count (WBC), blast count at diagnosis (blood and bone marrow), the prednisone response at day 8, the day 15 bone marrow response, and minimal residual disease (MRD) levels on day 15, 33 and 79. For the analysis of prednisone response patients with <1000 blasts/$\mu$l at initial diagnosis were excluded from the analysis as their response could not be adequately assessed. Group differences in exposure were evaluated for leukemia immunophenotype (T-cell or B-cell precursor), and available cytogenetic data (*ETV6-RUNX1*, *TCF3-PBX1*, *BCR-ABL*, hyperdiploidy, *KMT2A-AFF1* and *IKZF1-del*). Additionally, the AUC of unbound prednisolone was compared between patients who relapsed versus patient who did not.

*Statistical analysis*

Differences between groups were evaluated using Mann-Whiney U-test, ANOVA, Kruskal-Wallis and Fisher exact test. Relations between variables were evaluated using regression analysis and Spearman's rank correlation. Kaplan-Meier analysis was used to estimate relapse rate stratified by the exposure. A two-sided p-value <0.05 was considered significant. Statistical analysis was performed using R.

## Results

*Patients and samples*

Blood samples of 132 patients were available. Eight patients were excluded due to incomplete data and 124 patients were used for the PK analysis. Twenty-five prednisolone samples were excluded due to missing information (e.g., time of administration, sampling or dose time), sampling artefacts, or concentrations below the LLoQ. A total of 1028 unbound and total prednisolone concentrations were available. Patients had a median (range) age of 6.2 (0.4-17.7) years and a BSA of 0.86 (0.36-2.2) $m^2$. The population consisted of 37% girls and 63% boys. Eight patients were classified as prednisone poor responders (PPR) and 110 as prednisone good responders (PGR), and six unknowns. Thirty-two of the PGR patients had starting leukemic blasts <1000/$\mu$l and were excluded for the prednisone response analysis. The subset of patients in this study did not differ significantly from the total patients treated according to ALL-11 in the Netherlands, with respect to demographics, immunophenotype, risk group stratification, WBC and prednisone response ($p>0.05$). An overview of patients and sample characteristics can be found in table 5.1.

*Pharmacokinetic analysis*

A one-compartment model with first-order absorption best described the unbound prednisolone plasma concentration. Allometric scaling of the PK parameters improved the model significantly ($p<0.001$); the interindividual variability (IIV) in clearance (C$L$) and distribution ($V$) decreased from 54% to 31% and 73% to 33%, respectively. Prednisolone is reversibly metabolized into inactive prednisone, which was added to the model. The median percentage of bound prednisolone was 86% (range: 71%-99%). Implementing the ratio of total prednisolone/prednisone concentrations over time significantly improved the model ($p<0.001$). The affinity of the plasma protein corticosteroid binding globulin (CBG) for prednisolone ($_{cbg}$) could not be estimated and was fixed to 30 $\mu$M as found in literature, nor were CBG levels measured which were estimated by the PK model.[22] The final structural model was an allometrically scaled one-compartment model with first-order absorption, including plasma protein binding of prednisolone and the ratio of prednisolone/prednisone (figure 5.1). IIV was described for $CL$ and $V$ and the residual variability was best described using a proportional error.

**Table 5.1:** Patient characteristics

| | **Total** |
|---|---|
| Patients (n) | 124 |
| Female:male | 37% vs 63% |
| | **median (range)** |
| Age (y) | 6.0 (0.4-17.7) |
| Weight (kg) | 22 (7-86) |
| Height (cm) | 122 (68-188) |
| BSA (m2) | 0.86 (0.36-2.2) |
| | **median (range)** |
| Creatinin ($\mu$mol/L) | 29 (11-92) |
| ALAT (U/L) | 45 (5-99) |
| ASAT (U/L) | 29 (8-100) |
| Bilirubin ($\mu$mol/L) | 10 (1-77) |
| Urea (mmol/L) | 5.1 (1.6-51) |
| Albumin (g/L) | 38 (10-100) |
| Samples unbound + total (n) | 1028 |
| Samples per patient (n) | 4 (1-10) |
| Dose prednisolone (mg) | 16.5 (3-45) |
| B-cell (n) | 108 |
| Other (n) | 38 |
| *ETV6-RUNX1*(n) | 24 |
| *IKZF-del*(n) | 9 |
| *KMT2A-AFF1* (n) | 3 |
| *TCF3-PBX1* (n) | 2 |
| Hyperdiploid (n) | 30 |
| *BCR-ABL1* (n) | 2 |
| T-cell (n) | 16 |
| PPR:PGR (n) | 8:110 |
| SR:MR:HR (n) | 30:79:10 |
| | **median (range)** |
| WBC diagnosis ($L^{-1}$) | $12.1*10^9$ ($0.5*10^9$-$366*10^9$) |
| WBC day 8 ($L^{-1}$) | $1.9*10^9$ ($0.2*10^9$-$73*10^9$) |
| MRD day 15 | $5*10^{-3}$ ($0$-$2*10^{-3}$) |
| MRD day 33 | $1.5*10^{-4}$($0$-$0.7*10^{-4}$) |

PPR: prednisone poor responders; PGR: prednisone good responders; SR: ALL-11 standard risk; MR: ALL-11 medium risk; HR: ALL-11 high risk; WBC: white blood cell count; MRD: minimal residual disease.

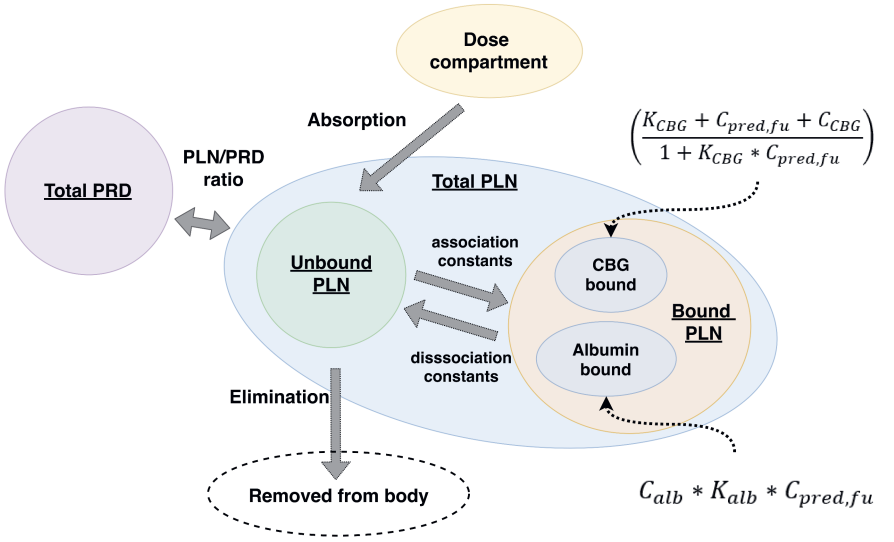


**Figure 5.1:** A representation of the final PK model. PLN: prednisolone; PRD: prednisone; CBG: corticosteroid binding globulin; $C_{alb}$: albumin concentration; $K_{alb}$: albumin affinity constant; $C_{pred,fu}$: unbound prednisolone concentration; $K_{cbg}$: CBG affinity constant; $C_{cbg}$: CBG concentrations.

The structural model was used for the covariate analysis. In a univariate analysis an association between ALAT, ASAT, bilirubin and treatment phase and both $CL$ and $V$ was observed (p<0.01), whereas albumin (p<0.05) solely correlated with $CL$. The plasma protein binding of prednisolone to CBG was associated with patient age, ASAT and treatment phase (p<0.01). After iterative forward inclusion, both ASAT and treatment phase on $V$, and age on CBG concentration remained. ASAT had a positive correlation with $V$; high ASAT was associated with higher $V$ (p<0.001). The estimated CBG concentration decreased with age (p<0.001). $V$ was slightly lower in the treatment phase after week one with concomitant chemotherapy (p<0.001). The fraction of unbound prednisolone versus age over the concentration range is shown in figure 5.3. No correlation between clearance of unbound prednisolone (corrected for BSA) and age was observed. An overview of the final parameter estimates can be found in table 5.2.

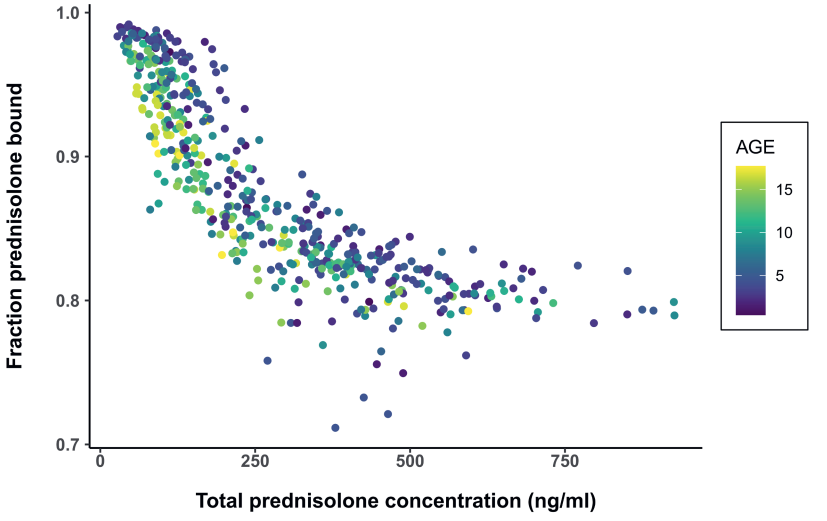


**Figure 5.2:** The fraction of total prednisolone bound to plasma proteins versus the total prednisolone concentration. The colors indicate age, from young to old patients respectively purple to yellow. Younger patients seems to have a higher fraction of prednisolone bound to proteins compared the older patients.

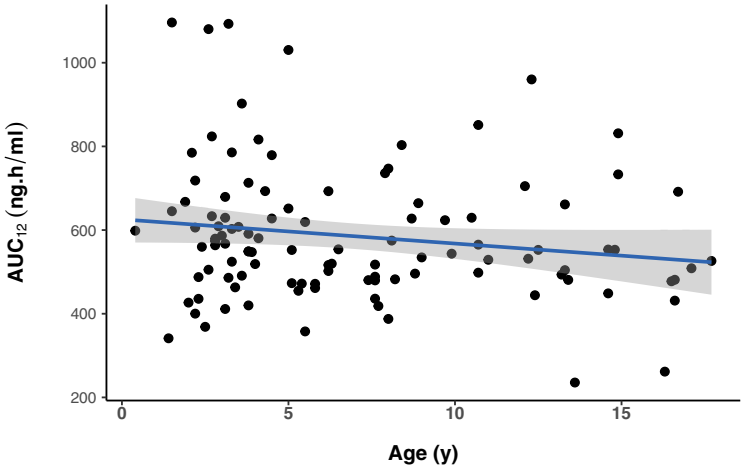


**Figure 5.3:** Dose corrected unbound $AUC_{0\text{-}12}$ versus the age of the patients. No correlation was found between the AUC and patients' age. It shows a large variability in the AUC (p=0.13) . The blue line is the regression line.

In the nonparametric bootstrap procedure 480 of the 500 runs were successful and model estimates were in accordance with the results from the bootstrap replicates, indicating the robustness of the model (table 5.2). The VPCs for both free and total prednisolone and the goodness-of-fit plots demonstrate the adequacy of developed model (supplement 3). Peak concentrations were slightly underpredicted especially for the unbound prednisolone concentrations, probably due to limited samples in the absorption phase. Patients with high peak concentrations were significantly younger.

*Pharmacodynamic analysis*

Individual *post hoc* estimates of the final model were used to evaluate differences in exposure between and within subgroups of the population. Patients with PPR (n=8) seemed to have a slightly lower unbound AUC than patients with PGR (n=78); the difference was however not statistically significant (p=0.2) with median AUC values 520 (IQR 451-577) and 553 (IQR 487-650) ng*h/ml, respectively. No differences in AUC between were observed between highest and lowest quartiles of WBC, blasts at diagnosis (both day 8 blood or 15 bone marrow), and MRD at day 15, 33 and 79, nor in the more resistant subgroups (T-cell phenotype and combined B-cell

**Table 5.2:** Population parameter estimates and nonparametric bootstrap

| | NONMEM | | | | | Bootstrap | | |
|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Estimate** | **RSE (%)** | **95% CI (lower)** | **95% CI (upper)** | **Shrink. (%)** | **Median** | **95% CI (lower)** | **95% CI (upper)** |
| CL/F (L/h/70kg) | 100 | 5 | 91 | 109 | - | 100 | 91 | 109 |
| V/F (L/70kg) | 589 | 9 | 490 | 688 | - | 583 | 499 | 684 |
| Ka (-h) | 4 | 2 | 3.9 | 4.2 | - | 4.0 | 2.0 | 7.1 |
| Ccbg ($\mu$M) | 0.83 | 6 | 0.73 | 0.92 | - | 0.84 | 0.74 | 0.95 |
| Kalb ($\mu M^{-1}$) | 0.002 | 20 | 0.001 | 0.003 | - | 0.002 | 0.001 | 0.003 |
| ASAT ~V | 0.15 | 52 | 0.0 | 0.29 | - | 0.16 | 0.0 | 0.34 |
| Age ~CBG | -0.15 | 40 | -0.27 | -0.03 | - | -0.15 | -0.27 | -0.03 |
| Ratio ~CL | -0.48 | 21 | -0.68 | -0.28 | - | -0.49 | -0.67 | -0.27 |
| Block ~V | 0.87 | 5 | 0.78 | 0.96 | - | 0.88 | 0.78 | 1.0 |
| IIV CL (%) | 26.6 | 25 | 19 | 34 | 22 | 41 | 29 | 53 |
| IIV V (%) | 39.2 | 17 | 33 | 48 | 24 | 50 | 33 | 67 |
| Prop res error | 0.46 | 4 | 0.42 | 0.49 | 7 | 0.40 | 0.35 | 0.45 |

*CL/F*: the apparent clearance; *V/F*: apparent volume of distribution; *ka*: absorption rate constant; Ccbg: corticoid binding globulin concentration; Kalb: affinity constant for prednisolone to albumin; ASAT~*V*: covariate ASAT on *V*; Age~CBG: covariate age on prednisolone binding to CBG; Ratio~*CL*: covariate total prednisolone over prednisone ratio on *CL*; Block~*V*: covariate treatment block on *V*; IIV: interindividual variability; Prop res err: proportional residual error of free and total prednisolone. RSE: relative standard error. CI: confidence interval

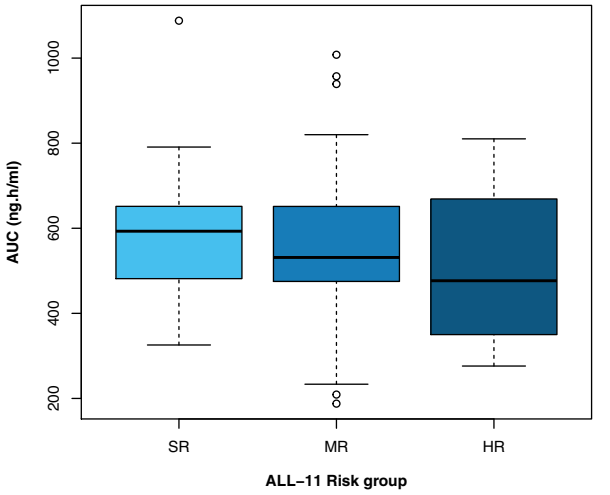


**Figure 5.4:** In this figure the AUC of unbound prednisolone was compared between patients stratified in different ALL-11 risk groups, standard risk (SR; n=30), medium risk (MR; n=79) and high risk (HR; n=10). Median AUC SR: 593 (IQR 482-651), MR: 531 (IQR 475-651) and HR: 477 (IQR 379-652) ng*h/ml (not statistically different; p=0.20).

genetic subtypes *IKZF-del*, *KMT2A-AFF1* and *BCR-ABL1*), where we assumed the effect of exposure might be greater due to cellular resistance. Additionally, the AUC was compared between the different ALL-11 risk groups, standard risk (SR, n=30), medium risk (MR, n=79) or high risk (HR, n=10). Although AUC seems to decrease with risk, no significant differences were found; median (IQR): 593 (482-651), 531 (475-651) and 477 (379-652) ng*h/ml respectively (figure 5.4). No difference was observed between BCP (n=108) and T-cell (n=16) immunophenotype in dose normalized AUC nor between the various B-cell precursor genetic subtypes (figure 5.5). However, the majority of the patients were *ETV6-RUNX1*, hyperdiploid and B-other, the number of patients in other subtypes was too limited for subgroup analysis.

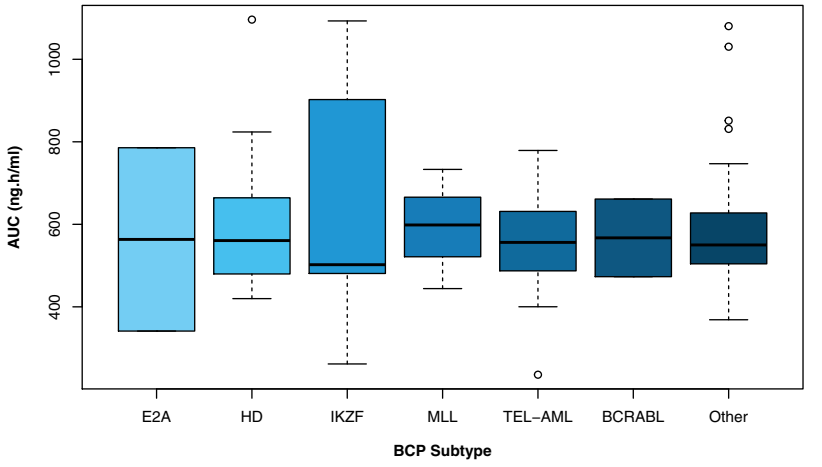


**Figure 5.5:** This figure shows the patients' AUC for different B-cell genotypes. No difference in AUC was found between the groups. Three of the subtypes only had a very limited number of patients *TCF3-PBX1* (n=2), *KMT2A-AFF1* (n=3), *BCR-ABL1* (n=2). Hyperdiploid (HD; n=30), *ETV6-RUNX1* (n=24), *IKZF-del* (n=9), B-other (n=38).

The Kaplan-Meier shows the probability of relapse free survival stratified by low (Q1), mid (Q2-Q3) and upper quartile (Q4) AUC of unbound prednisolone. Nine patients suffered from relapse, of which four had an AUC in the lowest quartile, five in the middle, and none in the highest quartile (figure 5.6). The subgroups were however very small and therefore only large group effects could be observed. To determine whether the difference in exposure and prednisone response, HR verses MR and SR, and relapse free survival would require over respectively 979, 495 and 364 patients. This however does not take into account whether this is a clinically relevant difference. The latter is probably not the case due to the large observed variability in exposure in all groups and adjusting the exposure would most likely not result in an improved clinical outcome.

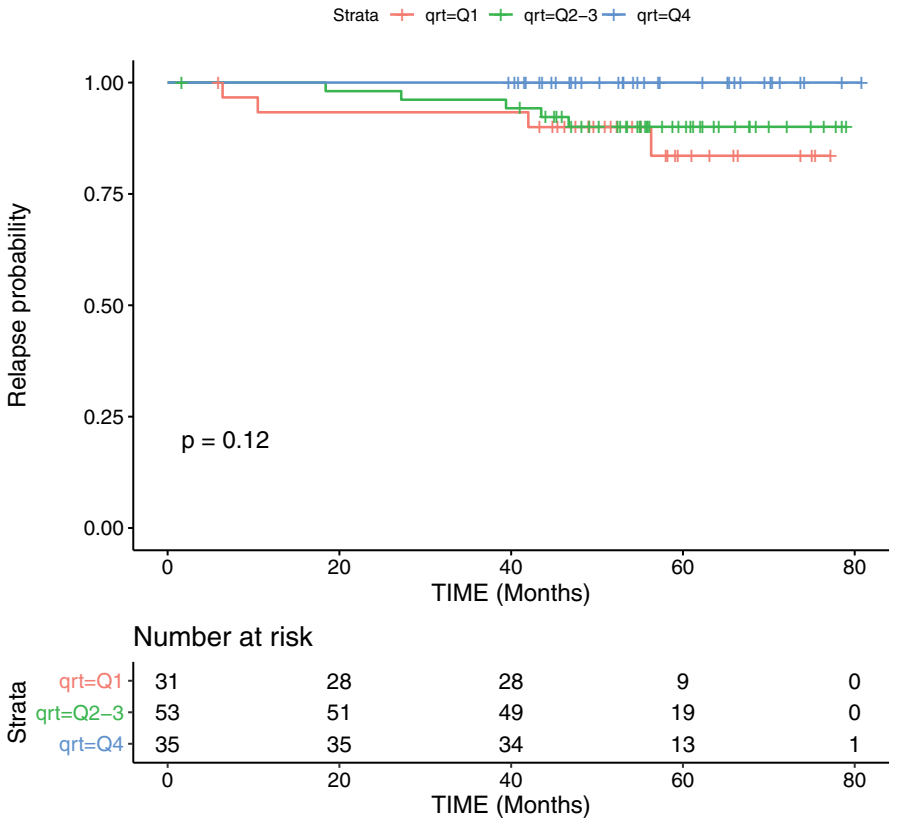


**Figure 5.6:** Kaplan-Meier of relapse (a) and survival (b) for patients within different AUC quartiles, lower quartile (Q1), mid quartiles (Q2 and Q3) and the upper quartile (Q4) with the highest AUC. Nine patients relapsed of which four in Q1 and five in Q2-3. Four patients deceased of which three in Q1 and one in Q2-3.

## Discussion

Glucocorticoids have an important place in the treatment of pediatric ALL. Patients who are more resistant to prednisolone experience worse outcome.[5,6] The occurrence of resistance to prednisolone in pediatric ALL has been extensively studied. However, the possible association between *in vivo* prednisolone exposure and outcome has not been studied so far. Kawedia *et al.* reported a higher clearance of dexamethasone in younger children, which could result in lower exposure when compared to older children.[14] This might also be the case for prednisolone, and might advocate dose modifications in younger patients. However, in this study no correlation was found between exposure and age. A complicating factor in the treatment with prednisolone may be the concentration dependent plasma protein binding (from 95% at low concentrations to 60% at high concentrations).[13,21,22] Hence, in this study, unbound, pharmacologically active, prednisolone was measured in plasma and related to different disease parameters.

The PK of unbound prednisolone was best described by a one-compartment model with first-order absorption and allometric scaling. This model included the protein binding of prednisolone to the plasma proteins CBG and albumin, and the prednisolone/prednisone ratio. $V$ was smaller in treatment phases with concomitant chemotherapy (>week 1). This might be due to patients receiving hyperhydration in the first week of treatment to prevent tumor lysis syndrome and no asparaginase. In addition, a positive correlation between ASAT and $V$ was observed. ASAT was significantly higher in the first week compared to subsequent weeks with chemotherapy (median 32 versus 23.5 U/L respectively; p=0.02). The addition of ASAT resulted in a significant improvement of the PK model on top of the treatment phase (P<0.01). ASAT might be used as a marker for liver function, however it would be expected that high ASAT correlates with a smaller $V$, due to less plasma protein binding. Therefore, it is more likely that the association between $V$ and ASAT reflects the collinearity between the latter and the treatment phase, e.g., due to cell decay.

The estimated CBG concentration showed a positive correlation with patient age, with lower CBG concentrations and lower protein binding in older patients. The affinity was set to a fixed value (30 $\mu$M as seen in Ionita *et al.*).[22] However, a wide array of affinity constants can be found in the literature.[13,21–23] Due to the fixed affinity of prednisolone and CBG its role between and within patients could not be evaluated. Prednisone and cortisol bind to CBG as well although this does not have a large effect on prednisolone.[21,23]

In this study, the clearance of unbound prednisolone per $m^2$ did not correlate with age, which is different from results found with dexamethasone clearance, where younger age was associated with higher clearance.[19] Prednisolone has some distinct pharmacokinetic differences compared to dexamethasone. Prednisolone exhibits nonlinear binding to plasma proteins whereas dexamethasone does not. The fraction of unbound prednisolone increases when the concentration of total prednisolone increases. Although small differences were observed in protein binding with age and peak concentrations, our data demonstrated that the AUC of the unbound prednisolone was similar throughout age.

The relationship between prednisolone exposure and clinical outcome parameters were studied. No differences in exposure were observed between the prednisone poor and good responders. This might suggest that the response is predominantly influenced by the cellular sensitivity to prednisolone and not due to lower exposure to prednisolone. This is also supported by the fact that no difference was found between the AUC quartiles of the more prednisolone resistant phenotype and B-cell genetic subtypes and treatment response. Hence, this study shows no effect of prednisolone exposure on treatment response after a high dose of 60 mg/$m^2$/day.

The exposure for patients in the ALL-11 risk groups seemed to slightly decrease with increasing risk. Four patients (44%) of the nine patients who relapsed had AUC values in the lowest quarter and none in the upper quarter (Q4). The Kaplan Meier estimates did not show a significant difference between the AUC quartiles and cumulative incidence of relapse (figure 5.6). However, the number of patients in high risk and more resistant subgroups was small, and differences between exposure and outcome within these subgroups could not be well determined.

## Conclusion

A PK model was developed to describe the time profile of unbound prednisolone plasma concentration in pediatric ALL patients. A one-compartment model with allometric scaling and a combined saturable and linear protein binding described the data adequately. Protein binding was slightly higher in younger patients. However, the AUC of unbound prednisolone did not differ with age. In this study, no differences were observed between exposure and disease outcomes for

good responders, including day 8 prednisone response, blast counts, and MRD. The sensitivity to prednisolone is probably the prominent factor regarding prognosis and individualized dosing in this group might not improve outcome. Regarding the prednisolone poor responders, relapse and high-risk patients, the numbers were small. Future studies might look at whether a combination of increased prednisolone dosing either in combination with sensibilization to prednisolone (e.g. MEK inhibitor) is feasible and beneficial in a hard to treat subset of patients.

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# SUPPLEMENT

## S1. Equations

Implementation of prednisolone protein binding as implemented in the final model.

$$C_{pred,tot} = C_{pred,fu} + \left(\left(\frac{K_{CBG} + C_{pred,fu} + C_{CBG}}{1 + K_{CBG} * C_{pred,fu}}\right) + C_{alb} * K_{alb} * C_{pred,fu}\right) \quad (5.1)$$

$C_{pred,tot}$ is the total prednisolone concentration, $C_{pred,fu}$ is the concentration of unbound prednisolone, $K_{CBG}$ is the affinity constant of corticosteroid binding globulin (CBG), $C_{CBG}$ is the concentration of CBG, $C_{alb}$ is the concentration of albumin and $K_{alb}$ is the affinity constant of albumin for prednisolone.

Implementation of continuous covariates (equation 5.2) and categorical covariates (equation 5.3) as example for the PK parameter clearance including allometric scaling:

$$P_i = CL_{pop} * \left(\frac{COV}{median\ COV}\right)^{\theta_{COV}} * \left(\frac{WT}{70}\right)^{0.75} * exp^{\eta_i} \quad (5.2)$$

$$P_i = CL_{pop} * {\theta_{Cat}}^{FLAG} * \left(\frac{WT}{70}\right)^{0.75} * exp^{\eta_i} \quad (5.3)$$

$P_i$ is the individual parameter estimate, $\Theta_{pop}$ is the population parameter estimate. COV is the continuous covariate, $\Theta_{cov}$ is the estimated exponent parameter of the continuous covariate. $\Theta_{cat}$ is the estimated fraction parameter of the categorical covariate. FLAG is either 1 (covariate present) or 0 (not present). WT is patients' body weight. $\eta_i$ is the individual deviation from the population estimate. Missing covariates were replaced by the median value of the covariate.

The calculation of the individual clearance and volume of distribution in the final model:

$$CL_i = CL_{pop} * \left(\frac{WT}{70}\right)^{0.75} * \left(\frac{ratio}{8.25}\right)^{-0.48} * 0.87^{Block} * exp^{\eta_i} \tag{5.4}$$

$CL_i$ is the individual clearance, $CL_{pop}$ the population clearance, WT the patients' body weight, ratio is the ratio of total prednisolone over total prendnisone, block is the treatment block and $\eta_i$ is the individual deviation from the population estimate.

$$V_i = V_{pop} * \left(\frac{WT}{70}\right)^{0.75} * \left(\frac{ASAT}{35}\right)^{0.15} * exp^{\eta_i} \tag{5.5}$$

$V_i$ is the individual clearance, $V_{pop}$ the population clearance, WT the patients' body weight and $\eta_i$ is the individual deviation from the population estimate.

$$C_{CBG,i} = C_{CBG,pop} * \left(\frac{AGE}{5.3}\right)^{-0.15} * exp^{\eta_i} \tag{5.6}$$

$C_{CBG,i}$ is the individual CBG concentration, $C_{CBG,pop}$ is the population CBG concentration, Age is patients' weight and $\eta_i$ is the individual deviation from the population estimate.

# S2. DCOG ALL-11 protocol risk stratification

**Standard risk (SR) group:**

- MRD-negativity at TP1 (day 33) and at TP2 (day 79 before start of Protocol M) AND
- no CNS involvement or testis involvement at diagnosis AND
- no prednisone poor response at day 8 AND
- absence of any HR criterium

**Medium risk (MR) group:**

- inconclusive/missing MRD results or MRD-positivity at TP1 and/or at TP2, but MRD level at day 79 < 10–3 AND
- absence of any HR criterium

**High Risk (HR) group:**

- MRD level > 10-3 or unknown at TP1 and MRD level of ≥ 10–3 at TP2, OR
- presence of the t(4;11)(q11;q23) translocation or the corresponding fusion gene MLL/AF4, OR
- no complete remission at day 33
- Note: children with Down syndrome that fulfill the HR criteria are assigned to the MR group

# S3. Figures

## VPC for unbound and total prednisolone concentrations

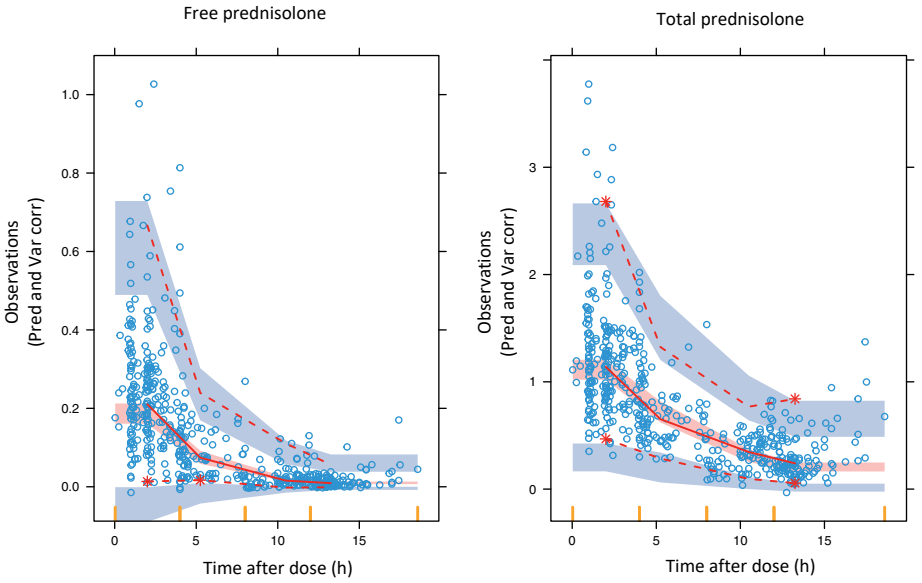
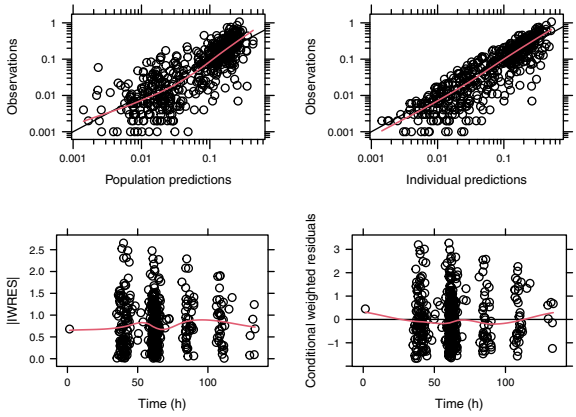



**Figure S1:** Visual predictive checks for unbound prednisolone (left) and total prednisolone (right) concentrations. The plots show the fit of the predicted concentrations versus the observed concentrations of the final model. The red solid line indicates the median observed concentrations and the surrounding opaque red area the simulation based 95% interval for the median. The red dashed lines indicates the observed 5% and 95% percentiles and the surrounding opaque blue areas show the simulated 95% confidence intervals for the corresponding predicted percentiles.

## Goodness-of-fit plots for unbound prednisolone

A. Total prednisolone concentrations:




B. Unbound prednisolone concentrations:

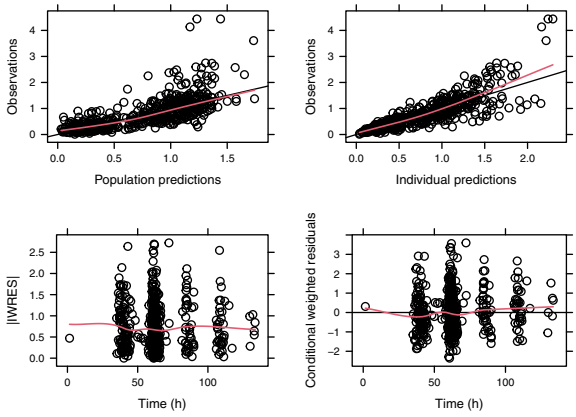



**Figure S2:** Goodness-of-fit plots for total prednisolone (A) and unbound prednisolone (B) of the final model Predicted population concentrations versus observed concentrations of the final model (upper left); Predicted individual concentrations versus observed concentrations of the final model (upper right). Individual weighted residuals versus time in hours (lower left), conditional weighted residuals versus time in hours (lower right).

## Half life versus age groups

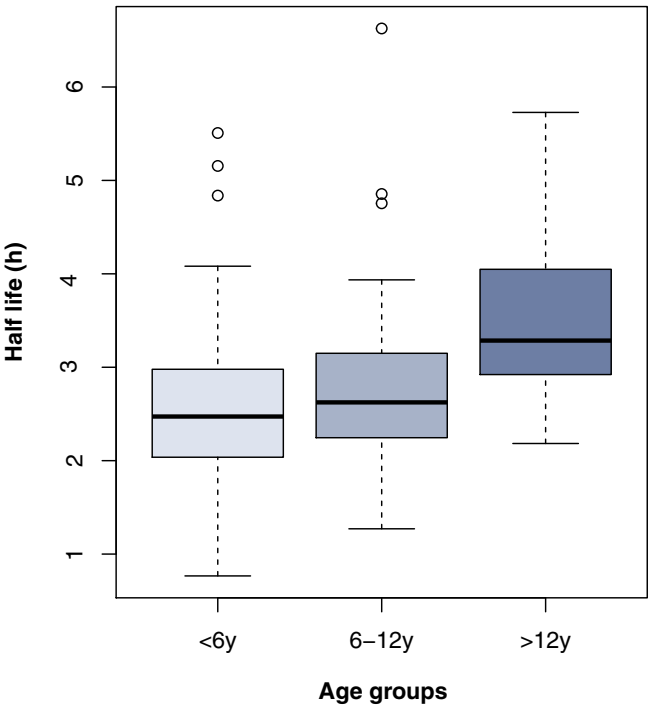



**Figure S3:** Half life stratified per age group showing incremental half life with age.

## AUC of unbound prednisolone versus day 8 prendisone response

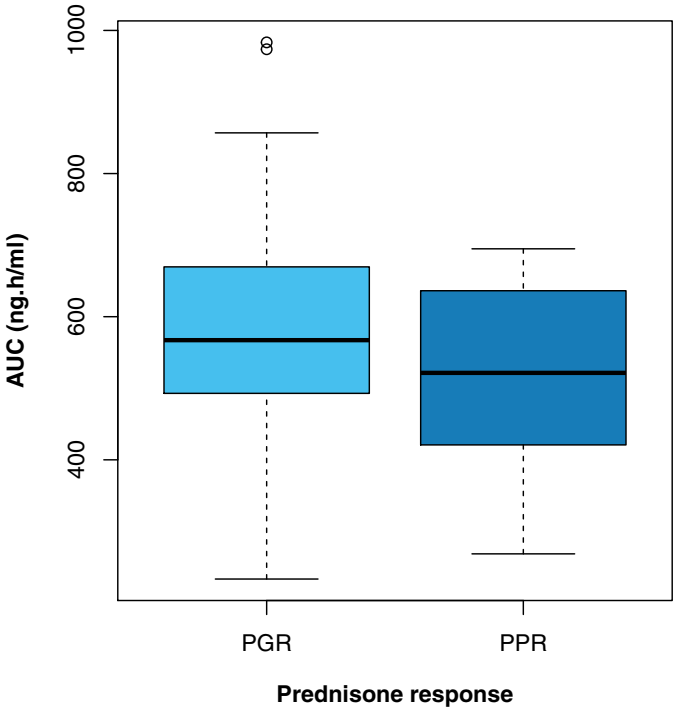



**Figure S4:** AUC of unbound prednisolone was compared between patients with prednisone good response (PGR; n=78) and prednisone poor response (PPR; n=8). Within this cohort no statistically significant difference was found between the two groups (p=0.32).

## Fraction of prednisolone vs total prednisolone concentration and age

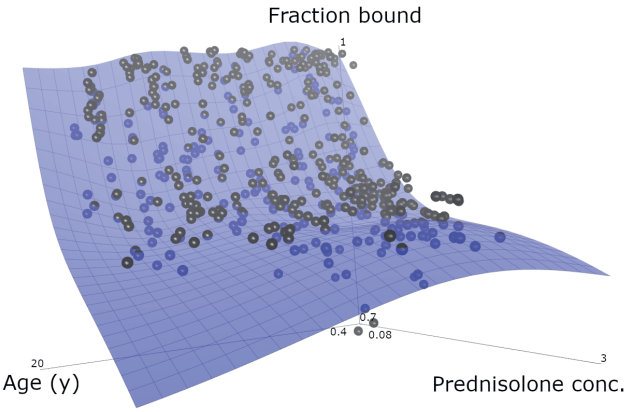



**Figure S5:** Plot showing the change in prednisolone concentrations (x-axis), the fraction of prednisolone bound to plasma proteins (y-axis) and the patients' age (z-axis).

## S4. Model

```
; 1. Based on: 071
; 2. Description: Prednisolon
; x1. Author: SDTSassen

$PROBLEM PK model

$INPUT
      CENSOR
      AORTA=DROP
      ID
      DATE=DROP
      TIME
      Center=DROP
      AMT=DROP
      AMT
      VORM
      ROUTE
      EVID
      MDV
      CMT
      TAD
      DV=DROP
      DV=DROP
      DV=DROP
      DV              ; Molar unbound and total (in umol/L)
      TOFR            ; Unbound = TOFR1 & Total = TOFR 2
      Type=DROP
      SEX
      AGE
      HT
      WT
      BLOK
      ALB
      Creat
      ALAT
      ASAT
      Bili
      Ureum
      Hyper
      REC
      POP             ; Ratio prednisolone over prednisone
```

```
$DATA DB_PLN_290220.CSV IGNORE=C

$SUBROUTINES
      ADVAN6 TOL=3

$MODEL
      COMP (ABSORB,DEFDOSE)
      COMP (CENTRAL)
      COMP (AUC)

$PK
      SIZE = (WT/70)

      IF(ALB.EQ.99999) THEN
           ALBX = 620
      ELSE
           ALBX = ALB/0.066430
      ENDIF

      IF(ASAT.EQ.99999) THEN
           ASTX = 35
      ELSE
           ASTX = ASAT
      ENDIF

      FLAG1 = 0
      IF(BLOK.EQ.2) FLAG1 = 1

      COV1 = 1
      IF(POP.GT.0) COV1 = (POP/8.25)**THETA(8)
      COV2 = (ASTX/35)**THETA(9)
      COV3 = (AGE/5.3)**THETA(10)
      COV4 = THETA(11)**FLAG1

      KA = THETA(3)

      TVCL = LOG(THETA(4)*(SIZE**0.75)*COV1)
      MU_1 = TVCL
      CL = EXP(MU_1+ETA(1))

      TVV =LOG(THETA(5)*(SIZE**1)*COV2 *COV4)
```

```
      MU_2 = TVV
      V = EXP(MU_2+ETA(2))

      TVCcbg = LOG(THETA(6)*COV3)
      MU_3 = TVCcbg
      Ccbg = EXP(MU_3 + ETA(3))

      Kalb = THETA(7)

      S2=V/1000
      K20=CL/V

$THETA
      (0, 0.646)          ;1 prop
      (0, 0.375)          ;2 prop
      (0, 3.95)           ;3 Ka
      (0, 102)            ;4 CL
      (0, 552)            ;5 V
      (0, 0.81)           ;6 Ccbg
      (0, 0.00197)        ;7 Kalb
      (-1, -0.457)        ;8 Metab
      (-1, 1)             ;9 V-ASAT
      (-1, 1)             ;10 CBG-Age
      (-1, 1)             ;11 V-blok

$OMEGA BLOCK(3)
      0.05                ; IIV-CL
      0.01 0.05           ; IIV-V
      0.01 0.01 0.03      ; IIV-Ccbg

$DES
      DADT(1)=-KA*A(1)
      DADT(2)=A(1)*KA-A(2)*K20
      DADT(3) = A(2)/S2     ; AUC

$SIGMA
      1 FIX
      1 FIX

$ERROR
      CALLFL=0

      ; CBG affinity 30
```

```
      CPU = A(2)/S2
      CP = CPU+((30*CPU*Ccbg/(1+30*CPU))+ ALBX*Kalb*CPU)


      W1 = (CPU**2*THETA(1)**2)**0.5
      IF (W1.EQ.0) W1 = 1

      W2 = (CP**2*THETA(2)**2)**0.5
      IF (W2.EQ.0) W2 = 1

      IF(TOFR.EQ.1) THEN
            IPRED = CPU
            IRES = DV-IPRED
            IWRES = IRES/W1
            Y= IPRED+W1*EPS(1)
      ELSE
            IPRED=CP
            IRES= DV-IPRED
            IWRES=IRES/W2
            Y=IPRED+W2*EPS(2)
      ENDIF

      AUC = A(3)


$EST METHOD=1 MAXEVAL=99999 SIG=3 NOABORT POSTHOC INTERACTION

$COV PRINT=E UNCONDITIONAL

$TABLE ID TAD PRED IPRED IWRES CWRES NOPRINT ONEHEADER FILE=SDTAB
$TABLE ID CL V NOPRINT ONEHEADER FILE=PATAB
$TABLE ID WT ALBX NOPRINT ONEHEADER FILE=COTAB
$TABLE ID NOPRINT ONEHEADER FILE=CATAB
```

# Chapter 6

## General discussion

# General discussion, conclusions and future perspectives

The prognosis for children with ALL has improved significantly over the last decades with current overall survival rates exceeding 90%.[1–3] This has been achieved through treatment intensification, identification of clinical and genetic risk factors, sophisticated early response measurements to refine risk stratification and risk adapted treatment protocols. The treatment has become more individualized. However, with respect to individualized dosing of medication, information remains scarce. It is often unknown whether exposure differs between and within patients and whether a relationship exists between the exposure and clinical response. Hence, the scope of this thesis was to fill this knowledge gap by studying the pharmacokinetics (PK) and pharmacodynamics (PD) of different drugs currently used in the treatment of pediatric ALL. Prednisolone was studied to determine whether younger children differ from older patients with respect to exposure and whether differences in exposure between patients had an effect on the clinical outcome. If this would be the case, patients or subsets of patients might benefit from individualized dosing. The PK of *Erwinia* asparaginase was studied to determine whether current starting dosages could be optimized to achieve target trough concentrations in all patients without using unnecessarily high dosages, taking into consideration the toxicity, high costs and scarcity of the drug. Ciprofloxacin was studied to get a better understanding of the use of ciprofloxacin as prophylactic treatment. The PK was studied to determine whether target exposure was achieved in all patients. The PK model was used to study the exposure of ciprofloxacin and its correlation to the incidence of infections and emergence of resistant cultures.

*Considerations on experimental design and methods*

As shown throughout this thesis, the use of modern PK/PD analysis and modeling techniques overcome many of the hurdles encountered when performing classic PK analyses in a pediatric population like dense/frequent sampling, rigid sampling times and high blood volumes. The sensitivity of high-end liquid chromatography-mass spectrometry (LC-MS) allows the quantification of multiple drugs in only one drop of blood. This technique was used for the analysis of total and unbound concentrations of prednisolone and ciprofloxacin in plasma. Due to the limited required volume of 250 $\mu$l plasma, it was possible to use finger prick blood sampling when venipuncture was not possible. Trough levels were taken for all three drugs, and samples at additional time points after dosing of prednisolone and ciprofloxacin were obtained. Erwinia asparaginase is dosed to achieve target trough levels of ≥100 IU/L, therefore prediction of trough levels would suffice.[4]

For prednisolone and ciprofloxacin, the area under the curve (AUC) was used to evaluate the correlation with the effect, which explains why additional sampling time points were required. In addition to the small volume of blood required, nonlinear mixed-effects modeling (NONMEM®) was used, which enables opportunistic and sparse sampling, and the use of heterogeneous data. With this technique, sampling is not limited to fixed times (flexible sampling), and reliable results can be achieved even with limited number of samples (as low as one to three samples per patient), as long as the time of sampling and drug administration are well documented. However, the intended use or research question should be taken into consideration for the design of the sampling schedule. If information concerning peak concentrations, absorption or multiple distribution compartments is required, samples taken during these phases should be available, as using only trough samples will not provide the necessary information to answer these questions. For the PK analysis of prednisolone and ciprofloxacin the first samples were taken at half an hour to an hour after administration. This made the estimation of the absorption PK parameters difficult (lag time, rate of absorption), as only few samples were available during the absorption phase (first 30 to 60 minutes after administration). More samples during this phase would have been preferred if the rate of absorption was the main interest. Additionally, no information concerning food intake around the time of administration of the drug was available. Food intake may affect drug absorption. However, this study focused on the prediction of overall exposure and trough concentrations.

In our experience most patients did not object to the additional blood withdrawals and liked to participate in the study. patients in the outpatient clinic however considered the extended stay in the hospital to obtain a sufficient number of blood withdrawals burdensome. In future studies, the use of dried blood spots (DBS) may be considered, which enables patients to collect blood samples at home. DBS is performed by collecting drops of blood onto dedicated paper cards. This has the advantage of the sampling of even smaller volumes of blood, less invasive sample collection, and easier logistics compared to venipuncture. However, when using DBS, it is important to take additional factors into account including the volume of blood, hematocrit levels, whether the blood is properly collected on the paper, and the use of paper designed specifically designed for DBS applications. Not surprisingly considering the aforementioned factors, a higher variability has been observed in concentrations from DBS collection compared to venipuncture.[5–7] This increased variability may therefore decrease the precision of the models' prediction power. Another options for monitoring of corticosteroids PK is the determination in saliva, which is even

less invasive. [8,9]

In general, limited numbers of pediatric oncology patients are available for enrollment in (PK) studies. This becomes more difficult if the incidence is low or the target population (for example relapsed patients in a new agent study, infants, etc.) is small. The population size may be increased by combining samples from other hospitals and/or studies. This facilitates the ability to study rare diseases and treatments as nonlinear mixed effects modeling (NLME) can handle heterogeneous data well. Another possible approach would be to study the drug in other populations, for instance healthy, volunteers or proper models for the extrapolation from adults to children. As chemotherapeutic agents cannot be ethically administered to healthy volunteers the use of microdosing (phase 0 studies) might provide a solution. With microdosing ultra-low (subtherapeutic) amounts of radio labeled drugs are administered safely to healthy subjects. Ultra-low dosages result in ultra-low concentrations therefore require highly sensitive analytical methods for quantification in the pico- to femtogram range. [10] Accelerator Mass Spectrometry (AMS) is one of the few techniques able to quantify ultra-low sub pharmacological doses of $^{14}$C-labeled drugs with high sensitivity and selectivity. One major concern when using microdosing in healthy volunteers is how well it extrapolates to therapeutic concentrations in the target population. The linearity should be evaluated in the concentration range of ultra-low dosages compared to therapeutic dosages prior to extrapolation of the PK from one to the other. Also, the PK in healthy subjects might differ from patients. Especially if we look at diseases like leukemia, where patients are treated with different combinations of chemotherapeutic agents which could cause drug-drug interactions during certain parts of the treatment. Also, altering state of disease can affect the PK for example due to disease-related changes in the body like protein-binding capacity or membrane permeability. [11]

*Population pharmacokinetics in pediatric oncology*

As shown in the examples throughout chapter two results from PK studies are currently being used in the clinic to tailor treatment and to improve outcome. However, in addition to the limited available PK studies in pediatric oncology patients, there often remains a gap between the performed PK studies and the translation of their results into the clinic. This is probably due to the technical nature of the studies and complex formulas to describe the behavior of the drug which makes it often not feasible or unclear how to implement it directly into the clinic. The examples provided in the chapter show a variety of studies where PK research was used to improve

the therapy in pediatric cancer patients.

As with the medication studied in the context of this thesis, large inter- and intraindividual differences in plasma concentration is often observed, which might result in subtherapeutic or toxic concentrations. In order to improve the treatment, it is necessary to quantify this variability and identify patient characteristics or clinical parameters that explain this variability. With population PK modeling valuable information concerning the variability between patients, within patients and between occasions is acquired through stochastic models and covariate analysis. These variabilities can be quantified providing insight into what kind of different concentrations one can expect after a certain dose (for example after administration of a similar dose inter-patient variability of PK parameters may cause a variability in exposure ranging from 50 to 500%). However, although the quantification of the variability is valuable, it does not determine whether dose adjustments are preferred and how the dose should be adjusted. With the covariate analysis in the population PK analysis multiple factors are tested in order to (partially) explain variability. The identified sources of variability (e.g. age, poor metabolizers, treatment block) can be used to adjust the dose of medication on an individual basis to optimize individual drug exposure by increasing or decreasing the dose during certain treatment blocks, comedication or patient characteristics. For example, if poor metabolizers exhibit reduced drug clearance, the dose could be decreased while retaining the same exposure in these patients. The potential benefit of PK based precision dosing was illustrated by Evans *et al.*[12] In this study, individualized treatment of high-dosed methotrexate based on patients' clearance and AUC resulted in a significant increase in the percentage of patients with pediatric B-lineage ALL remaining in continuous complete remission.

Population pharmacokinetic models can provide valuable information, but if it is not translated into the clinic, it may be of limited value. On the other hand, it may not always be useful to implement PK models in the clinic. For example, tailoring the dose to correct for changes in PK and therefore exposure may not be very beneficial if the relation between the concentration and effect (or side-effects) is unknown, or in case of a wide therapeutic window, which allows for a large range of acceptable concentrations. Indeed, other factors can play a role like limited availability of a drug, and/or costs to avoid spillage and financial burden. Additionally, identified covariates which result in altered PK which require dose adjustments should be available in clinical routine. For example, a dose adjustment based on the changes in patients' albumin levels, the clinician should have access to the albumin levels of that patient. If these covariates consist of ex-

otic measurements, it would not be feasible to use this in the daily clinic.

*Prednisolone*

The glucocorticoid prednisolone is used for its antileukemic properties which is caused by the induction of apoptosis in malignant cells.[13] The glucocorticoids prednisolone and dexamethasone form the backbone of the treatment, and the response to a prephase with prednisolone is an important prognostic indicator in ALL.[14–16] However, to date, limited studies are available on the pharmacokinetics of glucocorticoids in pediatric ALL patients. Many studies have addressed the *in vitro* cellular prednisolone sensitivity/resistance, but very little is known about the *in vivo* relationship between prednisolone dose, concentration and leukemic response.[17–19] A large variability (up to a tenfold) in prednisolone concentrations and PK parameters has been observed between patients after receiving the same body surface adjusted dose, similar to the variability in dexamethasone.[20–22] Studies showed a higher weight normalized clearance of dexamethasone in younger children which could lead to lower exposure in this group.[20,21,23] In chapter five the PK/PD of prednisolone was studied to determine whether the exposure is age related and whether young children require higher dosages to obtain similar exposure to older children. Additionally, the popPK model was used to identify other causes of variability that could affect clearance and exposure via covariate analysis. Subsequently the relation between exposure and outcome treatment outcome was evaluated, hence whether patients or subset of patients could benefit from individualized dosing.

A population PK model was developed to describe the concentrations-time relation during steady-state treatment of prednisolone in individual patients using NONMEM®. A complicating factor for the PK analysis was the presence of nonlinear binding of prednisolone to plasma proteins, which has also been previously described.[20,24,25] This phenomenon was also observed in our study. Moreover, the prednisolone binding was found have a negative correlation with age. Studying the unbound concentration is important, as only free, unbound drug exerts pharmacological activity. Prednisolone is highly bound at lower concentrations and protein binding decreases with increasing concentrations (the range observed in our study was 71%-99%).[20,24] Especially with a high percentage of protein binding, small changes can have large effects. If 99% of the drug is bound to plasma proteins, a change to 97% means tripling the amount of unbound pharmacologically active drug. In contrast to low protein bound drugs, where a change from 30% to 28% has far less of an impact. Protein binding can vary within patients and between patients, for example

due to differences in plasma protein concentrations or comedication.

The PK model for prednisolone, as described in chapter five, describes the binding of prednisolone to plasma proteins albumin and corticosteroid binding globulin (CBG). Describing the drug protein binding requires knowledge of the amount of available protein (protein concentration) and the binding affinity (affinity constant of drug and protein). However, only the patients' albumin concentrations were available. The concentration of CBG and the affinity of prednisolone to CBG and albumin were not measured during the study, and were either fixed to values reported in the literature (affinity constants), or estimated in the PK model (CBG concentration). For future research it is recommended that CBG concentrations are measured as well.

After the constructing of the PK model, the exposure to unbound prednisolone of the individual patients was determined and correlated to disease parameters and outcome. Overall, the total exposure to unbound prednisolone over the age range did not differ, despite the negative correlation between plasma protein binding and age, which might have been negated by higher initial concentrations in younger patients. The exposure was not significantly different for patients with poor prednisone response (PPR) versus patients with good prednisone response (PGR). Neither were statistically significant differences found in unbound prednisolone exposure and relapse or survival. However, all patients with relapse were in either the lowest quartile (n=4) or mid quartiles (n=5) of exposure and none in the highest quartile. The patients in the high-risk group had a slightly lower median exposure of 477 ng*h/ml compared to 593 and 531 ng*h/ml for respectively standard and medium risk, but this was not statistically significant (p=0.2). However, the total number of poor responders (n=8), patients in the high-risk group (n=10) and patients who relapsed (n=9) were small. The study was therefore underpowered to estimate whether the small differences in exposure levels were significantly different and/or clinically relevant. Without a strong correlation between dose-concentration-effect, adjustments of the therapy based on pharmacokinetics will most likely not be very beneficial. The majority (>90%) of the patients in this study responded well to prednisolone and exposure might achieve such levels that the fluctuations in PK and concentrations do not affect clinical outcome. Whether the more glucocorticoid resistant subtypes could be sensitized by increasing steroid exposure is not clear as these groups were too small.

Dexamethasone is often used in pediatric ALL due to its higher potency compared to pred-

nisolone. Kaspers *et al.* showed about sixteen fold higher *in vitro* LC50 concentrations (lethal to 50% of ALL cells) for prednisolone compared to dexamethasone, although the LC50 was highly variable.[26] A wide range of equivalent concentrations for dexamethasone to prednisolone has been published in the literature. This ranged from fivefold to over sixteen fold higher concentrations of prednisolone to establish a similar effect.[26,27] Möricke *et al.* found that T-ALL patients with good prednisone response treated with in the 10 mg/m$^2$ dexamethasone compared to 60 mg/m$^2$ prednisolone had better event-free survival, relapse reduction and overall survival (ratio 1:6). However, the dexamethasone arm also showed a higher incidence of induction-related treatment deaths and may result in severe complications such as osteonecrosis.[28] Mitchell *et al.* showed an improved event-free survival with 6.5 mg/m$^2$ dexamethasone compared to 40 mg/m$^2$ prednisolone but not in overall survival (ratio circa 1:6). However, the incidence of toxicity was also significantly higher in the dexamethasone group.[29] Although prednisolone and dexamethasone are both glucocorticoids used for their antileukemic activity, there are distinct differences in PK/PD. Prednisolone has a higher binding to plasma proteins compared to dexamethasone and a nonlinear binding, which dexamethasone has not.[30] Dexamethasone has a longer biological half-life resulting in a prolonged effect compared to prednisolone.[27,30] Dexamethasone has higher penetration into CSF probably related to lower plasma protein binding and decreased isolated CNS relapse.[29,30] Taken all of this into consideration a sixfold dose of prednisolone (60 mg/m$^2$) over dexamethasone (10 mg/m$^2$) might be less potent due to relative higher plasma binding, shorter half-life and possibly a lower potency than a 1:6 ratio. This might explain the better response but also higher incidence toxicities of dexamethasone in the study of Möricke *et al.* and Mitchell *et al.* using this one to six ratio.[28,29] Domenech *et al.* showed that dexamethasone at 6 mg/m$^2$ was equally effective as induction therapy compared to 60 mg/m$^2$ of prednisolone (1:10 ratio) except for incidence of central nervous system relapse. The incidence of the toxicity in both groups was similar as well.[31]

Additionally, increased rates of osteonecrosis have been found in pediatric patients with ALL due to treatment with corticosteriods.[32–35] Lower exposure to corticosteroids might reduce these long term effects. However, lower exposure should not be detrimental to the anti-leukemic effect of the corticosteroids and might only apply to patients with good sensitivity to steroid treatment. It could be studied whether lower exposure in steroid sensitive patients is noninferior to standard exposure while reducing side effects. Only one infant was available for the PK analysis in our ciprofloxacin and prednisolone studies. Infants can exhibit vastly different PK compared to older patient hence it should be studied whether dose should be adjusted in this group of patients.

Future research might look into the hard-to-treat subpopulations of ALL (like poor prednisone responders) and whether a correlation can be found between exposure and clinical outcome in this subset of patients. If this correlation exists, patients might benefit from higher dosages. On the other hand, as previously stated, patients with good response might currently achieve higher concentration than necessary. These patients might benefit from lower exposure, with possible fewer side effects without compromising the anti-leukemic effect.

*Erwinia asparaginase*

In chapter three the PK of Erwinia asparaginase was studied. *Erwinia* asparaginase (derived from the *Erwinia* chrysanthemi bacteria) is a chemotherapeutic agent, which is used for the depletion of the amino acid asparagine. Leukemic cells require extracellular asparagine because, in contrast to healthy cells, leukemic cell cannot synthetize asparagine. *Erwinia* asparaginase is indicated if patients develop hypersensitivity to the *E. coli* derived PEGylated asparaginase. To ensure complete asparagine depletion, trough concentrations of asparaginase should be above the threshold of 100 IU/L.[36–38] Currently, the *Erwinia* asparaginase starting dose is 20,000 IU/m$^2$ and is administered at a two or three daily interval. If necessary, dose adjustments are done based on the measured trough concentration. A population PK model was developed to describe the PK of *Erwinia* asparaginase in order to better predict the *Erwinia* asparaginase concentrations and to evaluate the current starting dose with respect to trough concentrations and target threshold. The PK model was built using trough samples which were collected in context of standard clinical care.

In contrast to PEGylated *E. coli* asparaginase, which exhibits time-dependent pharmacokinetics where clearance changes over time, *Erwinia* asparaginase exhibits linear PK.[39] A two-compartment PK model with allometric scaling was developed to predict *Erwinia* asparaginase trough concentrations. A decreased clearance was observed later in the treatment compared to the first month of treatment, resulting in higher concentrations of *Erwinia* asparaginase after the first month. Subsequently, the PK model was used for simulations to predict the trough concentrations in different patients and whether the target threshold was achieved. The simulations showed that patients with low weight might require higher starting dosages to achieve similar concentrations compared to patients with higher weight.

The question remains whether to increase the starting dose for patients with a lower body weight. A higher starting dose for all patients might result in unnecessarily high concentrations in

a large group of patients and unnecessary use of *Erwinia* asparaginase which is scarce and expensive. Also, the clearance in the first month was higher, therefore concentrations will increase later in the treatment without increasing the dose. Currently the target trough level is 100 IU/L, however studies have shown complete asparagine depletion at lower concentrations.[38,40,41] It would be interesting for future studies to study whether total asparagine depletion is achieved for patients with trough concentrations below 100 IU/L (for example during routine TDM). Considering the administration interval, costs and scarcity of *Erwinia* asparaginase the place of *Erwinia* asparaginase will most likely be exclusively for patients with silent inactivation or allergic reactions to PEGylated asparaginase.

*Ciprofloxacin*

The antibiotic ciprofloxacin is used as prophylaxis to prevent infections with gram-negative bacteria in this immunocompromised population. Studies have shown the effectiveness of antimicrobial prophylaxis, however there is no specific PK/PD target concerning the prophylactic dose.[42–44] Therefore, during prophylactic treatment a therapeutic target level is used, e.g a ratio of $AUC_{24}$/MIC of ≥125.[45,46] The ratio reflects the 24-hour exposure to ciprofloxacin (area under the curve; AUC) over the minimal inhibitory concentration (MIC). Besides proficient prophylaxis to prevent infections, the emergence of microbial resistance to antibiotics is an area of concern as this will limit treatment options. Successful treatment with antibiotics depends mainly on the achieved concentration in conjunction with the sensitivity of the bacteria for the antibiotic. The importance of concentrations is not limited to growth inhibition of the bacteria but also to avoid enrichment of resistant mutant subpopulations selectively (in the mutant selection window).[47–49] Hence, studying the PK and assessment of the exposure to antibiotics is valuable. In chapter four the PK of ciprofloxacin was studied in pediatric ALL patients to evaluate potential factors affecting the PK of ciprofloxacin and whether the target $AUC_{24}$/MIC was achieved.

In the developed population PK model associations were found between clearance and glomerular filtration rate, bilirubin and age. However, none of these associations reached statistical significance. In the final model, one factor that remained was the difference in PK for different treatment phases. Exposure to ciprofloxacin showed a two-fold increase throughout the treatment from induction (prior to chemotherapy) to periods with concomitant chemotherapy. This increase in exposure was also observed for prednisolone and *Erwinia* asparaginase albeit to a lesser extent. Different treatment phases consist of different combinations of chemotherapy which could alter

PK through drug interactions. However, a subanalysis for comedication was performed and could not clearly explain the difference in treatment phase. Hence, other factors are involved which affect the PK and therefore the concentrations in the different treatment phases besides the tested covariates for example, feeding, fluid intake, mucositis, infections, disease state or a combination of these.

The PK model was used for Monte Carlo simulations in order to estimate whether patients reached target threshold ratio of $AUC_{24}$/MIC. Simulations of 15 mg/kg with a maximum of 500 mg were performed for different MIC values in a range of 0.0156 to 1 mg/L. Only 38% of the patients in the first treatment phase and 1% in the second treatment phase achieved target ratio for bacteria with a MIC value of 0.25 mg/L, which is still considered susceptible. This was also found in other studies.[46,50,51] It could be that the target is higher than clinically necessary, as the optimal dose and target for ciprofloxacin prophylaxis are not known. Studies have shown the effectiveness of antimicrobial prophylaxis with fluoroquinolones in pediatric leukemia.[42–44] The required $AUC_{24}$/MIC have been shown to differ between strains and fluoroquinolones.[47,49] Felsenstein *et al.* observed a significant reduction in infections caused by gram-negative rods but a higher proportion of gram-positive bacterial and fungal infection with ciprofloxacin in pediatric AML patients.[52] Sung *et al.* used levofloxacin with higher gram-positive sensitivity.[42]

The concentrations are measured in plasma; however, the concentration differs at the site of infection or target pharyngal and fecal flora, which might lead to emergence of resistance.[53,54] Fantin *et al.* studied the emergence of resistance in fecal flora and pharyngeal flora and found emergence of resistance mainly when local concentrations were below MIC, but they did not find significant differences in plasma exposure between subjects in whom resistance was selected versus no resistance.[54] In our study, we did not find a correlation between ciprofloxacin exposure and emergence of resistance in fecal and throat swabs in overall treatment. However, in the first week of prophylaxis, which was the period with the lowest exposure, a significant correlation was found between exposure (AUC) and development of resistant gram-negative bacteria ($p=0.024$; $n=12$). Whether the target $AUC_{24}$/MIC ratio was achieved in these patients cannot be stated with certainty as exact MIC values were not available. Based on the simulations target ratios will be achieved in 65% and 1% of the patients in the first week of treatment compared to 81% and 18% later in treatment for MIC values of 0.125 and 0.25 mg/L respectively.

For future studies it would be interesting to study the concentrations at sites other than plasma to gain better understanding of the antibiotic exposure to the bacteria locally and bacterial response. Also, the target for prophylactic dose regimen remains unclear and whether this can or should be different from the therapeutic dosing regimen, not only to prevent infections but also to avoid selection of resistant mutants. The prophylactic treatment with ciprofloxacin in pediatric ALL is given for prolonged periods of time due to recurrent neutropenia and increased risk of infections. However, the musculoskeletal safety of ciprofloxacin has not been studied in this setting. Irreversible cartilage toxicity has been observed in animal studies, especially in weight baring joints and was therefore contra-indicated in patients under the age of 18 years.[55–59] Currently a study is being performed (Dutch Trial Registry nr NTR3623) comparing the cartilage of the knee of patients who received ciprofloxacin prophylaxis during pediatric ALL treatment compared to ciprofloxacin naive patients.

*Conclusion and future perspectives*

The studies in this thesis demonstrate how PK/PD research can be applied to gather insight in therapy and to optimize drug therapy in pediatric ALL patients. All studies in this thesis used limited sampling with mostly two to four samples per patient in a treatment phase. Samples were taken at flexible times therefore limiting clinical interference. Blood sampling volume was limited to only 250 $\mu$l to determine both total and unbound concentrations by using liquid chromatography tandem mass spectrometry (LC-MS/MS). With limited sampling both in numbers and volume we were able to assess the pharmacokinetics, to evaluate covariates to explain variability in concentrations and to relate the exposure to the effects. Our studies support the use of population PK analysis as the ideal method for PK/PD studies in children.

One recurrent variable affecting the PK to a significant effect in all studied medication in this thesis was treatment phase. This might indicate dose adjustments throughout treatment. This is not surprising as patients receive different combinations of medication during treatment and disease state over time as well. However, medication is often not adjusted during treatment other than due to toxicities or when therapeutic drug monitoring guided dosing is indicated. Hence, in addition to differences in PK and the ensuing dose adjustments between patients, adjustments within a patient throughout the treatment might be recommended as well. Identification of the underlying causality of PK alterations throughout treatment are not yet clarified and require further research.

Could patients benefit from dose adjustments or individualized dosing? Large differences in exposure does not necessarily mean PK based dosing would be beneficial, for example if the concentrations remain within the therapeutic window or if the concentration-effect relationship is unknown. Candidates for PK guided dosing would be drugs with a small therapeutic window, exhibiting large variability (between and within patients) and exhibit highly unpredictable pharmacokinetics (like saturation processes). If a drug exhibits high interindividual variability and low intraindividual variability, one sample may suffice to determine the optimal dose each for the individual patient with a good prediction model. Covariates which are identified in the covariate analysis could serve as a correction factor when dosing medication. For example, if younger patients have a higher clearance, age may be used to adjust the dose. In this case younger children might benefit from an increased dose. Other factors which could be considered are inflammation, comedication, disease severity, renal function, genetic mutations, treatment phase, etc. *Erwinia* asparaginase is currently adjusted based on trough concentrations and could benefit from a population prediction model, where better predictions can be made to determine which dose is required to reach target trough concentrations for each individual patient and without using unnecessary amounts of *Erwinia* asparaginase. PK based dosing could also aid the dosing of ciprofloxacin to achieve concentrations above MIC and outside the mutant selection window. However, the PK/PD target, especially for prophylactic dosing, is unclear, and we did not see emergence of resistance in patients who did not achieve the current target $AUC_{24}$/MIC of ≥125. Lower targets and therefore exposure might suffice and might avoid possible (long term) side effects. Prednisolone however did not show a correlation between exposure and effect with the current dosing regimen. In general PK based dosing of prednisolone might not prove to be beneficial. However, future studies into the concentration-response relation in steroid-resistant subpopulations (PK/PD interaction) might show otherwise for those specific groups.

PK analysis comes with disadvantages as well. The complexity of the analysis makes it difficult to perform, evaluate and translate prior to implementation into the clinic. PK models have to be validated prior to use in a new population. Often the exact model (code of the control stream) that was used for the publication is not available and the model has to be rebuild based on the reported parameters and description, which becomes more difficult with increasing model complexity. Extrapolation of PK models from one population to another has to be done with care. Before using a PK prediction model an external validation with an independent dataset should be performed

in order to evaluate whether it is suitable for the target population. Large differences can occur if the population used for model development is different from the population of its intended use like critically ill versus healthy patients, different ethnical groups and children versus adults.[60]

Physiologically based models or semi mechanistic models might be the next step in patient medication simulation. Physiology based PK (PB/PK) predicts exposure in different parts of the body using predefined multi-compartment models based on anatomical and physiological parameters (e.g., organ volume and composition, metabolism, perfusion, transporters) mostly connected via blood flows.[61,62] This is in contrast to the standard compartmental PK modeling where the compartments do not reflect an actual anatomical or physiological structure. The physicochemical properties of studied substances like lipophilicity, molecular weight, pH dependency, are used to predict the behavior of the substance within the body. These types of analyses require highly sophisticated models. Models are indeed a simplified version of the numerous possible variables however with the increasing complexity of the model the predictive power continues to improve. Currently physiology-based PK (PB/PK) software is available like Simcyp™[Certara] and PK-sim® [Bayer] to simulate different concentrations in different organs using a variety of libraries of anatomical and physiological parameters, drug properties, ADME processes, ontogeny, demographics, etc. PB/PK can be very useful for example in first-in-human studies, formulation design, drug-drug interactions, effect of intrinsic and extrinsic factors, and dose stratification for different populations.[63] Another technique that might become more prevalent is machine learning which can be used for example in the identification of risk factors in a complex tumor microenvironment relying on multiple interactions between genomic mutations.[64,65] The computational power enables these kinds of techniques that were previously unfeasible and might open up a new level of knowledge.

Due to regulatory changes and incentives studies (like the requirement of pediatric investigation plans for the approval of new drugs) more studies in children are performed. Previously, dosing regimens for children were generally extrapolated from their use in adults, which often assumed equal PK in children and adults. Due to the incremental availability of PK studies and knowledge, we know now that the this is often not the case and decreasing the dose purely on the size of the child might not result in optimal treatment. Medication becomes more precise with respect to drug targets; however, this should also apply to dosing regimen; precision medicine with precision dosing. Precision dosing still has a long way to go when considering all factors that

can influence the pharmacokinetics and pharmacodynamics of a drug. Every patient is different and every situation is unique, ideally so would be their treatment. Implementation of dose adjustments based on renal function or genotype polymorphism is not uncommon currently. However, this is only a fraction of what is possible. With all the research being done our knowledge will increase and hopefully we will be able to develop prediction models with the ability to tailor each treatment to each individual patients with high efficacy and efficiency.

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# Chapter 7

## Summary/Samenvatting

## Summary

Despite the cure rate, which has increased from a five-year overall survival rate of 10-30% in the seventies to over 90% in recent years, cancer remains the leading cause of death due to disease in children.[1–3] Acute lymphoblastic leukemia (ALL) is the most common form of childhood malignancy with an incidence of two to four cases per 100,000 person years in children and young adults of 0-19 years old.[4,5] Treatment has undergone major improvement as reflected in the overall survival rate and has become more adjusted to the individual, for example based on risk adaptation.[5,6] Every patient is unique and therefore the principle of "one dose/treatment fits all" does seem sub-optimal. ALL treatment consists of combinations of chemotherapeutic agents with supportive care. Adequate exposure to the agents is of utmost importance in order to achieve optimal efficacy and avoid toxicities. However, few studies are available on the dose-concentration and concentration-response relationships in this group of patients. This thesis focused on the pharmacokinetics (PK) and pharmacodynamics (PD) of chemotherapeutic agents and supportive care medicine currently used in the treatment of pediatric ALL. In the performed PK/PD studies the relationships between dose, concentration and effects was assessed, to gather insight in the current treatment and whether patients or subgroups of patients could benefit from individualized dosing in order to further optimize treatment.[7–9]

Due to the mathematical complexity PK/PD research study results are often difficult to interpret and to translate into the daily clinical care. Chapter two focused on how PK/PD research is currently involved in pediatric oncology and why population PK (popPK) is probably the ideal method to study the dose concentration relation in this group of patients. Classic PK research often required dense sampling at fixed times which can interfere with the daily clinical care. However, population PK is very flexible and works well with sparse sampling, random time points and heterogeneous data. This chapter describes how popPK is able to use limited sampling (as little as two to four per patient) to calculate typical PK parameters while retaining information about the individual and the quantification and identification of variability between and within patients. The chapter provides common terminology and concepts often observed in popPK research in order to improve the understanding of popPK research and therefore facilitate the implementation of popPK research into daily clinical care.

In chapter three the popPK approach was applied to study the PK of *Erwinia* asparaginase in

pediatric ALL patients. Asparaginase is an important component in the treatment where the *E. coli* derived PEG-asparaginase is the first choice. However, some patients develop hypersensitivity to PEG-asparaginase or have silent inactivation and switch to *Erwinia* asparaginase.[10–12] Large variability in serum concentrations has been observed and dose adjustments are made based on trough concentrations in order to ensure adequate asparaginase activity (trough concentrations ≥100 IU/L). Little is known about the PK of intravenous *Erwinia* asparaginase, which differs from the other forms of asparaginase.[13–18] In a multi-center prospective study 714 blood samples were collected from 51 children (1-17 years) with ALL. A popPK model was developed to evaluate the starting dose of 20,000 IU/m$^2$ thrice weekly. A two-compartment linear model with allometric scaling best described the data. The interindividual variability (IIV) and inter-occasion variability (IOV) of clearance were respectively 33% and 13%. The clearance in the first month of treatment was 14% higher ($p<0.01$), results in higher concentrations after the first month. The PK model was used to perform Monte Carlo simulations which showed that patients with a low weight require higher dosages to achieve similar concentrations compared to patients with high weight. The current starting dose of 20,000 IU/m$^2$ might result in inadequate concentrations especially for the smaller patients and dose adjustments based on individual derived clearance is recommended.

Ciprofloxacin is used as antimicrobial prophylaxis in pediatric ALL to prevent infections with gram-negative bacteria. However, there are no clear guidelines concerning the prophylactic use. In chapter four the PK of ciprofloxacin is studied and a popPK model was developed to determine the individual exposure. With the use of the PK model it was evaluated whether the target area under the concentration time curve over minimal inhibitory concentration ($AUC_{24}$/MIC) ratio of ≥125 was achieved in all patients.[19–21] Additionally, the emergence of resistance was studied during prophylactic treatment. A total of 615 samples were collected from 129 children with ALL in the age range of 0-18 years old in a multicenter prospective study. A one-compartment PK model with zero-order absorption and allometric scaling best described the data. No significant covariates were identified ($p<0.01$). A significant difference was found in the AUC between the first week of treatment versus later in the treatment with concomitant chemotherapy, respectively 16.9 mg*h/L versus 29.3 mg*h/l. Overall, 100%, 81% and 18% of the patients achieved target $AUC_{24}$/MIC ratios for MIC values of respectively 0.063, 0.125 and 0.25 mg/L. The prophylactic treatment seems to be adequate with limited emergence of resistance and few bacteremia. If the current $AUC_{24}$/MIC ratio of 125 is correct, the MIC cut-off of 0.25 mg/L might be too high for

prophylactic treatment as only 18% of the patients achieved the target ratio. However, if the treatment suffices for MIC 0.25 mg/L as well, the target ratio might be too high. Therapeutic drug monitoring might be recommended with increasing MIC values in order to achieve sufficient exposure and prevent emergence of resistance.

Glucocorticoids are the backbone of the treatment in pediatric ALL. Many studies have been performed on the PD aspects of steroid resistance and sensitivity.[22–26] However, the knowledge concerning the PK parameters and its relation to response is very limited. Studies showed increased weight-normalized clearance in younger children, which raises the question whether younger children might benefit from higher dosages.[27–29] Additionally, asparaginase was shown to potentially alter the PK of glucocorticoids. Hence, the PK of prednisolone was studied and its relation with clinical outcome in chapter five.[27] A total of 1028 total and unbound prednisolone plasma concentrations were available from 124 children with ALL in the age range of 0-18 years old. Prednisolone exhibits nonlinear PK where plasma protein binding depends on prednisolone concentration.[28,30,31] Hence, both total and unbound prednisolone was studied. The PK was best described with allometric scaling and both linear and saturable binding to plasma proteins. Plasma protein binding decreased with age. However, the overall AUC did not differ between younger and older children. No effect of exposure clinical treatment outcome parameters was observed. The apparent volume of distribution ($V$) was smaller in treatment phases with concomitant chemotherapy (>week 1), which might be due to hyperhydration in the first week of treatment to avoid tumor lysis syndrome and no asparaginase. Also, a positive correlation between ASAT and $V$ was observed. In this study no correlation was found between the exposure and clinical treatment outcome. Poor responders, high risk and relapsed patients showed a trend towards lower exposure compared to good responders. However, the group of poor responders was small and requires further research.

The studies performed within the scope of this thesis show that PK/PD studies are very well feasibly in pediatric oncology patients. We have shown that with limited and flexible sampling both in numbers and volume we were able to develop popPK models, quantify variability, identify covariates in order to understand inter- and intrapatient variability, and correlate the exposure to the effects. A recurrent covariate in all analyses was treatment phase. Large differences were observed in PK parameters and therefore exposure throughout treatment. *Erwinia* asparaginase concentrations were lower in the first month, ciprofloxacin exposure was almost half of the exposure later in treatment similar to prednisolone where lower concentrations were observed in the

first week of treatment. Whether individualized dose adjustments are recommended depends on the correlation with the effect. Overall PK/PD studies can provide valuable information to fine tune individual dosing in order to improve the treatment.

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# Samenvatting

Ondanks het genezingspercentage dat is gestegen van een algehele overlevingskans van 10-30% in de jaren zeventig tot meer dan 90% in de afgelopen jaren, blijft kanker de belangrijkste doodsoorzaak als gevolg van ziekte bij kinderen.[1–3] Acute lymfatische leukemie (ALL) is de meest voorkomende vorm van maligniteiten bij kinderen met een incidentie van twee tot vier gevallen per 100,000 persoonsjaren bij kinderen en jonge volwassenen in de leeftijd van 0-19 jaar.[4,5] De behandeling is door de jaren sterk verbeterd wat blijkt uit de algehele overlevingskans en is steeds meer aangepast aan het individu bijvoorbeeld door risico adaptatie.[5,6] Elke patiënt is uniek waardoor het principe van 'één dosis/behandeling voor iedereen' mogelijk kan leiden tot suboptimale therapie. De ALL-behandeling bestaat uit combinaties van chemotherapeutische middelen met supportive care zorg. Adequate blootstelling aan de middelen is essentieel om een optimale werkzaamheid te bereiken en toxiciteit te voorkomen. Er zijn echter weinig studies beschikbaar over de dosis-concentratie en concentratie-respons relaties bij deze groep patiënten. Vandaar dat dit proefschrift zich heeft gefocust op de farmacokinetiek (PK; de studie naar de relatie tussen dosis en concentratie) en farmacodynamiek (PD; de studie naar de relatie tussen concentratie en effect) van chemotherapeutische middelen en supportive care middelen die momenteel worden gebruikt bij de behandeling van ALL bij kinderen. In de uitgevoerde PK/PD-onderzoeken zijn de relaties tussen dosis, concentratie en effecten beoordeeld om inzicht te krijgen in de huidige behandeling en of patiënten of subgroepen van patiënten baat kunnen hebben bij geïndividualiseerde dosering ter verdere optimalisatie van de behandeling.[7–9]

Door de mathematische complexiteit zijn de resultaten van PK/PD onderzoek vaak moeilijk te interpreteren en te vertalen naar de dagelijkse klinische zorg. Hoofdstuk twee richtte zich derhalve op het huidige gebruik van PK/PD-onderzoek binnen de kinderoncologie en waarom populatie PK (popPK) de ideale methode is om de dosis-concentratierelatie in deze groep patiënten te bestuderen. Klassiek PK-onderzoek vereist vaak frequente bloedafnames op gezette tijdstippen die de dagelijkse klinische zorg kunnen verstoren. PopPK is echter zeer flexibel en werkt goed met schaarse bloedsamples, flexibele bloedafnametijden en heterogene data. In het hoofdstuk wordt uitgelegd hoe popPK in staat is om met beperkte steekproeven (slechts twee tot vier per patiënt) PK-parameters te berekenen met behoud van informatie over het individu en de kwantificering en identificatie van inter,- en intrapatiënt variabiliteit. Het hoofdstuk beschrijft de algemene termi-

nologie en concepten die vaak worden gebruikt in popPK-onderzoek om het begrip van popPK-onderzoek te verbeteren en zo de implementatie van popPK-onderzoek in de dagelijkse klinische zorg te vergemakkelijken.

In hoofdstuk drie werd de popPK benadering toegepast om de PK van *Erwinia* asparaginase bij pediatrische ALL-patiënten te bestuderen. Asparaginase is een belangrijk onderdeel van de behandeling waarbij de *E. coli* PEG-asparaginase de eerste keuze is. Echter sommige patiënten ontwikkelen overgevoeligheid voor PEG-asparaginase of hebben *'silent inactivation'* waardoor het middel niet meer werkt en worden om die reden overgezet op *Erwinia* asparaginase.[10–12] Asparaginase vertoont grote variabiliteit en derhalve worden er tijdens de therapie dosisaanpassingen gedaan op basis van dalspiegels om een adequate asparaginase-activiteit te bewerkstelligen (dalconcentraties ≥ 100 IE/L). Er is echter weinig bekend over de PK van intraveneuze *Erwinia* asparaginase, die verschilt van de andere vormen van asparaginase.[13–18] In een multicenter prospectief onderzoek werden 714 bloedmonsters verzameld bij 51 kinderen met ALL (1-17 jaar). Er is een popPK-model ontwikkeld om de startdosis van 20.000 IE/$m^2$ driemaal per week te evalueren. Een lineair model met twee compartimenten en allometrische schaling beschreef de gegevens het best. De interindividuele patiënt variabiliteit (IIV) en *'interoccasion'* variabiliteit (IOV; de variabiliteit tussen de verschillende momenten binnen de therapie) van de klaring waren respectievelijk 33% en 13%. De klaring was in de eerste maand van de behandeling 14% hoger (P<0,01), wat dus resulteert in hogere concentraties na de eerste maand van de behandeling. Het PK-model werd vervolgens gebruikt om Monte Carlo simulaties uit te voeren, waaruit bleek dat patiënten met een laag gewicht hogere doseringen nodig hebben om vergelijkbare concentraties te bereiken ten opzichte van met patiënten met een hoog gewicht. De huidige startdosis van 20,000 IE/$m^2$ kan leiden tot suboptimale concentraties, vooral voor de kleinere patiënten, en dosisaanpassingen op basis van individueel afgeleide klaring is aanbevolen.

Ciprofloxacine wordt gebruikt als antimicrobiële profylaxe bij kinderen met ALL om infecties met gramnegatieve bacteriën te voorkomen. Er zijn echter geen duidelijke richtlijnen voor profylactisch gebruik. In hoofdstuk vier werd de PK van ciprofloxacine bestudeerd en is een popPK-model ontwikkeld om de individuele blootstelling te bepalen. Met behulp van het PK-model werd geëvalueerd of de target van de blootstelling (het oppervlak onder de concentratie-tijdcurve) over de minimaal remmende concentratie ($AUC_{24}$/MIC) van ≥125 bij alle patiënten werd bereikt.[19–21] Daarnaast werd het ontstaan van resistentie tijdens profylactische behandeling bestudeerd. In een

multicenter prospectief onderzoek werden in totaal 615 monsters verzameld van 129 kinderen met ALL in de leeftijd van 0-18 jaar. Een PK-model met één compartiment, nulde-orde absorptie en allometrische schaling beschreef de data het best. Er werden geen significante covariabelen geïdentificeerd ($p<0,01$). Wel werd er een significant verschil gevonden in de AUC tussen de eerste week van behandeling ten opzichte van later in de behandeling met gelijktijdige chemotherapie, respectievelijk 16,9 mg*h/l versus 29,3 mg*h/l. In totaal bereikten 100%, 81% en 18% van de patiënten de beoogde $AUC_{24}$/MIC-ratio's voor MIC-waarden van respectievelijk 0,063; 0,125 en 0,25 mg/L. De profylactische behandeling lijkt effectief met beperkte opkomst van resistentie en weinig bacteriëmieën. Als de huidige $AUC_{24}$/MIC-ratio van 125 correct is, is de MIC-cut-off van 0,25 mg/L mogelijk te hoog voor profylactische behandeling, aangezien slechts 18% van de patiënten de beoogde ratio bereikte. Als de behandeling echter ook voldoende is voor MIC 0,25 mg/L, is de streefratio mogelijk te hoog zijn. Therapeutische medicatiecontrole kan worden aanbevolen bij toenemende MIC-waarden om voldoende blootstelling te bereiken en het ontstaan van resistentie te voorkomen.

Glucocorticoïden vormen de ruggengraat van de behandeling bij pediatrische ALL. Er zijn veel onderzoeken gedaan naar de PD-aspecten van steroïderesistentie en gevoeligheid.[22–26] Echter de kennis over de PK-parameters en de relatie met respons is zeer beperkt. Studies toonden een toegenomen gewicht genormaliseerde klaring aan bij jongere kinderen, wat de vraag doet rijzen of jonge kinderen baat kunnen hebben bij hogere doseringen.[27–29] Bovendien bleek asparaginase mogelijk de PK van glucocorticoïden te beïnvloeden. De farmacokinetiek van prednisolon en de relatie met de klinische uitkomst werd bestudeerd in hoofdstuk vijf.[27] In totaal waren er 1028 totale en ongebonden prednisolon plasmaconcentraties beschikbaar van 124 kinderen met ALL in de leeftijd van 0-18 jaar. Prednisolon vertoont niet-lineaire kinetiek waarbij de plasma-eiwitbinding afhangt van de prednisolon concentratie.[28,30,31] Om deze reden werd zowel totaal als ongebonden prednisolon bestudeerd. De PK werd het best beschreven met allometrische schaling en zowel lineaire als verzadigbare binding aan plasma-eiwitten. De plasma-eiwitbinding nam af met de leeftijd. De totale AUC verschilde echter niet tussen jongere en oudere kinderen. Het schijnbare distributievolume ($V$) was kleiner in behandelfasen met gelijktijdige chemotherapie (>week 1), wat te wijten kan zijn aan hyperhydratie in de eerste week van de behandeling om tumorlysesyndroom te voorkomen en in de eerste week wordt er geen asparaginase toegediend. Ook werd een positieve correlatie tussen ASAT en $V$ waargenomen. In deze studie werd geen correlatie gevonden tussen de blootstelling en de klinische behandeluitkomst. Patiënten met een slechte respons, een hoog

risico en recidiverende patiënten vertoonden een trend naar een lagere blootstelling in vergelijking met patiënten die goed reageerden, maar deze groepen waren klein en vereist verder onderzoek.

De onderzoeken die in het kader van dit proefschrift zijn uitgevoerd, laten zien dat PK/PD-onderzoeken zeer goed haalbaar zijn binnen de kinderoncologie. We hebben aangetoond dat we met beperkte en flexibele bloedafnames zowel in aantal als in volume in staat waren om popPK-modellen te ontwikkelen, variabiliteit te kwantificeren, covariaten te identificeren om de inter- en intra-patiëntvariabiliteit te begrijpen en de blootstelling aan de effecten te correleren. Een terugkerende covariabele in de analyses was de behandelfase. Er werden grote verschillen waargenomen in farmacokinetische parameters en blootstelling tijdens de duur van de behandeling. *Erwinia* asparaginaseconcentraties waren lager in de eerste maand, blootstelling aan ciprofloxacine was bijna de helft van de blootstelling in vergelijking met later in de behandeling. Dit was vergelijkbaar met prednisolon, waar lagere concentraties werden waargenomen in de eerste week van de behandeling. Of individuele dosisaanpassingen worden aanbevolen, hangt af van de correlatie met het effect. Kortom PK/PD-onderzoeken kunnen waardevolle informatie opleveren om de individuele dosering te verfijnen om de behandeling te verbeteren.

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## About the author

Sebastiaan Dinand Theodor Sassen was born on July 29th, 1984 in Gouda. After graduating secondary school (Coornhert Gymnasium) in 2002, he started studying Pharmacy at Utrecht University. During his studies he performed clinical research at the University of California San Diego Medical Center about the role selective dopamine 3 receptor in schizophrenia. During and after his studies he also worked at Research and Development department on the development of bone cement and surgical glue. After obtaining the degree of doctor of Pharmacy he started working in the Erasmus MC and NKFK on the development of the Dutch children's formulary (Kinderformularium). During his work he developed an interest in the pharmacokinetics and the role of computers in medical research and clinical practice. He got an opportunity to write grants and start his PhD research under the supervision of Prof. dr. C.M. Zwaan and Prof. dr. R.A.A. Mathôt at the department of pediatric oncology at the Erasmus Medical Center in Rotterdam and the department of hospital pharmacy at the Amsterdam Medical Center in Amsterdam. From 2017 to 2020 he worked at a medical software company as head of clinical affairs. Currently he is working as a pharmacometrician at the department of hospital pharmacy at the Erasmus Medical Center in Rotterdam.

| | Year | Workload (ECTS) |
|---|---|---|
| **General courses** | | |
| Research integrity | 2015 | 0.3 |
| Research management | 2014 | 1.0 |
| BROK | 2012 | 1.0 |
| BROK herregistratie | 2016 | 1.0 |
| **Specific courses** | | |
| Basic course on R | 2013 | 0.7 |
| Basic and Translational oncology | 2013 | 1.8 |
| Course Graphpad Prism | 2015 | 0.3 |
| MRI safety course | 2013 | 0.3 |
| Matlab+Sparck | 2014-16 | 1.0 |
| NONMEM | 2012-16 | 2.0 |
| Python course | 2016 | 1.0 |
| **Seminars and workshops** | | |
| NONMEM meeting (AMC) | 2012-16 | 2.0 |
| Pharmacology meeting (EMC) | 2012-13 | 1.0 |
| Pharmacometrics Network Benelux | 2013 | 1.0 |
| Sophia research days | 2014 | 1.0 |
| Pharmacometrics Leiden | 2015 | 1.0 |
| Promeras PhD days | 2013 | 1.0 |
| Qcat | 2014 | 1.0 |
| **Presentations** | | |
| FIGON DMD (poster) | 2015 | 1.0 |
| Masterclass ALL SKION days (oral) | 2013 | 1.0 |
| KiKa/Go4Children site visit (oral) | 2014 | 1.0 |

| | Year | Workload (ECTS) |
|---|---|---|
| SKION retreat (oral) | 2015 | 1.0 |
| Presentation Onco minor | 2016 | 1.0 |
| NONMEM meeting (oral) | 2012-16 | 4.0 |
| ASH Haematology Orlando, FL | 2015 | 1.0 |
| **Teaching** | | |
| Liaison NIH Principles of clinical pharmacology | 2012-13 | 2.0 (56h) |
| Lecture for medical students | 2016 | 0.5 |
| **Total** | | 30.9 |