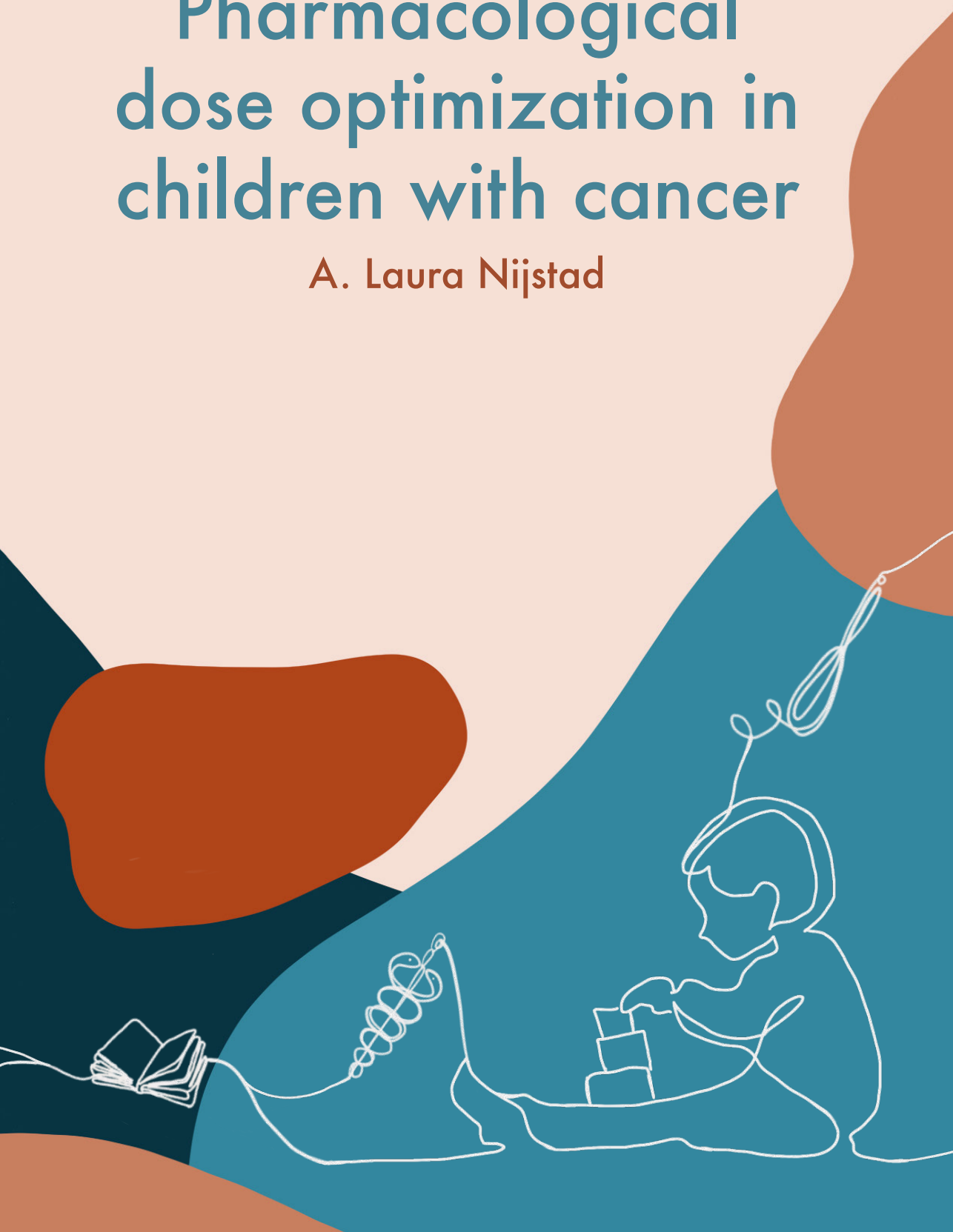
Pharmacological dose optimization in children with cancer

A. Laura Nijstad



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Farmacologische dosisoptimalisatie in kinderen met kanker

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. H.R.B.M. Kummeling, ingevolge het besluit van het college voor promoties, in het openbaar te verdedigen op donderdag 13 oktober 2022 des middags te 2.15 uur

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Promotoren:	Prof. dr. A.D.R. Huitema Prof. dr. C.M. Zwaan
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If your heart is in your dream,
no request is too extreme

- Jiminy Cricket

CONTENT

Preface & Thesis outline

PREFACE

Every year, 550-600 children in the Netherlands and approximately 400.000 children worldwide are diagnosed with cancer. About 10% of them is younger than 1 year of age. Types of cancer that occur in children differ from those seen in adults, and are divided in solid tumors, brain tumors and hematological malignancies. Leukemia and brain and other central nervous system tumors are the most common cancer types in children, followed by neuroblastoma, Wilms tumor and non-Hodgkin lymphoma.[1–4] Survival rates of children with cancer have been improved to 80% the past years by intensifying therapy, but this is hampered by the risk of toxicity. Moreover, the rates vary widely between tumor type and age category.[5]

Pediatric patients with cancer are treated with chemotherapy, and/or surgery, and/or radiation, depending on the disease and treatment protocol. One of the most common side effects during treatment is chemotherapy induced nausea and vomiting. To control for these side effects, patients are additionally treated with anti-emetics such as 5-HT_3 receptor antagonists, dexamethasone and aprepitant, but the anti-emetic response to these agents is worse than in adults.[6]

Chemotherapeutic and anti-emetic treatment of neonates, infants and older children can be challenging due to physiological differences in terms of physical size, body composition, physiology and biochemistry between neonates and infants compared to older children. These developmental changes can influence the drug absorption, distribution, metabolism and excretion (ADME, known as pharmacokinetics (PK)), see **Figure 1,** and need to be taken into account when establishing dosing strategies for children of different ages.[7–11] Moreover, age can influence the pharmacodynamics of a drug, for example in terms of toxicity. Younger patients might be more sensitive for side effects, which should be considered when developing dosing strategies for children as well.

Figure 1. Developmental changes that can influence the drug absorption, distribution, metabolism and excretion (ADME)

Precision medicine, the science of finding the right drug and dose for each patient, is especially important in pediatric healthcare. Clinical data usually originate from studies in adults, and are extrapolated to the pediatric population. However, knowledge on age-related differences in ADME is needed to derive an accurate dose for all age groups. Without this information, there is a risk of under- or overdosing the patients, which increases the risk of ineffective treatment or severe side effects, respectively. Several commonly used drugs, for which the pediatric dose is not linearly correlated with body weight, have been identified in the past decades.[12] For example, when chloramphenicol is linearly scaled according to body weight, a 5-fold higher exposure in neonates is observed as compared to adults, resulting in severe side effects. For carbamazepine, on the other hand, higher relative doses for children are needed to achieve comparable exposure as adults. Clinical pharmacological knowledge can be useful to understand such age-related differences between the age groups.

In 2007 the European Medicines Agency (EMA) introduced new guidelines for drug development for pediatric purposes. Since then, a pediatric investigation plan (PIP), ensuring that data from studies in children is obtained, is necessary for marketing authorisation of new drugs, new indications or new pharmaceutical forms of a drug, if a disease also occurs in children.[13] The purpose of a PIP is to support the approval of a drug in children and it supports data collection on age-related differences in ADME. Introduction of PIP's brought an increase in clinical trials in children, new pediatric drugs, -formulations and/or -indications, that will lead to the development of adequate pediatric dosing guidelines. However, it has been shown that the use of PIP's in hemato-oncology still needs improvement. Out of 48 new drugs with hemato-oncological indications that have been marketed since 2016, only 20 drugs did have a PIP, and none of them were completed within in the agreed

study period.[14] Moreover, for drugs that were marketed before establishing the guideline, age-related information on PK and pharmacodynamics is often lacking.[15] Population PK- and physiologically based PK studies, using real life collected PK data, can offer a solution for understanding the age-related differences in PK for those, frequently off-label used, drugs.

THESIS OUTLINE

This thesis focuses on age-related differences in PK and pharmacological dose optimization of chemotherapeutic (**Part I**) and anti-emetic (**Part II**) agents in (young) children with cancer.

Part I outlines the PK of chemotherapeutic agents in pediatric patients. **Chapter 1** describes the case of a pediatric patient with hepatoblastoma, receiving peritoneal dialysis. This case underlines the relevance of PK research in pediatric patients. In **Chapter 2**, clinical pharmacological evidence for the use of chemotherapeutic agents in neonates and infants is summarized and practical, evidence based dosing guidance is presented. This literature review highlights chemotherapeutics where dose guidance can be provided based on available evidence, as well as chemotherapeutics where evidence is lacking. **Chapter 3** discusses the population PK of clofarabine for allogeneic hematopoietic cell transplantation in pediatric patients. In **Chapter 4,** the PK of vincristine and its saturable binding to β-tubulin is described using a population PK model. A physiologically based PK model of vincristine is presented in **Chapter 5**. In this chapter, vincristine binding to blood cells and the age-dependent differences in the binding capacity between patients was presented. In **Chapter 6** a population PK modelling approach was used to compare several doxorubicin dosing regimens for young children.

Part II focuses on the PK of the anti-emetics agents, aprepitant and dexamethasone, in children with cancer. **Chapter 7** describes the development of a combined liquid chromatography tandem-mass spectrometry assay for the quantification of aprepitant and dexamethasone. This assay is used to support PK studies in pediatric patients. In **Chapter 8,** the PK parameters of a simple extemporaneous oral suspension of aprepitant, which yields sufficient exposure in children, are presented. This extemporaneous suspension was used during the period that the commercial suspension was unavailable worldwide. The effect of aprepitant on dexamethasone PK in children, is investigated in **Chapter 9**. This chapter focuses on the exposure to dexamethasone, in order to optimize dexamethasone dosing guidelines for pediatric patients when used concomitantly with aprepitant.

In summary, this thesis describes the clinical pharmacology of chemotherapeutic agents and anti-emetics in pediatric patients, with the aim of optimizing the doses for individual patients.

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PART I

Pharmacokinetics of chemotherapeutic agents in pediatric patients

1

Cisplatin and carboplatin pharmacokinetics in a pediatric patient with hepatoblastoma receiving peritoneal dialysis

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ABSTRACT

Purpose

Cisplatin and carboplatin are frequently used drugs in the treatment of pediatric hepatoblastoma. Dosing guidelines for these drugs in children requiring peritoneal dialysis are lacking. Here we describe the case of a three year old boy with pre-existing end-stage renal disease on peritoneal dialysis, requiring treatment with cisplatin and carboplatin for hepatoblastoma.

Methods

Pharmacokinetic data was generated to support clinical dosing decisions, with the aim of adequate exposure and minimal toxicity. In the first chemotherapy cycle, 25% of the standard cisplatin dose and 75% of the carboplatin dose, calculated using the pediatric Calvert formula, were administered. Free platinum concentrations were determined in plasma ultrafiltrate and dialysate samples drawn after administration of cis- and carboplatin.

Results

Cisplatin was well tolerated and the observed area under the curve (AUC) of cisplatin was 15.3 and 14.3 mg/L*h in cycle 1 and 3, respectively. The calculated AUC of carboplatin in cycle 1 (9.8 mg/mL*min) exceeded target AUC of 6.5 mg/mL*min and toxicity was observed, therefore, the dose was reduced in cycle 2 and 3. The observed AUC in cycle 2 and 3 were 5.4 and 5.7 mg/mL*min respectively. Platinum concentrations in the dialysate showed that 3-4% of the total dose of cisplatin and 10-12% of the total dose of carboplatin was excreted via peritoneal dialysis. Chemotherapy enabled extended hemihepatectomy and complete remission was achieved.

Conclusion

This report shows that it is feasible to measure AUCs for both drugs and to individualize the dose of these drugs according to the pharmacokinetic results and clinical parameters. Our advice for future cases would be to calculate the starting dose of carboplatin using the (pediatric) Calvert formula, assuming a dialytic clearance of zero, and to adjust the dose if required, based on therapeutic drug monitoring.

INTRODUCTION

Hepatoblastoma is the most common malignant liver tumor in the pediatric population.[1] In most of the cases, (neo)adjuvant chemotherapeutic treatment of hepatoblastoma in children consists of platinum-based therapy, like cisplatin and carboplatin. After administration, these drugs bind irreversibly to proteins and tissue. Free platinum is considered the active form in terms of antitumor effect and toxicity. Free platinum is mainly eliminated by the kidneys.

In rare cases, children with hepatoblastoma have concomitant kidney failure or a type of kidney disease. Impairment of renal function diminishes platinum clearance, causes an increase of the toxicity of platinum compounds and thereby complicates the treatment of children with hepatoblastoma. Dosing guidelines recommend a reduction of the dose or even omitting therapy with platinum derivatives in patients with renal impairment to prevent further nephrotoxicity. With the exception of a few case reports[2–4], there is no information available on the dosing of cisplatin and carboplatin in children with end-stage renal disease requiring peritoneal dialysis. If platinum drugs are excluded, treatment options are scarce, so more information about the dosing of these agents in patients on renal replacement therapy is needed.

As recommended by Labaki et al.[5], drugs undergoing significant renal elimination should be administered with caution in peritoneal dialysis patients with close monitoring of adverse events, dose reductions should be applied when using anti-cancer treatments that are typically excreted by the kidneys, and pharmacokinetic (PK) studies should be performed when available and dose adjustments applied when necessary, even in the absence of any toxicity.

This report describes the treatment of hepatoblastoma with cisplatin and carboplatin in a pediatric patient with pre-existing end-stage renal disease on nocturnal intermittent peritoneal dialysis (NIPD).

PATIENT AND METHODS

Patient

The patient is a three year old boy, with end-stage renal disease due to an atypical haemolytic uremic syndrome (HUS) following an influenza A infection. He remained anuric and peritoneal dialysis was started at the age of 5 months. He presented with an unexplained decline of his haemoglobin level for which an abdominal ultrasound was performed. This revealed a large mass in the left lobe of the liver of approximately 12 x 6 x 13 cm. An abdominal CT confirmed the presence of a large hepatic mass involving segments 2, 3 and 5 of the liver, staging to a PRETEXT III. There were no distant metastases detected. Alpha-fetoprotein (AFP) was elevated to 12,500 µg/L (reference range 0.8–4.5 µg/L). Biopsy of the mass revealed epithelial hepatoblastoma.

Dialysis prescription

The patient was on a nocturnal intermittent cycler-assisted peritoneal dialysis schedule. The dialysis prescription was not changed for the oncological treatment, but dialysate glucose composition was adapted dependent on fluid status of the patient. Total dialysate volume prescribed was 4200 mL/day (9.24 – 9.35 L/1.73 m^2/day). Dialysis time was 12 hours/treatment, with 7 exchanges per treatment. Daily ultrafiltration varied between 223 and 521 mL/treatment. Dialysis adequacy was monitored during treatment by measurement of K.t/V for urea and creatinin clearance. K.t/V was 2.38/week and creatinin clearance was 36 L/week. The patient had no residual renal function.

Treatment

He commenced with chemotherapy according to the intermediate group of the 'Pediatric Hepatic International Tumor Trial' (PHITT) SIOPEL 3 high risk (HR) treatment regimen as there was some concern about extrahepatic extension and this regimen was deemed less toxic than others.

The proposed treatment schedule included cisplatin, carboplatin and doxorubicin. The cisplatin dose according to protocol was 80 mg/m^2 on day 1 and the carboplatin dose was 500 mg/m^2 on day 15 as an intravenous (iv) infusion. In view of the impaired renal clearance and based on the case report of Sebestyen et al.[4], the dose of cisplatin was reduced to 20 mg/m^2 (25% of the recommended dose) and administered over 6 hours. The carboplatin dose was calculated using the formula of Newell et al.[6] In adults, the carboplatin dose is calculated using the Calvert formula.[7] Calvert showed that the renal clearance of carboplatin is linearly related to the glomerular filtration rate (GFR) and designed a formula to calculate an individual dose using a target area under the curve (AUC). Newell et al. developed a similar formula which is suitable for children:

$$\text{Carboplatin dose (mg)} = \text{target AUC} \times \left(\text{GFR} + (0.36 \times \text{BW})\right)$$

where BW equals the body weight in kg. According to Newell et al. a carboplatin dose of 500 mg/m^2 equals an AUC of approximately 6.5 mg/mL*min. With the formula and an estimated dialysis creatinin clearance of 5 mL/min, a dose of 64 mg was calculated, which was reduced to 75% (48 mg, which equals 75 mg/m^2). This was done for extra safety, since it was unknown if the estimated dialysis creatinine clearance exactly corresponded to the carboplatin clearance. Carboplatin was administered intravenously in 1 hour. Free plasma concentrations of cisplatin and carboplatin were measured after the platinum cycles to further individualize the dose. In the first cycle, doxorubicin was given in a 100% dose of 30 mg/m^2 on days 15 and 16. Treatment related toxicities were graded conform Common Terminology Criteria for Adverse Events version 5.0 [8], grade 3 toxicity or higher was recorded.

Pharmacokinetic analysis

Blood samples for the determination of platinum PK were collected at 3 or 4 time points after infusion of cis- and carboplatin up to 24 h after administration. For this purpose, ultrafiltrate was prepared from plasma samples. In addition, samples were taken from the dialysate after the first NIPD cycle after administration of platinum. Free and total platinum concentrations were measured in this ultrafiltrate and dialysate using a validated Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method as described by Brouwers et al.[9] $AUC_{0\text{-inf,free}}$ for each cycle was calculated using the trapezoid method. R (version 3.6.1) was used for data handling and visualization.[10]

RESULTS

Treatment and dose adjustment

Blood samples were collected after 2 cisplatin cycles and 3 carboplatin cycles. The PK parameters of each cycle are displayed in Table 1. Plasma concentration time curve are displayed in Figure 1. The $AUC_{0\text{-inf,free}}$ of cisplatin following the first dose of 20 mg/m^2 was 15.3 mg/L*h. No significant side effects were noted after this first cycle of cisplatin. The $AUC_{0\text{-inf,free}}$ of carboplatin following the first dose of 48 mg (75 mg/m^2) was found to be 9.8 mg/mL*min, which exceeds the target AUC of 6.5 mg/mL*min. Subsequently, severe clinical toxicity was observed, consisting of grade 3 mucositis.

During the next chemotherapy cycles, the patient was treated with the same cisplatin dose as the first cycle. The cisplatin dose was not escalated because the patient had just recovered from the toxicity after the carboplatin dose of cycle 1. For cisplatin, an $AUC_{0\text{-inf,free}}$ of 14.3 mg/L*h was measured in chemotherapy cycle 3. Because of the high carboplatin AUC and observed clinical toxicity in cycle 1, the dose of carboplatin was reduced to 50% of the previous dose for cycle 2 and 3. After two carboplatin doses of 24 mg (37.5 mg/m^2) $AUC_{0\text{-inf,free}}$ values of 5.4 and 5.7 mg/mL*min were measured in cycle 2 and 3, respectively. The second and third chemotherapy cycles were well tolerated.

Doxorubicin was administered in a dose of 30 mg/m^2 on the day of carboplatin and the day after. During the first course 100% of the recommended doxorubicin dose was administered, since the renal clearance of this drug is minimal. After development of toxicity in cycle 1, the dose of doxorubicin was reduced to 75% in cycle 2 and 3. Plasma concentrations of doxorubicin were not measured.

NIPD was started 3-4 hours after end of infusion of cisplatin and 8-9 hours after end of infusion of carboplatin. Platinum concentrations in the dialysate showed that platinum can be excreted by peritoneal dialysis. In total, approximately an amount of 0.4-0.5 mg cisplatin and 3-5 mg carboplatin was excreted after the first NIPD course after administration of cisplatin resp. carboplatin. This equals 3-4% of the total dose of cisplatin and 10-12% of the total dose of carboplatin.

Table 1. Pharmacokinetic parameters and tolerance for cisplatin and carboplatin

	Total dose (mg)	Dose (mg/m²)	$AUC_{0\text{-inf,free}}$	Tolerance
Cisplatin				
Cycle 1	11.75	20	15.3 mg/L*h	No side effects
Cycle 3	11.75	20	14.3 mg/L*h	No side effects
Carboplatin				
Cycle 1	48	75	9.8 mg/mL*min	Grade 3 mucositis
Cycle 2	24	37.5	5.4 mg/mL*min	No side effects
Cycle 3	24	37.5	5.7 mg/mL*min	No side effects

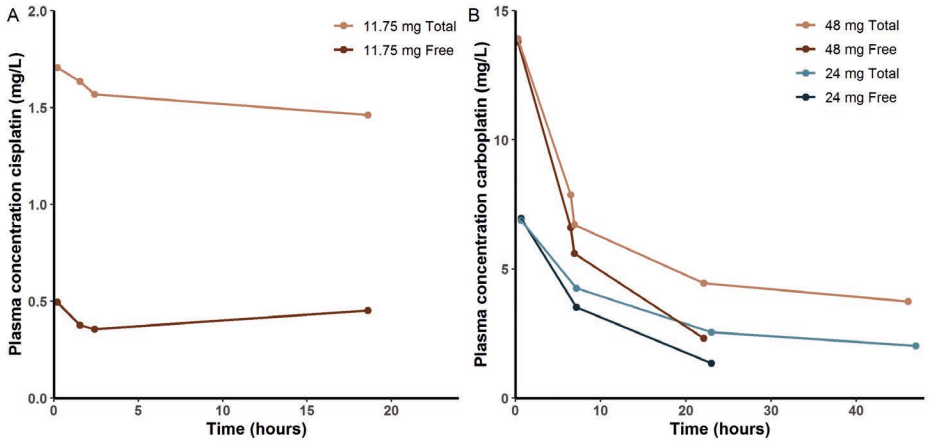

Figure 1. Plasma concentration-time curves for cisplatin (A) and carboplatin (B)

Treatment response and toxicity

The first response evaluation was performed using an MRI scan of the liver showing stable disease. Overall, the chemotherapy was well tolerated, but the patient developed grade 3 mucositis after the first cycle of carboplatin/doxorubicin. The AFP was reduced to 16,000 µg/L (after rise before treatment to 51,000 µg/L). Two additional courses of cisplatin and carboplatin/doxorubicin were given, his clinical condition improved during this period and NIPD was continued.

Thereafter, an uncomplicated left sided extended hemihepatectomy was performed. NIPD was converted to continuous venovenous hemodiafiltration for four weeks postoperatively, after which NIPD was resumed.

Histology showed multifocal pure epithelial hepatoblastoma with fetal (well differentiated, crowded and pleomorphic) and embryonal differentiation. There was extensive necrosis with also vital tumor tissue (40% approx.) and macroscopic angio-invasion. Radical resection was obtained. Currently, he has finished his hepatoblastoma treatment and is in complete remission. Six months after end of treatment, no signs of platinum related neurotoxicity or ototoxicity have been observed.

DISCUSSION

This case report describes the PK of cis- and carboplatin in a child requiring peritoneal dialysis. These results show that it is feasible to measure AUCs for both drugs and to individualize the dose of these drugs according to the clinical parameters and PK results, even though consensus for the target AUC of cisplatin is lacking. In this case, AUCs of cisplatin of 15.3 resp. 14.3 mg/L*h were well tolerated. Previously published, well tolerated, AUCs vary from 5.3 to 79.3 mg/L*h in infants with hepatoblastoma (both with and without hemodialysis).[11,12] In this case, we chose not to escalate the dose, since the patient had just recovered from severe toxicity following the first carboplatin cycle.

Sebestyen et al.[4] reported a case of cisplatin treatment in a 2-year old patient receiving peritoneal dialysis. For this patient an AUC of 64.1 and 66.6 mg/L*h was measured after a dose of 25 mg/m^2 and 29.7 mg/L*h after a dose of 8.7 mg/m^2. According to their data, peritoneal dialysis contributed less than 1% to total body clearance of cisplatin. They compared the values to data of Dominici et al.[13], who reported an AUC of 15.5 ± 9.1 mg/L*h in children with normal kidney function. The results of the AUC in our patient closely match the AUC of Dominici et al. However, the AUC values from the study of Dominici et al. were normalized to a dose of 100 mg/m^2, while our patient was supposed to receive a cisplatin dose equal to 80 mg/m^2.

Carboplatin was dosed according to the Newell formula.[6] Newell et al. developed a formula suited for GFR-based carboplatin dosing in children. The carboplatin dose is calculated using the target AUC, estimated GFR and body weight. For the first cycle an estimated creatinine clearance of 5 mL/min, was included in the Newell formula. A dose of 48 mg was given. This resulted in an AUC of 9.8 mg/mL*min, 1.5-fold higher than the target AUC of 6.5 mg/mL*min. At the same time toxicity (grade 3 mucositis) was observed, so the dose was reduced to 50% of the previous dose. When dialytic clearance was actually measured, it was shown to be 3.6 mL/min (36 L/week).

A previous case, reported by English et al.[2], also used the Newell formula to calculate the carboplatin dose for a 4.3-year old girl, diagnosed with Wilms tumor, receiving peritoneal dialysis. This patient had a residual renal clearance of 5 mL/min/1.73 m^2 as determined by ^{51}Cr EDTA clearance. According to their data, peritoneal dialysis did not contribute to

carboplatin clearance. In our case, assuming no dialytic clearance of carboplatin would have led to a starting dose of 33 mg. In retrospect, this would have been more appropriate in this case, however, it can lead to under-dosing in patients who have residual renal function. Our advice for future cases would be to assume a dialytic clearance of zero to calculate the starting dose of carboplatin, and to adjust the dose if required, based on therapeutic drug monitoring.

The first carboplatin dose led to severe toxicity, which can be explained by the high AUC. Since carboplatin was administered during the same course as doxorubicin it is hard to distinguish whether the toxicity was caused by carboplatin or doxorubicin. The second and third courses, with reduced doses of carboplatin and doxorubicin, were well tolerated.

In addition, platinum concentrations in the dialysate were measured. These results show that 3-4% of the total dose of cisplatin and 10-12% of the total dose of carboplatin was excreted by peritoneal dialysis. This supports our hypothesis that free platinum can be excreted via peritoneal dialysis. The difference in amount between these compounds can be explained by the fact that cisplatin rapidly binds to proteins, faster than carboplatin. The half-lives of free cisplatin and carboplatin are approximately 0.5-1 h and 3-5 h, respectively. Furthermore, the time between administration of the platinum drugs and start of peritoneal dialysis is important. If peritoneal dialysis starts directly after end of infusion, it is expected that more free platinum can be excreted then when it starts several hours after end of infusion, since most of the drug will be bound to proteins.

Clinical course of this three year old patient with hepatoblastoma on NIPD has been astounding. These 'tailored made' and targeted chemotherapy courses enabled extended hemihepatectomy. He is currently off treatment and in complete remission.

In conclusion, this report shows that treatment with cis- and carboplatin in patients requiring peritoneal dialysis is possible and that PK monitoring contributes to the knowledge about the dosing of these drugs in patients requiring peritoneal dialysis.

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2

Clinical pharmacology of cytotoxic drugs in neonates and infants: providing evidence-based dosing guidance

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ABSTRACT

Cancer in neonates and infants is a rare but challenging entity. Treatment is complicated by marked physiological changes during the first year of life, excess rates of toxicity, mortality and late effects. Dose optimisation of chemotherapeutics may be an important step to improving outcomes. Body size-based dosing is utilised for most anticancer drugs used in infants. However, dose regimens are generally not evidence based and dosing strategies are frequently inconsistent between tumour types and treatment protocols.

In this review we collate available pharmacological evidence supporting dosing regimens in infants for a wide range of cytotoxic drugs. A systematic review was conducted and available data ranked by a level of evidence (1-5) and a grade of recommendation (A-D) provided on a consensus basis, with recommended dosing approaches indicated as appropriate.

For 9 out of 29 drugs (busulfan, carboplatin, cyclophosphamide, daunorubicin, etoposide, fludarabine, isotretinoin, melphalan and vincristine) grade A was scored, indicating sufficient pharmacological evidence to recommend a dosing algorithm for infants. For busulfan and carboplatin, sufficient data were available to recommend therapeutic drug monitoring in infants. For 8 drugs (actinomycin D, blinatumomab, dinutuximab, doxorubicin, mercaptopurine, pegaspargase, thioguanine and topotecan) some pharmacological evidence was available to guide dosing (graded as B). For the remaining drugs, including commonly used agents such as cisplatin, cytarabine, ifosfamide and methotrexate, pharmacological evidence for dosing in infants was limited or non-existent: grades C and D were scored for 10 and 2 drugs, respectively.

The review provides clinically relevant evidence-based dosing guidance for cytotoxic drugs in neonates and infants.

INTRODUCTION

Cancer in neonates and infants under one year of age is a rare entity posing unique challenges. Not only do infants develop different types of cancer; the clinical behaviour, aetiology, biology and prognosis of these cancers differ from older children.[1] Treatment challenges include physiological changes in the first year of life influencing pharmacokinetics, with excess rates of toxicity, mortality and late effects observed in this vulnerable age group.[2–5]

Reported incidence of all cancers in the first year of life ranges from 194-243 per million, accounting for around 10% of cancer in 0-15 year olds.[6–11] The most common tumours in this age group are neuroblastoma, leukaemia, CNS tumours, retinoblastoma and renal tumours (Table 1), with some variation amongst geographic and ethnic groups.[6,7,9–12] Overall survival of infant cancers has improved to around 80% in the last two decades.[7,9,10,13] Survival varies widely between tumour groups, with survival above 80-90% consistently reported in retinoblastoma, neuroblastoma and renal tumours in this age group, but below 50-65% in leukaemias and CNS tumours.[7,9,10,13–15] Historically, efforts to improve survival have relied on intensifying therapy, which is hampered by amplifying the risks of acute toxicity and late effects. Childhood cancer survivors, regardless of age at diagnosis, have increased rates of chronic disease, mental health problems and early death, reduced fertility and lower rates of employment and marriage compared to age matched controls or siblings.[16,17] Certain late effects, including second neoplasms, need for special education, and impaired growth occur significantly more frequently amongst children diagnosed at a younger age.[16–19]

The clinical and biologic features of cancer in infancy differ from their older paediatric counterparts. For example, neuroblastoma in older children is typically an aggressive disease, but an infant subtype (stage 4S) exists which can spontaneously regress, even in the presence of widespread dissemination and is associated with markedly better survival.[20,21] Leukaemia and tumours of the CNS are associated with inferior prognosis and unique treatment challenges in infants. Lymphoid leukaemia occurs more frequently than myeloid leukaemia, although acute myeloid leukaemia (AML), which represents only 16% of all childhood leukaemia, accounts for 35% of infant leukaemia.[6,22] *KMT2A* (previously known as *MLL*) rearrangements occur in up to 80% of acute lymphoblastic leukaemia (ALL) and 50% of AML in infants, compared with 5% and 15% of older children, respectively.[22] Survival in ALL is markedly worse in infants than older children (47% vs 85%), despite the development of novel treatment protocols.[22,23] In contrast, infant EFS in AML approximates that of older children at around 60%, despite marked biological differences.[24]

Table 1. Frequency of cancer types in infants <1 year of age reported across registries in France, Israel, Australia, the United States and the United Kingdom[6,7,9–12]

	Incidence per million	% of all diagnoses <1 year
Neuroblastoma	41-58	21-35
Leukaemia	37-40	14-21
CNS tumours	27-34	8-16
Retinoblastoma	18-27	8-13
Renal tumours	17-18	8-11
Germ Cell tumours	14	6-9
Liver tumours	5-9	3-5
Total	189-243	

The treatment of infants and neonates with cancer can be challenging, reflected by a 4-fold increase in deaths within 30 days of diagnosis in this age group.[25–27] Increased mortality is in part due to the aggressive biology and advanced presentation of infant tumours, but also due to increased toxicity of treatment in this age group. Toxicity is multi-factorial, including immaturity of the immune system, organ development and metabolic function. Infectious deaths related to treatment in AML occurred in 13% of children under 2 years of age as compared to 6% of older children. In the early stages of the CCG1953 ALL study, infectious deaths were seen in 50% of children under 3 months, compared to 18% of 6-12 month olds, leading to dose modifications of daunorubicin.[28,29] Historically, infants with Wilm's tumour or ALL were treated under the same chemotherapy regimens as older children, leading to significantly more multi-organ toxicity in infants.[30,31] This effect was ameliorated by empirical dose reductions, and efforts have since focussed on exploring the pharmacokinetics of chemotherapeutic agents in infants to optimise chemotherapy dosing.[32,33]

There are well established physiological differences between neonates and infants as compared to older children that have the potential to significantly impact on drug disposition, and these differences have been comprehensively covered in previous publications.[34–36] These differences include age-dependent changes in gastrointestinal tract structure and function which may impact on drug absorption, developmental changes in percentages of total body water and body fat alongside differences in plasma protein binding affecting drug distribution, changes in metabolic capacity related to the ontogeny of enzymes involved in drug metabolism, and physiological developmental changes in kidney function impacting drug elimination. Clearly these differences need to be taken into account when considering dosing of chemotherapeutics in the neonate and infant patient population.

Infants with cancer represent a unique group with different biological drivers to cancer in older children. Many of these cancers are aggressive and require unique treatment approaches. At the same time, these children are uniquely vulnerable to the effects of

treatment. Developing approaches to optimise exposure to chemotherapeutic drugs may represent an important step to improving outcomes in this challenging group. The chemotherapeutic agents used in the commonest infant cancers are listed in Table 2.

Table 2. Common infant cancers and current first line chemotherapy agents

Cancer type	Chemotherapy drugs used
Acute Lymphoblastic Leukaemia	Cyclophosphamide, cytarabine, daunorubicin, dexamethasone, etoposide, mercaptopurine, methotrexate, PEGasparaginase, Prednisone, thioguanine, vincristine, triple intrathecals (methotrexate, cytarabine, prednisone)
Acute Myeloid Leukaemia	Cytarabine, fludarabine, gemtuzumab, idarubicin, mitoxantrone
Neuroblastoma	Busulfan, carboplatin, cisplatin, cyclophosphamide, dinutuximab, doxorubicin, etoposide, isotretinoin, melphalan, topotecan, vincristine
Retinoblastoma	Carboplatin, etoposide, vincristine, intrathecal cytarabine
Wilms Tumour	Actinomycin D, carboplatin, cyclophosphamide, doxorubicin, etoposide, vincristine

CURRENT APPROACHES TO DOSING AND THE APPLICATION OF PHARMACOLOGICAL DATA

For the vast majority of anticancer drugs used in neonates and infants, dosing regimens based on body weight are utilised in the clinic. This is partly a practical consideration as body surface area (BSA) is more challenging to predict accurately in this population as compared to body weight, and partly because of the tendency to overdose neonates and infants, since the developmental changes in pharmacokinetic parameters do not change proportionally with BSA. However, the body weight-based doses incorporate a discrepancy in dose as compared to the equivalent BSA-based dose administered to children above 1 year of age, or >10 or 12 kg, depending on the drug and clinical protocol on which the child is being treated. Dose adjustments for infants are frequently used inconsistently between tumour types and treatment protocols, with additional dose reductions of 33-50% commonly recommended for children <6 months or <5 kg, for example. This subject has been previously discussed in a number of well written review papers, highlighting the lack of clinical pharmacological data supporting many current dosing regimens and the marked dose increases implemented for many anticancer drugs when infants cross a dosing threshold boundary of 12 kg or 1 year of age.[4,33,37] As an example of the current state of play for the widely used anticancer drug vincristine, Table 3 provides examples of dosing regimens and recommended dose reductions for infants and neonates across a range of tumour types. As can be seen, clear inconsistencies exist between tumour type as to the most

appropriate dosing regimens and adjustments for infant cancer patients of varying ages as compared to the standard BSA-based dosing in older children. The one thing that is likely to be consistent across treatment protocols is that none of the dose reductions stipulated for infant patients are based on any kind of meaningful pharmacological rationale. In order to avoid the current situation whereby marked dose increments are introduced when infants cross defined weight or age boundaries, the COG Chemotherapy Standardization Task Force has recently recommended the use of dosing tables for infants to gradually transition from body weight to BSA-based dosing.[32] While potentially useful, these guidelines are, as acknowledged by the authors, a temporary solution designed to improve the current infant dosing situation in the absence of more rational-based adaptive dosing approaches.

There are good reasons why dose reductions may be needed in the infant cancer patient, either related to a reduced drug clearance associated with the early development of kidney and liver function in the first weeks and months of life, or due to an increased susceptibility to adverse drug effects in the developing child. However, with the critical importance of getting the balance right between efficacy and toxicity in this patient population, it would be prudent to consider pharmacological evidence to either support or refute current dosing regimens where this is available. A good example of how data generated from clinical pharmacological studies can be utilised to improve dosing practices is provided by the use of 13-cis-retinoic acid in a high-risk neuroblastoma setting. A study designed to investigate the feasibility of using therapeutic drug monitoring (TDM) approaches to 13-cis-retinoic acid dosing showed marked variability in drug exposures between patients and highlighted that children <12 kg who were receiving a body weight based drug dose were achieving consistently low and potentially sub-therapeutic drug levels.[38] The findings from this study led to removal of body weight-based dosing regimens for the younger patients, with all patients across Europe now receiving the standard BSA-based dose, with no reported issues in terms of tolerability. The study also had the added benefit of stimulating research that led to the recent development of an infant friendly liquid formulation of the drug.[39]

While more prospective studies are needed in this area, incorporating relevant pharmacokinetic and pharmacodynamic endpoints to generate data that can inform the selection of dosing regimens in neonates and infants, it is also important to scrutinise the currently available literature to investigate what current evidence is available. This information should be looked at alongside patient characteristics that may be used to determine more rational dosing regimens in neonates and infants. Such characteristics may include gestational or postnatal age, ontogeny information relating to metabolic and elimination processes, renal function measurements and body weight.

Table 3. Vincristine dosing regimens and dose adjustments across a range of tumour types

Tumour type	Dose	Route	Dose Adjustment	Absolute dose for a child of:		
				2 months, 5.5 kg, 0.30 m^2	**6 months, 8 kg, 0.39 m^2**	**12 months, 10 kg, 0.46 m^2**
Ependymoma (Post-operative intensive chemotherapy) and Infant ependymoma	1.5 mg/m^2	IV Infusion (1h)	Children >12 months: use full BSA-based dose (1.5 mg/m^2) For children 6-11 months and over: use 75% of BSA-based dose (1.125 mg/m^2) For children 6 months and under: use 50% BSA-based dose (0.75 mg/m^2)	0.22 mg	0.44 mg	0.69 mg
Low grade glioma (Induction therapy)	1.5 mg/m^2	IV bolus	For children <10 kg: 0.05 mg/kg/day For children <6 months: further dose reduction of 33%	0.18 mg	0.40 mg	0.69 mg
Low grade glioma (Consolidation therapy)	1.5 mg/m^2	IV bolus	For children <10 kg: 0.05 mg/kg/day For children <6 months: further dose reduction of 33%	0.18 mg	0.40 mg	0.69 mg
Low risk medulloblastoma	1.5 mg/m^2	IV bolus	For children 12 months and over: use full BSA-based dose (max 1.5 mg/m^2) For children 6-11 months and over: use 80% of BSA-based dose (1.2 mg/m^2) For children 6 months and under: use 66% BSA-based dose (0.99 mg/m^2)	0.30 mg	0.47 mg	0.69 mg
Non-metastatic rhabdomyosarcoma	1.5 mg/m^2	IV bolus	For children <12 months or <10 kg: 0.05 mg/kg/day	0.28 mg	0.40 mg	0.69 mg
Relapsed/ refractory rhabdomyosarcoma	1.5 mg/m^2	IV bolus	For children <10 kg: 0.05 mg/kg/day	0.28 mg	0.40 mg	0.69 mg

Table 3. [Continued]

Tumour type	Dose	Route	Dose Adjustment	Absolute dose for a child of:		
				2 months, 5.5 kg, 0.30 m^2	**6 months, 8 kg, 0.39 m^2**	**12 months, 10 kg, 0.46 m^2**
High-risk neuroblastoma	1.5 mg/m^2	IV bolus	For children <12 kg, use 0.05 mg/kg For infants <5 kg, a further 33% reduction is recommended	0.28 mg	0.40 mg	0.50 mg
High-risk neuroblastoma (second-line schema)	2 mg/m^2	Continuous IV infusion (48h)	For children <12 kg: use 0.033 mg/kg/day For infants < 5kg: a further 33% reduction is recommended	0.36 mg	0.52 mg	0.66 mg
Relapsed/ progressive high-risk neuroblastoma	1 mg/m^2	Continuous IV infusion (48h)	For children <12 kg: use 0.033 mg/kg	0.18 mg	0.26 mg	0.33 mg
Low/ intermediate-risk neuroblastoma	1.5 mg/m^2	IV bolus	For children <10 kg: use 0.05 mg/kg For infants below 5 kg: reduce by a further 33%	0.28 mg	0.40 mg	0.69 mg

PHARMACOKINETICS OF SELECTED CHEMOTHERAPEUTICS IN NEONATES AND INFANTS

Many chemotherapeutic agents are used in infants, despite pharmacological evidence for the dosing regimens utilised being scarce or even non-existent for the majority of anticancer drugs. For the current study, we investigated and collated the available pharmacological evidence supporting dosing regimens in infants and neonates for a wide range of clinically relevant cytotoxic drugs. A graphical summary of the workflow is shown in Figure 1, with levels of evidence and grades of recommendation inspired by the Oxford Centre for Evidence-Based Medicine (CEBM) system, as outlined in the detailed methods provided in the Supplementary Methods. All available pharmacological evidence was ranked based on the level of evidence (1-5) (Supplementary Table S5). Subsequently, a grade of recommendation (A-D) and a recommended dose per chemotherapeutic agent was derived by consensus opinion. For grade C or D agents, no dose advice is given since the pharmacological evidence was insufficient to come to a recommendation. Figure 2 gives an overview of the available pharmacological evidence per level for each chemotherapeutic agent of interest, alongside the total number of infants studied in the available papers.

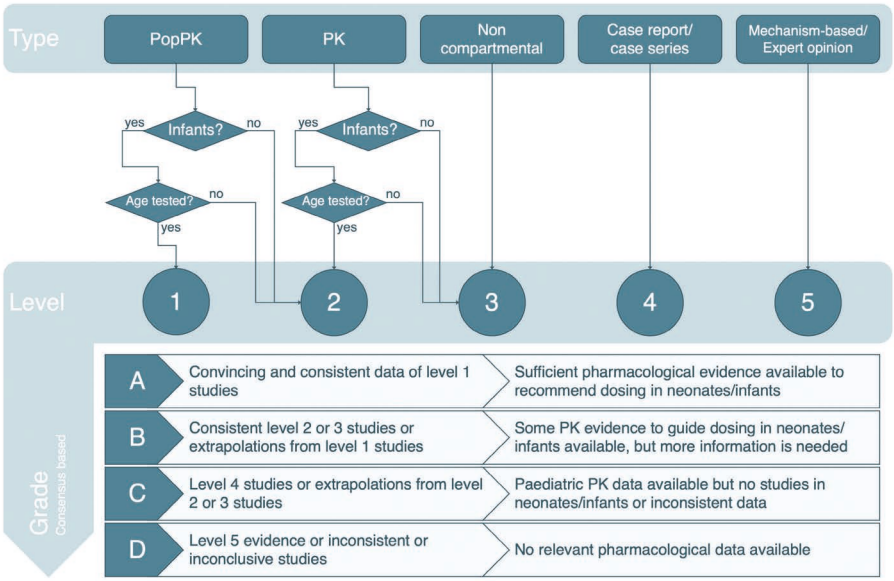

Figure 1. Graphical summary of the methods used for labelling articles with a specific level, and grading of the chemotherapeutic agents.

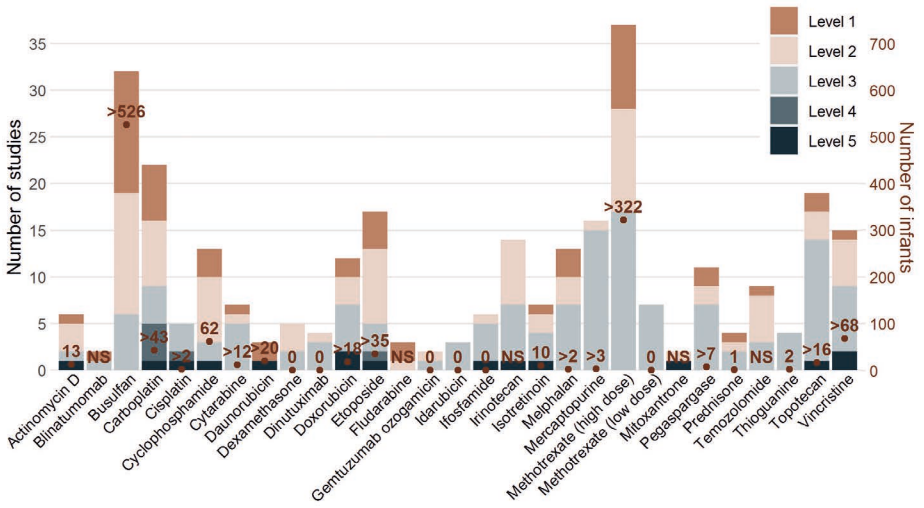

Figure 2. Bar plot displaying the number of studies for each chemotherapeutic agent per evidence level (primary y-axis), as well as the number of infants included in total in all published studies (secondary y-axis).

Our recommendations for dosing regimens for chemotherapeutic agents in neonates and infants are summarised in Table 4 and discussed below for each of the grades of classification. A comprehensive list of publications and reference details for each drug are provides in Supplementary Table S5 with key references included within the sections below. To provide examples of how the results from clinical pharmacology studies can positively impact on clinical practice, we describe in detail two drugs (carboplatin and busulfan) classified as grade A, for which pharmacokinetic data are well understood and are utilised to provide 'gold standard' treatment. Both drugs meet the criteria for TDM (e.g. narrow therapeutic index, a clear relation between exposure and clinical outcome, substantial interpatient variability and small intrapatient variability) and evidence shows that TDM practices can be successfully used to optimize the treatment for neonate and infant patients.

Grade A

For nine agents (busulfan, carboplatin, cyclophosphamide, daunorubicin, etoposide, fludarabine, isotretinoin, melphalan and vincristine) a grade A recommendation was given, for which sufficient pharmacological evidence is available to recommend dosing in infants. Details on busulfan and carboplatin are discussed separately below.

For daunorubicin, etoposide, isotretinoin and melphalan, sufficient and consistent level 1 pharmacokinetic studies including infants have been published (Supplementary Table S5). For all these drugs, no effect of age on pharmacokinetics has been observed and a full (mg/m^2) dose is recommended.

Several studies on the pharmacokinetics of cyclophosphamide in children have been published, including a total of 62 infant patients. No structural effect of age was found on pharmacokinetic parameters, but in two level 1 population pharmacokinetic analyses including a total of 54 infants, a higher clearance was observed in younger children, resulting in a greater exposure to active metabolites.[40,41] Therefore, a recommendation to use the mg/m^2 dose and reduce the dose by 20% in younger infants (<6 months) is supported, as proposed by Campagne et al.[40]

While relatively few studies reporting on fludarabine pharmacokinetics in children have been published, and the number of infants included in these studies was unspecified, the quality of the analyses was high (two level 1 studies) and all studied the effect of age. No effect of age on fludarabine pharmacokinetics was found, but estimated glomerular filtration rate (eGFR) was included as significant covariate for clearance in all studies.[42–44] A recommendation to administer the full (mg/m^2) dose is supported, with dose adaptation based on eGFR in cases of renal impairment.

Several vincristine pharmacokinetic studies in children have been conducted over the past 25 years, with a recently published level 1 population study focusing on drug disposition in neonates and infants, including 21 patients aged <1 year of age.[45] No significant difference in BSA-normalized clearance between infants and older children was found in this study, however there was a trend towards lower clearance in neonates (0-4 weeks) as compared to infants (1-12 months). Doses of <0.05 mg/kg resulted in significantly lower AUC values than observed in neonates and infants receiving doses of ≥0.05 mg/kg and older children receiving a dose of 1.5 mg/m^2. No significant differences in vincristine exposures between younger patients receiving vincristine doses of ≥0.05 mg/kg and older children (1.5 mg/m^2) were observed. These findings are supported by previously published level 2 and 3 studies which did not find an effect of age on vincristine pharmacokinetics. These recent data support a recommendation of either full (mg/m^2) dosing, or mg/kg dosing at doses of ≥0.05 mg/kg, with the latter approach potentially more appropriate for neonate patients (0-4 weeks of age).

Grade B

For a total of eight drugs (actinomycin D, blinatumomab, dinutuximab, doxorubicin, mercaptopurine, pegaspargase, thioguanine and topotecan) the available pharmacological evidence to guide dosing in infants was classified as grade B.

For actinomycin D, some pharmacokinetic data in children are available, with 13 infants included across two studies.[46,47] However, conclusions drawn on the effect of age on the pharmacokinetics of actinomycin D are inconsistent. This could be due to different analytical methods used or the limited number of infants included. More information is needed to provide an evidence-based actinomycin D dose recommendation. Until such information is available, it is recommended that the full (mg/m^2) dose is administered. This is based on

the findings of a non-compartmental analysis, where Skolnik et al. found that clearance, corrected for BSA, was not related to age.[47]

Some pharmacokinetic studies have been published in children focusing on the monoclonal antibody drugs blinatumomab and dinutuximab. For blinatumomab, a limited number of infants were included and no effect of age on pharmacokinetics was found.[48,49] For dinutuximab, no infants were studied and the effect of age was not investigated in the majority of studies, although there was a suggestion that dinutuximab clearance may be higher in younger patients.[50] While the pharmacokinetic behavior of antibodies in infants in general is reasonably well studied, specific information on the pharmacokinetics of blinatumomab and dinutuximab is limited. In accordance with current practice, a full (mg/m^2) dose for blinatumomab and dinutuximab is recommended in infants and neonate patients.

The pharmacokinetics of doxorubicin have been investigated in infants, with a lower clearance of doxorubicin observed in younger patients.[51–54] However, a limited number of infant patients were included in these studies. In addition, pharmacokinetic simulations using a published population pharmacokinetic model were performed by Siebel et al.[55] Equations for individualization of the doxorubicin dose based on age and BSA were published, accompanied by the advice to reduce the peak concentrations in very young children by prolonging drug infusion. Since this analysis is based on a population pharmacokinetic model including only 4 infants, it is recommended that these findings are confirmed in a larger infant patient cohort.

Mercaptopurine and thioguanine pharmacokinetic studies in children have been published, although the number of infant patients included is limited to a handful of studies.[51,56,57] No effect of age on the pharmacokinetics of mercaptopurine has been found, with the effect of age on the pharmacokinetics of thioguanine not investigated in most of the studies. More information on the pharmacokinetics of both of these drugs in infants and neonates is needed, to further elucidate the effect of age on drug disposition. Based on current practice, a full (mg/m^2) dose is recommended, with dose adjustments based on white blood cell count.

For pegaspargase, while several pharmacokinetic studies in children have been published, numbers of infant patients included are limited and the effect of age was not investigated in most cases. Although preliminary data would suggest no effect of age on pegaspargase pharmacokinetics[58–61], more studies including infant patients are needed to provide evidence-based dosing advice. In the meantime, full (mg/m^2) doses are recommended, with dose adjustments based on TDM approaches.

Topotecan represents an anticancer drug well studied in children. However, the effect of age was not investigated in the majority of published studies. Two level 1 studies including

infants, both describing topotecan disposition using population pharmacokinetic models, show conflicting results. Schaiquevich et al. found a correlation between age and BSA-normalized clearance and volume of distribution of the central compartment, whereas Roberts et al. did not observe any effect of age after normalizing for BSA.[62,63] Previous level 3 studies that studied the effect of age, but did not include infants, did not find a correlation between age and pharmacokinetic parameters. There is currently insufficient evidence to recommend changes to currently accepted dosing regimens, which may be based on BSA or BW for different tumour types.

Grade C

Drugs classified as grade C represent those for which paediatric data are available, but where no pharmacological studies have been conducted in infants, or where the published data are inconsistent. No dose advice can be provided for these ten agents (cisplatin, cytarabine, dexamethasone, gemtuzumab ozogamicin, idarubicin, ifosfamide, irinotecan, low dose methotrexate, prednisone and temozolomide) based on a pharmacological rationale.

For cisplatin, some level 3 pharmacokinetic studies including infant patients have been published and one case report in a neonate.[64–66] The level of evidence for a specific dose regimen is low. Clearance may be lower in younger children, but this needs to be verified in a cohort including infant patients.

Some pharmacokinetic studies of cytarabine in children have been published, however, the number of infants included are limited and the effect of age not studied in the majority of cases. The studies that did look into the effect of age reported conflicting results. While a level 1 study included age as covariate on all pharmacokinetic parameters, a level 2 study failed to observe a change in drug clearance in infants compared to older children.[51,67] Population pharmacokinetic analyses looking into the effect of age on the pharmacokinetics of cytarabine (and metabolites) are needed.

For dexamethasone and prednisone, some pharmacokinetic studies in children have been published in an oncology setting, however, no infant patients were included. While it has been suggested that dexamethasone clearance may be higher in younger patients[68,69], this finding needs to be verified utilising a population pharmacokinetic model approach in a study including infants. No correlation between age and BSA-normalized prednisone clearance was reported in a level 1 study incorporating a population pharmacokinetic modelling approach and including a single infant patient.[70] However, plasma protein binding of prednisone to corticosteroid-binding globulin was associated with patient age. These findings need to be examined in a larger cohort of infant patients in order to provide evidence-based dosing advice.

For gemtuzumab ozogamicin, idarubicin and ifosfamide, only small numbers of pharmacokinetic studies have been published in children, with no infant patients included. No effect of age on the pharmacokinetics of gemtuzumab ozogamicin were observed in two separate studies[71,72] and a single level 3 study on the pharmacokinetics of idarubicin similarly observed no effect of age.[73] The only published ifosfamide population pharmacokinetic model failed to look into the effect of age and the published level 3 studies did not find an effect of age on ifosfamide pharmacokinetics.[74,75] These findings require verification in population pharmacokinetic studies including infant patients.

Irinotecan pharmacokinetic studies in children, including infant patients, have been published, but frequently not investigating the effect of age. Clearance of the metabolite may be higher in younger children[76], but this finding needs to be verified through studies incorporating population pharmacokinetic model approaches across the paediatric age spectrum.

For low dose methotrexate, several studies investigating pharmacokinetics in children have been published, but no infants were included and the effect of age was not studied in the majority of cases. Level 3 non-compartmental studies that did look into the effect of age on pharmacokinetics did not find an effect[77–80], however population pharmacokinetic analyses for low dose methotrexate are needed. Again, for temozolomide, some pharmacokinetic studies have been published in children, but numbers of infant patients were limited or not specified and the effect of age was not investigated in most cases. The results of one level 1 population pharmacokinetic study, suggesting that age has an effect on BSA-normalized clearance and volume of distribution, did not match the results of two level 3 non-compartmental analyses, which indicated no effect of age on BSA-normalized drug clearance.[81–83] Population pharmacokinetic analyses looking into the effect of age on the pharmacokinetics of temozolomide are needed.

Grade D

The remaining two agents, high dose methotrexate and mitoxantrone, were classified as grade D, with no relevant pharmacological data currently available or conflicting results published.

Numerous studies on the pharmacokinetics of high dose methotrexate have been published and many of these studies included infants. However, the results of these studies are conflicting. Several level 1 and level 2 studies describe no effect of age on pharmacokinetics after including other covariates such as body weight (using allometric scaling), SLCO1B1 polymorphism, serum creatinine and/or treatment with dexamethasone.[84–90] Nevertheless, some level 1 studies did report an effect of age on high dose methotrexate clearance or volume of distribution of the central compartment, even after normalizing for body size.[91–94] In addition, one-, two- and three-compartment models have been published, suggesting a lack of consensus between studies.[84,85,94–99,86–93] These conflicting results could be related to

variations in method of drug analysis or differences in sampling times between the studies, with many models based on data obtained for routine patient care, e.g. blood samples taken every 24 hours to monitor the plasma concentrations for rescue therapy. The development of population pharmacokinetic models incorporating more intensive sampling times in children and infants is recommended.

For mitoxantrone, only one pharmacokinetic study has been published in children[100] and the effect of age on the mitoxantrone pharmacokinetics has not been studied. No relevant data is available to give an evidence-based dosing regimen.

Carboplatin

Carboplatin is a platinum based chemotherapeutic agent used to treat a variety of tumour types. It represents a cytotoxic drug for which TDM is well established, with defined target exposures for different tumour types and chemotherapy regimens.[101,102] There is a clear understanding from both adult and paediatric studies of the correlation between exposure of free carboplatin and toxicity/response[103,104], which can be utilised to obtain optimal exposure and limit the occurrence/severity of side effects in patients.

Carboplatin elimination is highly dependent on renal function. The GFR is used to calculate the dose administered to patients, as proposed by Calvert and Newell.[102,105] These dosing equations are described in greater detail in a recent review by Barnett et al.[106] However, carboplatin dosing based on renal function poses a substantial challenge in neonates and infants, since a reliable estimate of GFR is often unavailable. In addition, there is no standardised method of GFR determination across treatment centres, which can lead to marked variations in dose calculation.[107] Therefore, alternative strategies, such as dosing based on BSA have been developed, where an AUC of 1.325 mg/mL*min is typically achieved per 100 mg/m^2 dosed.[102] This mg/m^2 dosing approach is common for carboplatin paediatric dosing regimens within the UK.[106]

Several studies have highlighted that this strategy might, however, not be appropriate for neonates and infants. Allen et al. demonstrated that, in children with retinoblastoma, doses of carboplatin were generally higher in those dosed according to mg/m^2 relative to GFR.[108] Moreover, children who were dosed according to BSA were 3 times more likely to require a platelet transfusion. It was noted that there was a greater difference in the doses calculated using GFR versus mg/m^2 for the younger children recruited onto the study. This reflects the marked changes in GFR that occur within the first few months of life and the important role renal function plays in carboplatin elimination.

Barnett et al. recently compiled a summary of carboplatin dosing regimens utilised for various tumour types within the UK, including the dose reductions that are applied for the treatment for infants/neonates.[106] To illustrate, for low/intermediate risk neuroblastoma standard carboplatin dosing is 200 mg/m^2 to achieve a target AUC of 2.6 mg/mL*min

per day. However, for patients less than 10 kg a dose of 6.6 mg/kg is administered, and for infants less than 5 kg this dose is reduced further to 4.4 mg/kg. Therefore, patients <10 kg on 6.6 mg/kg dosing receive 41-67% of the carboplatin dose that would have been administered using 200 mg/m^2 dosing. These mg/kg adjustments are required for neonates/infants as obtaining accurate estimates of BSA and GFR can be challenging and mg/m^2 dosing has been shown to substantially over-estimate the dose required during the first few months of life.[108] In this respect, Qaddoumi et al. also showed that younger patients (<6 months) had a higher incidence of ototoxicity relative to older patients, most likely as a result of BSA-based carboplatin dosing.[109] Therefore, an emphasis has been placed on the avoidance of this approach in younger patients (<10 kg), in favour of mg/kg dosing.[110]

Although dosing in mg/kg can lead to more appropriate doses for carboplatin in neonates and infants, it is not without its limitations. Veal et al. showed marked differences in carboplatin clearance between neonates of a similar age and weight over several cycles of treatment.[111] For these patients treated over 3 cycles, carboplatin clearance increased to a higher magnitude than body weight. Therefore, markedly higher doses than those based solely on changes in body weight were frequently required to achieve carboplatin target AUC, demonstrating the importance of TDM for neonates in order to attain optimal carboplatin exposures.

Given the limitations, in the UK, carboplatin TDM is now routinely used for infant neuroblastoma and retinoblastoma patients, as recommended by national treatment guidelines. Details of how this process is carried out have recently been summarised.[106] Target carboplatin exposure depends on tumour type and/or risk group, with doses adjusted accordingly over multiple days of treatment in order to achieve these targets. For standard carboplatin chemotherapy in neonates and infants, the target AUC typically ranges from 5.2-7.8 mg/mL*min over 3 days. In addition to variations in dose due to tumour type, dose reductions are often applied to children <6 months of age, <12 months of age or less than 10 kg.

Busulfan

Busulfan is an alkylating agent used in conditioning regimens to prepare for both autologous and allogeneic haematopoetic stem cell transplantation (HSCT). It's pharmacokinetics, pharmacodynamics and pharmacogenetics in this paediatric population have been extensively reviewed by ten Brink et al.[112] Several studies published over many years have shown that busulfan dosing can be optimized by performing TDM.[112] Busulfan, combined with TDM guided dosing, is associated with higher event-free survival rates due to fewer graft failures or relapses and lower toxicity. The most appropriate target busulfan AUC has been studied in several papers[113–117] and has been optimised over many years, leading to consensus on a target AUC of 78-101 mg/L*h when combined with fludarabine.[116]

Busulfan is one of relatively few agents that has been thoroughly studied in infants (Supplementary Table S5) and demonstrates a U-shaped relationship between age and clearance. In one of the first busulfan pharmacokinetic papers in infants, Dalle et al. describe that exposure in infants can be higher than in older children after similar dosing regimens.[118] In contrast, several papers, published some years later, showed a higher clearance (corrected for body size) of busulfan in children younger than 4 years of age.[119–121] More recent population pharmacokinetic models indicate that clearance (corrected for body size) of busulfan increases after birth until the age of 2-12 years (depending on the pharmacokinetic model) and then begins to decline to adult levels.[122–126] Besides growth, one of the explanations for this increase in the first years of age is maturation of glutathione by glutathione S-transferase (GST) enzymes. Busulfan is extensively metabolized by GST enzymes, predominantly GSTA1. The GST enzymes involved can undergo significant changes in activity and/or expression, increasing gradually over the first 2 years of life.[127,128] In addition, several investigators studied the effect of GSTA1 genetic variations on the pharmacokinetics of busulfan and GSTA1 genetic variations were incorporated into population pharmacokinetic models in children and adults.[129–135]

These insights into the pharmacokinetics of busulfan in children and, in particular, infants have led to the development of several age based dosing strategies for initial busulfan dosing regimens (in mg/kg), whereafter the dose is adjusted based on TDM.[122,136–140] Current dosing recommendations from the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) for the use of busulfan in children are based on the nomogram of Nguyen et al., which is based on a pharmacokinetic model that takes only body weight into account, although more recent population pharmacokinetic models suggest that maturation should also be considered.[136,141]

Studies pointed out that there is no difference in pharmacokinetics between dosing once-daily or multiple times per day.[114,124,142] The exposure to busulfan can be adequately calculated based on 2-4 plasma levels, which is minimally invasive and does not exceed the limits of blood withdrawal in infants.[112,143]

FUTURE DIRECTIONS

In the current review we have collated data from clinical pharmacological studies incorporating pharmacokinetic data of cytotoxic agents in neonates and infants, with many of these studies involving the recruitment of only small numbers of individuals in the very young age category. While we have attempted to use this information to provide guidance for future dosing of infants with the selected drugs, there is still clearly much more work needed to further develop this area and hopefully provide the required level of evidence for making dosing recommendations that will positively impact patient treatment. In this respect, there are positive signs that progress is being made. In the US, plans are underway

to conduct a prospective study to validate the recently proposed COG Chemotherapy Standardization Task Force recommendations for the use of dosing tables for infants to gradually transition from body weight to BSA-based dosing.[32] In the UK and the Netherlands there are ongoing studies designed to investigate drug disposition in neonate and infant cancer patients, incorporating TDM and adaptive dosing approaches as appropriate, which have the potential to generate a wealth of data in this understudied patient population (https://www.isrctn.com/ISRCTN10139334 and https://www.trialregister.nl/trial/7527). Alongside the conduct of well-planned population pharmacokinetic studies in neonates and infants, the advancement of minimal sampling techniques for conducting such studies and the utility of physiologically-based pharmacokinetic model development, to investigate physiological factors that may influence pharmacokinetics and evaluate contrasting dosing regimens in this patient population, there are clear indications that advancements in this field are gathering pace.[144–148]

It is hoped that active research over the coming years will allow us to redefine dosing regimens for selected anticancer drugs as well as identify additional drugs that may benefit from adaptive dosing. In this way we may be able to truly optimise dosing regimens in a patient population where pharmacokinetic parameters can be difficult to predict and may be rapidly changing with time. In the meantime, it is hoped that the pharmacological information collated in the current study, acts as a temporary solution in providing a clinical tool to support dosing decisions in this challenging patient population.

Table 4. Results of the studied chemotherapeutic agents with the recommendations for dosing regimens in neonates and infants

Chemotherapeutic agent	PK findings/remarks	Recommended dosing regimen and dose adjustments for infants	Grade of recommendation
Actinomycin D	Some PK studies in children have been published, one including infants. The results on the effect of age on the PK are not consistent.	Full (mg/m^2) dose	B
Blinatumomab	Two PK studies in children have been published. No effect of age on the PK of blinatumomab has been found. However, the PK behavior of antibodies in infants is known.	Full (mg/m^2) dose	B

Table 4. [Continued]

Chemotherapeutic agent	PK findings/remarks	Recommended dosing regimen and dose adjustments for infants	Grade of recommendation
Busulfan	Busulfan has been thoroughly studied in infants. It demonstrates an U-shaped relationship between age and clearance. TDM guided dosing is associated with higher event-free survival rates due to fewer graft failures or relapses and lower toxicity.	Dose for day 1 (in case of a target AUC of 90 mg/l*h), after which the dose is adjusted based on TDM: **BW (kg) / Dose 1dd (mg/kg)** 3 / 3.8 5 / 4.7 7 / 5.1 8-13 / 5.2 15-16 / 5.1	A
Carboplatin	The PK of carboplatin in children has been studied thoroughly. The results on the effect on age on the PK are not consistent in all studies. TDM guided dosing has been successfully implemented in the UK.	Use TDM approach to achieve target AUC. If not available dose based on mg/kg or GFR.	A
Cisplatin	Some PK studies in children have been published, including one case report in an infant. However, the level of evidence for a specific dose regimen is low. CL might be lower in younger patients.	*No advice*	C
Cyclophosphamide	PK studies in children (including infants) have been published. A higher CL had been found in younger children, resulting in a higher exposure to metabolites.	Use mg/m^2 dose, reduce by 20% in young infants (<6 months)	A

Table 4. [Continued]

Chemotherapeutic agent	PK findings/remarks	Recommended dosing regimen and dose adjustments for infants	Grade of recommendation
Cytarabine	Some PK studies in children have been published, however, the number of infants was limited and the effect of age was not studied in most of the studies.	*No advice*	C
Daunorubicin	Two PK studies including infants have been published. No effect of age on the PK of daunorubicin observed.	Full (mg/m^2) dose	A
Dexamethasone	Some PK studies in children have been published, however, no infants were included. CL might be higher in younger patients.	*No advice*	C
Dinutuximab	Some PK studies in children have been published, however, no infants were included and the effect of age was not studied in most of the studies. However, the pharmacokinetic behavior of antibodies in infants is known. CL might be higher in younger patients.	Full (mg/m^2) dose	B
Doxorubicin	The PK of doxorubicin has been investigated in infants. A lower CL of doxorubicin has been found in younger patients, however, the number of infants included are low.	Adapt the dose based on age and BSA and duration of infusion, according to equations in Siebel et al (2020).[55]	B

Table 4. [Continued]

Chemotherapeutic agent	PK findings/remarks	Recommended dosing regimen and dose adjustments for infants	Grade of recommendation
Etoposide	PK studies including infants have been published. No effect of age on the PK of etoposide has been found.	Full (mg/m^2) dose	A
Fludarabine	Some PK studies in children have been published, however, the number of infants was limited. No effect of age on the PK of fludarabine has been found.	Full (mg/m^2) dose. Consider dose adaptation based on eGFR in case of renal impairment.	A
Gemtuzumab ozogamicin	Some PK studies in children have been published, however, no infants were included. No effect of age on the PK of gemtuzumab ozogamicin observed.	*No advice*	C
Idarubicin	Some PK studies in children have been published, however, no infants were included and the effect of age was only studied once. No effect on the PK of idarubicin has been found.	*No advice*	C
Ifosfamide	Some PK studies in children have been published, however, no infants were included and the effect of age on the PK parameters was only studied once. No effect on the PK of ifosfamide has been found.	*No advice*	C

Table 4. [Continued]

Chemotherapeutic agent	PK findings/remarks	Recommended dosing regimen and dose adjustments for infants	Grade of recommendation
Irinotecan	PK studies in children (including infants) have been published. The effect of age was not studied in most of the studies. CL of the metabolite might be higher in younger children.	*No advice*	C
Isotretinoin	Some PK studies including infants have been published. No effect of age on the PK of isotretinoin has been found.	Full (mg/m^2) dose	A
Melphalan	PK studies in children (including infants) have been published. No effect of age on the PK of melphalan has been found.	Full (mg/m^2) dose	A
Mercaptopurine	PK studies in children have been published, however, the number of infants was limited. No effect of age on the PK of mercaptopurine has been found.	Full (mg/m^2) dose, adjust based on WBC.	B
Methotrexate (high dose)	The PK of high dose methotrexate has been investigated in infants. However, these studies show conflicting results.	*No advice*	D
Methotrexate (low dose)	Some PK studies in children have been published, however, no infants were included and the effect of age was not studied in most of the studies. No effect of age on the PK of methotrexate low dose has been found.	*No advice*	C

Table 4. [Continued]

Chemotherapeutic agent	PK findings/remarks	Recommended dosing regimen and dose adjustments for infants	Grade of recommendation
Mitoxantrone	One PK study in children has been published (unknown number of infants). The effect of age on the PK of mitoxantrone has not been studied.	*No advice*	D
Pegaspargase	PK studies in children have been published, however, the number of infants was limited and the effect of age was not studied in most of the studies. No effect of age on the PK of pegaspargase has been found.	Full (mg/m^2) dose, adjust based on TDM.	B
Prednisone	Some PK studies in children have been published, however, no infants were included. No effect of age on the PK of prednisolone has been found.	*No advice*	C
Temozolomide	Some PK studies in children have been published, however, the number of infants was limited and the effect of age was not studied in most of the studies.	*No advice*	C
Thioguanine	Some PK studies in children have been published, however, the number of infants was limited and the effect of age was not studied in most of the studies. No effect of age on the PK of thioguanine has been found.	Full (mg/m^2) dose, adjust based on WBC.	B

Table 4. [Continued]

Chemotherapeutic agent	PK findings/remarks	Recommended dosing regimen and dose adjustments for infants	Grade of recommendation
Topotecan	PK studies in children (including infants) have been published, however, the effect of age was not studied in most of the studies. The studies on the effect of age on the PK of topotecan show conflicting results.	Full (mg/m^2) dose	B
Vincristine	PK studies in children have been published, however, the number of infants was limited. Most studies did not find an effect of age on the PK.	Full mg/m^2 or mg/kg dose (≥0.05mg/kg). For neonates (0-4 weeks of age), use mg/kg dose (≥0.05mg/kg).	A

AUC Area under the curve, *BSA* Body surface area, *BW* Body weight, *CL* Clearance, *GFR* Glomerular filtration rate, *PK* Pharmacokinetics, *SCT* Stem cell transplantation, *TDM* Therapeutic drug monitoring, *WBC* White blood cell count.

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SUPPLEMENTARY METHODS

Methods

Literature search and study selection
Studies were identified by searching the electronic Pubmed database using the following query:

((pharmacokinetic*[Title/Abstract]) AND ((***drug***[Title/Abstract]) OR (***drug synonym***[Title/Abstract])) AND ((child*[Title/Abstract]) OR (pediatric*[Title/Abstract]) OR (infant*[Title/Abstract])))

Studies reporting pharmacokinetics of the chemotherapeutic agents of interest in infants and children were eligible for inclusion. The drugs of interest are presented in Table S1 and eligibility criteria in Table S2. The Pubmed results were subsequently screened by title, abstract and full text, respectively. Relevant articles that were found via references of identified studies were added to database.

Supplementary Table S1. Chemotherapeutic agents of interest

Chemotherapeutic agent		
Actinomycin D	Doxorubicin	Mercaptopurine
Blinatumomab	Etoposide	Methotrexate (high and low dose)
Busulfan	Fludarabine	Mitoxantrone
Carboplatin	Gemtuzumab ozogamicin	Pegaspargase
Cisplatin	Idarubicin	Prednisolone
Cyclophosphamide	Ifosfamide	Temozolomide
Cytarabine	Irinotecan	Thioguanine
Daunorubicin	Isotretinoin	Topotecan
Dexamethasone	Melphalan	Vincristine
Dinutuximab		

Supplementary Table S2. Eligibility criteria for selection of relevant articles.

Inclusion criteria	Exclusion criteria
Articles about the pharmacokinetics of the drug in children with cancer Case reports on PK of the drug in children <4 years of age Full text available English manuscript	Studies that did not include any patients below the age of 4 years Studies about patients with impaired renal/hepatic function

Data collection

Two reviewers extracted the following data from the included studies in the database: PK methods (type of PK analysis, covariates for PK parameters), number of patients and infants included in the study, age and age-related findings. Two reviewers checked the extracted data.

Levels of evidence

Inspired by the Oxford Centre for Evidence-Based Medicine (CEBM) levels of evidence, five levels of evidence for PK studies were established[1,2]. The criteria for the levels of evidence are presented in Table S3. The reviewers categorized the included studies from 1 to 5 for the level of evidence.

Supplementary Table S3. Levels of evidence[1,2]

Level	Criteria
1	Population PK model including infants
2	PK model including infants Poor quality population PK model (effect of age not studied) Population PK model without infants
3	Non compartmental PK study Poor quality PK model (effect of age not studied) PK model without infants
4	Case-report/-series
5	Mechanism-based reasoning Expert opinion

Grades of recommendation

Grades of recommendations were subsequently established based on the grades of recommendation of the CEBM[1,2], see Table S4. The PK findings for all the individual drugs were summarized in Table 4, and a grade for each chemotherapeutic agent was derived. Five reviewers individually graded all the drugs and disagreements were resolved by discussion between the reviewers. Thereafter the reviewers agreed on a recommended dose or dose adjustment for neonates and infants for every chemotherapeutic agent.

Supplementary Table S4. Grades of recommendation[1,2]

Grade	Criteria	Clinical meaning
A	Convincing and consistent data of level 1 studies	Sufficient evidence available to recommend dosing in neonates/infants
B	Consistent level 2 or 3 studies or extrapolations from level 1 studies	Some evidence to guide dosing in neonates/infants available, but more information is needed
C	Level 4 studies or extrapolations from level 2 or 3 studies	Paediatric data available but no studies in neonates/infants or inconsistent data
D	Level 5 evidence or troublingly inconsistent or inconclusive studies of any level	No relevant data available

References Supplementary Methods

1. Oxford Centre for Evidence-Based Medicine: OCEBM Levels of Evidence Working Group*, "The Oxford 2011 Levels of Evidence". Accessed via: https://www.cebm.ox.ac.uk/resources/levels-of-evidence/ocebm-levels-of-evidence.
   * OCEBM Levels of Evidence Working Group = Jeremy Howick, Iain Chalmers (James Lind Library), Paul Glasziou, Trish Greenhalgh, Carl Heneghan, Alessandro Liberati, Ivan Moschetti, Bob Phillips, Hazel Thornton, Olive Goddard and Mary Hodgkinson
2. Oxford Centre for Evidence-Based Medicine: Levels of Evidence (March 2009). Accessed via: https://www.cebm.ox.ac.uk/resources/levels-of-evidence/oxford-centre-for-evidence-based-medicine-levels-of-evidence-march-2009

SUPPLEMENTARY MATERIAL

Supplementary Table S5. Overview of PK publications

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Actinomycin D						
Veal (2005)	Three compartment popPK model	31 / 0	7 (1-20)	Effect of age was not studied.	2	[1]
Mondick (2008)	Three compartment popPK model Allometric scaling for BW.	33 / 0	NS (1.6-20.3)	No age-related effects independent of BW.	2	[2]
Edwards (2012)	Three compartment popPK model Allometric scaling for BW. Age was included as covariate on V1.	36 / 0	NS (1.6-20.3)	Age was included as covariate on V1, indicating a lower V1, corrected for BW, in older patients.	2	[3]
Hill (2014)	Three compartment popPK model Allometric scaling for BW.	117 / 9	4.6 (0.3-19.8)	Effect of age on PK not discussed. No effect of age on toxicity grade.	1	[4]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Skolnik (2021)	Non-compartmental pharmacokinetics	53 / 4	NS (0.46-16.7)	Variability in $t_{1/2}$, AUC and CL was high in children <1 years. The median AUC for children <1 years was approximately 50% lower than for older children (relatable to the more than 2-fold higher BSA-normalized dose in children ≥ 1 year. Dose did not appear to be related to AUC and CL corrected for BSA did not appear to be related to age.	3	[6]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Blinatumomab						
Von Stackelberg (2016)	Non-compartmental pharmacokinetics	49 / NS - Phase I 44 / NS - Phase II	6 (<1-16) - Phase I 10.5 (<1-17) -Phase II	Pharmacokinetics consistent between age groups.	3	[7]
Clements (2020)	One compartment popPK model BSA was included as covariate on CL.	674 / >2 (Fig. 1b)	41.0 (0.6-80)	No effect of age on BSA-normalized CL (L/h/m^2). Dosing should be based on BSA in younger patients.	1	[8]
Busulfan						
Dalle (2003)	Non-compartmental pharmacokinetics	14 / 14	0.4 (0.06-1)	Cumulative dose of 16 mg/kg leads to increased exposure in infants compared to adults. No correlation between age and AUC in the complete cohort. A trend for correlation between age and AUC in the female subgroup was seen.	3	[9]
Nguyen (2004)	One compartment popPK model	24 / 3	6.0 (0.45-16.7), mean (range)	A log-linear relationship between BW and CL was demonstrated with no further age-dependency. A novel dosing regimen (mg/kg dose adjusted to discrete weight categories) for a better AUC targeting was developed.	1	[10]
Tran (2004)	One compartment popPK model	20 / 1	5.5 (0.8-14.9)	CL (mL/min/kg) was higher in children <6 years than in patients >6 years.	2	[11]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Oechtering (2005)	One compartment popPK model	19 / 1	4 (0.9-17.3)	No effect of age on exposure.	2	[12]
Kletzel (2006)	Non-compartmental pharmacokinetics	30 / 8	8 (0.11-16)	Effect of age was not studied.	3	[13]
Zwaveling (2006)	One compartment PK model	18 / 1	7.0 (0.5-16)	CL (L/h/kg) proved to be dependent on age, whereas CL (L/h/m^2) was age-independent.	2	[14]
Booth (2007)	One compartment popPK model Allometric scaling for BW.	24 / NS	6.3 (0.25-16.7), mean (range)	No effect of age on CL after adjusting for BW.	1	[15]
Schechter (2007)	One compartment popPK model	45 / 13	3.0 (0.25-16.2)	CL (mL/min/kg) was significantly higher in children <4 yrs. However, CL (mL/min/m^2) was significantly lower in children <1 yrs and <4 yrs. V (L/kg) was significantly higher in children <1 year or <4 years.	2	[16]
Nath (2008)	One compartment PK model	40 / 7	3.2 (1.5-9.1)	Age correlated with CL (L/h) and CL (L/h/kg), but not with CL (L/h/m^2). AUC did not correlate with age.	2	[17]
Kim (2009)	Non-compartmental pharmacokinetics	21 / NS	8 (0.25-18), mean (range)	CL (mL/min/kg) was significantly higher in children <4 yrs.	3	[18]
Wall (2009)	Non-compartmental pharmacokinetics	24 / 3	3.3 (0.5-16.7)	No effect of age on CL (mL/min/kg).	3	[19]
Trame (2011)	One compartment popPK model Allometric scaling for BW.	94 / NS	9.2 (0.4-18.8)	Effect of age was not described.	2	[20]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Bartelink (2012)	Two compartment popPK model Allometric scaling for BW.	245 / NS	3.33 (0.1-26)	Non-linear relationship between BW and CL (L/h): An increase in BW in neonates results in a larger increase in CL than an increase in BW in older children or adults.	1	[21]
Bartelink (2012)	Two compartment popPK model Allometric scaling for BW.	403 / NS	4.00 (0.1-35)	Non-linear relationship between BW and CL (L/h): An increase in BW in neonates results in a larger increase in CL than an increase in BW in older children or adults.	1	[22]
Michel (2012)	One compartment popPK model	67 / 9	4.0 resp. 7.5 (autoSCT resp. alloSCT) (0.3-17.2)	Non-linear relationship between BW and clearance.	1	[23]
Paci (2012)	One compartment popPK model Allometric scaling for BW.	205 / NS	2.5 (0.03-15)	The higher allometric exponent for CL accounted for a larger increase of CL in children <9 kg. For infants <9 kg, the model predicted a 2.4-fold increase in CL for a doubling in BW, whereas a 1.7-fold increase in CL was associated for the same BW growth in children ≥9 kg.	1	[24]
Veal (2012)	One compartment popPK model Allometric scaling for BW.	38 / NS	3.6 (0.7-13.1), mean (range) for iv subgroup	Effect of age was not described.	2	[25]
Le Gall (2013)	Non-compartmental pharmacokinetics	49 / 3	7.4 (0.2-18.4), mean (range)	CL (mL/min/kg) was significantly higher in children <4 yrs.	3	[26]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
McCune (2013)	Non-compartmental pharmacokinetics, one or two compartment PK model Allometric scaling for BW.	729 / NS	5.0 (0.1-20.0)	Non-linear relationship between age and CL (mL/min/kg).	2	[27]
Savic (2013)	One compartment popPK model Allometric scaling for BW. CL corrected for maturation.	149 / NS (20 <0.5 yrs)	0.94 (0.08-3.3)	CL increases by approximately 1.7-fold between 6 weeks and 2 years of life due to maturation.	1	[28]
Diestelhorst (2014)	One compartment popPK model Allometric scaling for BW.	82 / NS	NS (0.4-18.8)	Effect of age was not described.	2	[29]
McCune (2014)	Two compartment popPK model Allometric scaling for BW. CL corrected for maturation and sex V1 and V2 corrected for sex	1610 / 256	9.8 (0.1-66), mean (range)	The maturation of CL reaches 50% of adult values at 6 weeks after birth assuming a full-term gestational age of 40 weeks. Size-standardized CL reaches 95% of adult values at 2.5 yrs.	1	[30]
Okamoto (2014)	One compartment popPK model BW was included as covariate on CL and V.	25 / 5	6 (0.4-17)	Effect of age was not described.	2	[31]
Long-Boyle (2015)	One compartment popPK model Allometric scaling for BW. CL corrected for maturation.	90 / NS	7 (0.1-24)	CL (adjusted for BW) increases up through 12 yrs and then begins to decline to adult levels.	1	[32]
Neely (2016)	One compartment popPK model Allometric scaling for BW. CL and V corrected for age.	53 / NS	7.8 (0.2-19), mean (range)	CL (adjusted for BW) increases up through ~7 yrs and then begins to decline to adult levels.	1	[33]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Nava (2018)	One compartment popPK model Allometric scaling for BW. CL corrected for maturation and GSTA1 diplotypes	112 / 20	5.4 (0.1-20)	CL was corrected for maturation.	2	[34]
Alsultan (2020)	One compartment popPK model Allometric scaling for BW.	59 / NS	6.1 (0.16-13), mean (range)	Effect of age was not described.	2	[35]
Marsit (2020)	One compartment popPK model Allometric scaling for BW. CL and V corrected for age.	136 / NS	6.6 (0.2-20.7), mean (range)	Age was included as covariates on all parameters.	1	[36]
Poinsignon (2020)	One compartment popPK model Allometric scaling for BW. CL and V were corrected for maturation.	540 / 162	1.8 (0.02-24.1), mean (range)	All parameters were corrected for maturation.	1	[37]
Yuan (2021)	One compartment popPK model CL corrected for BSA, GSTA1 diplotypes and ASAT. V corrected for BSA.	69 / NS	4.90 (0.50-15.18)	No effect of age after including BSA as covariate.	2	[38]
Neroutsos (2021)	Non-compartmental pharmacokinetics	76 / NS (12 <2 years)	6.5 (0.5-19)	Effect of age was not studied.	3	[39]
Ben Hassine (2021)	Two compartment popPK model Postmenstrual age dependent allometric scaling of BW on CL. CL corrected for day of therapy, GSTA1 diplotypes and regimen including fludarabine.	302 / NS	5.2 (0.1-20.1)	CL was corrected for maturation.	1	[40]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Carboplatin						
Riccardi (1992)	Case series, two compartment PK model (total and free platinum)	5 / 0	6 (1.5-7)	Effect of age was not studied.	4	[41]
Madden (1992)	Two compartment popPK model (total and free platinum)	18 / 0	7.7 (2.9-23)	No effect of age on CL after normalization to BW	2	[42]
Newell (1993)	Two compartment PK model (total and free platinum)	22 / 4	3.8 (0.3-15.8)	Effect of age was not studied only the impact of EDTA CL on dosing. Significant correlation between EDTA CL and carboplatin CL.	3	[43]
Riccardi (1994)	Two compartment PK model (free platinum)	35 / NS	7 (0.8-17)	Effect of age was not studied but PK parameters comparable to those reported in adults.	3	[44]
Peng (1995)	Two compartment popPK model (total and free platinum)	23 / 3	3.7 (0.08-18.4)	Effect of age was not studied.	2	[45]
Chatelut (1996)	Two compartment popPK model (free platinum) CL corrected for BW, creatinine and nephrectomy	57 / NS	5 (0.17-18)	No additional effect of age on PK parameters that was not explained by BW or BSA.	1	[46]
Tonda (1996)	One compartment popPK model (free platinum)	21 / 4	1.7 (0.2-4.2)	Median CL (mL/min/m^2) in infants lower than median for older patients.	1	[47]
Doz (1998)	Two compartment popPK model (free platinum)	15 / 1 16 / 1	5.0 (0.9-10.7) 4.6 (0.75-17.5)	Effect of age was not studied.	2	[48]
Thomas (2000)	Two compartment popPK model (free platinum)	38 / NS	3.5 (0.4-16.3)	No effect of age on dosing method (renal function vs BSA dosing).	1	[49]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Patoux (2001)	Two compartment popPK model (free platinum) Serum creatinine was identified as the most significant covariate on CL.	117 / NS	6 (0.1-18.4)	No effect of age on CL relative to other covariates tested.	1	[50]
Rubie (2003)	Two compartment popPK model (free platinum)	12 / 0	3.4 (2.8-17.3)	Effect of age was not studied.	2	[51]
Kangarloo (2004)	Non-compartmental pharmacokinetics and PK model (total and free platinum)	10 / 2	11 (0.25-16)	Effect of age was not studied.	3	[52]
Veal (2007)	Two compartment popPK model (free platinum)	28 / 0	11.7 (1-21)	Effect of age on PK was not studied. No age effect on platinum adduct formation.	2	[53]
Levy (2009)	Non-compartmental pharmacokinetics (free platinum)	28 / 0	8.5 (1-21)	Effect of age was not studied.	3	[54]
Picton (2009)	Case series, two compartment PK model (free platinum)	1 / 1	Gest. age 32 weeks	CL (mL/min) increased by 2-fold over 7 weeks.	4	[55]
Veal (2010)	Two compartment popPK model (free platinum) BW was included as covariate on PK parameters.	19 / 16	0.8 (0.2-1.2)	CL (mL/min/kg) higher in infants <12kg compared to children >12kg.	1	[56]
Veal (2015)	Therapeutic drug monitoring in preterm and full-term neonates. CL and AUC were calculated using the PK model of Veal (2007) and Peng (1995).	9 / 9	3 weeks (0.4-24 weeks) (gest. age 40 weeks (35-52 weeks))	CL (mL/min and mL/min/kg) increased with increasing age (normalized to full-term gestation).	1	[57]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Veal (2016)	Case series, non-compartmental pharmacokinetics (free platinum)	1 / 1	8 weeks (gest. age 40 weeks)	CL normalized for BW comparable with reported values.	4	[58]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Duong (2019)	Two compartment popPK model (free platinum) Allometric scaling for BW.	46 / 0	3.5 (1.7-8.3)	No effect of age on the PK parameters after including BW into the model.	2	[59]
Hawley (2019)	Case series, non-compartmental pharmacokinetics (free platinum)	1 / 1	5 weeks (gest. age 40 weeks)	Effect of age not discussed, but under standard dosing regimen patient would have been under-dosed	4	[60]
Hong (2020)	Non-compartmental pharmacokinetics and one compartment popPK model (total platinum) eGFR was included as covariate on CL.	25 / 0	6 (1-17)	Effect of age was not studied.	2	[61]
Cisplatin						
Crom (1981)	Two compartment PK model (total platinum) and non-compartmental pharmacokinetics (free platinum)	28 / 0	11.4 (1.7-20.5)	The elimination rate constant of total platinum was larger in older children (more rapid elimination).	3	[62]
Dominici (1989)	Non-compartmental pharmacokinetics (total and free platinum)	14 / NS	NS (0.8-13)	Effect of age was not studied.	3	[63]
Murakami (1990)	One compartment PK model (free platinum)	6 / 0	9.4 (1.7-15.7)	Younger patients showed a lower CL and a higher V.	3	[64]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Peng (1997)	Non-compartmental pharmacokinetics and one compartment PK model (free platinum)	21 / 1	9.1 (0.5-19.3)	Effect of age was not studied.	3	[65]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Thomas (2018)	Case report, non-compartmental pharmacokinetics	1 / 1	2 weeks (gest. age 37 weeks)	CL was correlated to age and BW. CL (mL/min/kg) increased with increasing age.	4	[66]
Cyclophosphamide						
Juma (1984)	Non-compartmental pharmacokinetics Kenyan patients	8 / 0	NS (1-4)	Effect of age not studied in this patient group. However, CL (mL/h/kg) values here are higher than those reported in adults.	3	[67]
Tasso (1992)	Non-compartmental pharmacokinetics	9 / 2	4.3 (0.7-16.8)	Effect of age not studied in this patient group. However, CL (L/h/m^2) values here are higher than those reported in adults.	3	[68]
Yule (1996)	One compartment popPK model	38 / 5	4 (0.2-18)	No additional effect of age on PK parameters that was not explained by BSA.	1	[69]
Yule (1997)	PopPK model (compartments not described)	14 / 1	8.5 (0.7-17)	Effect of age on PK was not studied.	2	[70]
Yule (2001)	One compartment popPK model	13 / 0	4 (2-17)	No effect of age on PK parameters.	2	[71]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Yule (2004)	One compartment popPK model	36 / 0	8 (2-16)	Effect of age on PK was not studied.	2	[72]
McCune (2009)	Multicompartment popPK model (one compartment for cyclophosphamide, three compartments for metabolites)	22 / 0	3.16 (1.3-9.37)	Cyclophosphamide AUC associated with patient age and BSA.	2	[73]
Balasubramanian (2012)	Two compartment popPK model (cyclophosphamide) One compartment popPK model (metabolites) BW, age and CYP2C19 polymorphism were included as covariates for CL	55 / 0	7.3 (2-14)	Decrease in CL (L/h/kg) with patient age.	2	[74]
Navid (2013)	Two compartment popPK model	19 / 0	9.2 (1.2-24.5)	Effect of age was not studied.	2	[75]
Veal (2016)	Two compartment popPK model Allometric scaling for BSA. CYP2B6 polymorphism and eGFR correlated with CL.	49 / 0	11.7 (3.5-18.7)	No effect of age on CL (L/h/m^2).	2	[76]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Campagne (2020)	Two compartment popPK model (cyclophosphamide) One compartment popPK model (metabolites) Age, phenobarbital treatment and CYP2B6 polymorphism correlated with PK.	171 / 42	1.8 (0.07-4.9)	Infants had higher exposures of the active metabolite compared to older children. Suggested to decrease dose by 20% in young infants to achieve similar exposures to older children.	1	[77]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Barnett (2021)	Two compartment popPK model Allometric scaling for BSA. CYP2B6 polymorphism and eGFR were included as covariates for CL.	25 / 12	1 (0.33-2)	No effect of age on PK within this study. However, CL (mL/min/m^2) of cyclophosphamide and AUC of metabolites in children <2 years old significantly higher than those reported in older children.	1	[78]
Cytarabine (ARA-C)						
Avramis (1987)	Non-compartmental pharmacokinetics (ARA-C and ARA-U)	20 / 0	3 (1.5-19)	AUC (ARA-C) higher in children compared to adults. Patients <2 yrs had higher ARA-C concentrations than older patients	3	[79]
Avramis (1989)	Two compartment PK model (ARA-C) One compartment PK model (ARA-U) Age and BSA were included as covariates on all parameters.	8 / 1	6.5 (0.58-16)	Age was included as covariate on all parameters.	3	[80]
McLeod (1992)	One compartment PK model	67 / 3	NS (0.64-19)	CL (mL/min/m^2) is not different in infants.	2	[81]
Periclou (1996)	Two compartment popPK model (ARA-C) One compartment popPK model (ARA-U) Age and BSA were included as covariates on all parameters.	52 / NS	NS (0.2-19)	All parameters increase with increasing age and BSA. Conversion of ARA-C to ARA-U is age dependent (lower rate of conversion in infants).	1	[82]
Avramis (1998)	Non-compartmental pharmacokinetics (ARA-C)	20 / 0	8 (1-19)	Effect of age was not studied.	3	[83]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Evans (1998)	One compartment PK model	182 / 8	NS (0.3-18.8)	Effect of age was not studied.	3	[84]
Ozkaynak (1998)	Non-compartmental pharmacokinetics (ARA-C and ARA-U)	13 / 0	7 (1-17)	Effect of age was not studied.	3	[85]
Daunorubicin						
Hempel (2010)	Two compartment popPK model	33 / 20	NS (0.05-18.8)	No correlation between PK parameters, dose normalized for BSA, and age.	1	[86]
Thompson (2014)	Two compartment popPK model	98 / NS	12 (0.5-20.4)	No correlation between PK parameters and age or body composition.	1	[87]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Dexamethasone						
Spektor (2008)	Non-compartmental pharmacokinetics	25 / 0	4 (1-14)	Effect of age was not studied. But $t_{1/2}$ comparable to data reported in adults.	3	[88]
Yang (2008)	One compartment popPK model	214 / 0	NS (1-18.8)	Negative correlation between patient age and CL/F (normalized to BSA). Higher CL in younger patients.	2	[89]
Kawedia (2012)	Not stated	339 / 0	NS	CL (L/h/m^2) higher in patients less than 10 years old	3	[90]
Inaba (2018)	One compartment popPK model	409 / 0	NS (1-18)	Effect of age on PK was not studied.	2	[91]
Jackson (2019)	One compartment popPK model Allometric scaling of CL for BW.	161 / 0	NS (1.3-18.7)	No effect of age on PK.	2	[92]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Dinutuximab						
Uttenreuther-Fischer (1995)	Two compartment PK model	10 / 0	6.4 (2.1-11.4)	Effect of age was not studied. $t_{1/2}$ values lower than those reported in adults	3	[93]
Ladenstein (2013)	Non-compartmental pharmacokinetics	16 / 0	7.6 (3.8-17.3)	Effect of age was not studied.	3	[94]
Desai (2014)	Non-compartmental pharmacokinetics and Two compartment PK model	14 / 0	4.3 (1.2-7.3)	CL normalized to BW shows a negative correlation with age. Younger children exhibit higher CL values.	3	[95]
Marachelian (2016)	Two compartment popPK model BW included as an allometric covariate on CL and V in the model.	28 / 0	4 (2-7) Arm 1 4 (1-9) Arm 2, mean (range)	Effect of age not studied.	2	[96]
Doxorubicin						
McLeod (1992)	Non-compartmental pharmacokinetics (doxorubicin)	60 / 4	NS (0.17-20)	No difference in CL (mL/min/kg) between infants and older children, but there was a trend toward a lower CL (mL/min/m^2) in infants compared to older children.	3	[81]
Eksborg (2000)	Non-compartmental pharmacokinetics (doxorubicin)	31 / NS	5.4 (0.73-15.3)	No correlation between age and Cmax after dose normalization for body size.	3	[97]
Frost (2002)	Non-compartmental pharmacokinetics (doxorubicin and doxorubicinol)	112 / 5	4.7 (for pts >1 yr) (0.3-17.3)	Children 4-6 yrs had the highest dose normalized steady state plasma concentration, followed by children 2-4 yrs, younger and older pts showed similar plasma concentrations.	3	[98]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Hempel (2002)	Non-compartmental pharmacokinetics (doxorubicin)	27 / 0	4.13 (1.56-19.99)	Age had no effect on Cmax	3	[99]
Palle (2006)	Non-compartmental pharmacokinetics (doxorubicin)	37 / 1	9.2 (0.63-17.7)	No sign differences in CL (mL/min/ m^2) between children <2 yrs and older children	3	[100]
Thompson (2009)	Three compartment popPK model (doxorubicin) One compartment popPK (doxorubicinol) Allometric scaling for BSA.	22 / 0	15.0 (3.3-21.5)	No correlation between PK parameters and age after adjusting for BSA.	2	[101]
Köntny (2013)	Three compartment popPK model (doxorubicin) One compartment popPK (doxorubicinol) Allometric scaling for BSA.	82 / 0	21 (3-73)	After adjusting for BSA: No correlation between CL of doxorubicin and doxorubicinol and age. A trend towards a smaller V1 and a larger Vm in younger patients was observed.	2	[102]
Völler (2015)	Three compartment popPK model (doxorubicin) One compartment popPK (doxorubicinol) Allometric scaling for BSA. Age was included as covariate on CL of doxorubicin.	94 / 4	5.32 (0.2-17.7)	Age was included as covariate on CL of doxorubicin, indicating a lower CL, corrected for BSA, in younger patients.	1	[103]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Krischke (2016)	Three compartment popPK model (doxorubicin) One compartment popPK (doxorubicinol) Allometric scaling for BSA. Age was included as covariate on CL of doxorubicin.	101 / NS	5.3 (0.2-17.7)	Age dependence of CL of doxorubicin: <3 years having a statistically significant lower CL than older children after correcting for BSA.	1	[104]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Kunarajah (2017)	Three compartment popPK model (doxorubicin) One compartment popPK (doxorubicinol) Allometric scaling for BSA. Age was included as covariate on CL of doxorubicin.	17 / 0	7.50 (3.4-14.7)	Age was included as covariate on CL, indicating a lower CL, corrected for BSA, in younger patients.	2	[105]
Siebel (2020)	Pharmacokinetic simulations using the popPK model of Völler (2015).	94 / 4	5.32 (0.2-17.7)	Conclusions: treatment strategies for young children should adapt both the dose (based on age and BSA) and the duration of infusion.	5	[106]
Etoposide						
Evans (1982)	Two compartment PK model	9 / NS	10 (0.25-18)	CL comparable with adults.	2	[107]
McLeod (1992)	Two compartment PK model	25 / 2	NS (0.5-18)	CL (mL/min/kg) was lower in infants compared to older children. However, no difference in CL (mL/min/m^2).	2	[81]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Lowis (1993)	Two compartment PK model	33 / NS	4.8 (0.4-16.1)	Effect of age was not studied.	2	[108]
Rodman (1994)	Two compartment PK model	22 / 0	6.7 (1.6-23.9)	No effect of age on CL after adjusting for body size.	3	[109]
Boos (1995)	Evaluation of steady state plasma concentrations	40 / 11	2.4 (0.25-28)	Dosing in mg/kg let to significantly lower Css values than dosing in mg/m^2. No effect of age on CL ($mL/min/m^2$ and mL/min/kg).	3	[110]
Würthwein (1999)	Two compartment popPK model	18 / 1	10.5 (0.8-17)	Effect of age was not studied.	2	[111]
Eksborg (2000)	One or two compartment PK model	16 / 1	8.3 (0.3-22)	AUC normalized to BSA was not age dependent. $t_{½}$ was not age dependent.	2	[112]
Lacayo (2002)	Two compartment popPK model Pharmacokinetic interaction with cyclosporine	38 / NS	NS (0.7-17)	Effect of age was not studied.	2	[113]
Würthwein (2002)	Three compartment popPK model	31 / 1	8.0 (0.8-23.7)	No correlation between CL ($mL/min/m^2$) and age. A significant correlation between CL (mL/min/kg) and age.	1	[114]
Kato (2003)	One compartment PK model	18 / 1	7.4 (0.3-18.0, mean (range)	No effect of age on $t_{½}$, V or AUC.	2	[115]
Palle (2006)	Non-compartmental pharmacokinetics	45 / 2	10.3 (0.5-17.7)	No effect of age on CL ($mL/min/m^2$).	3	[116]
Veal (2010)	Two compartment popPK model Allometric scaling for BW	11 / 7	0.8 (0.2-1.2)	No effect of age on CL after including BW into the covariate model.	1	[56]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Urien (2011)	Two compartment popPK model Allometric scaling for BW	67 / 8	3.5 (0.3-16.7)	No effect of age on CL (using a sigmoid Emax model relating the post-menstrual age) after including BW into the model.	1	[117]
Baheti (2013)	One compartment popPK model Allometric scaling for BW	26 / 0	8.5 (2-19), mean (range)	Including age as covariate on CL and V did significantly reduce the objective function during stepwise forward addition process, but was not significant when backward elimination was performed.	1	[118]
Veal (2016)	Case series, non-compartmental pharmacokinetics	1 / 1	8 weeks (gest. age 40 weeks)	CL and AUC comparable with reported values.	4	[119]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Duong (2019)	Two compartment popPK model Allometric scaling for BW.	51 / 0	3.5 (1.7-8.3)	No effect of age on the PK parameters after including BW into the covariate model.	2	[59]
Fludarabine						
Ivaturi (2017)	Two compartment popPK model Allometric scaling for BW. eGFR was included as covariate on CL.	133 / NS	5 (0.2-17.9)	No effect of age on CL, normalized to BW.	1	[120]
Chung (2019)	Two compartment popPK model Allometric scaling for BSA. eGFR was included as covariate on CL.	43 / 0	11.8 (1.3-18.5)	No effect of age on CL, normalized to BSA.	2	[121]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Langenhorst (2019)	Three compartment popPK model Allometric scaling for BW. eGFR was included as covariate on CL.	258 / NS	18 (0.3-74)	After accounting for covariates, no size-independent effect of age was identified.	1	[122]
Gemtuzumab ozogamicin						
Buckwalter (2004)	Non-compartmental pharmacokinetics	29 / 0	9.6 (1-16), mean (range)	No statistical difference in PK parameters across age groups	3	[123]
Masters (2018)	Two compartment popPK model Allometric scaling for BW.	29 / 0	12 (1.2-16.9)	No age-related effects independent of BW.	2	[124]
Idarubicin						
Tan (1987)	Non-compartmental pharmacokinetics (idarubicin, idarubicinol)	7 / 0	NS (1-19)	Effect of age was not studied.	3	[125]
Reid (1990)	Non-compartmental pharmacokinetics (idarubicin, idarubicinol)	21 / 0	NS (1-21)	No effect of age on PK parameters of idarubicin or idarubicinol	3	[126]
Dreyer (2003)	Non-compartmental pharmacokinetics (idarubicin, idarubicinol)	14 / 0	7.2 (2.2-19)	Effect of age was not studied.	3	[127]
Ifosfamide						
Boddy (1993)	Non-compartmental pharmacokinetics	16 / 0	4 (1-17)	No additional effect of age on PK parameters that was not explained by BW/BSA	3	[128]
Prasad (1994)	Non-compartmental pharmacokinetics	5 / 0	14 (3-15)	Effect of age was not studied.	3	[129]
Boddy (1996)	Non-compartmental pharmacokinetics	11 / 0	NS (1-16)	Positive correlation between patient age and exposure	3	[130]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Kerbusch (2001)	One compartment popPK model	32 / 0	NS (1-18)	Effect of age was not studied.	2	[131]
Willits (2005)	Non-compartmental pharmacokinetics	19 / 0	10 (1-19)	Effect of age on PK parameters not studied. No correlation between age and DNA damage	3	[132]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Irinotecan						
Crews (2002)	PopPK model (irinotecan and metabolites, compartments not described)	31 / 0	NS (3-21)	Effect of age was not studied.	2	[133]
Gajjar (2003)	Non-compartmental pharmacokinetics	35 / 0	NS (3-21)	Effect of age was not studied.	3	[134]
Vassal (2003)	Non-compartmental pharmacokinetics	77 / NS	8 (0.9-18.6)	Effect of age was not studied. But CL values (L/h/m^2) in these children were higher than those reported in adults	3	[135]
Wagner (2004)	One compartment popPK model (additional compartments for metabolites)	12 / 0	12.5 (1-23)	Effect of age was not studied.	2	[136]
Bomgaars (2006)	Non-compartmental pharmacokinetics	9 / 0 (Stratum 1) 9 / 0 (Stratum 2)	11 (4-17) S1 6 (2-15) S2	Effect of age was not studied.	3	[137]
Furman (2006)	One compartment popPK model (additional compartments for metabolites) (after oral administration)	39 / 0	10 (3-19)	Effect of age was not studied.	2	[138]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Rodriguez-Galindo (2006)	One compartment popPK model (additional compartments for metabolites)	11 / 0	10 (3-19)	Effect of age was not studied. Comparable PK to children in other studies.	2	139
Bomgaars (2007)	Non-compartmental pharmacokinetics	79 / 0	10 (2-23)	Effect of age was not studied.	3	140
Stewart (2007)	Multicompartment PopPK model (after i.v. and oral administration)	74 / 0	10.4 (3.2-21.6)	Effect of age was not studied.	2	141
Thompson (2008)	Two compartment popPK model (additional compartments for metabolites) Allometric scaling for BW. Age and bilirubin included as covariates on SN-38 CL	82 / 0	NS (1-21)	Age and bilirubin significant covariates in SN-38 CL. CL (L/h) of SN-38 greater in patients <10 years old	2	142
Furman (2009)	Two compartment popPK model (additional compartments for metabolites)	29 / 0	9 (1-21)	Effect of age was not studied.	2	143
Levy (2009)	Non-compartmental pharmacokinetics	28 / 0	8.5 (1-21)	Effect of age was not studied.	3	54
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	5
Jannier (2020)	Non-compartmental pharmacokinetics	42 / 0	10.5 (2-18)	Effect of age was not studied.	3	144
Isotretinoin						
Villablanca (1995)	Non-compartmental pharmacokinetics	51 / 0	4 (2-12)	Effect of age was not studied.	3	145
Khan (1996)	One compartment PK model	31 / 0	4 (2-12)	Effect of age could not be determined due to age distribution in patient cohort.	3	146

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Veal (2007)	One compartment popPK model	29 / 0	3.2 (1.1-18.7)	Higher weight and age associated with higher exposures.	2	[147]
Veal (2013)	One compartment popPK model	103 / 10	4.3 (0.8-20.5)	No effect of age on PK parameters.	1	[148]
Gota (2016)	Non-compartmental pharmacokinetics	35 / 0	5 (1-13)	No effect of age on exposure.	3	[149]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Veal (2021)	Two compartment popPK model Allometric scaling for BW.	20 / 0	4.3 (1-1.66)	No additional effect of age on PK parameters that was not explained by BW.	2	[150]
Melphalan						
Taha (1983)	One compartment PK model	10 / 0	5.5 (2.5-16)	No effect of age on PK parameters	3	[151]
Ninane (1985)	Two compartment PK model	9 / 1	4.1 (1.1-10)	Effect of age was not studied.	3	[152]
Ardiet (1986)	Two compartment PK model	26 / 0	12.2 (1.3-57)	No difference in PK between adults in children. But a trend towards lower exposures in children due to shorter half-life.	3	[153]
Gouyette (1986)	Two compartment PK model	20 / 0	4.4 (1.8-14)	Effect of age was not studied.	3	[154]
Horowitz (1988)	Non-compartmental pharmacokinetics	26 / 1	11 (<1-21)	Effect of age was not studied.	3	[155]
Vassal (2001)	Two compartment popPK model	21 / NS	4.1 (0.7-14.2)	Effect of age on PK not studied. Correlation between age and neutropenia/platelet recovery. Younger age associated with prolonged neutropenia and delayed platelet recovery.	2	[156]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Nath (2005)	Non-compartmental pharmacokinetics	52 / NS	5.6 (0.3-18)	No additional effect of age on PK parameters that was not explained by BW.	3	[157]
Nath (2007)	Two compartment popPK model BW, eGFR and previous carboplatin treatment were included as covariate on CL. BW was included as covariate on V.	59 / NS	NS (0.3-17.6)	No effect of age on PK parameters. BW, creatinine CL important covariates for CL and BW for V.	1	[158]
Schaiquevich (2012)	Two compartment popPK model BW was included as covariate on the PK parameters.	17 / NS	1.8 (0.6-6.2)	Inter-individual variability partly explained by age, BW and BSA.	1	[159]
Taich (2014)	Two compartment popPK model The dose was normalized to BW.	21 / NS	1.7 (0.5-6.2)	No additional effect of age on PK parameters that was not explained by BW.	1	[160]
Mizuno (2018)	Two compartment popPK model Allometric scaling for BW. eGFR was included as covariate on CL.	5 / 0	5.6 (1.5-16.5)	No effect of age on PK parameters.	2	[161]
Duong (2019)	Two compartment popPK model Allometric scaling for BW.	51 / 0	3.5 (1.7-8.3)	No effect of age on the PK parameters after including BW into the covariate model.	2	[59]
Zhao (2021)	Non-compartmental pharmacokinetics	5 / 0	5.6 (1.5-16.8)	Effect of age was not studied.	3	[162]
Mercaptopurine (6-MP)						
Lennard (1986)	Non-compartmental pharmacokinetics (plasma 6-MP, RBC 6-TGN)	19 / 0	6.5 (3-16), mean (range)	No effect of age on any of the PK parameters was found.	3	[163]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Sulh (1986)	Non-compartmental pharmacokinetics (plasma 6-MP)	20 / 0	7.5 (2-16), mean (range)	No effect of age on AUC or elimination half-life.	3	[164]
Lennard (1989)	Non-compartmental pharmacokinetics (RBC 6-TGN)	120 / 0	5.3 (2-17)	No effect of age on the 6-TGN effect was found.	3	[165]
Koren (1990)	Non-compartmental pharmacokinetics (plasma 6-MP)	23 / 0	Means were 3.9 and 4.3 for two groups. Range NS.	Effect of age was not studied.	3	[166]
Lennard (1990)	Non-compartmental pharmacokinetics (RBC 6-TGN)	95 / 0	5.3 (2-17)	No effect of age on the 6-TGN concentration was found.	3	[167]
Kato (1991)	Non-compartmental pharmacokinetics (plasma 6-MP)	8 / 0	7.7 (3.6-15.1), mean (range)	No effect of age on AUC was found.	3	[168]
Zuccaro (1991)	Non-compartmental pharmacokinetics (plasma 6-MP)	18 / 0	6.9 (3-15), mean (range)	Effect of age was not studied.	3	[169]
McLeod (1992)	Non-compartmental pharmacokinetics (RBC 6-TGN)	110 / 3	NS (0.58-19)	No difference in RBC 6-TGN concentrations between infants and older children.	3	[81]
Welch (1997)	Non-compartmental pharmacokinetics (RBC 6-MMP, RBC 6-TGN)	7 / 0	4 (2-6)	Effect of age was not studied.	3	[170]
Balis (1998)	Non-compartmental pharmacokinetics (plasma 6-MP, RBC 6-TGN)	89 / 0	4.6 (1.1-17.3)	No effect of age on CL (mL/min/m^2) of 6-MP or RBC 6-TGN levels.	3	[171]
Erb (1998)	Non-compartmental pharmacokinetics (RBC 6-TGN)	32 / 0	NS (1-18)	Effect of age was not studied.	3	[172]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Mawatari (2001)	Non-compartmental pharmacokinetics (RBC 6-TGN)	12 / 0	7 (2-13)	Effect of age was not studied.	3	[173]
Bell (2004)	Non-compartmental pharmacokinetics (RBC 6-MMP, RBC 6-TGN)	226 / NS	4.4 (3.1-6.8), median (quartiles)	No effect of age on metabolite levels.	3	[174]
Hawwa (2008)	One compartment popPK model (RBC 6-MMP, RBC 6-TGN) TPMT mutation was included as covariate on formation of the 6-TGN metabolite BSA was included as covariate on CL of 6-TGN.	19 / 0	10 (3-17)	No effect of age on the PK parameters.	2	[175]
Hanff (2013)	Non-compartmental pharmacokinetics (RBC 6-MMP, RBC 6-TGN)	20 / 0	4.1 resp 4.3 (1.9-14.6)	Effect of age was not studied.	3	[176]
Larsen (2020)	Non-compartmental pharmacokinetics (plasma 6-MP)	12 / 0	5(3.75-9.25), median (quartiles)	The relative performance of two formulations (tablet vs. liquid) with respect to dose normalized AUC was not associated with age.	3	[177]
Methotrexate (MTX) (low dose)						
Pinkerton (1982)	Non-compartmental pharmacokinetics (MTX p.o. and i.v., plasma MTX concentrations)	28 / 0	NS (3-16)	No effect of age on absorption of MTX.	3	[178]
Sonneveld (1986)	Non-compartmental pharmacokinetics (MTX p.o., plasma MTX concentrations)	19 / 0	6.3 (3-14), mean (range)	Effect of age was not studied.	3	[179]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Pearson (1987)	Non-compartmental pharmacokinetics (MTX p.o. and i.m., plasma MTX concentrations)	127 / 0	NS (1-14)	No effect of age on absorption or CL (L/h/m^2) of MTX.	3	[180]
Balis (1988)	Non-compartmental pharmacokinetics (MTX p.o. and s.c., plasma MTX concentrations)	8 / 0	8 (3-19)	Effect of age was not studied.	3	[181]
Koren (1989)	Non-compartmental pharmacokinetics (MTX p.o., plasma MTX concentrations)	16 / 0	6.26 (3.75-16.75), mean (range)	A weak correlation between AUC and age was observed.	3	[182]
Skoglund (1994)	Non-compartmental pharmacokinetics (MTX p.o., plasma MTX concentrations)	17 / 0	7.3 (3-12), mean (range)	Effect of age was not studied.	3	[183]
Balis (1998)	Non-compartmental pharmacokinetics (MTX p.o., plasma MTX concentrations)	89 / 0	4.6 (1.1-17.3)	No effect of age on CL (mL/min/m^2) of MTX.	3	[171]
Methotrexate (high dose)						
Goh (1979)	Non-compartmental pharmacokinetics (plasma MTX)	24 / 0	NS (1.3-20)	Effect of age was not studied.	3	[184]
Ettinger (1982)	Non-compartmental pharmacokinetics (plasma MTX)	16 / 0	NS (1.75-14)	Effect of age was not studied.	3	[185]
Evans (1984)	Non-compartmental pharmacokinetics (plasma MTX)	108 / NS	4.1 (0.4-17)	No effect of age on CL (mL/min/m^2).	3	[186]
Parker (1986)	Non-compartmental pharmacokinetics (plasma MTX)	12 / 0	6 (2-18)	No effect of age on plasma concentrations.	3	[187]
Slørdal (1987)	Non-compartmental pharmacokinetics (plasma MTX)	5 / 0	6.2 (2.5-12), mean (range)	Effect of age was not studied.	3	[188]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Borsi (1987)	Non-compartmental pharmacokinetics (plasma MTX)	58 / 0	4 (1-19)	Children <4 years had a lower steady state concentration, higher V (L/m^2) and higher CL ($mL/min/m^2$) than older children.	3	[189]
Wolfrom (1990)	Non-compartmental pharmacokinetics (plasma MTX, 7OH-MTX)	5 / 0	NS (2-7)	Effect of age was not studied.	3	[190]
Borsi (1990)	Non-compartmental pharmacokinetics (plasma MTX, 7OH-MTX)	58 / 0	4 (1-19)	A correlation between metabolic index and age: Younger patients had higher metabolite concentrations.	3	[191]
McLeod (1992)	Two compartment PK model (plasma MTX)	112 / 4	NS (0.26-19)	CL ($mL/min/m^2$) tended to be lower in infants compared to older children (not significant).	2	[81]
Najjar (1993)	Non-compartmental pharmacokinetics (plasma MTX)	10 / 0	4.8 (3-12)	No effect of age on CL ($mL/min/m^2$).	3	[192]
Murry (1995)	Two compartment PK model (plasma MTX)	18 / NS	7.8 (0.2-17.2)	Effect of age was not studied.	3	[193]
Donelli (1995)	Two compartment PK model (plasma MTX)	122 / 7	NS (0.25-15)	Patients <10 years had a higher CL ($L/h/kg$ and $L/h/m^2$). A faster elimination in infants was observed.	2	[194]
Seidel (1997)	Non-compartmental pharmacokinetics (plasma MTX)	42 / NS	NS (0.8-13.1)	Effect of age was not studied.	3	[195]
Rask (1998)	Non-compartmental pharmacokinetics (plasma MTX, 7OH-MTX)	13 / 0	6.7 (3.3-12.9)	No effect of age on AUC.	3	[196]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Odoul (1999)	Two compartment PK model (plasma MTX)	23 / 4 (Fig. 4)	6 (0.75-15), mean (range)	There was a correlation between V (L) and age and between CL (L/h) and BW.	2	[197]
Wall (2000)	Two compartment popPK model (plasma MTX)	24 / 0	12.5 (1.5-22)	Patients <6 years had a higher CL (mL/min/m^2).	2	[198]
Seidel (2000)	Non-compartmental pharmacokinetics (plasma MTX)	42 / 1	NS (0.83-13)	Variability of plasma concentrations was higher in younger patients.	3	[199]
Crews (2004)	Two compartment PK model (plasma MTX)	140 / 0	14.5 (3.2-24.1)	Age did not influence Cmax, AUC or CL (mL/min/m^2).	2	[200]
Aumente (2006)	Two compartment popPK model (plasma MTX) Age and BW were included as covariate on CL and V1.	49 / 2	5.0 (0.5-17)	Age was incorporated as categorical covariate on CL and V1 (≤10 yrs and >10 yrs).	1	[201]
Thompson (2007)	Non-compartmental pharmacokinetics (plasma MTX)	61 / 61	0.63 (0.17-1), mean (range)	CL (mL/min/m^2) in infants of 0-6 months was lower than in infants of 7-12 months. Older infants showed similar CL values compared to older children.	3	[202]
Piard (2007)	Two compartment popPK model (plasma MTX) BW was included as covariate on V1.	79 / 0	6.9 (2-16), mean (range)	Significant correlation between V1 and BW.	2	[203]
Lönnerholm (2009)	Non-compartmental pharmacokinetics (plasma MTX)	103 / 85	0.69 (0.16-1.16)	CL increases with age	3	[204]
Chládková (2010)	Two compartment popPK model (plasma MTX, 7OH-MTX)	10 / 0	8.5 (2.9-16), mean (range)	Effect of age was not studied.	2	[205]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Martelli (2011)	Two compartment popPK model (plasma MTX)	69 / 0	6.7 (1-15)	Effect of age was not described.	2	[206]
Jönsson (2011)	Two compartment popPK model (plasma MTX) BW was included as covariate on the PK parameters.	340 / NS	5.0 (0.44-17.83)	After including BW, no effect of age on the parameters was observed.	1	[207]
Rühs (2012)	Two compartment popPK model (plasma MTX) Age- and gender-normalized creatinine clearance was included as covariate on CL.	494 / 0	5.42 (1.03-18.85)	Effect of age was not studied.	2	[208]
Csordas (2013)	Non-compartmental pharmacokinetics (plasma MTX, 7OH-MTX)	153 / 0	6.4 (1.0-17.9), mean (range)	Children >14 years had a significantly higher MTX concentration at 48 h than children <6 years of age	3	[209]
Wright (2015)	Two compartment popPK model (plasma MTX) Age was included as covariate on CL and V1.	75 / 22 (Fig. 2)	1.6 (0.02-3.5)	CL and V1 increased with age.	1	[210]
Lucchesi (2016)	Non-compartmental pharmacokinetics (plasma MTX)	8 / 8	0.4 (0-0.75)	No significant correlation between age and CL (L/h/m^2)	3	[211]
Beechinor (2019)	Two compartment popPK model (plasma MTX) Allometric scaling for BW.	71 / 71	0.71 (0.24-1.08)	No effect of age on the PK parameters.	1	[212]
Medellin-Garibay (2019)	Two compartment popPK model (plasma MTX) BSA was included as covariate on CL.	50 / 0	5 (1-15)	No effect of age on the PK parameters.	2	[213]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Hui (2019)	Two compartment popPK model (plasma MTX) For ALL patients: BW and creatinine clearance were included as covariate on CL. Age was included as covariate on Q. For osteosarcoma patients: Height was included as covariate on CL and V1. Dose/BSA and creatinine clearance were included as covariate on CL. BW was included as covariate on Q. Age was included as covariate on V2.	52 / 0	NS (1.3-19)	No effect of age after including covariates.	2	[214]
Kawakatsu (2019)	Two compartment popPK model (plasma MTX) Allometric scaling for BW. eGFR was included as covariate on CL. Age and ALT were included as covariate on V1. ALT was included as covariate on V2.	320 / NS	16.4 (0.6-78.9)	Age was included as covariate on V1, indicating a lower V1, corrected for BW, in older patients.	1	[215]
Panetta (2020)	Two compartment popPK model (plasma MTX) PK parameters were normalized to BSA. eGFR and treatment with dexamethasone or vancomycin were included as covariates on CL.	178 / 55	1.8 (0.02-4.7)	CL in infants was lower than in older children.	1	[216]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Shi (2020)	One compartment popPK model (plasma MTX) BW, creatinine clearance and treatment with dexamethasone were included as covariates on CL.	105 / NS	3 (0-15), mean (range)	No effect of age after including covariates.	1	[217]
Schulte (2021)	Two compartment popPK model (plasma MTX) Allometric scaling for BW. SLCO1B1 polymorphism was included as covariate on CL.	106 / 2	10.1 (0.6-27.6)	No effect of age after including covariates.	1	[218]
Gao (2021)	Three compartment popPK model Allometric scaling for BW. Serum creatinine was included as covariate on CL.	311 / NS	5.0 (0.75-15.2)	No effect of age or maturation after including covariates.	1	[219]
Mitoxantrone						
Lacayo (2002)	Three compartment popPK model Cyclosporine treatment was included as covariate on CL.	12 / NS	NS (0.7-17)	Effect of age was not studied.	2	[113]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Pegaspargase						
Muller (2000)	Comparison of asparaginase activity after single i.v. dose using a popPK model (not published).	70 / NS	6 (0.4-17)	Effect of age was not studied.	3	[220]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Avramis (2002)	One compartment popPK model (pegaspargase i.m.)	59 / 0	NS (1-9)	There was no correlation between V, the half-lives of absorption or elimination and BSA or age.	2	[221]
Vieira Pinheiro (2002)	Non-compartmental pharmacokinetics (pegaspargase i.v.)	271 / 1	NS (0.9-19)	No clear effect of age on PK was described.	3	[222]
Vieira Pinheiro (2006)	Non-compartmental pharmacokinetics (pegaspargase i.v.)	70 / 0	4.6 (1.7-14)	Effect of age was not studied.	3	[223]
Appel (2008)	Non-compartmental pharmacokinetics (pegaspargase i.v.)	57 / 0	4.9 (1.4-15.1)	No correlation with peak levels of pegaspargase and age was observed.	3	[224]
Hempel (2010)	One compartment popPK model (pegaspargase i.v.) BSA was included as covariate on CL and V. CL increased with time.	168 / 2 (Fig. 4)	6.7 (0-20)	After including BSA as covariate on CL and V, the influence of age was not significant. However, a trend towards higher V in the older patients was found (not significant).	1	[225]
Tram Henriksen (2017)	Non-compartmental pharmacokinetics (pegaspargase i.m.)	97 / 0	4 (1-17)	Effect of age was not studied.	3	[226]
Würthwein (2017)	CL increasing with time, described by using a transit compartment model (pegaspargase i.v.)	1342 / 0	5.2 (1.0-17.9)	Effect of age was not studied.	3	[227]
Albertsen (2019)	One compartment popPK model (pegaspargase i.m.)	11 / 4	At diagnosis: 0.72 (0.29-0.94)	The half-life and V (L/m^2) being the same in infants and children, the higher mean asparaginase activity value reported in this study reflects a 200 and 250% higher dose. CL seems to be the same in these age groups.	1	[228]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Schore (2019)	Non-compartmental pharmacokinetics (pegaspargase i.v.)	48 / 0	10.7 (1.08-23.49)	Effect of age was not studied.	3	[229]
Kloos (2020)	One compartment popPK model (pegaspargase i.v.) Significant covariates for CL were: BSA, infection and treatment phase	120 / 0	NS (3.3-12.5)	No effect of age on the PK parameters.	2	[230]
Prednisolone						
Choonara (1989)	Non-compartmental pharmacokinetics	6 / 0	4.75 (2.8-12.4)	No effect of age on PK in these 6 patients. But values here for CL (mL/min/kg) are slightly higher than those reported in studies with older children.	3	[231]
Hill (1990)	Non-compartmental pharmacokinetics	43 / 0	10 (2-50), mean (range)	Significant negative correlation between BSA-normalized CL and age. Children <12 years old had higher CL than older children and adults.	3	[232]
Petersen (2003)	Two compartment popPK model BSA was included as covariate on CL. BW was included as covariate on V1 and V2.	23 / 0	5.4 (2.4-15.2)	No additional effect of age on PK parameters that was not explained by BSA or BW.	2	[233]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Sassen (2021)	One compartment popPK model Allometric scaling for BW. Plasma protein binding of prednisolone and the ratio of prednisolone/prednisone was included as covariate.	124 / 1 (Fig. 3)	6.2 (0.4–17.7)	The plasma protein binding of prednisolone to corticosteroid-binding globulin was associated with patient age. The estimated corticosteroid-binding globulin concentration decreased with age. No correlation between CL of unbound prednisolone (corrected for BSA) and age was observed.	1	[234]
Temozolomide						
Panetta (2003)	One compartment popPK model BSA and age were included as covariates on CL and V.	39 / NS	7.1 (0.7-21.9)	Impact of increasing age and BSA on CL (L/h) and V (L).	1	[235]
Riccardi (2003)	Non-compartmental pharmacokinetics	22 (children) / 0 8 (adults)	40 (3-16) / 30 (19-54), mean (range)	No effect of age on CL. BSA-normalized CL values comparable between children and adults.	3	[236]
Wagner (2004)	One compartment popPK model	12 / 0	12.5 (1-23)	Effect of age was not studied.	2	[136]
Broniscer (2005)	One compartment popPK model	33 / 0	6.4 (3.1-15)	Effect of age was not studied.	2	[237]
Kirstein (2005)	One compartment popPK model	38 / NS	NS	Effect of age was not studied.	2	[238]
Horton (2007)	Non-compartmental pharmacokinetics and compartmental PK model	16 / 0	11 (1-19)	Effect of age was not studied.	3	[239]
Broniscer (2007)	One compartment popPK model	44 / NS (Stratum 1) 26 / 0 (Stratum 2)	8.6 (0.4-20.2) S1 11.3 (2.4-18.6) S2	Effect of age was not studied.	2	[240]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Meany (2009)	Non-compartmental pharmacokinetics	21 / 0	11.4 (3.0-21.3)	No effect of age on BSA-normalized CL	3	[241]
Rubie (2010)	One compartment popPK model	16 / 0	8.5 (3-19)	Effect of age was not studied.	2	[242]
Thioguanine						
Erb (1998)	Non-compartmental pharmacokinetics (RBC 6-TGN)	22 / 0	NS (1-18)	Effect of age was not studied.	3	[172]
Lancaster (2001)	Non-compartmental pharmacokinetics (plasma and RBC 6-TGN)	11 / 0	4 (1.5-7)	Effect of age was not studied.	3	[243]
Lowe (2001)	Non-compartmental pharmacokinetics (plasma and RBC 6-TGN)	35 / 0	3 (1-9)	No effect of age on AUC of plasma 6-TGN was found.	3	[244]
Palle (2009)	Non-compartmental pharmacokinetics (RBC 6-TGN)	46 / 2	11.0 (0.5-17.7)	No effect of age on RBC 6-TGN concentrations have been found.	3	[245]
Topotecan						
Blaney (1993)	Non-compartmental pharmacokinetics	14 / 0	16 (1-23)	Effect of age was not studied.	3	[246]
Pratt (1994)	Two compartment PK model	14 / 0	10 (2-20)	Effect of age was not studied.	3	[247]
Stewart (1994)	Two compartment PK model	20 / 0	8 (3.5-18.0)	No correlation between age and PK parameters.	3	[248]
Baker (1995)	Three compartment PK model including CSF	17 / 0	12 (1-16)	Effect of age was not studied.	3	[249]
Furman (1996)	Two compartment popPK model	18 / 0	10.8 (1.3-20.1)	Effect of age was not studied.	2	[250]
Tubergen (1996)	Two compartment PK model	36 / 0	11.3 (3.1-20.6)	No correlation between age and PK parameters.	3	[251]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Frangoul (1999)	Non-compartmental pharmacokinetics	15 / 0	9 (2-18)	Effect of age was not studied.	3	[252]
Athale (2002)	Two compartment PK model	16 / 0	13 (1.5-21)	Effect of age was not studied.	3	[253]
Furman (2002)	Two compartment PK model	33 / 0	9.2 (1.9-20.4)	A correlation between age and CL ($L/h/m^2$) was found.	3	[254]
Santana (2003)	Two compartment PK model	15 / 0	12.8 (2.1-19)	Effect of age was not studied.	3	[255]
Daw (2004)	Two compartment popPK model (after oral administration)	20 / 0	10.6 (3.8-19.8)	Effect of age was not studied.	2	[256]
Stewart (2004)	Two (three including CSF) compartment PK model	36 / 0	7.3 (3.2-16.9)	Effect of age was not studied.	3	[257]
Santana (2005)	Two compartment PK model	30 / NS	3.1 (<0.08-16.9)	Effect of age was not studied.	3	[258]
Freeman (2006)	Two (three including CSF) compartment PK model	6 / 0	4.9 (3.2-8.4)	Effect of age was not studied.	3	[259]
Schaiquevich (2007)	Two compartment popPK model Significant covariates for CL were age, renal function, and administration of phenytoin or dexamethasone. Significant covariates for V1 were age and administration of phenytoin.	162 / 16 or 17 (Fig. 2)	8.0 (0.04-22)	Age evaluated as a linear continuous variable was not a significant covariate. Age evaluated as a categorical variable (i.e., age <0.5 years and age >0.5 years) was a significant covariate for BSA-normalized CL and V1.	1	[260]
Hijiya (2008)	Two compartment PK model	23 / NS	12.7 (0.6-21.1)	Effect of age was not studied.	3	[261]
Rubie (2010)	Two compartment popPK model	16 / 0	8.5 (3-19)	Effect of age was not studied.	2	[242]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Roberts (2016)	One compartment popPK model (after oral administration) BSA was included as covariate on CL and V. ABCG2 polymorphism was included as covariate on the absorption rate constant.	61 / NS (20 <2 years)	2.37 (0.48-4.59)	No effect of age on the PK parameters.	1	[262]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Vincristine						
Crom (1994)	Two compartment PK model	54 / 2	4.3 (0.2-18)	No correlation between CL (mL/min/m^2) and age. A significant correlation between CL (mL/min/kg) and age. CL in children in higher than in adults, but CL in two infants was lower.	2	[263]
De Graaf (1995)	Two compartment PK model	17 / 0	3.8 (1.3-12.4)	CL (mL/min/m^2) in children appeared to be more than twice as large as in adults and $t_{1/2}$ in adults was much longer than in children.	3	[264]
Gidding (1999)	Two compartment PK model	32 / NS	4.6 (0-16)	CL (mL/min/m^2) in infants under 1 year is lower than in older children.	2	[265]
Groninger (2002)	Two compartment PK model	70 / 0	NS (1-16)	No effect of age on CL (mL/min/m^2).	3	[266]
Frost (2003)	Two compartment PK model	98 / 0	4.5 (1.3-17.3)	No effect of age on the PK parameters.	3	[267]
Plasschaert (2004)	Two compartment PK model	52 / 0	NS (1-16)	No effect of age on CL (mL/min/m^2).	3	[268]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Groninger (2005)	Two compartment PK model	54 / 0	NS (1-16)	Effect of age was not studied.	3	[269]
Lönnerholm (2008)	Two compartment PK model (with data of Frost 2003)	86 / 0	NS (1.2-17.4)	No effect of age on the PK parameters.	3	[270]
Guilhaumou (2011)	Two compartment popPK model	26 / 0	NS (2.0-16.0)	No effect of age on the PK parameters.	2	[271]
Moore (2011)	One compartment popPK model Allometric scaling for BW.	50 / 0	6.5 (1.0-16.25)	No effect of age on the PK parameters.	2	[272]
Balis (2017)	Mechanism-based development of dose bands based on BSA intervals	NA	NA	NA	5	[5]
Lee (2019)	Physiological-based PK model Intracellular binding to β-tubulin was included as covariate.	25 / NS	NS (0.4-9)	Simulating a higher hypothetical (4.9-fold) pediatric expression of β-tubulin relative to adult improved predictions PK.	5	[273]
Van de Velde (2020)	Two compartment popPK model PK parameters were normalized to BSA. Duration of infusion was included as covariate on Q and V2.	35 / 0	10.06 (NS)	Effect of age was not described.	2	[274]
Skolnik (2021)	Non-compartmental pharmacokinetics	132 / 9	NS (0.21-16.8)	Age had a minimal effect on variability of PK. Compared to children <1 year, the BSA-adjusted dose was 44-79% higher older children. Consistent with the differences in dose, the median AUC was lowest for children <1 year and highest in older children. CL did not appear to be related to age.	3	[6]

Author	Method	Number of patients/ infants (<1 yrs)	Age (yr), median (range)	Age-related findings	Level	Ref
Barnett (2021)	Two compartment popPK model Allometric scaling for BW. Age was included as covariate on V2	57 / 21	5.6 (0.04-17.2)	No significant difference in BSA-normalized CL between infants and older children. There was a trend towards lower CL in neonates (0-4 weeks) as compared to infants (1-12 months). Doses of <0.05mg/kg result in significantly lower AUC values than observed in neonates and infants receiving doses of ≥0.05mg/kg, and in older children receiving a dose of 1.5mg/m^2.	1	[275]

AUC Area under the curve, *BSA* Body surface area, *BW* Body weight, *CL* Clearance, *Cmax* Maximum plasma concentration, *eGFR* estimated glomerular filtration rate, *NA* Not applicable, *NS* Not specified, *PK* Pharmacokinetic(s), *popPK* Population pharmacokinetic(s), *Q* Intercompartmental clearance, *RBC* Red blood cell count, $t_{1/2}$ elimination half-life, *V* Volume of distribution.

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3

Population pharmacokinetics of clofarabine for allogeneic hematopoietic cell transplantation in pediatric patients

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ABSTRACT

Aim

Clofarabine has recently been evaluated as part of the conditioning regimen for allogeneic hematopoietic stem cell transplantation (HCT) in children. Pharmacokinetic (PK) exposure of different agents commonly used in conditioning regimens is strongly related to HCT outcome. Consequently, the PK of clofarabine may be important for outcome. This report describes the population PK of clofarabine in pediatric patients and one adult.

Methods

From 80 pediatric (0.5-18 years) and 1 adult patient (37 years), 805 plasma concentrations were included in PK analyses using non-linear mixed effects modelling.

Results

A two-compartment model adequately described the PK of clofarabine. Body weight and estimated glomerular filtration rate (eGFR) were included as covariates. Clearance was differentiated into non-renal and renal clearance (approximately 55% of total clearance), resulting in population estimates of 24.0 L/h (95% confidence interval (CI) 13.7-34.4) and 29.8 L/h (95% CI 23.9-36.1) for a patient of 70 kg with normal renal function, respectively. Unexplained interindividual variability in clearance was 17.8% (95% CI 14.6%-22.4%). A high variability in exposure was observed (range area under the curve$_{T0\text{-}inf}$ 1.8-6.0 mg/L*h) after body surface area (BSA) based dosing. Interestingly, children with low body weight had a lower exposure than children with a higher body weight, which indicates that the currently practiced BSA-based dosing is not adequate for clofarabine.

Conclusion

A clofarabine dosing algorithm based on this PK model, using body weight and eGFR, results in a more predictable exposure than BSA-based dosing. However, the exact target exposure needs to be further investigated.

INTRODUCTION

Clofarabine, a purine nucleoside analog with anti-tumor activity, is approved for the treatment of children (<21 years) with relapsed or refractory acute lymphoblastic leukemia (ALL).[1] In addition, clofarabine was recently added to the conditioning regimen for allogeneic hematopoietic stem cell transplantation (HCT) in pediatric hematological malignancies for its capacity to enhance the antileukemic effect in combination with busulfan and fludarabine (BuFlu).[2] These studies concluded that this strategy is safe and promising in high risk (myeloid) leukemia.

Clofarabine is a prodrug, metabolized intracellularly by phosphorylation to the active metabolite clofarabine-5'-triphosphate. Clofarabine-5'-triphosphate decreases cell replication and DNA-repair leading to cell death. Unchanged clofarabine is mainly renally cleared; approximately 60% is excreted with urine[1]. The half-life of clofarabine is approximately 5 hours, while the half-life of clofarabine-5'-triphosphate is around 24 hours.[1]

The pharmacokinetics (PK) of clofarabine in children and adults has been studied previously.[3–5] In patients with hematologic malignancies and solid tumors the clofarabine exposure increased with decreasing estimated glomerular filtration rate (eGFR) and a dose adjustment in case of moderate (eGFR 30-60 mL/min/1.73 m^2) and severe (eGFR <30 mL/min/1.73 m^2) renal impairment is suggested.[4] Recently, a population PK model of clofarabine used in conditioning regimens for HCT in children has been developed.[5] No effect of renal function on clofarabine clearance was seen. However, only patients with normal renal function (range eGFR 96-150 mL/min/1.73 m^2) were included, so the effect of impaired renal function could not be studied. Additionally, the previous population PK models of clofarabine all found that body weight was the best predictor of clofarabine clearance.[3–5] Taken together, this would indicate that dosing based on weight and renal function would lead to the best predictable exposure. However, at present clofarabine is still dosed based on body surface area (BSA).

Previous work on the PK of busulfan and fludarabine used in conditioning regimens for HCT in pediatric and adult patients showed that optimal individual exposure of both agents is needed to prevent graft failure and relapse and that over-exposure leads to an increase in toxicity and delayed immune reconstitution.[6,7] The same could to be expected for clofarabine. More knowledge on the PK of clofarabine used in conditioning regimens of HCT in children is needed to investigate whether the exposure relates to clinical outcome, which parameters predict the clofarabine exposure, and how to adjust the dose to achieve adequate exposure.

The aim of this study was to describe the population PK of clofarabine, using a large heterogeneous dataset of pediatric patients, in order to optimize the dosing regimen for clofarabine during conditioning prior to allogeneic hematopoietic cell transplantation in children.

METHODS

Patients and sampling

A retrospective PK analysis was performed with data from patients who received myeloablative conditioning before HCT, between October 2011 and January 2019, at the University Medical Centre Utrecht (UMCU) and the Princess Máxima Center for pediatric oncology in the Netherlands, and of whom PK samples were available. No restrictions were applied for comorbidities, age, and indication for HCT. Patients were included after written informed consent was acquired. Ethical approval by the institutional Medical Ethics Committee of the UMCU was obtained under protocol number 11/063.

The conditioning regimen consisted of 4 days of chemotherapy (administered from day -5 to day -2 relative to HCT). Patients were treated with a 1 h infusion of clofarabine directly followed by a 1 h infusion of fludarabine and a 3 h infusion of busulfan. In the unrelated donor HCT setting, rabbit ATG was added: 4 h infusions on 4 consecutive days from day -9 to day -6 relative to HCT (10 mg/kg <30 kg; 7.5 mg/kg >30 kg) till 2015. After that patients received lymphocyte count and weight based dosing of ATG from day -9 with a maximum of 10 mg/kg in 4 days.[8] Patients received a cumulative dose of 120 mg/m^2 clofarabine. Fludarabine was given intravenously in a cumulative dose of 40 mg/m^2 and busulfan was targeted to a myeloablative cumulative 4-day exposure of 90 mg/L*h (expressed as area under the curve for all doses [AUC_{T0-inf}]). For patients receiving ATG, clemastine, paracetamol, and 2 mg/kg prednisolone (with a maximum of 100 mg) were administered intravenously prior to ATG infusion.

Plasma concentrations of clofarabine were determined in PK samples taken for routine busulfan therapeutic drug monitoring (TDM). According to the local TDM protocol, plasma samples were drawn on the first or second day of the conditioning regimen. If considered necessary for busulfan TDM purposes, samples were also drawn on the following days. Additional samples were taken on the final day of conditioning (day 4). In general, plasma samples were taken at 5, 6, 7 and 8 h, after the end of the clofarabine infusion. For a subset of patients, additional samples were collected from 8 to 24 h post-infusion. From January 2016 onwards, additional samples were collected between the end of the fludarabine infusion and the start of the busulfan infusion, which equals approximately 1.5 h after the end of the clofarabine infusion. Clofarabine concentrations were measured using a validated liquid chromatography mass spectrometry method, with a lower limit of quantification (LLOQ) of 1 ng/mL, as described by Punt et al.[9]

Model development

Starting point for model development was a two-compartment model with first order elimination consisting of a renal and non-renal fraction.

Interindividual variability (IIV) was evaluated for all parameters, according to equation 1:

$$P_i = P_{pop} \times e^{(\eta_i)} \quad (1)$$

where P_i represents the individual parameter estimate for individual *i*, P_{pop} represents the typical population parameters estimate, and η_i is assumed to be normally distributed with a mean of zero and a variance of ω^2.

Since data of multiple days of therapy was available, interoccasion variability (IOV) was implemented similarly as IIV, with each dose and subsequent sampling defined as a separate occasion. This variability was evaluated for all parameters to diagnose potential time-dependent trends and to allow for random unaccounted variability between dosing moments.

Residual unexplained variability was evaluated as a proportional error model or as a combination of a proportional and additive error model.

Covariate analysis

Following structural model development, the influence of patient-specific factors for variability in PK parameters were evaluated. Assessed covariates included body weight (BW), body surface area (BSA), fat free mass (FFM), age and renal function. These continuous covariates were evaluated using both a linear function and a power function. To implement body size descriptors on PK parameters, standard allometric scaling was applied, with *p* fixed at 0.75 (BW, FFM) or 1 (BSA) for clearances, and 1 for distribution volumes (BW, BSA, FFM).[10]

Renal function was evaluated as covariate, since clofarabine is partly eliminated renally.[1] As creatinine levels were not measured daily, the most recent values of creatinine prior to infusion (maximum 10 days) were used. Subsequently, eGFR was calculated using the Cockcroft–Gault equation, which takes age into account.[11] eGFR for patients below the age of 17 years for women and 14 years for men was calculated using the Schwartz equation.[12] eGFR was capped to a maximum of 8.4 L/h/1.73 m^2 (140 mL/min/1.73 m^2) and was assumed to increase to this level from birth until the age of 1.5 years, starting at 2.1 L/h/1.73 m^2 (35 mL/min/1.73 m^2) (25% of maximum value). The absolute eGFR (in L/h) was standardized to 70 kg as shown in the equation. Relative renal function (RF) was normalized to a standard eGFR ($eGFR_{STD}$) of 6 L/h (100 mL/min):

$$RF = \frac{eGFR \times \frac{70}{BW}}{eGFR_{STD}} \quad (2)$$

where eGFR is the absolute estimated glomerular filtration rate in L/h, BW is body weight in kg and $eGFR_{STD}$ is a standard eGFR (6 L/h was used in this model).

RF was included in the model, using a linear independent combination of renal and non-renal CL parameters:

$$CL_{overall} = CL_{non\text{-}renal} + CL_{renal} \times RF \quad (3)$$

where $CL_{overall}$ is the overall population value of parameter for clearance. $CL_{non\text{-}renal}$ is non-renal clearance and CL_{renal} is renal clearance.[13,14]

Because the dataset contained several infants, the effect of maturation on clearance was implemented using the method described by Rhodin et al. They showed that maturation of renal clearance across the entire pediatric population was well described using postmenstrual age (PMA) with a sigmoidal Hill equation. The TM_{50}, the PMA at which clearance is 50% of the mature value, was estimated at 55.4 weeks and the Hill coefficient describing the slope of the sigmoidal curve at 3.33.[15] For our population, exact PMA was not known, so the PMA was estimated using age in weeks plus mean gestational age (40 weeks):

$$F_{CL} = \frac{(\text{Age in weeks} + 40)_i^{HILL}}{(\text{Age in weeks} + 40)_i^{HILL} + TM_{50}^{HILL}} \quad (4)$$

The Hill coefficient and TM50 were fixed to 3.92 and 54.2 weeks, respectively, according to published models.[16]

Model evaluation

Discrimination between models was guided by physiological plausibility, goodness-of-fit (GOF) plots, precision of parameter estimates and change in objective function value (dOFV). A drop of ≥3.84 points, corresponding to a $P<0.05$ (χ^2-distribution with 1 degree of freedom (df)), was considered a significant improvement of the fit for hierarchical nested models. The adequacy of the models was assessed by GOF plots and visual predictive checks (VPC).[17] Parameter precision was assessed by the sampling importance resampling (SIR) procedure.[18]

Software

Nonlinear mixed-effects modeling was performed using NONMEM (version 7.3.0, ICON development Solutions, Ellicott City, MD, USA) and Pearl-speaks-NONMEM (PsN, version 4.7.0) with First-Order Conditional Estimation with interaction (FOCE-I) as estimation

method.[19,20] Pirana (version 2.9.9) was used as graphical user interface for NONMEM.[21] R (version 3.4.3) was used for data handling and visualization.[22]

RESULTS

Patients and sampling

A total of 81 patients with a median age of 11.1 years (range 0.5-37.8) were included in this study. Five patients were younger than 12 months and one adult (37 years) was included. Of these patients, 805 PK samples were available for analysis. None of these samples were below the lower limit of quantification. Figure 1 displays the observed plasma concentrations over time. Detailed patient characteristics are shown in Table 1.

Model development

A linear two-compartment model with first order kinetics was appropriate to describe the PK of clofarabine. Final estimates and 95% confidence intervals (CI) are shown in Table 2. The model was parameterized in terms of volume of distribution of the central (V1) and peripheral (V2) compartment, clearance from the central compartment (CL) and intercompartment clearance between V1 and V2 (Q). BW was a priori included as covariate using allometric scaling on all PK parameters. The exponents for BW on clearance and volume of distribution were fixed to 0.75 and 1, respectively, prior to covariate analyses.

IIV was added on CL, V1 and Q. Inclusion of IOV on CL and V2 led to a significant improved model fit.

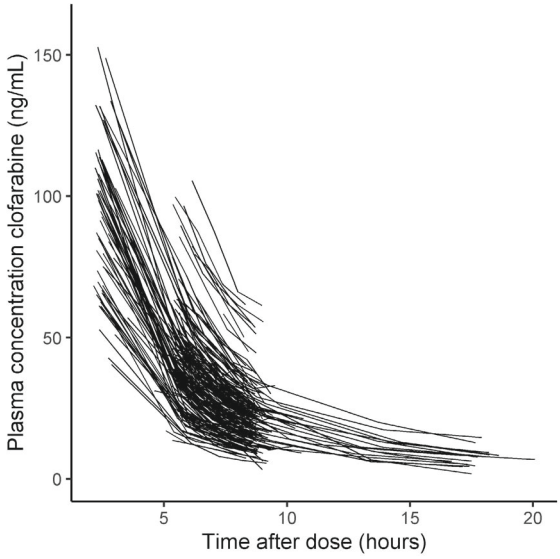

Figure 1. Clofarabine plasma concentrations versus time after dose. Each line corresponds to a single dose.

Table 1. Patient characteristics

	N=81 Median [range]
Available data	
Total no. of PK samples [n]	805
No of samples per patient	10 [3-20]
Patient characteristics	
Female sex [n (%)]	30 (37%)
Age at transplantation, years	11.1 [0.5-37.8[a], IQR 5.5-14.8]
Actual bodyweight, kg	36.6 [6.6-102.9, IQR 20.1-53.5]
Renal function, mL/min/1.73 m^2	140 [69.3-140, IQR 123.1-140]
Indication for transplantation [n (%)]	
ALL	40 (49%)
AML	28 (35%)
CML	2 (2%)
Myelodysplastic syndrome	8 (10%)
Other	3 (4%)
Transplant cell source [n (%)]	
Cord blood	46 (57%)
Bone marrow	35 (43%)

IQR interquartile range, *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *CML* chronic myeloid leukemia [a] *The population existed of 80 pediatric patients, aged 0.5-18 years, and one adult patient of 37.8 years.*

Table 2. Final population PK parameter estimates

PK parameter	Estimate	95% CI
$CL = (CL_{non\text{-}renal} + CL_{renal} \times RF) \times \left(\frac{BW}{70}\right)^{0.75}$		
$RF = \frac{eGFR\ (L/h) \times \frac{70}{BW}}{eGFR_{STD}}$		
$CL_{non\text{-}renal,70kg}$ (L/h)	24.0	13.7 – 34.4
$CL_{renal,70kg}$ (L/h)	29.8	23.9 – 36.1
$V1 = V1_{70kg} \times \left(\frac{BW}{70}\right)^{1}$		
$V1_{70kg}$ (L)	268	234.8 – 296.6
$V2 = V2_{70kg} \times \left(\frac{BW}{70}\right)^{1}$		
$V2_{70kg}$ (L)	186	165.4 – 210.7
$Q = Q_{70kg} \times \left(\frac{BW}{70}\right)^{0.75}$		
Q_{70kg} (L/h)	33.2	27.5 – 40.9
IIV CL (%)	17.8	14.6 – 22.4
IIV V1 (%)	12.6	6.8 – 18.1
IIV Q (%)	64.5	49.5 – 83.7
IOV CL (%)	9.7	7.8 – 11.5
IOV V2 (%)	39.1	29.2 – 53.7
Proportional residual error (%)	8.3	7.7 – 8.8

PK pharmacokinetics, CI confidence interval obtained by sampling importance resampling, CL clearance, RF relative renal function, BW body weight, V1 volume of distribution of the central compartment, V2 volume of distribution of the peripheral compartment, Q intercompartment clearance between V1 and V2, IIV interindividual variability, IOV interoccassion variability. Population estimates $CL_{renal,70kg}$, $CL_{non\text{-}renal,70kg}$, $V1_{70kg}$, $V2_{70kg}$, Q_{70kg} correspond to a subject weighing 70 kg and are adjusted to an individual value, according to the corresponding parameter formula in the table.

Covariate selection
BSA and FFM were evaluated as metrics for body size, but did not improve the model fit over BW.

Renal function was evaluated as covariate on CL. Renal clearance was differentiated from non-renal clearance by adding an extra parameter for renal-clearance, which was normalized to a standard eGFR. Adding renal function resulted in a significant improved model fit, with a drop of 34 points in OFV ($P<0.05$). The effect of maturation on CL was tested using the method described by Rhodin et al.[15] This did not result in a better fit of the model, so maturation was not included in the final model. In the final model CL_{renal} was estimated at 29.8 L/h for a typical patient, which correspondents to 55% of the total clearance in patients with normal renal function. The calculated alpha- and beta half-life ($t_{½\,\alpha}$ and $t_{½\beta}$) were 1.7 h and 8.1 h, respectively.

Including BW and eGFR in the model caused a decline in IIV CL from 46.2% to 17.8%.

Figure 2A depicts the variability in total exposure (observed $AUC_{T0\text{-}inf}$). As shown in Figure 1, plasma concentrations over time after dose were highly variable, leading to a wide range of observed $AUC_{T0\text{-}inf}$ (1.8-6.0 mg/L*h). Figure 2B and C show the exposure at different weight and renal function categories. Low BW seems to be correlated to low exposures, indicating that BSA-based dosing does not sufficiently account for variability. As expected, patients with a creatinine clearance below 80 mL/min/1.73 m^2 have a higher exposure than patients with a better renal function. The renal function of the patients weighing less than 20 kg varied from 70-140 mL/min/1.73 m^2, 14 patients (70%) had a creatinine clearance >120 mL/min/1.73 m^2. This shows that the lower exposure is partly, but not totally explained by a good renal function throughout this subgroup.

Model evaluation
The GOF plots (Supplementary Figure S1A and B) showed accurate population and individual predictions, without any signs for over- or underprediction. CWRES are evenly distributed over the whole plasma concentration range (Supplementary Figure S1C) and time interval (Supplementary Figure S1D). No trends were observed for CWRES vs. renal function (Supplementary Figure S1E) or actual body weight (Supplementary Figure S1F).

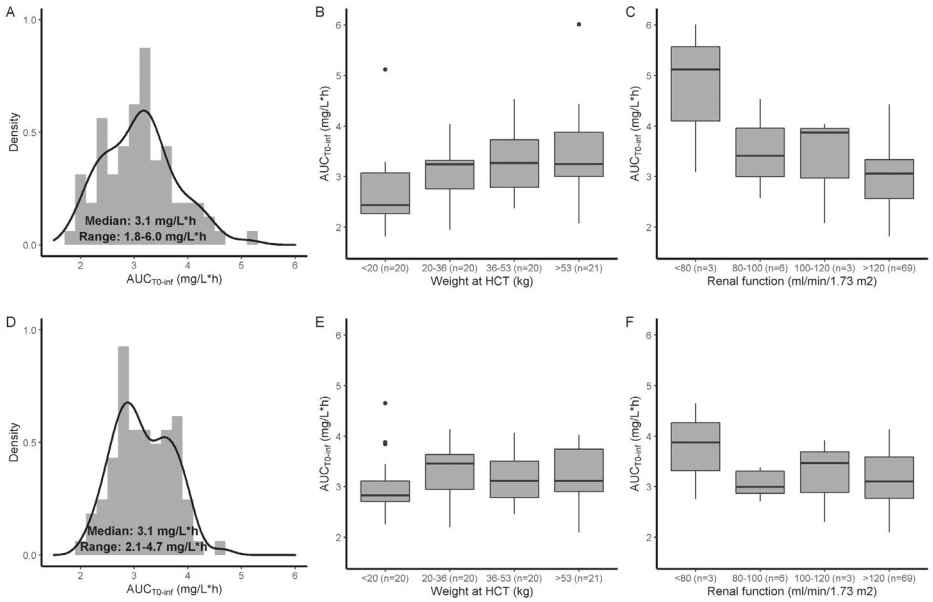

Figure 2. Exposure variability after dosing in the trial (A, B, C) and after dosing with suggested dosing algorithm (D, E, F). A Histogram (grey area) and density plot (black solid line) of the observed $AUC_{T0\text{-}inf}$. B Boxplots of the observed $AUC_{T0\text{-}inf}$ per body weight quartile. C Boxplots of the observed $AUC_{T0\text{-}inf}$ per renal function category. D Histogram (grey area) and density plot (black solid line) of the calculated $AUC_{T0\text{-}inf}$. E Boxplots of the calculated $AUC_{T0\text{-}inf}$ per body weight quartile. F Boxplots of the calculated $AUC_{T0\text{-}inf}$ per renal function category.

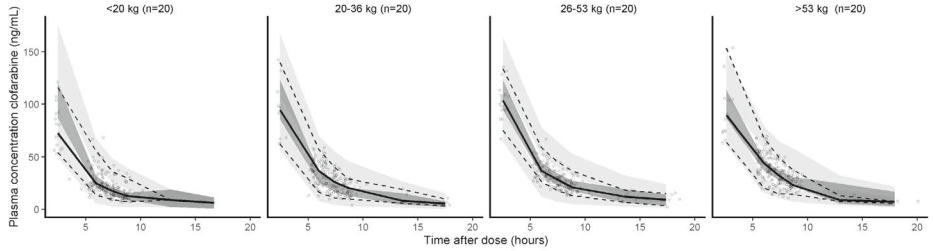

Figure 3. Body weight stratified prediction-corrected visual predictive check. Black lines depict the observed median (solid) and 2.5% and 97.5% percentile (dashed) concentrations. Dark- and light-grey areas represent 95% prediction intervals of the simulated mean and the 2.5 and 97.5% percentiles, respectively. Round dots represent observations.

The VPC demonstrated that the median and the 95% CI of the observed data were in line with those from the simulation-based predictions from the model for all age and BW strata (Figure 3). Except for the early time points, where the median and the 95% CI of the observations were slightly lower than the predictions, indicating underprediction. However, only 61 samples (7.6%) with a time after dose <4 h were included in the model.

Dosing regimen

As mentioned before, previous publications showed that exposure of busulfan and fludarabine used in conditioning regimens for HCT relates to clinical outcome.[6,7] Target AUCs for these agents have been established. Even though a target AUC for clofarabine has not yet been described, a dose algorithm could be extracted from this PK model:

$$\text{Dose} = \text{AUC}_{\text{Target}} \times \left(24.0 + 29.8 \times \frac{\text{eGFR} \times \frac{70}{\text{BW}}}{6} \right) \times \left(\frac{\text{BW}}{70} \right)^{0.75} \quad (5)$$

where dose is the cumulative clofarabine dose for four days in mg, $\text{AUC}_{\text{Target}}$ is the cumulative target AUC, eGFR is the absolute estimated glomerular filtration rate in L/h and BW is body weight in kg.

Using this algorithm and the median AUC of 3.1 mg/L*h, a new dose was calculated for each patient. Following this, the exposure using this new dose was calculated based on the individual estimated CL values of our patients. Figure 2D depicts the variability in total exposure under the new dosing regimen (calculated $\text{AUC}_{\text{T0-inf}}$). This figure shows that the range of the calculated $\text{AUC}_{\text{T0-inf}}$ was smaller than with BSA-based dosing (2.1-4.7 mg/L*h resp. 1.8-6.0 mg/L*h). Figure 2E and F show the calculated exposure at different weight and renal function categories. In Figure 4, a line plot of the 4 day cumulative clofarabine dose as a function of the body weight for several relative renal function values and an $\text{AUC}_{\text{target}}$ of 3.1 mg/L*h is presented.

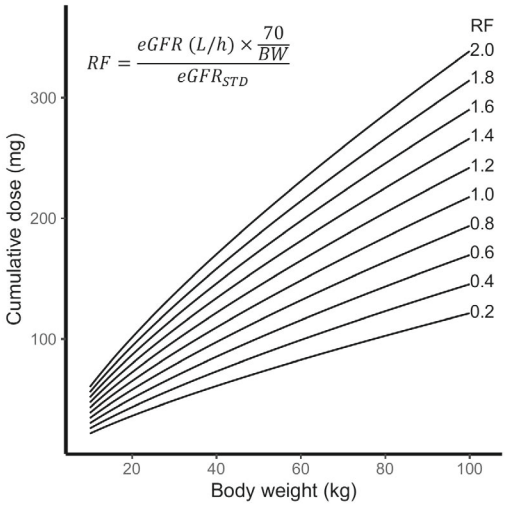

Figure 4. Line plot of the 4 day cumulative clofarabine dose as a function of the body weight (kg) for several relative renal function values (RF).

DISCUSSION

In this analysis, a population PK model was developed, using a large and diverse dataset including pediatric patients from the age of 0.5 years with a variety of hematological diagnoses requiring HCT. A two-compartment model was appropriate to describe the clofarabine PK in these patients, which is in line with previous published clofarabine PK models.[3–5]

Two covariates were identified as predictor for clofarabine CL. Body weight was included using allometric scaling, which is in line with the previous described PK models. In addition, renal function was included as covariate, which significantly improved the model fit and reduced the IIV CL significantly (from 46.2% to 17.8%). Bonate et al. also found a significant improvement of the model after including renal function as covariate, while Wang et al. did not find a significant effect.[4,5]

By including renal function into our model, we estimated that clofarabine is renally cleared for approximately 55% in a typical patient. In addition, we found that the exposure to clofarabine is higher in patients with a creatinine clearance below 80 mL/min/1.73 m^2. This is in accordance with information that can be found in literature[1], and is in line with the advice of Bonate et al. to reduce the dose in case of renal impairment.[3] However, we did not include any patients with moderate (eGFR 30-60 mL/min/1.73 m^2) or severe (eGFR <30 mL/min/1.73 m^2) renal impairment. Therefore, one must be careful translating the results of this population PK model to patients with moderate or severe renal impairment. On

the other hand, moderate and/or severe renal impairment is very exceptional in pediatric patients undergoing HCT, so the results of this PK model might be sufficient for this specific patient population.

The median clofarabine exposure was 3.1 mg/L*h with a of range 1.8-6.0 mg/L*h. This is in accordance with the median cumulative AUC of 3.3 mg/L*h (range 1.5-5.5 mg/L*h) after a cumulative dose of 120 mg/m^2 as described by Wang et al.[5] We observed a decreased exposure in children with low BW (<20 kg). Accordingly, younger children also seemed to have a lower exposure than older children. Previous population PK models found that CL increases with increasing BW or increasing age (for patients <20 years).[4,5] This is in line with the results of our study, but it does not correlate with a low exposure in children with low BW or age. Bonate et al. simulated the effect of age, BW and eGFR on clofarabine exposure after BSA-based dosing and showed that the exposure is lower in younger children than in older children with comparable eGFR.[4] In contrast, Wang et al. suggested a lower dose for younger children, based on their simulation of CL values.[5] Our results show, however, that reducing the dose in younger children could lead to underexposure of those patients. In contrast to the advice of Wang et al., younger children may require a higher clofarabine dose. Nevertheless, these results need to be carefully interpreted in case of very young infants. Especially up to the age of 3 months, the metabolic capacity and renal elimination undergo substantial developmental changes.[24] This population model did not include any patients below the age of 6 months, so lower doses might be needed for these children because of this maturation phase.

The results of this PK study show that renal function and body weight are two important covariates for clearance, and should, therefore, be considered as components to base the clofarabine dose on. Clofarabine is still dosed based on BSA, while renal function is not taken into account, apart from exceptional cases of renal impairment (according to the label of clofarabine for non-conditioning for HCT indications, a dose reduction of 50% needs to be made in patients with moderate renal impairment, while clofarabine is contraindicated in patients with severe renal impairment). The decrease in clofarabine clearance relating to renal function is a gradual process and even an eGFR below 120 mL/min/1.73 m^2 is associated with a decrease in clofarabine clearance and concomitant higher exposures[4], which makes a dose algorithm, taking renal function into account, more suitable to decide which dose should be administrated. This approach has been described previously for other drugs, for example carboplatin and fludarabine.[25–27] Such a dosing algorithm for clofarabine was derived from this PK model (equation 5), however, a target AUC is needed to calculate the conventional clofarabine dose. A target AUC for clofarabine during conditioning prior to HCT has not been determined yet. The relation between clofarabine exposure and clinical outcome after HCT in children needs to be studied in order to determine the target AUC, but this could be challenging since these children were treated with multiple agents (busulfan and fludarabine), all contributing to clinical outcome. Even tough, when the target AUC for clofarabine is set, the dose algorithm

can be used to calculate the conventional dose for an individual patient. The median of the observed AUC was used for calculations of the new dose and exposure in this paper. These calculations illustrate that the range of the clofarabine exposure is smaller when this dosing algorithm is used, while the median exposure is similar. The number of very young patients in our population was limited and, therefore, the benefits of weight and renal function based dosing in this population need further investigation.

CONCLUSION

In summary, the results of this clofarabine population PK model, using data from the largest pediatric dataset reported to date, showed that BW and eGFR appeared as important covariates influencing the clofarabine CL. Unexplained interindividual variability in exposure clearance was observed (17.8% (95% CI 14.6%-22.4%)). In the included population a high variability in exposure was observed (range $AUC_{T0\text{-}inf}$ 1.8-6.0 mg/L*h). Interestingly, children with low BW have a lower exposure than children with a higher BW, which indicates that BSA-based dosing is not adequate for clofarabine. Younger children may require higher doses than older children. A dosing algorithm based on this PK model, using BW and eGFR, was developed, which would result into a more predictable exposure than body surface area based dosing. However, the exact target exposure needs to be further investigated.

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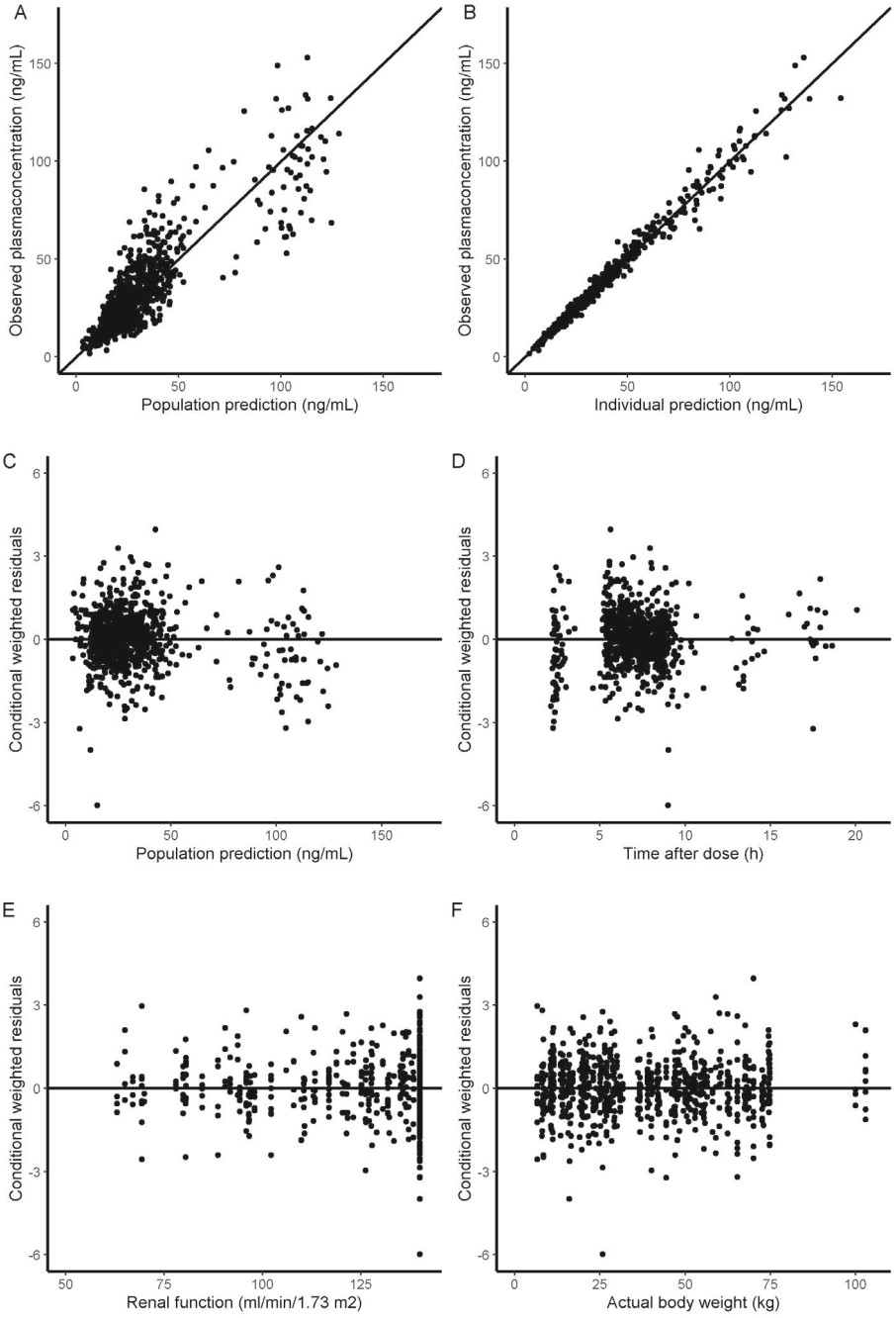
SUPPLEMENTARY MATERIAL



Supplementary Figure S1. Goodness of fit plots.

4

A population pharmacokinetic modelling approach to unravel the complex pharmacokinetics of vincristine in children

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ABSTRACT

Background

Vincristine, a chemotherapeutic agent that extensively binds to β-tubulin, is commonly dosed at 1.4-2.0 mg/m^2 capped at 2 mg. For infants, doses vary from 0.025-0.05 mg/kg or 50-80% of the mg/m^2 dose. However, evidence for lower doses in infants compared to older children is lacking. This study was conducted to unravel the complex pharmacokinetics of vincristine, including the effects of age, to assist optimal dosing in this population.

Methods

206 patients (0.04-33.9 years; 25 patients <1 years), receiving vincristine, with 1297 plasma concentrations were included. Semi-mechanistic population pharmacokinetic analyses were performed using non-linear mixed effects modelling.

Results

A three-compartment model, with one saturable compartment resembling saturable binding to β-tubulin and thus, saturable distribution, best described vincristine pharmacokinetics. Body weight and age were covariates significantly influencing the maximal binding capacity to β-tubulin, which increased with increasing body weight and decreased with increasing age. Vincristine clearance (CL) was estimated as 30.6 L/h (95% confidence interval (CI) 27.6-33.0), intercompartmental CL (Q) as 63.2 L/h (95%CI 57.2-70.1), volume of distribution of the central compartment as 5.39 L (95%CI 4.23-6.46) and of the peripheral compartment as 400 L (95%CI 357-463) (all parameters correspond to a patient of 70 kg). The maximal binding capacity was 0.525 mg (95%CI 0.479-0.602) (for an 18 year old patient of 70 kg), with a high association rate constant, fixed at 1300 /h and a dissociation constant of 11.5 /h.

Interpretation

A decrease of vincristine β-tubulin binding capacity with increasing age suggests that young children tolerate higher doses of vincristine.

INTRODUCTION

Vincristine is used in the chemotherapeutic treatment of various pediatric malignancies. Its effect is caused by binding to tubulin and inhibiting microtubule formation, causing arrest of the cell at metaphase. Treatment with vincristine is mainly hampered by risk of developing vincristine induced peripheral neuropathy (VIPN). VIPN pathogenesis involves nerve cell mitochondria, endothelium and microtubules and has been shown to be dose-dependent.[1,2] Younger children have been suggested to exhibit a lower risk of developing VIPN compared to adolescents, despite a higher dose per kg body weight administered, which underlines potential differences in pharmacokinetics (PK) and pharmacodynamics in the younger patient population.[3]

Vincristine is usually dosed based on body surface area (BSA) (doses vary from 1.4-2.0 mg/m^2).[4] Because of the dose-dependent VIPN, the absolute dose is capped to a maximum of 2 mg.[4] However, clear evidence for this maximum dose in children is lacking. Additionally, commonly used dose reductions in infants are not evidence based. For infants, several dosing regimens are used in current practice. Doses vary from 0.025-0.05 mg/kg or 50-80% of the usual dose per BSA[5–11], but none of these reductions are based on literature. Theoretically, younger children could be at risk for lower vincristine clearance values, due to incomplete maturation of cytochrome p450 (CYP) 3A4, however, findings on age-related differences in PK of vincristine in infants and children are not conclusive.

Recently, Barnett et al. did not report significant differences in BSA-normalized vincristine clearance values between infants and older children, apart from a trend towards lower clearance in neonates (0-4 weeks) as compared to infants (1-12 months).[12] They showed that doses of <0.05 mg/kg resulted in significantly lower area under the curve (AUC) values than observed in infants and children receiving doses of ≥0.05 mg/kg or 1.5 mg/m^2, showing that dose reductions to for example 0.025 mg/kg in infants could lead to underexposure. In a recently published in-depth literature review, based on these findings and the results of other PK studies that did not find a relationship between age and PK[13–21], we concluded that infants should be administered doses of 0.05 mg/kg or 1.5 mg/m^2.[11]

Even though age-related differences in the PK of vincristine have not been found in published studies, Lee et al. proposed that there is a 5-fold higher β-tubulin binding capacity in children compared to adults.[22] Using a physiologically based pharmacokinetic (PBPK) modelling approach, they suggested that binding to β-tubulin in healthy tissue could play a key role in vincristine distribution, which might explain differences in toxicity. An increased fraction of the vincristine dose bound to β-tubulin in healthy tissue may lead to lower amounts of free vincristine and thus a lower risk of VIPN. Indeed, it is well known that vincristine binds to β-tubulin. Moreover, β-tubulin is abundant in thrombocytes, and, decades ago, both *in vitro* and *in vivo* studies showed that vincristine rapidly binds to

thrombocytes, so it is hypothesized that thrombocyte levels could also have an effect on vincristine distribution.[23–29]

This current study was conducted to unravel the complex PK of vincristine, including the effects of age, using a semi-mechanistic population PK modelling approach. Unravelling the complex PK of vincristine in (very) young children, alongside key clinical pharmacology data recently published in this area[12], will promote more rational vincristine dosing in this patient population.

METHODS

Patients and sampling

A prospective observational study was performed in Princess Máxima Center for Pediatric Oncology in the Netherlands. Patients up to the age of 18 years with a central venous line in situ were eligible for inclusion after written informed consent was obtained. No restrictions for types of tumors or malignancies were formulated, but patients with Down syndrome were excluded. Ethical approval by the institutional Medical Ethics Committee of the Erasmus MC was obtained (NL63037.078.18). The data generated from this study were combined with data from an ongoing prospective observational study in 20 clinical cancer centers across the UK. In the UK study, patients with Ewing sarcoma up to 24 years of age with a central venous line in situ were eligible for inclusion after written informed consent was obtained. Patients with a glomerular filtration rate <60 mL/min/1.73 m^2 were excluded. Ethical approval by the National Research Ethics Service committee North East-Newcastle and North Tyneside 1 was obtained (EudraCT 2013-000052-17). Beside these two prospectively collected cohorts, data from three historical cohorts previously described by Lee et al.[22] (n=24; only UK patients were included), van de Velde et al.[30] (n=37) and Barnett el al.[12] (n=26) were included in this analysis. All previous studies included patients up to the age of 18 years.

All patients were treated with vincristine as standard of care, with doses according to local protocols. Doses, varying from 1-2 mg/m^2 with a maximum of 2 mg, with specific reductions for infants, were administered either as bolus or 1 hour infusion.

In total, 4-8 blood samples per patient were collected at various time points. Vincristine plasma concentrations were quantified using a previously described high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) method[31] or a validated LC-MS assay developed in Newcastle[32], with lower limits of quantification (LLOQ) of 0.25 ng/mL and 0.50 ng/mL respectively. Vincristine plasma concentrations of the Princess Máxima Center for Pediatric Oncology study were quantified using a validated LC-MS/MS method using 200 µL human plasma, with a LLOQ of 0.10 ng/mL.[33] First samples below LLOQ were included using ½ of the LLOQ value.

When data on covariates (age, body weight (BW) and height) were missing, values were imputed based on UK growth charts[34] and known variables. For all cases where age was missing, BW and height were documented, therefore, these values were used to find the corresponding age in the growth charts (using median BW and height curves). In the cases where BW and height were missing, age and BSA were available. The age and BSA were used to find the corresponding height, and this value and the BSA were used to calculate the BW, using the Du Bois equation.[35]

Model development

For the structural model, two- and three-compartment models with first order elimination were tested.

Saturable binding to β-tubulin was implemented by incorporating the maximal binding capacity (Bmax) in the differential equation as follows:

$$\frac{dA(bound)}{dt} = k_{on} \times A(Vc) \times \left(1 - \frac{A(bound)}{B_{max}}\right) - k_{off} \times A(bound) \qquad (1)$$

where k_{on} is the association rate constant, k_{off} is the dissociation rate constant, A(Vc) is the amount of vincristine in the central compartment Vc, A(bound) is the amount of vincristine bound to β-tubulin and Bmax is the maximal binding capacity to β-tubulin. Bmax was estimated. See Supplementary Table S1 for differential equations of other compartments.

Interindividual variability (IIV) was evaluated for all PK parameters, and implemented as follows:

$$P_i = P_{pop} \times e^{(\eta_i)} \qquad (2)$$

where P_i is the individual parameter estimate for individual *i*, P_{pop} is the typical population parameters estimate, and η_i is assumed to be normally distributed with a mean of zero and a variance of ω^2.

Since data of multiple cycles of therapy were available, interoccasion variability (IOV) was implemented similarly as IIV, with each dose and subsequent sampling defined as a separate occasion. This variability was evaluated for clearance parameters and Bmax to diagnose potential time-dependent trends and to allow for random unaccounted variability between dosing moments.

Residual unexplained variability was evaluated as a proportional error model or as a combination of a proportional and additive error model.

Covariate analysis

The influence of patient-specific factors for variability in PK parameters were evaluated following structural model development. Allometric scaling was applied to implement the impact of BW on PK parameters with a fixed exponent of 0.75 resp. 1 for clearances resp. volumes of distribution. PK parameters were normalized to a BW of 70 kg.[36] Other assessed covariates included age and thrombocyte levels, using a power function, normalizing to an age of 18 years and a thrombocyte level of 300x10^9/L, respectively.

Table 1. Patient characteristics (Median (range), unless specified otherwise)

	N=206
Available data	
Total no. of occasions	253
Total no. of PK samples [n]	1297
No. of occasions per patient	1 (1-5)
No. of samples per occasion	5 (1-8)
Patient characteristics	
Age, years	8.3 (0.04-33.9)
No. of patients 0-1 yrs [n]	25
Actual body weight, kg	27.1 (2.9-126.0)
Female sex [n (%)]	98 (48%)
Thrombocyte levels	
Available occasions [n (%)]	137 (54%)
Thrombocyte levels, x10^9/L	224 (5-1063)
Not available occasions [n (%)]	116 (46%)
Vincristine treatment	
Dose, mg	1.6 (0.1-2.0)
Dose, mg/m^2	1.4 (0.4-2.5)
Dose, mg/kg	0.05 (0.02-0.09)
Infusion duration [n]	
Bolus	214
15-113 min	39

PK Pharmacokinetic(s)

Model evaluation

Discrimination between models was guided by physiological plausibility, goodness-of-fit (GOF) plots, precision of parameter estimates and change in objective function value

(dOFV). A drop of ≥3.84 points, corresponding to a P<0.05 (χ^2-distribution with 1 degree of freedom (df)), was considered a significant improvement. The adequacy of the models was assessed by GOF plots and visual predictive checks (VPC).[37] The sampling importance resampling (SIR) procedure was used for the assessment of parameter precision.[38]

Software

Nonlinear mixed-effects modeling was performed using NONMEM (version 7.3.0, ICON development Solutions, Ellicott City, MD, USA) and Pearl-speaks-NONMEM (PsN, version 4.9.0) with First-Order Conditional Estimation with interaction (FOCE-I) as estimation method.[39,40] Pirana (version 2.9.9) was used as graphical user interface for NONMEM.[41] R (version 3.4.3) was used for data handling and visualization.[42]

RESULTS

Patients and sampling

In total, 206 patients with a median age of 8.3 years (range 0.04-33.9) were included. Detailed patient characteristics are presented in Table 1. 25 patients, with 25 vincristine cycles and 88 samples, were younger than 1 years of age (7 patients 0-3 months; 8 patients 3-6 months; 4 patients 6-9 months; 6 patients 9-12 months). In total, 1297 samples were available, of which 30 samples were below the LLOQ. Supplementary Figure S1 displays the observed plasma concentrations over time.

In total, for 8 patients the age was missing and for 2 patients BW and height were missing and were imputed based on UK growth charts. All these patients came from UK studies.

Model development

The base model that best described the data was found to be a three-compartment model with first order elimination. Allometric scaling using BW was a priori included on all PK parameters. Following structural model development, a saturable compartment was incorporated, resembling saturable binding to β-tubulin and thus, saturable distribution, to test the hypothesis of Lee *et al.*[22] They hypothesized that binding to β-tubulin has a significant impact on the PK of vincristine. This third, saturable compartment was incorporated as a compartment, driven by the concentration in the central compartment. Adjustment of the base three-compartment model to a three-compartment model containing one saturable compartment, resulted in a drop in OFV of 80 points. This model was parameterized in terms of volume of distribution of the central (Vc) and peripheral (Vp) compartment, clearance from the central compartment (CL) as well as intercompartmental CL between Vc and Vp (Q), Bmax, the association rate constant (k_{on}) and dissociation rate constant (k_{off}). k_{on} was considered to be too fast to estimate adequately, so was fixed at 1300 /h (the value that resulted in the lowest OFV). The model was further optimized by adding IIV on CL, Q, Vc, Vp, k_{on} and k_{off} and IOV on Bmax. No trends in IOV on Bmax vs. dosing occasions or age were observed.

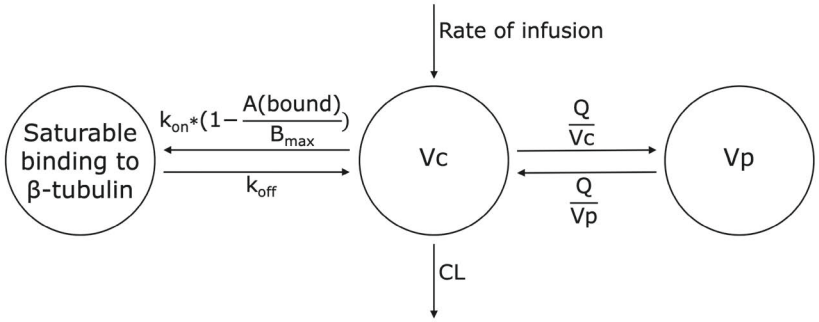

Figure 1. Graphical representation of the final model for vincristine. k_{on} is driven by the amount of vincristine bound to tubulin (A(bound)) and Bmax. *Bmax* Maximal binding capacity, *CL* Clearance, k_{off} Dissociation rate constant, k_{on} Association rate constant, *Q* Intercompartmental clearance, *Vc* Vincristine central compartment, *Vp* Vincristine peripheral compartment.

Covariate analysis

Subsequently, various covariates were tested for their influence on PK parameters and Bmax. In the PBPK model of Lee *et al.*[22], age was found to be a significant covariate for β-tubulin expression, defined as Bmax in our model. Furthermore, we expect Bmax to be dependent on BW, based on allometric scaling principles. For this reason, BW and age were tested as covariates on Bmax. Firstly, BW was included in Bmax using a power function with an estimated exponent. The exponent was estimated to be 0.707. This was thought to be a result of a combined, opposite effect of BW and age, where an increasing BW would lead to an increase in Bmax (allometric principles), but where an increase in age would lead to a decrease in Bmax (hypothesis Lee *et al.*[22]). For this reason, the exponent on BW was fixed to 1 (in accordance with allometric scaling for volumes of distribution) and age was included as covariate using a power function (normalization to a patient of 18 years), with an estimated exponent. This resulted in an exponent of -0.199 for age.

Furthermore, thrombocyte levels were tested as covariate on Bmax. Several studies showed that vincristine binds to thrombocytes, which is hypothesized to be related to tubulin, since β-tubulin isoforms are abundant in human thrombocytes[29]. Data on thrombocyte levels were not available for 46% of the occasions. When thrombocyte levels were not available, a thrombocyte count of $300x10^9$/L was imputed, plus IIV to allow for variability on this imputed value. An IIV of around 30% was found. However, adding thrombocyte levels as covariate on Bmax resulted in unstable models with divergent OFV values, very sensitive to initial estimate changes. In addition, the IOV on Bmax did not decrease and IIV's on other parameters increased. Therefore, thrombocyte levels were not included as covariate on Bmax in the final model.

A graphical representation of the final model is presented in Figure 1. Final PK parameters estimates are displayed in Table 2. Figure 2 displays the typical Bmax and CL vs. age for patients until the age of 2 years. Data was based on typical weight and height values according to WHO growth charts.[34] Absolute doses according to three different dosing regimens were included: A. All ages: 1.5 mg/m^2. B. Children <6 months: 50% of BSA dose (0.75 mg/m^2); Children 6-11 months: 75% of BSA dose (1.125 mg/m^2); Children ≥12 months: 1.5 mg/m^2. C. Children <10 kg: 0.05 mg/kg/day; Children ≥10 kg: 1.5 mg/m^2

Table 2. Vincristine PK parameters estimates of the final model

Parameter	Estimate	95% CI
CL_{70kg} (L/h)	30.6	27.6 – 33.0
Q_{70kg} (L/h)	63.2	57.2 – 70.1
Vc_{70kg} (L)	5.39	4.23 – 6.46
Vp_{70kg} (L)	400	357 – 463
$Bmax_{18yrs\ 70kg}$ (mg)	0.525	0.479 – 0.602
k_{on} (/h)	1300 fixed	
k_{off} (/h)	11.5	9.2 – 14.5
Age on Bmax	-0.199	-0.304 – -0.090
IIV CL (%)	47.7	41.0 – 54.3
IIV Q (%)	38.1	26.2 – 49.0
IIV Vc (%)	122.5	98.7 – 158.3
IIV Vp (%)	57.1	48.8 – 69.7
IIV k_{on} (%)	126.5	108.7 – 147.8
IIV k_{off} (%)	24.1	11.1 – 33.8
IOV Bmax (%)	59.1	50.7 – 66.1
Proportional residual error (%)	30.1	28.9 – 31.4

Bmax Maximal binding capacity, *CI* Confidence interval obtained by sampling importance resampling, *CL* Clearance, *IIV* Interindividual variability, *IOV* Interoccasion variability, k_{off} Dissociation rate constant, k_{on} Association rate constant, *PK* Pharmacokinetic(s), *Q* Intercompartmental clearance, *Vc* central compartment, *Vp* peripheral compartment. Bmax corresponds to a subject of 18 years weighing 70 kg, other population estimates correspond to a subject weighing 70 kg and are adjusted to an individual value using allometric scaling.

Model evaluation

The model performance was checked through GOF plots. Looking at population and individual predictions, conditional weighted residuals vs. plasma concentration and time after dose, no trends, or signs for over- of underprediction have been found (Supplementary Figure S2). Furthermore, the VPC showed no signs for structural over- of underprediction (Supplementary Figure S3). The PK parameters of the patients with imputed age or BW and height were not markedly different.

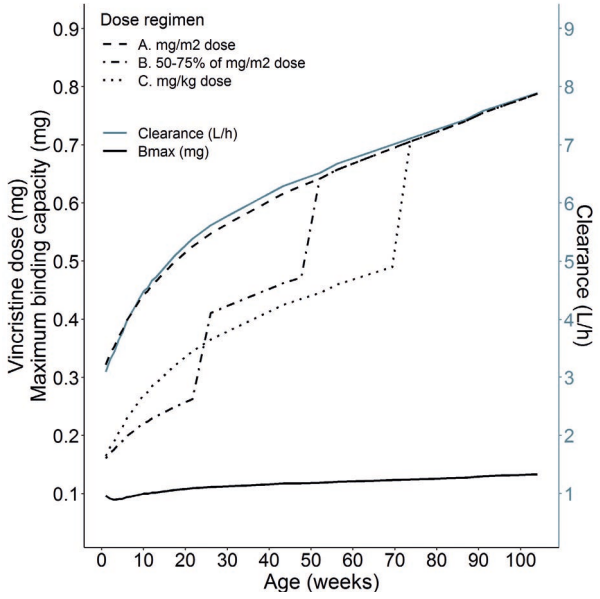

Figure 2. Vincristine clearance (solid grey line), maximum binding capacity (solid black line) and absolute vincristine dose for three different infant dosing regimen over age: A. All ages: 1.5 mg/m² (dashed line). B. Children <6 months: 50% of BSA dose (0.75 mg/m²); Children 6-11 months: 75% of BSA dose (1.125 mg/m²); Children ≥12 months: 1.5 mg/m² (dashdotted line). C. Children <10 kg: 0.05 mg/kg/day; Children ≥10 kg: 1.5 mg/m² (dotted line).

DISCUSSION

This study successfully implemented saturable binding to β-tubulin in a population PK model of vincristine in children. A three-compartment model, including one saturable compartment, was found to best describe the available data from 206 patients. This saturable compartment resembles saturable binding to β-tubulin and thus, saturable distribution of vincristine.

Two covariates were identified to account for variability in the binding capacity to β-tubulin. BW was included as covariate to Bmax using allometric principles. Additionally, age was added as covariate using a power function. BW and age were found to have an opposite effect on Bmax. By using allometric scaling for volumes of distribution, a rise in BW resulted in an increase in Bmax, while we found that a higher age led to a decrease in Bmax. This is in line with the findings of Lee *et al.*[22] With their PBPK analyses they showed that there is a 5-fold higher β-tubulin binding capacity in children compared to adults.

Clinical information on the difference in β-tubulin exposition between children and adults is not available, but it has been shown that immunohistochemical distribution of β2-tubulin was higher in tissue of neonates compared to older children and adults, and that the expression

decreased with increasing age.[43] The same is to be expected for other β-tubulin isotypes, since tubulin in microtubules play an important role in cell division, which is more prevalent in children. A higher β-tubulin expression in younger children is most likely the reason for a higher β-tubulin binding capacity of vincristine.

A higher β-tubulin binding capacity leads to a faster drop of the vincristine plasma concentration and lower amounts of free vincristine present in the central compartment thus a lower risk of VIPN, since we assume that free vincristine is able to distribute to peripheral tissue, where it causes VIPN. In children, with a higher β-tubulin binding capacity, a lower amount of free vincristine is available to distribute to peripheral tissue, while in adults, with a lower β-tubulin binding capacity, the amount of free vincristine is higher as well as the risk of developing VIPN. This hypothesis is consistent with the findings that younger children seem to tolerate higher doses with regards to development of VIPN compared to older children and adults.[1,3]

These findings also raise the question whether children should be administered higher doses than adults to achieve the same effect. Children are usually treated with doses of 1.5-2.0 mg/m^2, with a maximum of 2 mg. Essentially, the capped dose comes into play for patients with a BSA >1.3 m^2. It is unclear to what extend and over what age range the risk of developing VIPN is lower than in adults, so definitive advice for changing the maximum dose for children cannot be given. For younger children, however, we could make some remarks based on the current PK study and previous research. As mentioned previously, infants are treated with doses varying from 0.025-0.05 mg/kg or 50-80% of the usual dose per BSA.[5–11] Barnett et al.[12] showed that doses of <0.05 mg/kg result in significantly lower AUC values than observed in infants and children receiving doses of ≥0.05 mg/kg or 1.5 mg/m^2. These exposure data, combined with our current results, strongly suggest that dose adjustments for infants may not be justified.

A major concern with current dosing approaches for vincristine, is that using different dosing regimens for infants leads to disproportional increases in the dose when the patient reaches a specific age or weight (see Figure 2). While this widely used rudimentary approach to dosing is a concern for all drugs, it is of particular concern for a drug such as vincristine, for which no age-related differences in CL have been found. In the current study, we show that younger children have a higher β-tubulin binding capacity for vincristine. However, absolute Bmax values (taking BW and age into account) in patients up to 2 years of age, displayed in Figure 2, do not seem to change markedly over time, except for the first weeks of age. Furthermore, Figure 2 visually shows that a dose regimen of 1.5 mg/m^2 follows the curve of vincristine CL with increasing age. From a pharmacokinetic perspective, we would suggest administering the full mg/m^2 (e.g. 1.5 mg/m^2) dose to infants, except for neonates of 0-4 weeks (0.05 mg/kg according to Barnett et al.[12]).

Increasing the dose should, however, be done with caution. Besides VIPN, other adverse reactions, like vocal cord paralysis, respiratory distress or constipation, frequently occur. Preferably, a clinical trial in young patients investigating the exposure and toxicity profiles under the proposed mg/m^2 dosing regimen compared to the mg/kg dose is performed, before changing the dose in infants.

Furthermore, in order to decrease the IOV on Bmax, we aimed to look into the effect of thrombocyte levels on the β-tubulin binding capacity. We did not find an effect of thrombocytes levels on Bmax or the IOV of Bmax. This is probably due to missing thrombocyte counts in a large part of the dataset. Moreover, since β-tubulin is present in all cells (all types of blood cells as well as cells in peripheral tissue)[25,27,44,45], it is possible that thrombocytes account for just a small part of the β-tubulin expression throughout the body. This will be studied further using a PBPK modelling approach.

A limitation of the current study is that the data did not include the exact amount of β-tubulin in patients, since we were not able to measure β-tubulin exposition. We have explained the saturable distribution as being vincristine binding to β-tubulin, however, alternative explanations could be made. We have looked into other developmental changes that could explain the age-related findings of the current study, but did not find other pharmacological rationales,

Another limitation relates to the fact that the effect of CYP3A4/5-inductors and –inhibitors on the PK of vincristine was not studied, since information on the use of CYP3A4/5-inductors and –inhibitors was not available. Also, genetic variations in CYP3A4/5, which can vary with race, were not taken into account in the current study. Moreover, the metabolic capacity of CYP3A4/5 changes during the first years of life. However, it is to be expected that variations in activity of metabolising enzymes CYP3A4/5 only effect vincristine CL, and does not influence vincristine distribution, which is the main topic of the current study. Furthermore, previous research did not find an effect of CYP3A4/5 polymorphisms on vincristine PK.[16]

CONCLUSION

Vincristine binding to β-tubulin was found to be dependent of body weight and age. β-tubulin binding capacity decreases with increasing age, suggesting that children can tolerate higher doses of vincristine. Based on these results and previous literature we would suggest that administration of full mg/m^2 doses to infants from 4 weeks of age may be more appropriate than the currently used mg/kg dosing regimens.

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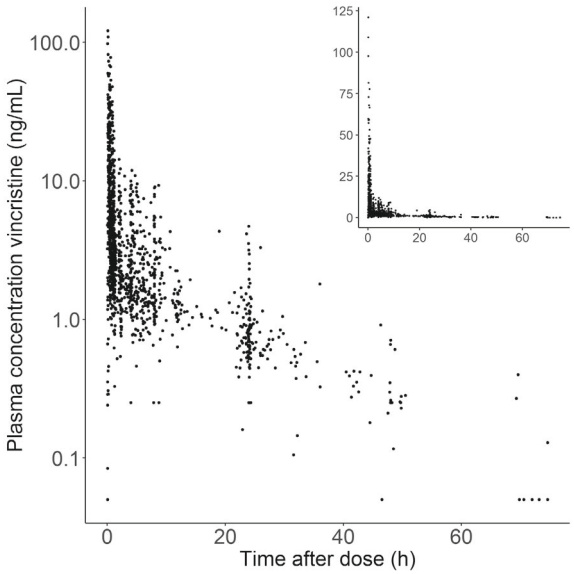
SUPPLEMENTARY MATERIAL

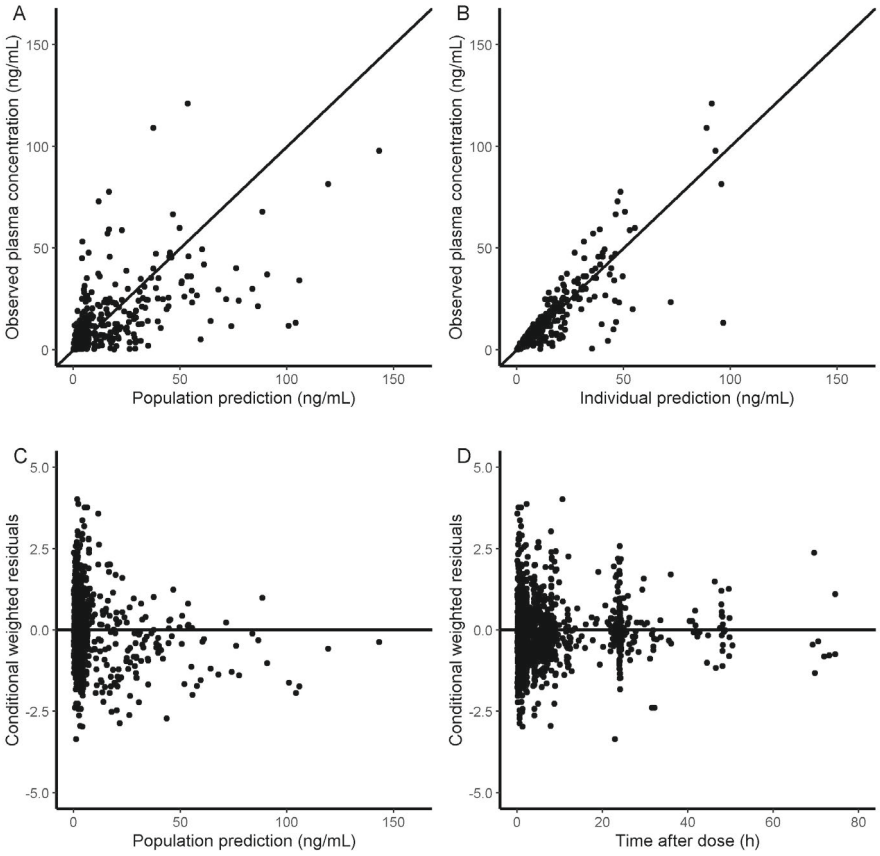
Supplementary Table S1. Differential Equations for the mass transport of vincristine between the compartments

Compartment	Differential equation describing compartment
Vincristine central	$\frac{dA(Vc)}{dt} = -\frac{CL}{Vc} \times A(Vc) - k_{on} \times A(Vc) \times \left(1 - \frac{A(bound)}{B_{max}}\right) + k_{off} \times A(bound) - \frac{Q}{Vc} \times A(Vc) + \frac{Q}{Vp} \times A(Vp)$
Vincristine peripheral	$\frac{dA(Vp)}{dt} = \frac{Q}{Vc} \times A(Vc) - \frac{Q}{Vp} \times A(Vp)$
Saturable binding to β-tubulin	$\frac{dA(bound)}{dt} = k_{on} \times A(Vc) \times \left(1 - \frac{A(bound)}{B_{max}}\right) - k_{off} \times A(bound)$

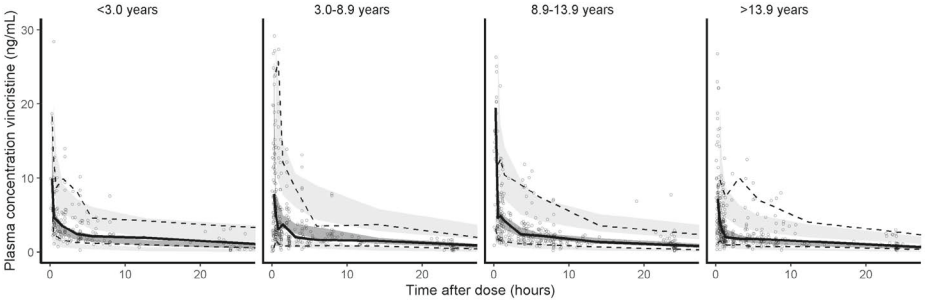
A(n) Amount in compartment n, B_{max} Maximal binding capacity, *CL* Clearance, k_{off} Dissociation rate constant, k_{on} Association rate constant, *Q* Intercompartmental clearance, *Vc* Vincristine central compartment, *Vp* Vincristine peripheral compartment.



Supplementary Figure S1. Vincristine plasma concentrations versus time after dose on a logarithmic and linear scale.



Supplementary Figure S2. Goodness-of-fit plots



Supplementary Figure S3. Age stratified prediction-corrected visual predictive check. Black lines depict the observed median (solid) and 5% and 95% percentile (dashed) concentrations. Dark- and light-grey areas represent 90% prediction intervals of the simulated mean and the 5 and 95% percentiles, respectively. Round dots represent observations.

5

Age-dependent vincristine binding to β-tubulin in tissue and blood cells explains non-linear pharmacokinetics of vincristine: a physiologically based pharmacokinetic approach

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ABSTRACT

Vincristine is widely used in pediatric oncology and acts by binding to the β-subunits of tubulin in tumor cells. The expression of β-tubulin in healthy tissue and blood cells is thought to be highly relevant for the distribution of vincristine throughout the body and is most likely age-dependent. The current physiologically-based pharmacokinetic (PBPK) study has been conducted to optimize a previously published PBPK model by including vincristine binding toβ-tubulin in blood cells and to assess age-related differences.

Data from 16 adults, 10 adolescents (13-16 years), 23 children (2-10 years) and 17 infants (0-1 years) were included in the model. Using PK-Sim, an adult PBPK model including metabolism and elimination by CYP3A4, CYP3A5 and P-gp and binding to β-tubulin in tissue and blood cells was developed. This model was successfully scaled to adolescents, children and infants. The pediatric model was optimized by simulating a 2.5-fold higher binding capacity of blood- and tissue-β-tubulin for infants (0-1 years), a 2-fold higher binding capacity for children (2-10 years) and a 1.5-fold higher binding capacity for adolescents (13-16 years) as compared to adults. Moreover, the model adequately described vincristine whole blood concentrations.

A higher binding capacity to β-tubulin leads to a more rapid decline in the vincristine plasma concentration during the first period after infusion. Reduced amounts of free vincristine in the central compartment will potentially lead to a lower risk of peripheral neuropathy, since it is assumed that free vincristine is able to distribute to peripheral tissue, where it causes peripheral neuropathy. The higher binding capacity of vincristine to β-tubulin in (young) children would explain the fact that children are able to tolerate higher relative doses of vincristine and the need for vincristine dose capping in adults. This study forms the basis for further optimization of pediatric dosing guidelines of vincristine.

INTRODUCTION

Vinca alkaloids are frequently used in the chemotherapeutic treatment of various malignancies. These substances bind to the β-subunits of tubulin, thereby inhibiting microtubule formation and causing arrest of the cell at metaphase. The vinca alkaloid vincristine is widely used in both adult and childhood cancer patients, and is usually dosed at 1.4-2.0 mg/m^2, with a maximum of 2 mg per dose.[1] The dose is maximized because of the risk of developing vincristine induced peripheral neuropathy (VIPN), which was found to be dose-dependent. However, these dosing capping recommendations have been questioned since they are mainly based on empirical experiences.[2,3] For infants, the dose is reduced to 50-80% of the usual body surface area (BSA)-based dose or a mg/kg dose (0.025-0.05 mg/kg) is given, even though a pharmacological rationale for these regimens is lacking.[4,5]

Optimizing vincristine dosage for individuals remains a challenge, mainly because of lack of knowledge on factors that might influence its pharmacokinetics (PK). This has most recently been highlighted in terms of the challenges of dosing vincristine in neonates and infants as compared to older children.[6] Vincristine is mainly metabolized through hepatic cytochrome P450 (CYP450) enzymes, particularly by the CYP3A4 and CYP3A5 isoforms, with biliary excretion through the hepatic efflux transporter P-glycoprotein (P-gp).[7] The plasma concentration-time profile of vincristine indicates a rapid distribution phase followed by a relatively long elimination phase, with initial and terminal half-life values of 5 minutes and 19-155 hours, respectively.[1] The PK of vincristine is most commonly described by a two- or three-compartmental model and is characterized by large interindividual variability. For example, in recent population PK studies of vincristine, interindividual variability in PK parameters ranged from 17% to 66% in adults, and 48% to 67% in children.[8,9] The effects of demographic, clinical, and biomedical variables, such as age, BSA, dose, and pharmacogenetics, on the PK of vincristine have been studied but no structural covariates on vincristine clearance (CL) or volume of distribution (Vd) have been identified.[3,4,6,8,10–17]

Binding of vincristine to β-tubulin in healthy tissue might be key in understanding its unexplained PK variability.[5,18] Saturable binding to β-tubulin was incorporated into a previously published population PK model.[5] Vincristine binding to β-tubulin was found to be dependent on body weight and age. β-tubulin binding capacity decreased with increasing age, which is in line with the PBPK results from Lee et al.[18] They concluded that binding to β-tubulin in healthy tissue plays a key role in determining differences in vincristine distribution, and a 5-fold higher β-tubulin binding capacity was observed in children as compared to adults.[18] However, a 5-fold higher β-tubulin binding capacity can be seen as physiologically implausible and the PBPK model of Lee et al. only included β-tubulin in tissue as a binding partner of vincristine, and did not take β-tubulin in blood (e.g. erythrocytes, leukocytes and thrombocytes) into account. Previously, it has been shown that several isoforms of β-tubulin are expressed in erythrocytes, leukocytes and thrombocytes, so it is necessary to take this into account in the PBPK model as well.[19–22]

The current PBPK study was conducted with the aim to improve understanding of the complex non-linear PK profile of vincristine, particularly to comprehend evidence-based dosing regimens for children. The previously published PBPK model of Lee et al.[18] was optimized by including vincristine binding to β-tubulin in blood cells and age-related differences were investigated.

METHODS

Patient data

Data for PBPK model development
Historical data comprising adults from two different PK studies (Villikka et al. and a Newcastle study described by Nijstad et al.) were used for the adult PBPK model development.[5,23] Data from Villikka et al.[23] were digitized using GetData Graph Digitizer (version 2.26.0.20). Plasma concentrations from Villikka et al.[23] were used for the adult training dataset, and plasma concentrations from the Newcastle study[5] for the adult evaluation dataset.

Data from pediatric patients originated from a prospective, observational study performed in the Princess Máxima Center for Pediatric Oncology in the Netherlands, previously described by Nijstad et al.[5] Data from patients aged 0-1 years, 2-10 years and 13-16 years, with at least 1 sample between 0 and 24 hours after dose, were included in this PBPK analysis. Regarding the infants, only patients treated with a dose of 0.05 mg/kg were included.

All patients were treated with vincristine as standard of care, with doses according to local protocols. Doses, varying from 1 to 2 mg/m^2 with a maximum of 2 mg or 0.05 mg/kg for infants, were administrated as bolus infusion. Vincristine plasma concentrations were quantified using a liquid chromatography (tandem) mass spectrometry (LC-MS/MS) assay.[5,23–26]

Vincristine whole blood concentrations
For a selection of patients included in the prospective, observational study performed in the Princess Máxima Center for Pediatric Oncology in the Netherlands, previously described by Nijstad et al.[5], vincristine whole blood concentrations were determined using a validated LC-MS/MS method (L.T. van der Heijden et al, manuscript in preparation).

Whole blood was collected using a Mitra® (10 µL, Neoteryx, Torrance, USA) device. The sponge of the Mitra® was transferred into a 2.0 mL reaction tube. Two grinding beads were add to all reaction tubes. A two-step extraction was performed. First, 400 µL 0.2% formic acid in water was added to all samples. The double blanks (CAL0/0) were spiked with 20 µL methanol. All other samples were spiked with 20 µL internal standard in methanol

(vincristine-d3, 50 ng/mL). The first extraction step was performed by placing all samples in a genogrinder for 10 minutes (1250 rpm). The second extraction step consisted of liquid-liquid extraction with acetonitrile prior to centrifuging (15,000 rpm for 5 minutes). The supernatant was collected and dried under a gentle stream of nitrogen (40ºC) prior to reconstitution with 100 µL methanol:acetonitrile:water (1:1:2, v/v/v). Continuing, the samples were vortex mixed and centrifuged (15,000 rpm for 5 minutes) before the supernatant was transferred to vials prior to analysis. The final extracts were analyzed with a validated LC-MS/MS method. The method was validated according to the EMA and FDA guidelines on bioanalytical method validation over a linear range of 1-50 ng/mL. The lower limited of quantification (LLOQ) was 1 ng/mL. The intra- and inter-assay bias and precisions were within ±10.9% and ≤5.2%.

Plasma concentrations for the corresponding time point were available as well. Concentrations in blood cells were calculated by subtracting the plasma concentration from the whole blood concentration, taking the hematocrit level into account:

$$C_{Blood\ cell} = \frac{C_{Whole\ blood} - C_{Plasma} \times (1 - Hematocrit)}{Hematocrit}$$

PBPK model development

A schematic work-flow for the PBPK model development is presented in Figure 1.

Adult base model

Firstly, an adult base model was built and evaluated. Physiochemical properties, binding partners, metabolism and elimination processes of vincristine were included based on Lee et al[18]. Expression levels of CYP3A4, CYP3A5, P-gp (ABCB1) and tissue-β-tubulin were incorporated using the genome expression arrays from ArrayExpress.[27] For tissue-β-tubulin, TUBB (Gene ID: 203068) was selected to represent overall tissue-β-tubulin binding, since it is expressed in most human tissues. The initial model was simulated based on virtual adults following a single bolus dose of 2 mg vincristine and fitted to the data of the adult training dataset.

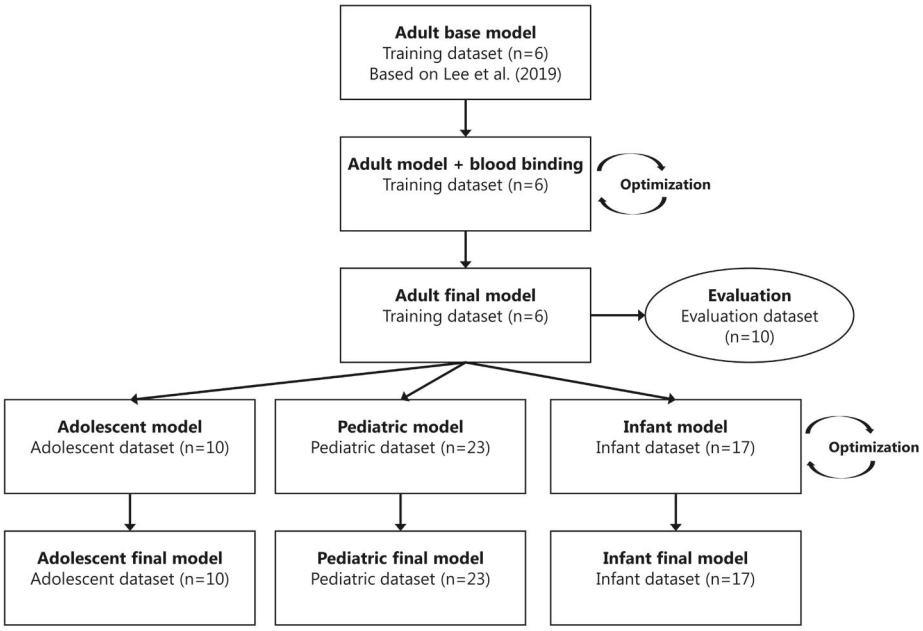

Figure 1. Schematic work-flow for the PBPK model development

Adult model including blood-β-tubulin as binding partner

After development of the base adult model, binding of vincristine to β-tubulin in blood cells was implemented. A dummy protein binding partner, only expressed in the blood cell compartment defined by PK-Sim, was added to the base model. Binding parameters (i.e. dissociation constant (K_D) and dissociation rate (k_{off})) of tissue-β-tubulin binding as presented by Lee et al. were used as initial estimates.[18]

Model fitting and evaluation

Model parameter optimization was conducted by fitting the model to the adult training dataset. The reference concentrations of blood-β-tubulin as well as tissue-β-tubulin and binding parameters of blood-β-tubulin were optimized. Within each step, the rationality of parameterization was evaluated by reported values in literature and served as the key determinant to include subsequent binding partners.

For model evaluation, a virtual adult population of 100 individuals was generated based on patient demographics of the adult evaluation dataset. Models were evaluated visually and were accepted if simulated concentration-time profiles fit the overall shape of observed profiles and most observed concentration data fell within the 95% prediction interval (PI) for simulated data.

Sensitivity analyses were performed in order to evaluate the impact of blood- and tissue-β-tubulin-binding on vincristine distribution. Input parameters related to blood- and tissue-β-tubulin-binding (K_D, k_{off} and reference concentration) were selected for sensitivity analyses performances and were evaluated with a 100% variation to determine their relative impact on the area under the concentration-time curve from 0-24h post-infusion $(AUC)_{0-24}$, AUC_∞, V_d, V_{ss}, CL and terminal half-life ($t_{1/2}$).

Pediatric model
The final adult population model was scaled to an infant, pediatric and adolescent population of 100 individuals based on patient demographics of the infant, pediatric and adolescent dataset. Age-dependent variations and maturations in anatomy, physiology, and biochemistry were implemented.[28–30] Age-dependent organ volumes, tissue compositions, blood flow rates, etc. were scaled by the implemented algorithm in PK-Sim within the limits of the International Commission on Radiological Protection (ICRP).[31] Ontogeny of CYP3A4 was included based on the built-in PK-Sim ontogeny function.

Virtual infants patients were dosed with a single vincristine bolus of 0.05 mg/kg and pediatric patients until the age of 12 years were dosed with a single vincristine bolus of 1.5 mg/m^2. For model evaluation, simulated concentration-time profiles were compared with plasma observations from the pediatric datasets.

Software
PBPK modeling was performed using PK-Sim (version 7.10, Open Systems Pharmacology Suite).[32] Simulations were carried out using the Schmitt partition coefficient calculation method. Model input parameter optimization was accomplished using the Levenberg-Marquardt algorithm implemented in PK-Sim. Sensitivity analyses were performed using the PK-Sim built-in tool for model evaluation. R (version 3.4.3) was used for data handling and visualization.[33]

RESULTS

Patient data
Data from 66 patients (16 adults, 10 adolescents (13-16 years), 23 children (2-10 years) and 17 infants (0-1 years)) were included. Plasma concentrations of 6 adult patients were used for the training dataset and the remaining 10 adult patients for the evaluation dataset. Patient characteristics are displayed in Table 1.

PBPK model development

Adult base model
An adult base model was built and evaluated. Metabolism and elimination processes were included identically to Lee et al[18]: CYP3A4, CYP3A5, P-gp (ABCB1). Tissue-β-tubulin was incorporated as binding partner of vincristine.

The fit of this model to the training dataset was assessed visually and optimized manually. An optimized tissue-β-tubulin reference concentration of over 10 μM was necessary to adequately describe vincristine distribution in the first few hours post-dose. The model with only tissue-β-tubulin as a specific binding partner of vincristine was, therefore, considered not physiological plausible to describe vincristine distribution. This was considered to indicate additional binding of vincristine by blood cells.

Adult model including blood-β-tubulin as binding partner
The adult base model was optimized by including blood-β-tubulin as a binding partner of vincristine. This model improved the description of the vincristine observations within the first 2 hours after administration (Figure 2A).

As a starting point, k_{off} was set to $1.93*10^{-3}$ /s and K_D to 0.05 μM based on literature of tissue-β-tubulin.[18] K_D was optimized to 0.20 μM. Optimized reference concentrations of tissue- and blood-β-tubulin were 1.0 μM and 1.2 μM, respectively.

Table 1. Patient characteristics

	Adults				
	Training dataset[23]	**Evaluation dataset[5]**	**Adolescents 13-16 years[5]**	**Children 2-10 years[5]**	**Infants 0-1 years[5]**
	N=6	**N=10**	**N=10 (12 OCC)**	**N=23 (27 OCC)**	**N=17**
Female sex [n (%)]	3 (50%)	3 (30%)	2 (20%)	10 (43%)	7 (41%)
Age, years [median (range)]	46.5 (40-54)	20.3 (18.3-33.9)	14.0 (13.2-15.3)	5.3 (2.9-10.0)	0.5 (0.04-0.98)
Body weight, kg [median (range)]	NA	90.1 (42.5-126)	43.8 (35.0-59.9)	20.2 (11.8-33.1)	5.8 (2.9-11.0)
BMI, kg/m^2 [median (range)]	24.5 (17-32)	NA	NA	NA	NA
Absolute dose, mg [median (range)]	2	2	2	1.2 (0.8-1.7)	0.4 (0.2-0.69)

BMI Body mass index, *NA* not applicable, *OCC* Occasions

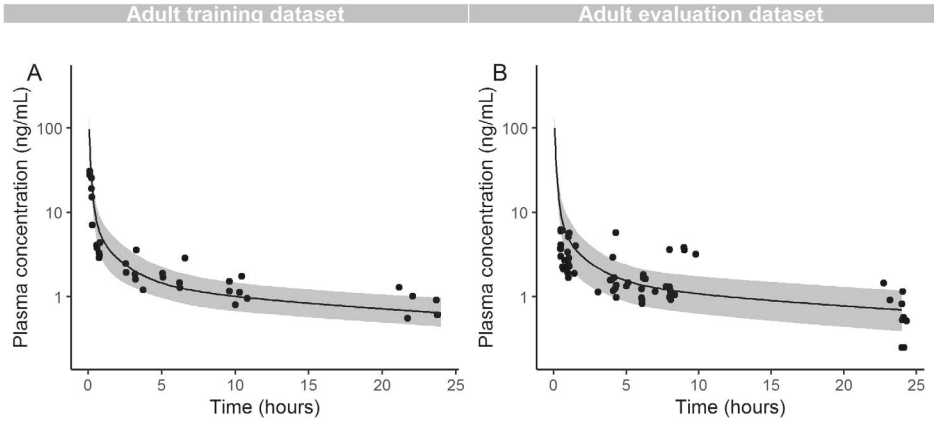

Figure 2. Simulated plasma concentration time curves for the adult training dataset (A) and the adult evaluation dataset (B). The solid line represents the simulated mean and the grey area the 95% prediction interval.

The model was evaluated with the adult evaluation dataset. Population-based simulations were performed for virtual adults with similar patient characteristics as the evaluation dataset (Table 1). As shown in Figure 2B, the majority of observed concentration data points were within the 95% PI of simulated vincristine plasma concentrations, indicating that the established model with blood-β-tubulin-binding was adequate.

The sensitivity analysis (Table 2) showed that most PK parameters were more sensitive to variations in blood-β-tubulin expression level than in tissue-β-tubulin expression level. For example, 100% increases in blood- or tissue-β-tubulin reference concentrations led to decreases in $AUC_{0\text{-}24}$ of 34% and 5%, and increases in V_{ss} of 88% and 11%, respectively. Changes in the K_D of vincristine-blood-β-tubulin binding also led to a greater impact on PK parameters than that of tissue-β-tubulin binding. Moreover, the effect of blood-β-tubulin-binding on PK parameters related to both the period shortly after infusion and the later time points, as suggested by the changes in V_{ss} and V_d. The CL of vincristine was marginally influenced by variations in either blood- or tissue-β-tubulin reference concentration, suggesting that the binding processes mostly affected vincristine distribution.

This adult PBPK model considering possible binding between vincristine and blood-β-tubulin demonstrated physiologically appropriate tissue-β-tubulin and blood-β-tubulin reference concentrations. Therefore, this model strengthened the relevant binding of vincristine to blood-β-tubulin in addition to tissue-β-tubulin.

Table 2. Sensitivity analyses

Binding partner	Input parameter	Relative change in PK parameter with a 100% variation in selected input parameters (%)					
		AUC_{0-24}	AUC_{∞}	V_{ss}	V_d	CL	$t_{1/2}$
Blood-β-tubulin	Ref Conc	-34%	-0.1%	88%	87%	0.1%	87%
	K_D	24%	7%	-54%	-56%	-7%	-50%
	k_{off}	-0.04%	-0.03%	-0.06%	-0.24%	0.03%	-0.26%
Tissue-β-tubulin	Ref Conc	-5%	-2%	11%	11%	2%	9%
	K_D	5%	2%	-11%	-11%	-2%	-9%
	k_{off}	-0.01%	-0.42%	-0.67%	-0.66%	0.42%	-1.00%

AUC area under the curve, *CL* total body clearance, K_D dissociation constant, k_{off} dissociation rate, *PK* pharmacokinetics, $t_{1/2}$ half-life, V_d volume of distribution during terminal phase, V_{ss} volume of distribution at steady state

Pediatric model

The final adult population model was scaled to an adolescent (13-16 years), a pediatric (2-10 years) and an infant (0-1 years) population of 100 individuals with characteristics similar to patient demographics of the corresponding datasets (Table 1).

For both the adolescent, the pediatric and the infant population, the simulated concentration-time curve overpredicted plasma concentrations in the first hours after administration (Figure 3A-C), suggesting that vincristine distribution was still not well described in the model, despite incorporating vincristine binding to blood-β-tubulin.

Lee et al. have shown the same misspecification when scaling the adult PBPK model to children aged 2 to 9 years, solving it by assuming a 5-fold higher tissue-β-tubulin expression in children.[18] However, as the developed adult PBPK model demonstrated the importance of blood-β-tubulin-binding in vincristine distribution, a higher vincristine binding capacity, which might be due to increasing tissue- or blood-β-tubulin affinity, could also attribute to the more extensive distribution in children. Therefore, a 2 to 5-fold increase with steps of 0.5 in both blood- and tissue-β-tubulin expression was evaluated. For adolescents, increasing the vincristine binding capacity of blood- and tissue-β-tubulin 1.5-fold led to improved overall predictions, whereas in children 2-10 years, increasing the blood- and tissue-β-tubulin reference concentrations 2-fold was considered optimal. For infants until 1 years of age, the initial distribution phase was even more pronounced. As a consequence, a 2.5-fold higher reference concentrations was considered optimal to fit the infant data. The final PBPK model evaluation in adolescents, children and infants are shown in Figure 3D-F and final model parameters in Table 3.

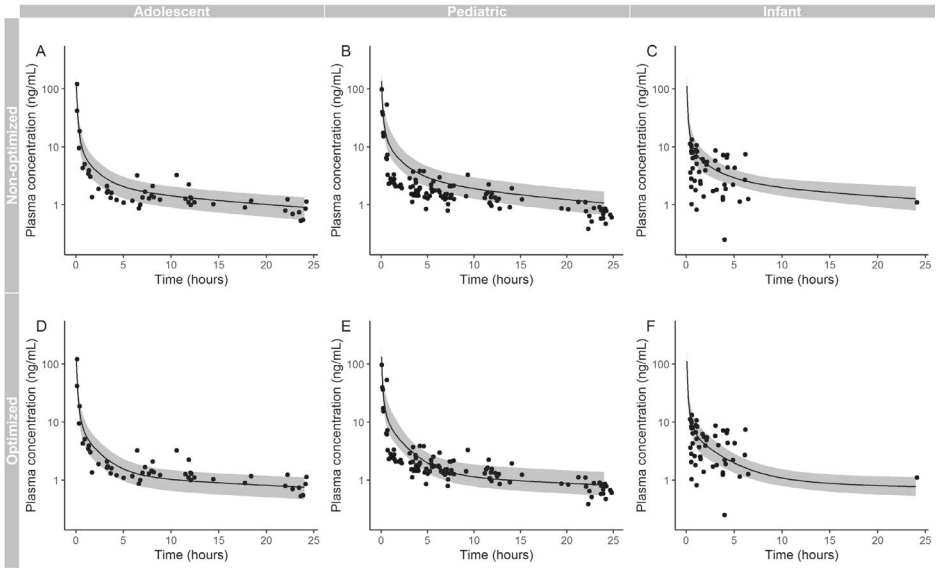

Figure 3. Non-optimized (upper panels A, B and C) versus and optimized (lower panels D, E and F) simulated plasma concentration time curves for the adolescent population (A and D respectively), pediatric (B and E respectively) and infant population (C and F, respectively). The solid line represents the simulated mean and the grey area the 95% prediction interval.

Vincristine whole blood concentrations

In total, 21 whole blood concentrations of 6 pediatric patients (1.0-17.6 years) were available. A nonlinear relationship between whole blood and plasma concentrations was observed. The simulated blood cell-, whole blood- and plasma concentration-time curves based on the final PBPK model are displayed in Figure 4. The observed concentrations closely followed the simulated curves.

Table 3. Final parameters used for the adult and pediatric vincristine PBPK model

Parameter	Value *
Molecular weight, g/mol	824.958
Solubility, mg/L	2.27
LogP	2.82
pK_a	5.00 and 7.4
f_u	0.51 (α-1-acid glycoprotein)
CYP3A4 metabolism	
V_{max} (pmol/min/pmol enzyme)	0.9
K_m (μM)	19.8
CYP3A5 metabolism	
V_{max} (pmol/min/pmol enzyme)	8.1
K_m (μM)	14.3
P-gp transport	
J_{max} (pmol/mL/min)	416.1
K_m (μM)	17.1
Binding to β-tubulin in tissue	
k_{off} (1/s)	$1.93 * 10^{-3}$
K_D (μM)	0.05
Reference concentration (μM)	1.00
Relative expression	1.0 (adult) 1.5 (adolescent) 2.0 (pediatric) 2.5 (infant)
Binding to β-tubulin in blood	
k_{off} (1/s)	$1.93 * 10^{-3}$
K_D (μM)	0.20
Reference concentration (μM)	1.20
Relative expression	1.0 (adult) 1.5 (adolescent) 2.0 (pediatric) 2.5 (infant)

*All parameters, except for binding to β-tubulin in blood, were based on the model of Lee et al.[18] f_u unbound fraction, J_{max} maximal rate of transport, K_D dissociation constant, K_m Michaelis-Menten constant, k_{off} dissociation rate, *LogP* partition coefficient, *P-gp* P-glycoprotein, V_{max} maximum rate of metabolism.

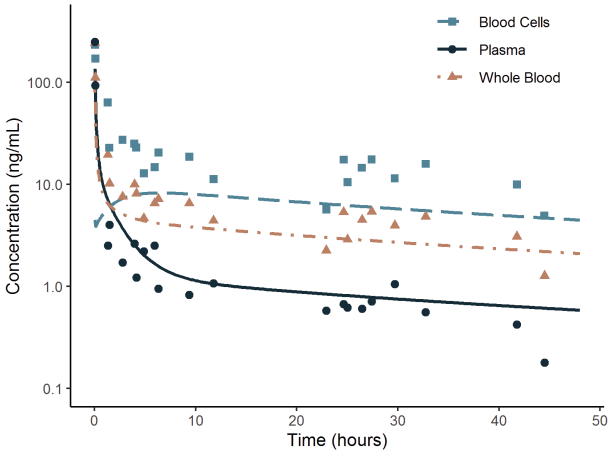

Figure 4. Simulated blood cell- (lightblue, dashed line), whole blood- (lightred, dotdashed line) and plasma (darkblue, solid line) concentration time-curves for the pediatric population.

DISCUSSION

In this current study, the complex interplay between binding of vincristine to β-tubulin including binding to blood components was successfully modelled using a PBPK approach. The age-dependent effects of β-tubulin binding on the PK of vincristine were incorporated, providing a mechanistic explanation for the observed age-dependency of plasma vincristine PK. We have shown that incorporation of blood-β-tubulin-binding improves the adult PBPK model as published by Lee et al. and explains the observed differences between children and adults.[18] Our final adult model incorporated metabolism by CYP3A4 and CYP3A5, elimination by P-gp and binding to β-tubulin in peripheral tissue and blood cells.

We have shown that vincristine binding to β-tubulin in blood mainly affects the distribution phase in the first hours after administration. This is explained by the fact that blood cells are the first component that are exposed to the drug after intravenous administration. Moreover, the findings on the non-linear binding in blood components show that vincristine binding to blood-β-tubulin explains the non-linear PK of vincristine and the rapid distribution in the first period after infusion. Binding of vinca alkaloids to blood cells has been previously shown in several *in vivo* and *in vitro* studies.[19,34–36] In addition, a high affinity to thrombocytes was previously described.[35]

In the first step of model development, the adult optimized PBPK model was scaled to children. Evaluation of this model on pediatric data indicated that children (2-10 years) had a 2-fold higher binding capacity of blood- and tissue-β-tubulin, while this was 1.5-

fold higher for adolescents (13-16 years) and 2.5-fold higher for infants (0-1 years) as compared to adults. Previously, Lee et al., found a 5-fold higher binding capacity of tissue-β-tubulin for children (0-12 years) as compared to adults, albeit without taking binding to blood-β-tubulin into account.[18] The expression of β-tubulin in the built-in expression database of PK-Sim is limited to peripheral tissue. The current study demonstrated that including blood-β-tubulin-binding on top of tissue-β-tubulin-binding not only provided an adequate description of the vincristine distribution phase for both plasma and whole blood concentrations, but also resulted in biologically more plausible parameter estimations. The nonlinearity between whole blood and plasma concentrations can be explained by saturable binding to blood-β-tubulin.

The observed age-dependent PK of vincristine is explained in our model by a higher expression of β-tubulin in tissue as well as blood cells in children as compared to adults. Tubulin in microtubules plays a significant role in cell division. Since cell division is more dominant in younger patients, a higher β-tubulin expression in children as compared to adults is highly likely. Even though evidence for age-related differences in the exposition of β-tubulin is not available, previous immunohistochemical research showed that expression of β2-tubulin was higher in neonatal tissue compared to tissue of older children and adults. Also, expression decreased with increasing age.[37] The same could be expected for other isotypes of β-tubulin. Our findings are in line with our previous observations from a vincristine population PK model in children.[5] Here, a decrease in maximal vincristine binding capacity to β-tubulin associated with an increase in age was identified.

A lower expression of β-tubulin for adults as compared to children would lead to a lower binding capacity of vincristine in blood and peripheral tissue, which would lead to higher amounts of free vincristine in the central compartment. This higher amount of free vincristine in the central compartment could have several implications. Firstly, higher amounts of free vincristine are available to distribute to peripheral tissue, where it could lead to VIPN. Secondly, higher amounts of vincristine are available to bind to tumor cells, and could, theoretically, be more effective in terms of treatment. This last implication would suggest that vincristine could be less effective in children. However, in current practice, children already receive a higher mg/m^2 dose than adults, due to the fact that children are able to tolerate higher doses of vincristine. Children are dosed based on BSA, while adults receive a standard capped dose of 2 mg, corresponding to lower mg/m^2 doses than administered to children with a BSA below 1 m^2. The difference in β-tubulin binding capacity between children and adults provides a physiologically plausible reason for higher relative doses of vincristine in children and capping the vincristine dose to 2 mg in adolescents and adults. This also explains why considerably higher doses of vincristine can be administered via a long continuous infusion. In that case, vincristine concentrations remain below the concentrations necessary to saturate the β-tubulin binding capacity.[38,39]

The higher expression of β-tubulin for infants as compared to children and adults strengthens the hypothesis that extra dose reductions for very young patients as compared to older children might not be justified.[5] However, we did observe a higher variability in vincristine plasma concentrations in the first 6 hours after infusion for infants than for children and adolescents, which might indicate that some variability in the distribution phase in this population is still unexplained.

Despite the fact that children are able to tolerate higher doses of vincristine, VIPN remains a serious side effect, which also occurs in children. To date, no convincing predictors for VIPN in children have been found.[40] However, a substantial interindividual variability (up to 7-fold) in β-tubulin VI expression in blood cells has been observed.[22] The high variability in β-tubulin VI expression could explain the variability in observed VIPN, which is still poorly understood.[40]

In addition, DNA missense variations in β-tubulin VI expression, altering myelosuppressive action, have been characterized in a previous study. One of these variations significantly decreased sensitivity to paclitaxel induced tubulin polymerization.[22] Moreover, these polymorphisms were thought to contribute to decreased myelosuppression, associated with the use of microtubule-binding drugs, like paclitaxel and vinca alkaloids. Genetic variations in other β-tubulin isoforms in tumor cells have also been described and are associated with resistance to microtubule-binding drugs.[41,42] These results lead to the question whether genetic variations in β-tubulin isoforms in neurons could explain variability in sensitivity to VIPN. However, evidence to support this hypothesis are lacking.

Previous studies have shown that children of African-American origin are at a lower risk of developing VIPN than children of Caucasian origin.[40] This difference is thought to be associated to differences in CYP polymorphisms between the populations. However, the current study indicates that it could possibly be related to differences in β-tubulin expression or polymorphisms in the encoding genes, but this hypothesis should be confirmed by studying the β-tubulin expression and/or genotype in various populations.

A limitation of the current study concerns explaining the variability in vincristine binding capacity. In the current study, we have shown that vincristine binding to blood cells impacts vincristine distribution throughout the body. It could be expected that variability in blood cell counts explains variability in vincristine PK. However, previous research[5] did not find an effect of thrombocyte levels on the maximal binding capacity of vincristine. Future studies could include data on blood cell counts (e.g. erythrocytes, leukocytes and thrombocytes) to study this effect.

In conclusion, the presented vincristine PBPK model describes the plasma and whole blood pharmacokinetics of vincristine in children and adults, and demonstrates a substantial effect of blood-β-tubulin-binding on vincristine distribution. A 2-fold higher β-tubulin

expression in children compared to adults was found for tissue as well as for blood cells, potentially explaining the fact that children are able to tolerate higher doses of vincristine. The previously reported, high variability in β-tubulin expression and DNA polymorphisms, altering sensitivity to vincristine, could explain the poor understood variability in VIPN between patients.

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6

A population pharmacokinetic approach to evaluate doxorubicin exposures in infants and children using different dosing regimens

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ABSTRACT

Background

Doxorubicin is widely used in the treatment of solid tumors and hematological malignancies in children. Doxorubicin has a small therapeutic window. To achieve exposure within this therapeutic window, several dosing regimens have been suggested for young patients recently. The current population pharmacokinetic (PK) study was initiated to investigate the age-related differences in the PK of doxorubicin and to evaluate exposures in young children with application of different dosing methods corresponding to a full dose of 30 mg/m^2 for older children and adults.

Methods

In total, 31 pediatric (0.44-17.9 years) and 59 adult patients (29.5-81 years) were included. PK analyses were performed using nonlinear mixed effects modelling.

Results

A three-compartment PK model was developed. All PK parameters were scaled to a body surface area (BSA) of 1.8 m^2. Doxorubicin clearance (CL) was estimated as 44.8 L/h (95% confidence interval (CI) 40.9-49.5), intercompartmental CL 2 (Q2) as 9.24 L/h (95% CI 7.16-11.7), Q3 as 44.3 L/h (95% CI 39.1-49.6), volume of distribution of the central compartment (V1) as 12.1 L (95% CI 10.9-13.7), V of the peripheral compartment 1 (V2) as 8.82 L (95% CI 5.96-14.5) and V3 as 611 L (95% CI 550-684). Achieved area under the plasma concentration-time curves (AUC) with different dosing regimens were predicted for all included patients. A full 30 mg/m^2 dose resulted in exposures comparable to literature for adolescent and adult patients (median AUC 1.30 mg/L*h and 1.03 mg/L*h, respectively). For young children, the exposure achieved with a mg/kg dose was lower than a BSA-based dose but comparable to the exposure achieved with a recently published dosing regimen based on BSA and age.

Conclusion

Doxorubicin exposure using the current mg/kg regimen leads to a slightly lower exposure in young patients than achieved with the corresponding dose in older patients, but recent attempts for optimizing the dose did not reduce pharmacokinetic variability. We recommend to keep using the simple mg/kg dosing approach for young patients, until more information on exposure-response and exposure-toxicity relationships is available.

INTRODUCTION

Doxorubicin, an anthracycline with chemotherapeutic properties, is widely used in the treatment of solid tumors and hematological malignancies in children. Almost 60% of the children with cancer are treated with anthracyclines.[1] Doxorubicin has a narrow therapeutic window, with myelotoxicity being the short term dose limiting toxicity and cardiotoxicity on the long term. Doxorubicin doses in children vary from 15-60 mg/m^2 per day, often on multiple days in one treatment block, depending on the diagnosis and treatment protocol. For infants, empirical dosing regimens are used in current practice; the full mg/m^2 dose (the dose that is given to older children and adults) is divided by 30 (the typical body weight for a child with a body surface area (BSA) of 1 m^2) to come to a mg/kg dose for children below the age of 1 year or with a body weight <10 kg. For example, conform the Dutch Childhood Oncology Group (DCOG) Neuroblastoma protocol (NBL 2009)[2], children are treated with 30 mg/m^2 in the N6 cycle. For infants below the age of 1 year, the dose is divided by 30, which gives a dose of 1 mg/kg, Given the narrow therapeutic window and interindividual variability, there is a high need to optimize dosing in children, most importantly in infants.

After intravenous administration, doxorubicin is rapidly distributed to tissue such as lungs, liver, heart, spleen, lymph nodes, bone marrow and kidneys. Thus, doxorubicin has a very large volume of distribution. It shows a triphasic elimination, with a terminal half-life of about 30 hours in adults. Doxorubicin is mainly metabolized hepatically into various metabolites, of which doxorubicinol is the most common.[3] Doxorubicinol is an active metabolite, and is held responsible for the cardiotoxicity of doxorubicin, one of the most severe and cumulative dose-limiting side-effects. The risk on cardiotoxicity after treatment with anthracyclines is thought to be age-dependent. It is supposed that younger children have a higher risk of cardiotoxicity.[4–7]

The effect of age on the doxorubicin pharmacokinetics (PK) was investigated the past years, in several population PK models in children. Contradictory results have been found. Two publications did not find a structural effect of age on clearance (CL)[8,9], but two other papers did observe a lower BSA-corrected CL for younger patients.[10,11] However, the number of infants included in previous doxorubicin PK analyses are low. The population PK model of Völler et al.[10] included only four infants, whereas Kunarajah et al.[11] did not include any infants. Even though a lower BSA-corrected CL for younger children was found in some population PK models, it is not known what exposures are reached with the current (empirical) dosing regimens.

Several attempts to optimize the dosing regimen of doxorubicin for young patients have been made. Firstly, a theoretical framework was published by the Children's Oncology Group's Chemotherapy Standardization Task Force.[12] Using BSA-based dose banding, a dose advice for children with a BSA <0.6 m^2 was given, taking the gradual process of maturation into account, see Table 1. Furthermore, dosing recommendations were made

by Siebel et al., based on PK simulations using the population PK model of Völler et al. and the consensus of an expert panel.[10,13] It was recommended to individualize the dose for young patients based on the age and BSA, targeting a uniform area under the plasma concentration-time curve (AUC). The dosing equations provided by Siebel et al.[13] are, however, not very easy to interpret. For example, units of used components are missing, which could lead to misinterpretation of the equations and thus, risk of over- or underdosing.

The current population PK analysis was initiated to further investigate the age-related differences in the PK of doxorubicin and to evaluate exposures in young children as compared to older children and adults, using different suggested dosing regimens.

METHODS

Patients and sampling

Patients up to 18 years of age with a central venous line in situ, receiving doxorubicin as standard of care treated were included in a prospective observational study in Princess Máxima Center for Pediatric Oncology in the Netherlands. No restrictions for tumors types or malignancies were formulated, but patients with Down syndrome were excluded. Patients were included after written informed consent was obtained. Ethical approval by the institutional Medical Ethics Committee of the Erasmus MC in Rotterdam was obtained (NL63037.078.18). PK data from this pediatric trial was combined with adult PK data from a previously reported study.[14]

Pediatric patients from the Princess Máxima Center study were treated with doxorubicin as standard of care, doses according to local protocols. In total, 4-6 blood samples per patient were collected at various time points up to 48 hours after infusion of doxorubicin. If doxorubicin was administered in the last 7 days, a trough level sample was collected before infusion of doxorubicin as well.

Doxorubicin plasma concentrations were quantified using a liquid chromatography tandem mass spectrometry (LC-MS/MS) method, with a lower limit of quantification (LLOQ) of 1.00 ng/mL. Daunorubicin was used as internal standard. The compounds were extracted from 50 µL plasma after protein precipitation with acetonitril:methanol (1:1, v/v). Isocratic chromatographic separation was performed on a Waters BEH C18 column (50 x 2.1mm ID, 1.7 µm). For detection an API5500 tandem mass spectrometer equipped with a turbo ionspray interface (TIS) was used, operating in the positive ion mode.

Model development

The doxorubicin model as published by Völler et al.[10] formed the basis for model development. For the structural model, a three-compartment model with first order elimination was developed. Scaling for body size was a priori included on all parameters.

Several methods for scaling were tested: Allometric scaling using body weight (BW) and scaling for BSA using either a linear function (similar to Völler et al.[10]) or power function. In the case of allometric scaling or a power function, we tried estimating and fixing the exponents, with the exponent fixed at 0.75 (BW) or 1 (BSA) for clearances (CL), and 1 for distribution volumes (BW, BSA).

Interindividual variability (IIV) was evaluated for all PK parameters, and implemented as follows:

$$P_i = P_{pop} \times e^{(\eta_i)} \quad (1)$$

where P_i is the individual parameter estimate for individual i, P_{pop} is the typical population parameters estimate, and η_i is assumed to be normally distributed with a mean of zero and a variance of ω^2. Residual unexplained variability was modelled as a proportional error model.

Covariate analysis

Since age was found to be a significant covariate for doxorubicin CL in previous models[10,11,15], age was evaluated as covariate for CL. Age was both tested using a power function, normalizing to an age of 18 years, and by including maturation as covariate using a sigmoid hyperbolic model:

$$F_{mat} = \frac{(\text{Age in weeks} + 40)_i^{HILL}}{(\text{Age in weeks} + 40)_i^{HILL} + TM_{50}^{HILL}} \quad (2)$$

The typical CL is multiplied with the factor F_{mat}. TM_{50} is the post menstrual age (PMA) in weeks at which CL is 50% that of the mature value and Hill is the coefficient that describes the slope of the sigmoidal curve. For our population, exact PMA was not known, so the PMA was estimated using age in weeks plus mean gestational age (40 weeks). The Hill coefficient and TM_{50} were fixed to 3.92 and 54.2 weeks, respectively, according to published models.[16]

Model evaluation

Discrimination between models was guided by physiological plausibility, goodness-of-fit (GOF) plots, precision of parameter estimates and change in objective function value (dOFV). A drop of ≥3.84 points, corresponding to a $P<0.05$ (χ^2-distribution with 1 degree of freedom (df)), was considered a significant improvement of the fit for hierarchical nested models. The adequacy of the models was assessed by GOF plots and visual predictive checks (VPC).[17] The sampling importance resampling (SIR) procedure was used for the assessment of parameter precision.[18]

Exposure

In order to evaluate achieved exposures using different dosing regimens, all included patients were virtually treated with different dosing regimens corresponding to a full dose of 30 mg/m^2. The first regimen was conform the Dutch Childhood Oncology Group (DCOG) Neuroblastoma protocol (NBL 2009)[2]: All patients were treated with 30 mg/m^2, except for patients <1 years of age or with a body weight <10 kg, who were treated with 1 mg/kg. Moreover, the suggested dosing regimens of Balis et al.[12] and Siebel et al.[13] were used. Lastly, a full mg/m^2 dose for patients of all ages was used. Table 1 shows the specifications of all dosing regimens. The AUC was calculated using the individual dose and individual CL.

Software

Nonlinear mixed-effects modeling was performed using NONMEM (version 7.3.0, ICON development Solutions, Ellicott City, MD, USA) and Pearl-speaks-NONMEM (PsN, version 4.9.0) with First-Order Conditional Estimation with interaction (FOCE-I) as estimation method.[19,20] Pirana (version 2.9.9) was used as graphical user interface for NONMEM.[21] R (version 3.4.3) was used for data handling and visualization.[22]

Table 1. Dosing regimens corresponding to a full dose of 30 mg/m^2

Regimen	Dose
Balis et al.[12]	0.25-0.29 m^2: 5.6 mg 0.30-0.34 m^2: 7.2 mg 0.35-0.39 m^2: 9.2 mg 0.40-0.44 m^2: 11.2 mg 0.45-0.49 m^2: 13.2 mg 0.50-0.54 m^2: 14.8 mg 0.55-0.59 m^2: 16.2 mg ≥ 0.6 m^2: 30 mg/m^2
Siebel et al.[13]	$\text{Absolute dose}_{\text{individual}} = \text{Absolute dose}_{18\text{yrs}} \times \frac{9.26 \times (1 + (\text{BSA} - 0.77) \times 1.3) \times \left(1 + \left(\frac{\text{AGE}}{5.32}\right)^{0.286}\right)}{\text{CL}_{18\text{yrs}}}$ Where $\text{Absolute dose}_{18\text{yrs}}$ is 54 mg and $\text{CL}_{18\text{yrs}}$ is 53.5 L/h
NBL2009	Patients <1 year or <10 kg: 1 mg/kg Other patients: 30 mg/m^2
Full mg/m^2	All ages: 30 mg/m^2

BSA Body surface area, *CL* Clearance

RESULTS

Patients and sampling

In total, 90 patients aged 0.44-81 years were included. In total, 31 of these patients were children with a median age of 6.0 years (range 0.44-17.9 years). Six and four patients, respectively, were younger than 2 and 1 years of age. Detailed patient characteristics are displayed in Table 2. In total, 364 plasma concentrations were available. None of these samples were below LLOQ. Figure 1 shows the observed plasma concentrations over time.

Model development

A three-compartment model with first order elimination was appropriate to describe the PK of doxorubicin. Final estimates and 95% confidence intervals (CI) are shown in Table 3. The model was parameterized in terms of volume of distribution of the central (V1) and peripheral (V2 and V3) compartments, clearance from the central compartment (CL) and intercompartment clearance between V1 and V2 (Q2) and between V1 and V3 (Q3). IIV was added on CL, V1, V2 and Q3.

Body size scaling using BSA resulted in a slightly better fit than allometric scaling for BW. Moreover, BSA scaling using a power function resulted in a similar OFV as compared to BSA scaling using a linear function, but resulted in a much more stable model. Furthermore, using the linear function, we were not able to normalize to a BSA of 1.8 m^2, because this resulted in negative parameters for the younger patients. Therefore, scaling for BSA using a power function was considered best for our population. BSA was included using a power function on all parameters. One estimated exponent was used for clearance parameters, which was estimated as 1.08 (95%CI 0.802-1.33), and one estimated exponent was used for volumes of distribution, estimated as 0.76 (95%CI 0.470-0.968).

Covariate analysis

The effect of age on CL was tested using a power function on top of the BSA-based scaling, but this did not result in an improved model and neither did inclusion of maturation as covariate on CL. Age or maturation were therefore not included in the final model.

Model evaluation

The GOF plots (Supplementary Figure S1) showed accurate population and individual predictions, without any signs for over- or underprediction. The VPC demonstrated that the median and the 95% CI of the observed data were mostly in line with those from the simulation-based predictions from the model (Supplementary Figure S2). There seemed to be some misspecification in the early time points for the 4h infusion group (existing of only pediatric patients). This was considered related to the fact the patients in this group did not have infusion durations of exact 4 h. The range of infusion duration in this group was 3.25-4.40 h.

Table 2. Patient characteristics (Median (range), unless specified otherwise)

	Pediatric N=31	Adult[14] N=59	Total N=90
Patient characteristics			
Female sex [n (%)]	15 (48%)	59 (100%)	74 (82%)
Age, years	6.0 (0.44-17.9)	55.7 (29.5-81)	48.9 (0.44-81)
Bodyweight, kg	22.5 (6.9-64.4)	70 (51-95)	63.0 (6.9-95)
Doxorubicin treatment			
Dose, mg/m^2	27.8 (10.3-56.7)	63.9 (50.3-66.3)	61.7 (10.3-66.3)
Available data			
Total no. of occasions [n]	37	59	96
Total no. of PK samples [n]	132	232	364
No. of occasions per patient	1 (1-2)	1 (1-1)	1 (1-2)
No. of samples per occasion	4 (1-6)	4 (3-5)	4 (1-6)

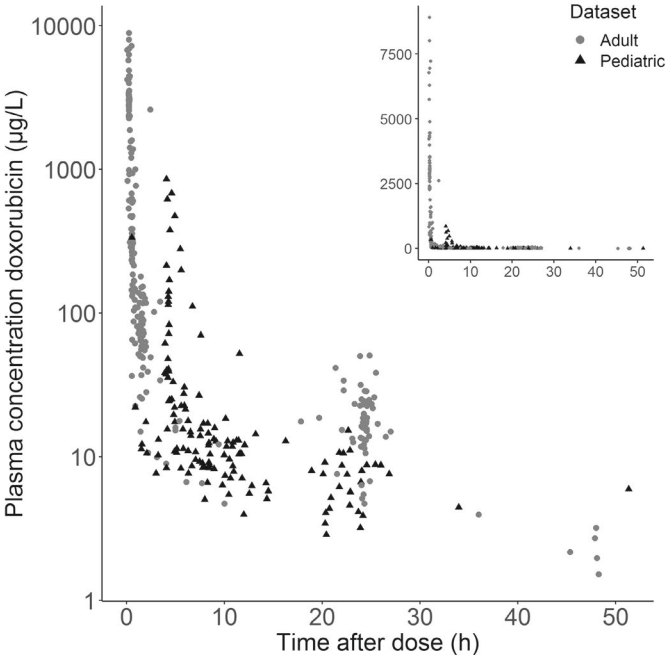

Figure 1. Doxorubicin plasma concentrations versus time after dose on a logarithmic and linear scale.

Exposure

For all included patients, the AUC for four different dosing regimens was calculated, see Table 4 and Figure 2. A median AUC of 1.30 mg/L*h (interquartile range (IQR) 0.97-3.72 mg/L*h) and 1.03 mg/L*h (IQR 0.93-1.33 mg/L*h) was achieved in patients 12.6-18.0 years and >18 years, respectively. Dosing according to Siebel et al.[13] resulted in a median exposure of 0.83 mg/L*h (IQR 0.71-0.98 mg/L*h) and dosing conform the NBL 2009 protocol (1 mg/kg for patients <1 years or <10 kg) in a median exposure of 0.92 mg/L*h (IQR 0.73-1.15 mg/L*h) for patients younger than 2.9 years of age. For one patient, in the group of patients <2.9 years, a remarkable lower clearance was observed, resulting in higher exposures as compared to other patients in this group. An explanation for this lower clearance could not be found, besides that this patient was treated with a higher dose than usual (40 mg/m^2 without reduction for infants).

Table 3. Final population PK parameter estimates

PK parameter	Estimate	95% CI
$CL_{1.8m2}$ (L/h)	44.8	40.9 - 49.5
$V1_{1.8m2}$ (L)	12.1	10.9 - 13.7
$Q2_{1.8m2}$ (L/h)	9.24	7.16 - 11.7
$V2_{1.8m2}$ (L)	8.82	5.96 - 14.5
$Q3_{1.8m2}$ (L/h)	44.3	39.1 - 49.6
$V3_{1.8m2}$ (L)	611	550 - 684
Exponent for BSA on CL and Q	1.08	0.802 - 1.33
Exponent for BSA on V	0.76	0.470 - 0.968
IIV CL (%)	55.0	48.0 - 62.5
IIV V1 (%)	29.6	21.0 - 37.8
IIV V2 (%)	71.8	44.3 - 103
IIV Q3 (%)	78.2	67.7 - 95.2
Proportional residual error (%)	25.5	20.9 - 28.4

PK pharmacokinetics, *CI* confidence interval obtained by sampling importance resampling, *CL* clearance, *V* volume of distribution, *Q* intercompartment clearance, *IIV* interindividual variability. Population estimates correspond to a subject with a BSA of 1.8 m^2.

Table 4. Predicted doxorubicin area under the curve (mg/L*h, median (interquartile range)) stratified for dosing regimen and age (pediatric patients were categorized based on quartiles).

	Area under the curve (mg/L*h) stratified for dosing regimen			
Age category	**Balis et al.**[12]	**Siebel et al.**[13]	**1 mg/kg**	**30 mg/m²**
<2.9 years	0.99 (0.96-1.37)	0.83 (0.71-0.98)	0.92 (0.73-1.15)	1.20 (1.06-1.47)
2.9-6.0 years	0.91 (0.83-1.04)	0.71 (0.61-0.98)	NA	0.91 (0.76-1.22)
6.0-12.6 years	NA	1.28 (0.93-2.00)	NA	1.37 (1.01-2.24)
12.6-18.0 years	NA	1.26 (0.96-3.57)	NA	1.30 (0.97-3.72)
>18.0 years	NA	1.29 (1.12-1.71)	NA	1.03 (0.93-1.33)

NA Not applicable

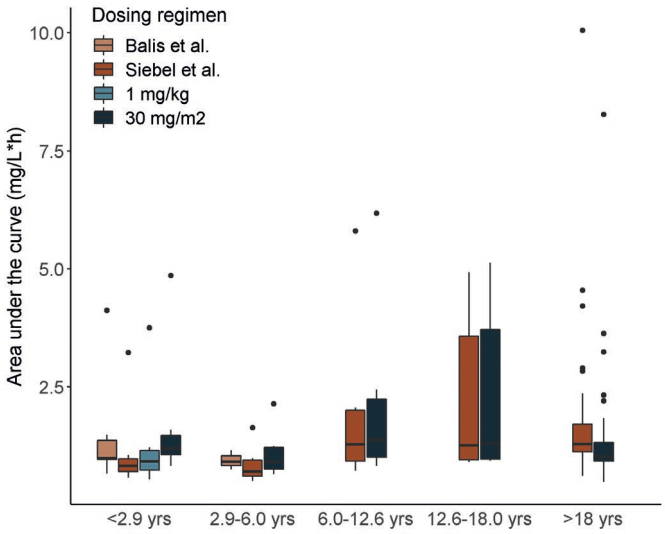

Figure 2. Predicted doxorubicin area under the plasmaconcentration-time curve (mg/L*h) stratified for dosing regimen and age (pediatric patients were categorized based on quartiles).

DISCUSSION

A combined pediatric and adult doxorubicin population PK model was developed, to give an accurate illustration of the PK of doxorubicin over the whole age range. A three compartment model for doxorubicin was developed, which is in line with previously published models in children and adults.[9–11,15,23,24]

Age or maturation was not included in our final model, since it did not improve the model fit when body size was already taken into account. In contrast, previously published BSA-scaled PK models in children have included age as a covariate on CL.[10,11,15] However, the two models including age show divergent results. Both models included age using a power function. The exponent for this age component was estimated to be 0.286 and 0.736 for the models of Völler et al. and Kunarajah et al., respectively.[10,11] Moreover, the estimated linear scaling factor of BSA on CL was different in both models (1.3 and 0.465 for the models of Völler et al. and Kunarajah et al., respectively[10,11]), most likely caused by the obvious correlation between body size and age components. In our model development process, we first included and optimized scaling for body size (in this case BSA), before we tested age as a covariate on CL. We have optimized scaling for BSA as compared to the models of Völler et al. and Kunarajah et al., by incorporating two different factors for the scaling of clearance parameters and volume parameters. Extensive literature on body size scaling of PK parameters highly suggests that clearance components and volume of distributions components do not scale similarly with body size.[25]

Our results regarding the estimated exponent for BSA-scaling of clearance parameters of 1.08 is in accordance with the principles of allometric scaling. However, the exponent for BSA-scaling of volumes of distribution (estimated as 0.76) is lower than expected. An exponent of 0.76 indicates that children have relative higher volumes of distribution as compared to adults. This could be related to the age-related differences in body composition. In infants, the total amount of body water is 80-90% of the body weight, while this amounts decreases over age to 55-60% for adults.[26] However, the lower exponent for BSA-scaling of volumes of distribution could also be a modelling artefact, related to the differences in infusions duration. All the adults received doxorubicin in 0.25 h, while most of the pediatric patients (n=27, 87%) received an infusion of about 4 h.

After scaling for BSA, we did not observe any unexplained age effects on CL on top of this. Moreover, we have tried to include age using a sigmoidal function (representing hepatic maturation in the first weeks after birth)[16], instead of including age as a power function, but this did not improve the model either.

Using our final model, the doxorubicin exposure with various dosing regimens was predicted, in order to compare the achieved exposure in young patients when the different dosing methods would have been applied. Dosing methods described by Balis et al. and

Siebel et al. and used in current practice (NBL 2009 protocol), were compared with a standard full mg/m^2 dose of 30 mg/m^2. The predicted AUC of adolescent patients after a full 30 mg/m^2 dose (1.30 mg/L*h) was comparable to the AUC of 1.28 mg/L*h, which was described previously.[13] Using the full mg/m^2 for the youngest patients resulted in comparable exposures as achieved in older patients. Dosing young patients according to the recommendations of Balis et al.[12] resulted in slightly lower median exposures and comparable interquartile range exposures as compared to the full mg/m^2 dose. The dosing method suggested by Siebel et al.[13] resulted in a lower exposure as compared to a full mg/m^2. However, the exposure was comparable to the exposure achieved by using a mg/kg approach conform the NBL 2009 protocol.

Both the mg/kg (according to the NBL 2009 protocol[2]) and the Siebel et al.[13] approach are supposed to result in a similar exposure as compared to the mg/m^2 dose for adult and adolescent patients. The current study shows that comparable exposures are not achieved. However, it has been shown that young age is a risk factor for anthracycline-associated cardiotoxicity.[4–7] So it might be precarious to strive for the same exposure in young patients without information on the exposure-response and exposure-toxicity relationships in these patient populations. In order to compose an evidence-based target AUC, exposure-response and exposure-toxicity relationships in specific populations need to be investigated.

To date, the most experience was gained with the mg/kg dose, which is current practice for most of the clinical protocols. We have shown that the exposure achieved with this dosing regimen is comparable to the exposure achieved with the newly composed regimen by Siebel et al.[13] However, the mg/kg approach is much easier to use and less multi-interpretable. Therefore, we recommend to keep using the simple mg/kg dosing approach for young patients, until more information on exposure-response and exposure-toxicity relationships is available. In addition, the option for prolonging the doxorubicin infusion in infants, as suggested by Siebel et al.[13], needs to be studied. They recommended to reduce peak concentrations in very young children, since the peak concentration is thought to relate to cardiotoxicity.[27] However, major differences in peak concentrations between children and adults have not been found.[28] Previously, an infusion duration of at least 1 h was recommended for children.[29] 97% of the children included in our current analysis received doxorubicin in at least 1 h.

The current study has some limitations. Firstly, the current dataset did only include 6 patients younger than 2 years. It could be possible that possible age effects were not observed due to the limited amount of infants. Preferably, the results of the current study will be confirmed with a dataset containing more infants. This could for example be accomplished by combining historical datasets with the current dataset. Secondly, previous studies included doxorubicinol, the active metabolite of doxorubicin, which is mainly responsible for cardiotoxicity, in the population PK model. For our patients, the doxorubicinol plasma concentrations were not available and could therefore not be included in the current model.

Future models could include the metabolite concentrations in order to give a full overview of the PK of doxorubicin.

In summary, this current study shows that doxorubicin clearance is dependent on BSA, but not on age. Doxorubicin exposure using the current mg/kg regimen leads to a slightly lower exposure in young patients than achieved with the corresponding dose in older patients, but recent attempts for optimizing the dose did not reduce pharmacokinetic variability. We recommend to keep using the simple mg/kg dosing approach for young patients, until more information on exposure-response and exposure-toxicity relationships is available.

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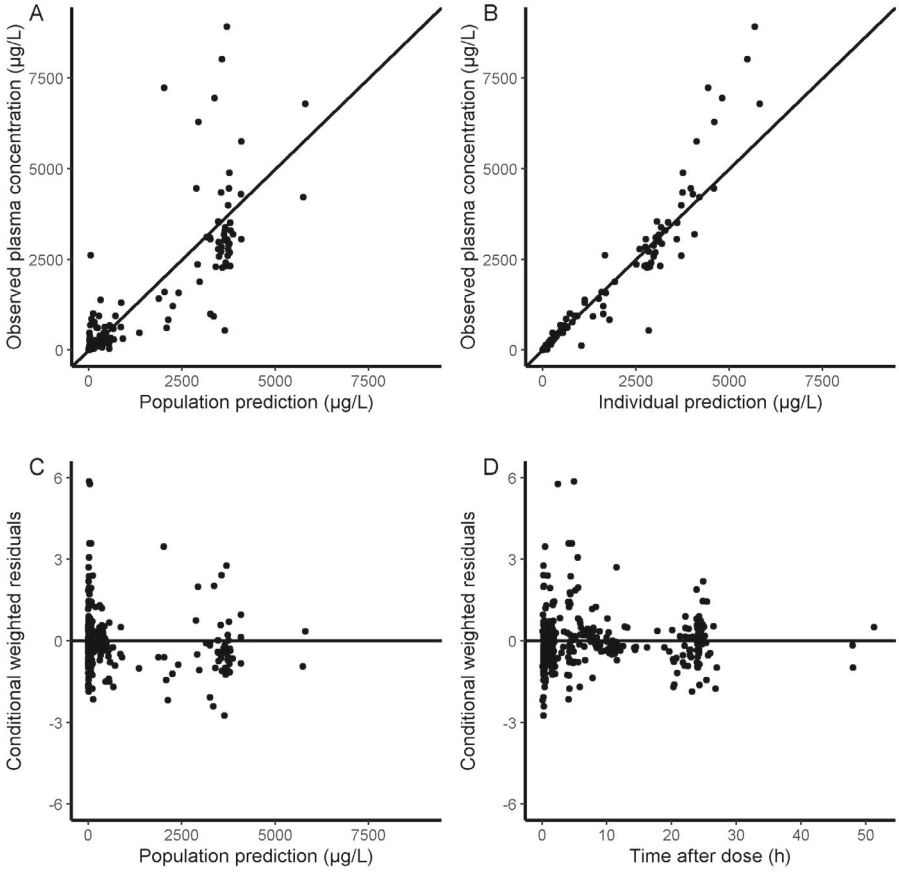
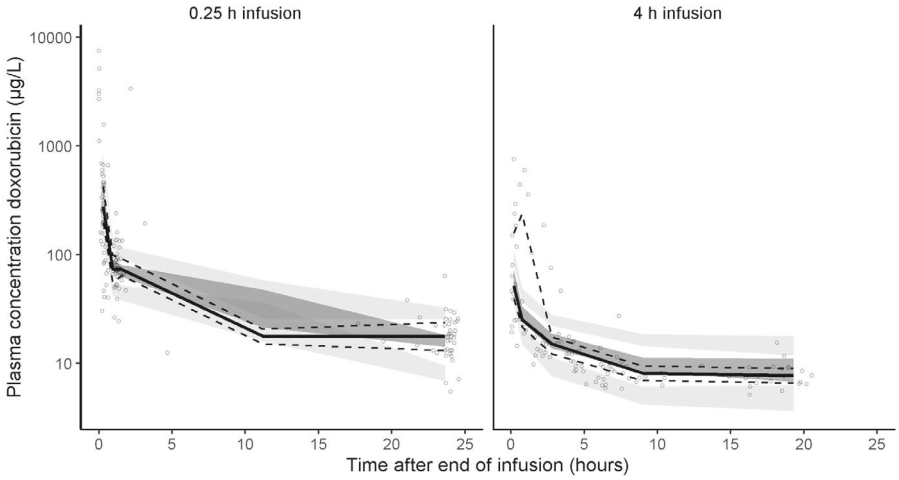
SUPPLEMENTARY MATERIAL



Supplementary Figure S1. Goodness-of-fit plots



Supplementary Figure S2. Prediction-corrected visual predictive check stratified for infusion duration. Black lines depict the observed median (solid) and 25 and 75% percentiles of observed (dashed) concentrations. Dark- and light-grey areas represent interquartile prediction intervals of the simulated mean and the 25 and 75% percentiles, respectively. Round dots represent observations.

PART II

Pharmacokinetics of anti-emetic agents in pediatric patients

7

Development and validation of a combined liquid chromatography tandem-mass spectrometry assay for the quantification of aprepitant and dexamethasone in human plasma to support pharmacokinetic studies in pediatric patients

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ABSTRACT

A pharmacokinetic study was set up to investigate the pharmacokinetics of the anti-emetic agents aprepitant and dexamethasone and the drug-drug interaction between these drugs in children. In order to quantify aprepitant and dexamethasone, a liquid chromatography-tandem mass spectrometry assay was developed and validated for the simultaneous analysis of aprepitant and dexamethasone.

Protein precipitation with acetonitrile-methanol (1:1, v/v) was used to extract the analytes from plasma. The assay was based on reversed-phase chromatography coupled with tandem mass spectrometry detection operating in the positive ion mode. The assay was validated based on the guidelines on bioanalytical methods by the US Food and Drug Administration and European Medicines Agency. The calibration model was linear and a weighting factor of $1/concentration^2$ was used over the range of 0.1-50 ng/mL for aprepitant and 1-500 ng/mL for dexamethasone. Intra-assay and inter-assay bias were within ±20% for all analytes at the lower limit of quantification and within ±15% at remaining concentrations. Dilution integrity tests showed that samples exceeding the upper limit of quantification can be diluted 100 times in control matrix. Stability experiments showed that the compounds are stable in the biomatrix for 25h at room temperatures and 89 days at -20°C.

This assay is considered suitable for pharmacokinetic studies and will be used to study the drug-drug interaction between aprepitant and dexamethasone in pediatric patients.

INTRODUCTION

Aprepitant is a selective neurokinin-1 receptor antagonist approved for the prevention of chemotherapy-induced nausea and vomiting (CINV) in adults and pediatric patients from the age of 6 months and for the prevention of postoperative nausea and vomiting in adults.[1] For the prevention of CINV, aprepitant is co-administered with the corticosteroid dexamethasone. Pharmacokinetic (PK) studies in adults have shown a drug-drug interaction between aprepitant and dexamethasone. The area under the plasma concentration-time curve (AUC) of dexamethasone increases approximately 2-fold when co-administered with aprepitant[2–4] and hence according to the product information of aprepitant the dexamethasone dose needs to be reduced by 50% when administered together with aprepitant.[1] In children this interaction has not been studied thoroughly.

Aprepitant has been shown to be effective for the prevention of CINV in pediatric patients as an adjuvant to ondansetron and dexamethasone.[5,6] However, the complete anti-emetic response to triple therapy in children is lower than in adults (approximately 50% and 70-80%, respectively)[7–9], which could be due to an incorrect dose of dexamethasone. It is possible that the influence of aprepitant on the PK of dexamethasone is different in children than in adults. To study the PK of aprepitant and dexamethasone and the drug-drug interaction between these drugs in children, a PK study has been set up.

PK studies in children require a sensitive assay to quantify aprepitant and dexamethasone as the volume of blood available is limited. In addition, a simultaneous quantification is preferred to reduce the amount of blood even more. Several methods to quantify aprepitant or dexamethasone plasma concentrations have been published[10–16], but no simultaneous analysis has been described hitherto. The previously developed methods for quantification of dexamethasone use 50-200 µl human plasma and could be suitable for quantification of dexamethasone in children[13–16], however, for quantification of aprepitant, plasma volumes up to 1 mL were needed[10,11], which is unacceptable in the pediatric population.

The aprepitant quantification method of Wu et al. was used as starting point for the development of a combined liquid chromatography tandem-mass spectrometry (LC-MS/MS) assay for the quantification of aprepitant and dexamethasone.[12] The development and validation of that combined LC-MS/MS assay in small sample volumes from pediatric oncology patients is described here. Its clinical applicability is demonstrated with the analysis of samples of children with cancer undergoing chemotherapy, treated with dexamethasone and aprepitant as anti-emetics.

MATERIALS AND METHODS

Chemicals and reagents

Aprepitant (≥98%) and $^{2}H_{4}$-aprepitant (≥98%, 97.6% $^{2}H_{4}$) were obtained from Toronto Research Chemicals (North York, ON, Canada). Dexamethasone (100%) was obtained from Sigma Aldrich (Zwijndrecht, the Netherlands) and $^{2}H_{4}$-dexamethasone (≥98.3%, 97.6% $^{2}H_{4}$) from Alsachim (Illkirch Graffen-staden, France). Acetonitrile, formic acid, methanol, isopropyl alcohol and water originated from Biosolve Ltd (Valkenswaard, The Netherlands). K_{2}EDTA plasma was obtained from BioreclamationIVT LLC (Hicksville, NY, USA). The chemical structures of the analytes are depicted in Figure 1.

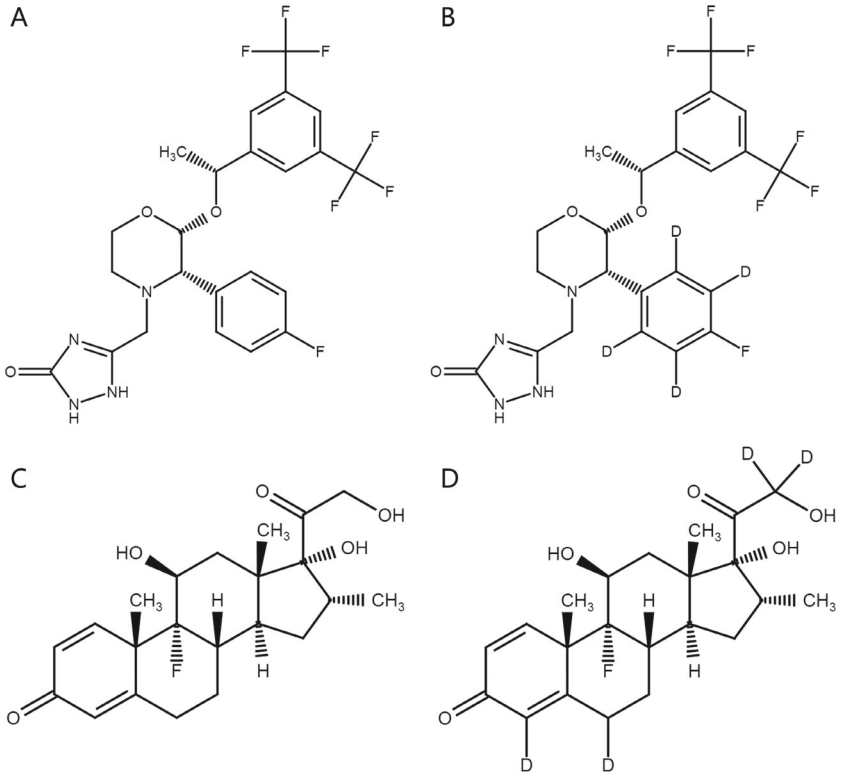


Figure 1. Chemical structures of aprepitant (A), $^{2}H_{4}$-aprepitant (B), dexamethasone (C) and $^{2}H_{4}$-dexamethasone (D)

Stock solutions and working solutions

Stock solutions were prepared at a concentration of 1 mg/mL in methanol for aprepitant and dexamethasone. The stock solutions were diluted with methanol to obtain working solutions, containing both analytes. For aprepitant the calibration working solutions were prepared

at concentrations of 2, 5, 20, 50, 200, 500, 800, 1,000, 10,000 and 100,000 ng/mL and at concentrations of 20, 50, 200, 500, 2,000, 5,000, 8,000, 10,000 and 100,000 ng/mL for dexamethasone. Two independent stock solutions and working solutions were prepared to spike the calibration standards and quality control (QC) samples. The QC stock- and working solutions were prepared at concentrations of 2, 6, 100, 700, 10,000 and 100,000 ng/mL for aprepitant and at concentrations of 20, 60, 1,000, 7,000 and 100,000 ng/mL for dexamethasone. Stock solutions (1,000 ng/mL) and a working solution (1 ng/mL) containing both internal standards (IS) were prepared in methanol. All stock- and working solutions were stored at -70°C

Calibration standards, quality control samples

A volume of 380 µL control human K_2EDTA plasma was spiked by 20 µL of the working solution to obtain the calibration standards in the range of 0.1 to 50 ng/mL for aprepitant and 1.0 to 500 ng/mL for dexamethasone. QC samples were prepared in control human K_2EDTA plasma at the lower limit of quantitation (LLOQ), at 3 times the LLOQ (LOW), at approximately midway between the high and low QC samples (MID) and at 75 to 90% of the highest calibration standard (HIGH). The QC samples were prepared by spiking either 150 or 300 µL of the separately prepared working solutions to either 2850 or 5700 µL control human K_2EDTA plasma (depending on the amount of volume needed) to obtain the final QC LLOQ, LOW, QC MID and QC HIGH concentrations. Both calibration standards and QC samples were subsequently stored in aliquots of 100 µL at -20°C. Back-calculated concentrations of the calibration standards were used for the determinations of the linearity of the calibration model, using the reciprocal of the squared analyte concentrations ($1/x^2$) as the weighting factor.

Sample preparation

A maximum of 7 whole blood samples of 1 mL were collected from each patient treated with dexamethasone with or without aprepitant. Directly after collection, samples were centrifuged for 5 min at 2000g at room temperature. Thereafter, plasma was obtained and stored at -70/-80°C until analysis. Before sample pretreatment, samples were thawed at room temperature and vortex-mixed for 10 s. To 100 µL of plasma, a volume of 10 µL IS working solution was added, except for the double blank samples. A volume of 200 µL acetonitrile-methanol (1:1, v/v) was used for protein precipitation (PP) to extract the analytes from plasma. Samples were vortex-mixed for 10 s and centrifuged at 21,500 g for 3 min at room temperature. The supernatant was transferred to an autosampler vial with insert.

Liquid chromatography-mass spectrometry equipment and conditions

An Acquity I class UPLC system with binary pump, integrated degasser, column oven and I class autosampler were used (Waters, Milford, MS, USA). The temperature of the autosampler and column were kept at 8°C and 40°C, respectively. Mobile phase A consisted of 0.1% (v/v) formic acid in water and mobile phase B consisted 0.1% (v/v) formic acid in acetonitrile. Gradient elution was applied at a flow of 300 µL/min through

an Acquity UPLC BEH C18 column (50 x 2.1 mm ID, 1.7 µm particle size) with an additional Acquity UPLC BEH C18 guard column (5 x 2.1 mm ID, 1.7 µm particle size) (both Waters). The applied gradient program was 30% B (0-1.0 min); 30-98% B (1.0-2.5 min); 98% B (2.5-4.0 min); 98-30% B (4.0-4.01 min); 30% B (4.01–6.0 min).

A QTRAP5500 triple quadrupole mass spectrometer (MS) equipped with a turbo ion spray interface, operating in positive ion mode was used (Sciex, Framingham, MA, USA). Multiple reaction monitoring (MRM) chromatograms were acquired and processed using Analyst™ software (Sciex, version 1.6.2). The MS operating parameters are summarized in Table 1.

Validation procedures

The validation of the assay was conducted in compliance with the most recent edition of the OECD Principles of Good Laboratory Practice, and based on the FDA and EMA guidelines on bioanalytical method validation.[17–19]

Ethical considerations

In compliance with ethical standards, patients were included after written informed consent was acquired. Ethical approval by the institutional Medical Ethics Committee of the Erasmus Medical Center was obtained under protocol number 2018-1578.

Table 1. Mass spectrometry settings for the analytes and their internal standards.

Parameter					
Run duration	6.00 min				
Ion spray voltage	4000 V				
Collison gas	8 au				
Curtain gas	30 au				
Temperature	500°C				
Dwell time	75 ms				
Specific Parameters Analyte	**Parention (m/z)**	**Production (m/z)**	**Collison energy (V)**	**Collision exit potential (V)**	**Declustering potential (V)**
Aprepitant	535.3	277.2	25	8	61
$^{2}H_{4}$-Aprepitant	539.0	281.1	27	10	86
Dexamethasone	393.0	355.2	17	12	96
$^{2}H_{4}$-Dexamethasone	397.0	359.3	17	6	116

Au arbitrary units

RESULTS AND DISCUSSION

Method development

Starting point of the method development was the aprepitant method of Wu et al.[12] Detector settings for all compounds were established by infusion of both analytes and internal standards. As organic component of the mobile phase, acetonitrile proved to have a far lower background than methanol. Variations in gradient compositions were tested to optimize elution times, peak shapes and the separation of the analytes from interferences. Subsequently sample processing was tested and protein precipitation with 200 µL acetonitrile-methanol (1:1, v/v) proved to give the lowest variation and best signal to noise. Chromatograms of a double blank sample, blank sample, plasma sample spiked at QC MID levels, blank patient sample and patient sample are shown in Figure 2. The method was developed using a small sample volume of 100 µL human plasma. The resulting method proved to be linear from 0.1-50 ng/mL for aprepitant and from 1 to 500 ng/mL for dexamethasone.

Table 2. Assay performance data for the analysis of aprepitant and dexamethasone, assessed in 3 different analytical batches, tested at 4 concentration levels analyzed in 5-fold.

		Intra-assay		Inter-assay	
Analyte	**Nominal concentration (ng/mL)**	**Bias (%)**	**CV, %**	**Bias (%)**	**CV, %**
Aprepitant	0.100	Within ±4.2	Within +7.5	+2.3	+2.1
	0.300	Within ±7.7	Within +3.8	+4.0	+3.8
	5.00	Within ±5.3	Within +4.2	+3.6	+0.9
	35.0	Within ±8.5	Within +1.4	+5.5	+3.3
Dexamethasone	1.00	Within ±4.6	Within +9.4	+0.9	+1.8
	3.00	Within ±6.0	Within +1.9	+4.4	+1.6
	50.0	Within ±3.4	Within +3.8	+2.2	- [a]
	350	Within ±5.8	Within +1.6	+3.0	+3.4

[a] No significant additional variation was observed due to the performance of the assay in different analytical runs (mean square within groups is larger than mean square between groups). *CV* coefficient of variation

Method validation

Accuracy and precision
In order to assess intra- and inter-assay accuracies and precisions, five replicates of QC samples were analyzed in three analytical runs at the LLOQ, 3 times the LLOQ, midrange and high concentrations. The accuracy (bias) was determined as relative difference between the mean measured concentration (per run for intra-assay bias and overall for inter-assay bias) and the nominal concentration and coefficient of variation (CV, %) were used to assess the intra-run precision. Analysis of Variance (ANOVA) was applied to assess the inter-run precision. The inter- and intra-assay accuracy and precision were ≤20% for the LLOQ and ≤15% for the other concentrations and thus within the acceptance criteria for all analytes. Details on the assay performance data are listed in Table 2.

Dilution integrity
High and variable concentrations in patient samples were expected for aprepitant based on previously published pharmacokinetic studies.[20] In order to extend the range for both analytes, a 100-fold dilution factor was validated. Five replicates of a plasma sample spiked with aprepitant and dexamethasone concentrations above the upper limit of quantification (ULOQ) (10,000 and 1000 ng/mL, respectively), were 100-fold diluted in control human plasma, prior to sample pre-treatment. Bias and CV for aprepitant were -0.1% and 3.4%, respectively. For dexamethasone, bias was 0.4% and CV was 1.4%, within the requirements of ±15% and a CV ≤15%.

Carry-over
The carry-over was determined in three analytical runs and no analyte peaks or IS peaks were observed in the first blank sample injected after an ULOQ sample. As a result the carry-over was considered acceptable (≤20% of the analyte peak area of the LLOQ sample and ≤5% of the peak area of the IS).

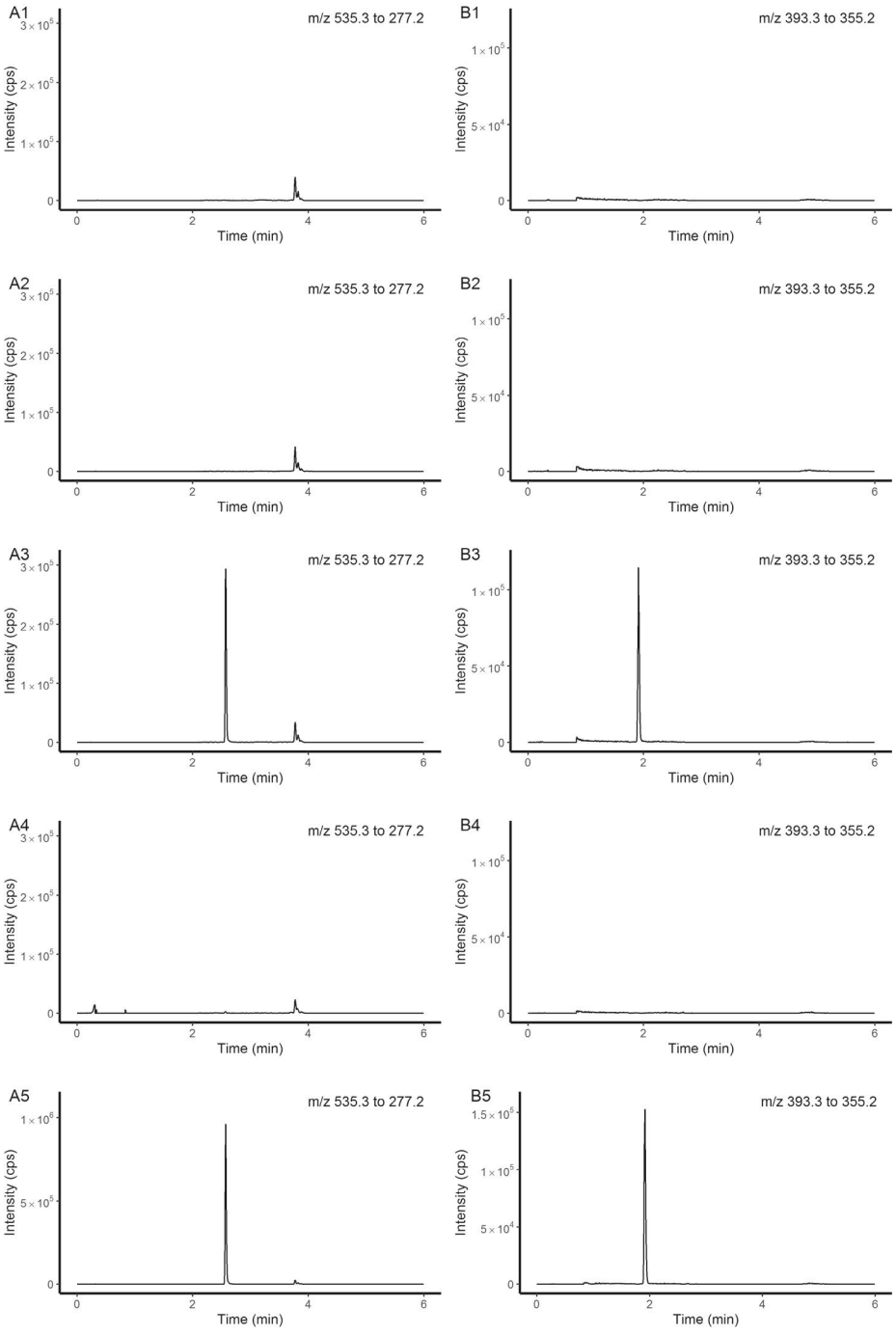
Specificity and selectivity
The selectivity of the method was determined by the analysis of six different batches of control human plasma. Double blank samples and LLOQ samples of each batch were processed and analyzed. The mean measured concentrations at LLOQ level were ±9.0% for aprepitant and ±16.1% for dexamethasone (requirement: within ±20% of the nominal concentrations) and no interferences were detected at the retention times for the analytes or IS. The cross analyte/IS interferences were determined by separately spiking the analytes and IS to control human plasma at their ULOQ levels and IS levels, respectively. The interferences from aprepitant, dexamethasone or IS at the other transitions were ≤20% of the peak area of the analytes at the LLOQ level and ≤5% of the peak area of the IS: 0.3% for dexamethasone in $^{2}H_{4}$-dexamethasone and 0% for all other components.

Matrix effect and recovery
Six batches of individual control human plasma at low and high concentrations in singular were prepared to determine the matrix effect. For both the analyte and IS, the matrix factor (MF) was calculated for each matrix lot by calculating the ratio of the peak area in the presence of matrix to the peak area in absence of matrix (working solution of the analyte). Furthermore, the IS normalized MF was calculated by dividing the MF of the analyte by the MF of the IS. At both tested QC concentration levels the CV of the IS-normalized matrix factor from the 6 batches ranged from 0.87 to 0.99 and CV was 3.0% for aprepitant and 5.2% for dexamethasone and were thus within the required ≤15% for both analytes.

The overall recovery was calculated by dividing the peak area of a processed sample by the peak area in absence of matrix. The overall recovery was 91.1±5.2% and 90.7±1.8% for aprepitant and its IS, respectively, and was 56.5±2.0% and 61.7±1.3% for dexamethasone and its IS, respectively.

Stability
The analytes were considered stable in the biomatrix when 85-115% of the nominal concentration was found. For stock solutions acceptance criteria of 95%-105% were applied. Plasma samples were stable for at least up to 25h at 20-25°C, 89 days at -20°C and after three freeze (-20°C)/thaw (20-25°C) cycles for both analytes at QC LOW and HIGH levels. The processed samples were stable for at least 15 days at 2-8°C. The stock solutions of dexamethasone were stable for at least 24 h at 20-25°C. For aprepitant stock solutions, an increase in concentration of 5.6% was observed after 24 h at 20-25°C, most likely explained by evaporation of solvent. The stock solutions of both analytes were stable at -70°C for at least 8 months and after three freeze (-70°C)/thaw (20-25°C) cycles.

Clinical application
The assay was used to determine plasma concentrations of two pediatric patients receiving dexamethasone with aprepitant. The patients (patient 1: male, 8.9 years; patient 2: female, 16.4 years) were treated with 1dd 3 mg/kg aprepitant (with a maximum 125 mg on day 1) and 4dd 3 mg/m^2 dexamethasone. Samples were taken at six time points: 0.5, 2, 4, 6, 12 and 24 h after the first administration of the agents. Samples were processed as described. The plasma concentration time curves of two patients receiving both aprepitant and dexamethasone are displayed in Figure 3.


A1
m/z 535.3 to 277.2
B1
m/z 393.3 to 355.2
A2
m/z 535.3 to 277.2
B2
m/z 393.3 to 355.2
A3
m/z 535.3 to 277.2
B3
m/z 393.3 to 355.2
A4
m/z 535.3 to 277.2
B4
m/z 393.3 to 355.2
A5
m/z 535.3 to 277.2
B5
m/z 393.3 to 355.2
Intensity (cps)
Time (min)

< Figure 2. Chromatograms of aprepitant (A-series) and dexamethasone (B-series) for a double blank sample (1), blank sample (2), plasma sample spiked at QC MID levels (3) (aprepitant: 5 ng/mL, dexamethasone: 50 ng/mL), blank patient sample (4) and patient sample (5) (aprepitant: 1590 ng/mL (diluted 100-fold), dexamethasone: 90.4 ng/mL). The traces of the internal standards are not shown (compounds co-eluted with the analytes).

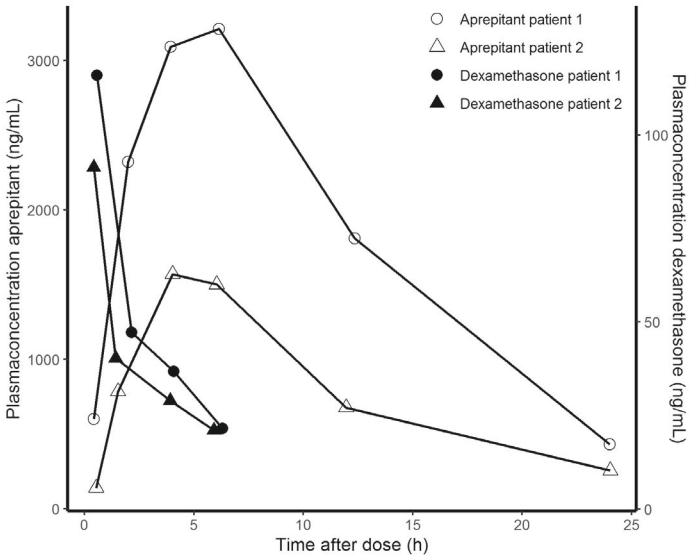


Figure 3. Plasma concentration time curves of two patients treated with aprepitant (first day: 1dd 3 mg/kg, maximum 125 mg) and dexamethasone (4dd 3 mg/m^2)

To our knowledge, this is the first study to describe a LC-MS/MS assay to simultaneously quantify aprepitant and dexamethasone in small volumes of human plasma (100 µL). This makes it possible to simultaneously study the pharmacokinetics of aprepitant and dexamethasone for instance in the pediatric population using low sample volumes. In addition, this assay allows to determine plasma concentrations of aprepitant at a lower level than previously described in literature (LLOQ of 0.1 ng/mL compared to 1 or 10 ng/mL in human plasma)[10–12], which might enable quantification of aprepitant in alternative matrices like cerebrospinal fluid. Since this method was validated for a 100-fold dilution, it will be even possible to use lower samples volumes than 100 µL for the quantification of aprepitant, which makes this method even more suitable for the quantification of aprepitant in the pediatric population. Previous published methods on the quantification of aprepitant report higher upper limits of quantification (ULOQ=1000 or 5000 ng/mL in human plasma).[10–12] However, with this described method it is possible to successfully measure higher aprepitant plasma concentrations in human plasma samples by diluting the plasma.

CONCLUSION

We successfully developed a sensitive LC–MS/MS assay for the simultaneous quantification of aprepitant and dexamethasone in small volumes of pediatric human plasma. The validated linear assay ranges are 0.1–50 ng/mL for aprepitant and 1–500 ng/mL for dexamethasone. Stability showed that both analytes were stable in human K_2EDTA plasma at room temperature for longer than 24 h. This assay is considered suitable for pharmacokinetic studies and will be used to study the drug-drug interaction between aprepitant and dexamethasone in pediatric patients.

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8

A simple extemporaneous oral suspension of aprepitant yields sufficient pharmacokinetic exposure in children

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ABSTRACT

Introduction

Aprepitant is used for the treatment of chemotherapy induced nausea and vomiting. A liquid formulation is needed for treatment of young children. However, the commercial (powder for) suspension was not available worldwide for a prolonged period of time and, therefore, a 10 mg/mL aprepitant oral suspension was extemporarily prepared to prevent suboptimal anti-emetic treatment. The current pharmacokinetic study was developed to investigate whether this extemporaneous oral suspension offers an appropriate treatment option.

Methods

From 49 pediatric patients (0.7-17.9 years) 235 plasma concentrations were collected. Patients were either treated with our extemporaneous oral suspension (n=26; 53%), commercially available capsules (n=18; 37%), or the intravenous prodrug formulation of aprepitant (fosaprepitant, n=5; 10%). Pharmacokinetic analyses were performed using nonlinear mixed effects modelling.

Results

A one-compartment model adequately described the pharmacokinetics of aprepitant in children. The bioavailability of the extemporaneous oral suspension was not significantly different to that of the capsules (P=0.26). The observed bioavailability throughout the total population was 83% (95%CI 69%-97%). The absorption of the extemporaneous oral suspension was 39.4% (95%CI 19.5-57.4%) faster than that of capsules (mean absorption time of 1.78 h (95%CI 1.32-2.35), but was comparable to that of the commercial oral suspension. The median area under the curve after (fos)aprepitant was 22.2 mg/L*h (range 8.9-50.3 mg/L*h) on day 1.

Conclusion

Our extemporaneous oral suspension is an adequate alternative for the commercially (un) available oral suspension in young children. An adequate exposure to aprepitant in children was yielded and the bioavailability of the extemporaneous suspension was comparable to capsules.

INTRODUCTION

Aprepitant is a selective neurokinin-1 (NK-1) receptor antagonist, which is orally available. Aprepitant enhances its effect by blocking the NK-1 receptor, preventing substance P from binding to the NK-1 receptors and, thereby, preventing chemotherapy induced nausea and vomiting (CINV).[1] Later, fosaprepitant, an intravenous formulation, was developed.[2] Fosaprepitant is a water soluble prodrug of aprepitant, which is rapidly converted to aprepitant after administration.

Aprepitant and fosaprepitant (hereafter, (fos)aprepitant) are used in children and adults for the treatment of CINV.[3,4] (Fos)aprepitant is added to the anti-emetic regimen when high emetogenic chemotherapy is given. The standard prophylaxis for highly emetogenic agents consists of a 5-HT3 receptor antagonist, dexamethasone and aprepitant. Fosaprepitant is used in cases were oral administration is not possible. Aprepitant and fosaprepitant are administered in a 3-day regimen using a dosing schedule of 3 mg/kg (aprepitant max 125 mg, fosaprepitant 115 mg) at day 1 and 2 mg/kg (aprepitant and fosaprepitant max 80 mg) at day 2 and 3.[1,2] In the Netherlands, dexamethasone is dosed four times daily 3 mg/m^2 (intravenous or oral) when combined with (fos)aprepitant, according to the national guideline of the Dutch Childhood Oncology Group (DCOG).

For aprepitant treatment of younger children (below 12 years of age) the required doses of 2 or 3 mg/kg cannot be administered using commercially available capsules of 40, 80 and 125 mg as lower individual weight-based doses are needed. For this purpose, a powder for suspension was developed by Merck Sharp & Dohme B.V.[1] Aprepitant has low water solubility (Log P 4.8 at pH 7.0[5]). To improve the bioavailability of aprepitant, a nano-particle formulation was developed by the manufacturer. The commercially available capsules and oral suspension both contain these aprepitant nano-particle-coated beads.[6,7] The pharmacokinetics (PK) of aprepitant in children, administered orally using commercially available capsules and commercially available oral suspension and intravenously using fosaprepitant, have been studied before.[8] The absorption of aprepitant was delayed in the case of capsules, but not when the oral suspension was used. The difference in bioavailability between capsules and commercial suspension was not tested.

For a longer period of time, the commercial (powder for) suspension was not available worldwide creating an urgent need for an alternative liquid formulation in order to treat younger patients with aprepitant. Therefore, we extemporized a 10 mg/mL aprepitant oral suspension using the commercially available capsules and a standard commercially available suspension base. Similar attempts with commercially available capsules and Orablend have been made. The relative bioavailability of this extemporaneous suspension, compared to capsules, was 82.3% in healthy adults.[9,10] Since the suspension is mainly used for treatment of young children, it is important to study the PK properties (e.g. bioavailability)

of the extemporaneous suspension in a pediatric population, in order to investigate whether our extemporaneous oral suspension offers an appropriate alternative for young children.

METHODS

Patients and sampling

Patients 0.5-18 years, treated with (fos)aprepitant and with a central venous line in situ for blood collection were included in a prospective observational study, studying the PK of aprepitant and dexamethasone, at the Princess Máxima Center for pediatric oncology in the Netherlands. There were no restrictions for chemotherapeutic treatment. Patients with Down syndrome were excluded. The use of CYP3A4-substrates and/or -inhibitors within seven days or CYP3A4-inducers within 30 days before the start of anti-emetic therapy was also reason for exclusion. Patients were included after written informed consent was obtained. Ethical approval by the institutional Medical Ethics Committee of the Erasmus MC was obtained. The study was registered in the Dutch Trial Registry as NTR7720. The current study describes the aprepitant part of this prospective observational study.

Patients were treated with (fos)aprepitant according to local protocol (doses as described in the introduction). Patients that could not be treated with the standard capsule formulation (80 mg or 125 mg) were treated with an extemporaneous oral suspension of 10 mg/mL, produced using the commercially available capsule filling[1] (Emend, Merck Sharp & Dohme B.V.) and a suspension base (Fagron). The suspension was prepared by grinding the aprepitant capsule filling, before mixing it with the suspension base as described by Dupuis et al.[9] Excipients of the suspension base were sucrose, colloidal magnesium-aluminum silicate, carboxymethylcellulose sodium salt, citric acid, methylparaben, propylparaben and banana essence. The self-life of the extemporaneous suspension was 1 month at room temperature.[9] Fosaprepitant was assumed to rapidly and completely convert to aprepitant after intravenous administration.

After administration of (fos)aprepitant on day 1, 2 or 3, a maximum of 6 blood samples of 1 mL over a time period of 24 hours (at t=0.5, 1.5, 4, 6, 12, 24 h) were taken from the central venous line. When samples were taken on day 2 or 3, a trough level sample before (fos)aprepitant administration was added. Plasma concentrations of aprepitant were measured using a validated liquid chromatography mass spectrometry method, with a lower limit of quantification (LLOQ) of 0.1 ng/mL, as described by Nijstad et al.[11] The first samples below LLOQ were included as a plasma concentration of 0.05 ng/mL (1/2 LLOQ).

Model development

Starting point for model development for aprepitant was a one-compartment model with first order absorption. Allometric scaling using body weight (BW) was a priori included on all parameters.[12] Subsequently, a two-compartment model was tested.

Since an absorption lag-time was found by Chain et al.[8], a transit compartment model was implemented to describe aprepitant drug absorption[13]. The number of transit compartments was optimized manually. The mean absorption time (MAT) was estimated.

Interindividual variability (IIV) was evaluated for all parameters, according to equation 1:

$$P_i = P_{pop} \times e^{(\eta_i)} \quad (1)$$

where P_i represents the individual parameter estimate for individual *i*, P_{pop} represents the typical population parameters estimate, and η_i is assumed to be normally distributed with a mean of zero and a variance of ω^2.

For some patients, data of multiple doses was available, so interoccasion variability (IOV) was implemented similarly as IIV, with each dose and subsequent sampling defined as a separate occasion. This variability was evaluated for all parameters to diagnose potential time-dependent trends and to allow for random unaccounted variability between dosing moments. Residual unexplained variability was modelled as a proportional error model.

Covariate analysis

The influence of formulation on the absorption related PK parameters was evaluated. Furthermore, age, as measure of maturation, was evaluated as covariate on clearance (CL), because Chain et al. found this to be a significant covariate on CL.[8]

Model evaluation

Discrimination between models was guided by physiological plausibility, goodness-of-fit (GOF) plots, precision of parameter estimates and change in objective function value (dOFV). A drop of ≥3.84 points, corresponding to a $P<0.05$ (χ2-distribution with 1 degree of freedom (df)), was considered a significant improvement of the fit for hierarchical nested models. The adequacy of the models was assessed by GOF plots and visual predictive checks (VPC).[14] Parameter precision was assessed by the sampling importance resampling (SIR) procedure.[15]

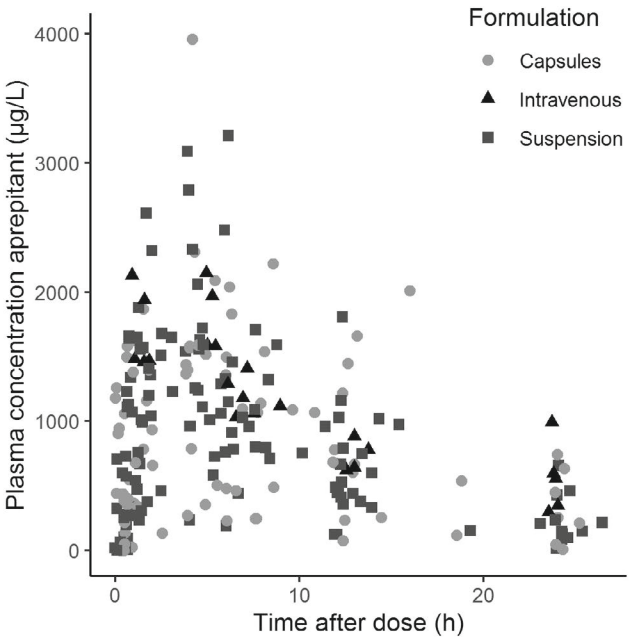

Figure 1. Aprepitant plasma concentrations versus time after dose

Exposure

Using the given dose and the estimated individual CL, an area under the curve (AUC) was calculated, according to equation 2:

$$AUC_i = \frac{F_i \times Dose_i}{CL_i} \quad (2)$$

where AUC_i represents the area under the curve for individual *i*, F_i represents the estimated individual bioavailability, $Dose_i$ represents the given dose to individual *i*, and CL_i represents the estimated individual CL.

Software

Nonlinear mixed-effects modeling was performed using NONMEM (version 7.3.0, ICON development Solutions, Ellicott City, MD, USA) and Pearl-speaks-NONMEM (PsN, version 4.7.0) with First-Order Conditional Estimation with interaction (FOCE-I) as estimation method.[16,17] Pirana (version 2.9.9) was used as graphical user interface for NONMEM.[18] R (version 3.4.3) was used for data handling and visualization.[19]

RESULTS

Patients and sampling

In total, 49 patients with a median age of 10.0 years (range 0.7-17.9) were available for inclusion in the aprepitant part of this study. 26 patients (53%) were treated with our extemporaneous oral suspension. In total, 235 aprepitant samples were available for analysis, of which 2 were below LLOQ. 86% of the patients were sampled during day 1 of (fos)aprepitant. Figure 1 displays the observed plasma concentrations over time. Detailed patient characteristics are shown in Table 1.

Model development and evaluation

A linear one-compartment model was appropriate to describe the PK of aprepitant. BW was a priori included as covariate using allometric scaling on all PK parameters. The exponents for BW on clearance (CL) and volume of distribution (V) were fixed to 0.75 and 1, respectively, prior to covariate analyses. A two-compartment model was tested based on a previously published model[8], but this did not improve the model, so further optimization was performed using a one-compartment model.

Table 1. Patient characteristics, median (range)

	Aprepitant capsules N=18 (37%)	**Aprepitant suspension N=26 (53%)**	**Fosaprepitant N=5 (10%)**	**Total N=49**
Patient characteristics				
Female sex [n (%)]	4 (22%)	15 (58%)	1 (20%)	20 (41%)
Age, years	14.4 (10.4-17.9)	6.0 (0.7-14.1)	13.3 (4.4-13.8)	10.0 (0.7-17.9)
Body weight, kg	50.7 (40.5-66.3)	22.1 (8.4-41.7)	41.7 (19.5-52.4)	32.5 (8.4-66.3)
Available data				
Total no. of PK samples [n]	88	123	24	235
No of samples per patient	5 (2-7)	5 (1-7)	5 (4-5)	5 (1-7)

Transit compartments were implemented to describe a delay in absorption of aprepitant. The optimal number of transit compartments was three. The MAT was estimated to be 1.78 h (95% CI 1.32-2.35 h), multiplied by 0.61 (95% CI 0.43-0.81) in the case of the extemporaneous oral suspension (absolute MAT of 1.08 h). This represents a faster absorption of the oral suspension compared to the capsules.

The absolute bioavailability (F1) for the whole population (capsules and extemporaneous oral suspension together) was estimated to be 83% (95% CI 69%-97%). The relative bioavailability of the oral suspension compared to the capsules was tested and estimated to be 87% (95% CI 71%-108%, p=0.26). This indicates that the bioavailability of the extemporaneous oral suspension is comparable to the bioavailability of the capsules.

In line with the published model of Chain et al.[8], maturation was tested as covariate on CL, but this did not improve our model. In addition, no trend for an age effect was found looking at (ETA) plots.

In the final model, IIV was included on CL, MAT and F1. IOV was included on CL. The final estimates and 95% confidence intervals (CI) are shown in Table 2.

The GOF plots (Figure on request) showed accurate population and individual predictions, without any signs for over- or underprediction. Conditional weighted residuals (CWRES) are evenly distributed over the whole plasma concentration range and time interval. The VPC demonstrated that the median and the 95% CI of the observed data were in line with those from the simulation-based predictions from the model (Supplementary Figure S1).

Table 2. Final population PK parameter estimates of aprepitant

PK parameter	Estimate	95% CI
CL_{70kg} (L/h)	5.83	5.02 - 7.20
V_{70kg} (L)	86.8	74.2 - 98.1
MAT (h)	1.78	1.32 - 2.35
Effect of suspension on MAT	0.606	0.426 - 0.805
F1 (%)	83	69 - 97
IIV CL (%)	25.1	7.2 - 39.5
IIV MAT (%)	58.9	47.3 - 72.5
IIV F1 (%)	33.0	24.7 - 41.9
IOV CL (%)	30.2	15.8 - 47.2
Proportional residual error (%)	33.2	28.8 - 37.0

PK pharmacokinetics, *CI* confidence interval obtained by sampling importance resampling, *CL* clearance, *V* volume of distribution, *MAT* mean absorption time, *F1* bioavailability, *IIV* interindividual variability, *IOV* interoccassion variability. Population estimates CL_{70kg}, V_{70kg} correspond to a subject weighing 70 kg and are adjusted to an individual value using allometric scaling.

Exposure
The median AUC after (fos)aprepitant was 22.2 mg/L*h (range 8.9-50.3 mg/L*h) on day 1 and 10.4 mg/L*h (range 4.3-35.3 mg/L*h) on day 2 or 3. The median AUC of day 1 was 24.3 mg/L*h (range 8.9-50.3 mg/L*h) for patients treated with capsules. Patients treated with the extemporaneous suspension achieved a median AUC of 20.1 mg/L*h (range 11.7-42.3 mg/L*h) on day 1. Intravenously administered fosaprepitant yielded a median aprepitant AUC of 28.4 mg/L*h (range 23.4-45.9 mg/L*h) on day 1.

DISCUSSION

This study describes the characterization of PK properties of aprepitant, administered as an extemporaneous oral suspension, by developing a population PK model of (fos) aprepitant in children. The bioavailability of the extemporaneous oral suspension was shown to be comparable to the bioavailability of commercially available aprepitant capsules and therefore offers an appropriate treatment option for young children. Extemporaneous preparation of an oral aprepitant suspension in the hospital pharmacy secures the availability of a highly needed drug in pediatric oncology, and solves supply continuity issues with the manufacturer. Eventually, this can be combined with dose banding strategies to enable safe and efficient administration of this important drug.

A one-compartment model was found to best describe the data. This is in line with a previous population PK model in adults[20], but in contrast to a large PK study in children by Chain et al[8]. However, a two-compartment model was inferior to describe our data and we did not find any trends in our GOF plots that would suggest that a two-compartment model would fit our data better. Another discrepancy between the model of Chain et al. and our model is that we could not find an effect of CYP3A4 maturation within our dataset. This could be explained by the fact that we included only 3 patients (6%) below the age of 2 years. Chain et al. included 30 patients (20%) <2 years.[8]

Using a transit compartment modelling approach, we observed that absorption of aprepitant from capsules is slower than absorption of aprepitant from our extemporaneous suspension (MAT of 1.78 h for capsules compared to a MAT of 1.08 h for the extemporaneous suspension). This is as expected, as the dissolution step is faster in case of treatment with suspension. This is also in accordance with previous literature.[8,20] However, both previous studies used lag-time to correct for the delayed absorption, which represents an abrupt increase in the absorption rate from a value of zero. From a physiological approach, one would expect a rather slow increase starting from time point zero. Using transit compartments, a more physiological drug absorption process is described by a multistep process.[13]

Furthermore, we can conclude that the bioavailability of aprepitant within our population is 83%. We observed that the bioavailability of our extemporaneous suspension is comparable to the bioavailability of the capsules. Previous studies showed a bioavailability range of 59-67% for the commercially available capsules, which is increased when administered in fed state.[1] We observed a higher bioavailability than the described range. However, we did not have data on the fed state of our patients, so that might explain the slightly higher bioavailability. Data on the bioavailability of the commercially available oral suspension is not available, but the previous study that investigated the bioavailability of an extemporaneous aprepitant suspension observed a relative bioavailability of 82%, which is comparable with our results.[10] Furthermore, we detected an IIV of 33% for F1, so variability throughout our population seems to be moderate.

This study has several limitations. Firstly, within this PK study, we did not investigate the anti-emetic effect of aprepitant. This means that within the current study, we were only able to compare the aprepitant AUC to historical cohorts, described in literature. The AUC that was found within our population was comparable with ranges described in literature.[1,3,8] Furthermore, as mentioned before, we did not collect data on fed state of the patients. The study protocol did not specify any dietary restrictions. Nevertheless, in the summary of product characteristics, the difference in exposure between fed and unfed state, was not considered clinically relevant.[1] Moreover, the acceptability of the extemporaneous suspension was not tested. However, we did not note any complaints about intake during the clinical study, so this may indicate that there were no problems with administration. Lastly, we did not investigate the chemical stability and the state of the nanoparticles in our extemporaneous formulation. The chemical stability of a similar extemporaneous suspension was investigated previously and appeared stable for at least 90 days by 4°C and 65 days at 4°C.[9] Information on stability of the aprepitant nanoparticles was not available, but since we did not experience any significant decline in bioavailability, we assume that the nanoparticles were still intact.

Overall, the results of the PK study show that the bioavailability of the extemporaneous oral suspension is comparable to capsules and that the extemporaneous oral suspension is an adequate alternative for the commercially available oral suspension in young children.

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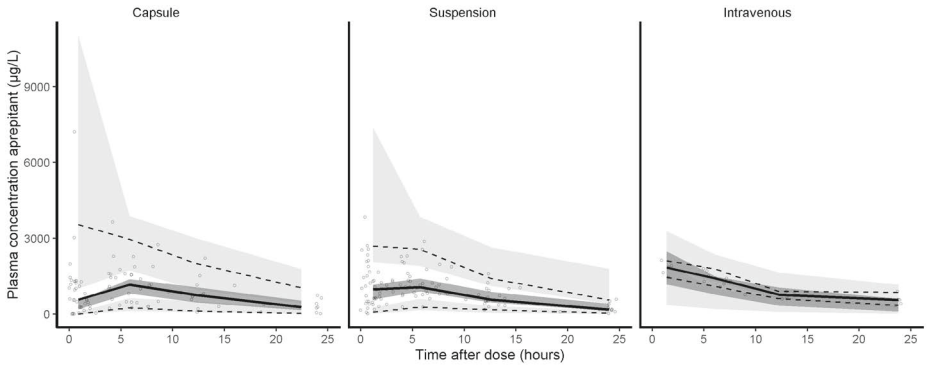
SUPPLEMENTARY MATERIAL



Supplementary Figure S1. Formulation stratified prediction-corrected visual predictive check. Black lines depict the observed median (solid) and 2.5% and 97.5% percentile (dashed) concentrations. Dark- and light-grey areas represent 95% prediction intervals of the simulated mean and the 2.5 and 97.5% percentiles, respectively. Round dots represent observations

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Overestimation of the effect of (fos)aprepitant on intravenous dexamethasone pharmacokinetics requires adaptation of the guidelines for children with chemotherapy induced nausea and vomiting

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ABSTRACT

Chemotherapy induced nausea and vomiting (CINV) are common side effects in pediatric oncology treatment. Besides 5-HT_3-antagonists, both dexamethasone and aprepitant are cornerstone drugs in controlling these side effects. Based on results of adult studies, the dexamethasone dose is reduced by 50% when combined with aprepitant, because of a drug-drug interaction, even though data on the interaction in children is lacking. The current study was developed to investigate the effect of aprepitant on dexamethasone clearance (CL) in children, in order to assess if dexamethasone dose reduction for concomitant use of aprepitant is appropriate in the current anti-emetic regimen.

In total, 65 children (0.6-17.9 years), receiving anti-emetic therapy (dexamethasone±aprepitant) as standard of care, were included. 305 dexamethasone plasma concentrations were determined using LC-MS/MS.

An integrated dexamethasone and aprepitant pharmacokinetic model was developed using non-linear mixed effects modelling in order to investigate the drug-drug interaction. In this population, dexamethasone CL in patients with concomitant administration of aprepitant was reduced by approximately 30% of the uninhibited CL (23.3 L/h (95% confidence interval 20.4-26.0)). Aprepitant reduced dexamethasone CL by approximately 30%. This result is not consistent with the results of adult studies (50% reduction). This difference was not age-dependent, but might be related to the route of administration of dexamethasone.

Future studies are needed to assess the difference in oral/intravenous dexamethasone. Nevertheless, when dexamethasone is given intravenously as a component of triple therapy to prevent CINV in children we advise to reduce the dexamethasone dose by 30% instead of 50%.

INTRODUCTION

Chemotherapy induced nausea and vomiting (CINV) are one of the most common side effects of pediatric oncology treatment. International guidelines for management and prevention of these side effects have been developed.[1–4] Different treatment levels are based on the emetogenicity classification of the Canadian POGO group.[1,5] The overall backbone of supportive care regarding anti-emetic agents consist of a 5-HT_3 receptor antagonist for low emetogenic chemotherapy, addition of dexamethasone for moderate emetogenic chemotherapy and, for highly emetogenic chemotherapy (HEC), addition of the NK1 receptor antagonist, aprepitant.

Despite standardized prophylaxis, children receiving HEC achieve 20-30% less control of CINV compared to adults.[6–8] Kang et al.[9] reported a double-blind randomized phase 3 trial, in which aprepitant turned out to be effective for the prevention of CINV in pediatric patients, as adjuvant of ondansetron with or without dexamethasone. However, still in 58% of the children, this therapy was not effective in the delayed phase of chemotherapy. Interestingly, the anti-emetic control was even lower in children receiving also the anti-emetic drug dexamethasone, which was administered as intravenous infusion in this trial (36 versus 56%, respectively). Since children received both aprepitant and dexamethasone during HEC protocols only, this was hypothesized to explain the difference in anti-emetogenic control.

An alternative explanation for inadequate CINV control could lie in suboptimal dexamethasone therapy. When dexamethasone is given concurrently with aprepitant, its dose is reduced by 50%. As a weak inhibitor of cytochrome P450-3A4 (CYP3A4), aprepitant therapy can result in higher plasma levels of CYP3A4 metabolized agents (e.g. dexamethasone). In adult studies, it has been shown that concurrent use of aprepitant resulted in a lower dexamethasone clearance (CL) and a higher exposure to dexamethasone of approximately 2-fold, and thus a 50% dose reduction of dexamethasone is recommended.[10–13] However, for children, this reduction is based on extrapolation of adult data, and it is not known if the magnitude of this interaction is the same.[14]

Therefore, in this study, we evaluated the drug-drug interaction between aprepitant and dexamethasone in pediatric oncology patients. We studied the effect of aprepitant on dexamethasone pharmacokinetics (PK) in children, in order to assess if dexamethasone dose reduction for concomitant use of aprepitant is appropriate in the current anti-emetic regimen.

METHODS

Patients, sampling and bioanalysis

A prospective observational study was performed in Princess Máxima Center for Pediatric Oncology in the Netherlands. Patients aged 0.5-18 years with a central venous line, treated

with dexamethasone with/without (fos)aprepitant were eligible for inclusion after written informed consent was obtained. Patients could be included twice: once for oral aprepitant and once for intravenous fosaprepitant, if applicable. Patients using CYP3A4-substrates and/or -inhibitors within seven days or CYP3A4-inducers within 30 days before the start of anti-emetic therapy were excluded (Supplementary Table S1). Patients with Down syndrome were excluded. Ethical approval by the institutional Medical Ethics Committee of the Erasmus MC was obtained. The study was registered in the Dutch Trial Registry as NTR7720.

Table 1. Dose regimens for dexamethasone and (fos)aprepitant

Emetogenicity	Dexamethasone[1]	Aprepitant[2]	Fosaprepitant[3]
Moderate	<0.6 m^2: 2 mg 2dd iv/po >0.6 m^2: 4 mg 2dd iv/po		
High	3 mg/m^2 4dd iv/po (or 6 mg/m^2 4dd iv/po without aprepitant)	Day 1: 3 mg/kg 1dd po (max 125 mg) Day 2-3: 2 mg/kg 1dd po (max 80 mg)	Day 1: 3 mg/kg 1dd iv (max 115 mg) Day 2-3: 2 mg/kg 1dd iv (max 80 mg)

[1] Dexamethasone was administered as commercially available tablets, oral solution or solution for injection, using locally available products. [2] Aprepitant was administered as 80 mg or 125 mg capsules (Merck Sharp & Dohme B.V.[36] or generic equivalent) or as an extemporaneous oral suspension of 10 mg/mL (prepared using the commercially available aprepitant capsule filling and a suspension base as described by Nijstad et al.[19]). [3] Fosaprepitant was administered using the commercially available iv aprepitant formulation (Ivemend, Merck Sharp & Dohme B.V.[37]).

Patients were treated with dexamethasone with/without (fos)aprepitant according to the anti-emetic guidelines of the DCOG, as detailed in Table 1. Before and after administration of dexamethasone with/without (fos)aprepitant on day 1, 2 or 3, a maximum of respectively one and six blood samples of 1 mL over a time period of 24 hours were taken from the central venous line (Supplementary Table S2). Plasma concentrations of dexamethasone and aprepitant were measured using a validated liquid chromatography mass spectrometry method, with a lower limit of quantification (LLOQ) of 1 µg/L and 0.1 µg/L for dexamethasone and aprepitant respectively, as described previously.[15] The first post-dose samples below LLOQ were included as a plasma concentration of 0.5 µg/L (1/2 LLOQ for dexamethasone), all other samples below LLOQ were omitted.[16]

Sample size calculation

Children ≤18 years were divided in three age groups (≥0.5-6 years; ≥6-12 years and ≥12 years) treated with dexamethasone with/without (fos)aprepitant. We planned to include 30 children in each age category, with the aim to distinguish 15 children using dexamethasone without co-medication of (fos)aprepitant and 15 children using dexamethasone in combination with (fos)aprepitant. We planned to evaluate at least 5 children across all age groups.

Dexamethasone model development

Starting point for model development for dexamethasone were one- and two-compartment models with first order absorption. Allometric scaling using body weight (BW) was a priori included on all parameters.[17]

Interindividual variability (IIV) was evaluated for all parameters, using an exponential function.[18] Since data of multiple doses were available, interoccasion variability (IOV) was implemented similarly as IIV, with each dose and subsequent sampling defined as a separate occasion. This variability was evaluated for all parameters to diagnose potential time-dependent trends and to allow for random unaccounted variability between dosing moments. Residual unexplained variability was evaluated as a proportional error model or as a combination of a proportional and additive error model.

Dexamethasone covariate analysis

Following structural model development, the influence of patient-specific factors for variability in PK parameters was evaluated. Assessed covariates included age and aprepitant treatment. Continuous covariates were evaluated using a power function. Aprepitant treatment was described as a binary categorical variable: 1 when aprepitant was administered concomitantly and 0 when no aprepitant was administered. Aprepitant as a covariate was tested on CL as follows:

$$P_i = P_{pop} \times P_{cov}^{\text{aprepitant}}$$

where P_{cov} represents the estimated proportional factor by which CL changes at a specific covariate value.

Integrated dexamethasone and aprepitant model development

In order to thoroughly investigate the drug-drug interaction between the agents, an integrated PK model of dexamethasone and (fos)aprepitant was developed, The PK of (fos)aprepitant was described using a one-compartment model with absorption transit compartments, as described by Nijstad et al.[19]

Two (hypothetical) enzyme compartments ($enzyme_{active}$ and $enzyme_{inactive}$) were added as previously described by Huitema et al.[20] Elimination of dexamethasone was directly proportional to the amount of $enzyme_{active}$ present in the compartment. The amount of $enzyme_{active}$ was set to 1 and $enzyme_{inactive}$ to 0 at t=0. The conversion of $enzyme_{active}$ to $enzyme_{inactive}$ was driven by the amount of aprepitant in the central compartment.

Drug-drug interaction between dexamethasone and aprepitant

Since patients in both groups (dexamethasone with or without (fos)aprepitant) were not treated with the same dexamethasone doses (see Table 1), it is difficult to compare the observed areas under the curve (AUCs) between the two groups, in order to assess the

influence of (fos)aprepitant on the AUC of dexamethasone. For this reason, simulations were carried out. Patients included in this study were used for the simulations and individuals were replicated to come to a total of 2000 patients per group. All patients where hypothetically treated, once with dexamethasone alone and once with dexamethasone and aprepitant. In the first simulations, the hypothetical dexamethasone dose in both groups was the same (100%), to compare AUCs. Subsequently, dexamethasone dose reductions were tested to test which dose regimen would lead to comparable AUCs.

Model evaluation

Discrimination between models was guided by physiological plausibility, goodness-of-fit (GOF) plots, precision of parameter estimates and change in objective function value (dOFV). A drop of ≥3.84 points, corresponding to a $P<0.05$ (χ^2-distribution with one degree of freedom (df)), was considered a significant improved fit for hierarchical models. The adequacy of the models was assessed by GOF plots and visual predictive checks (VPC).[21] Parameter precision was assessed by the sampling importance resampling (SIR) procedure.[22]

Software

Nonlinear mixed-effects modeling was performed using NONMEM (version 7.3.0, ICON development Solutions, Ellicott City, MD, USA) and Pearl-speaks-NONMEM (PsN, version 4.7.0) with First-Order Conditional Estimation with interaction (FOCE-I) as estimation method.[23,24] Pirana (version 2.9.9) was used as graphical user interface for NONMEM.[25] R (version 3.4.3) was used for data handling and visualization.[26]

RESULTS

Patients and sampling

In total, 65 of 93 (70%) patients with a median age of 8.8 years (range 0.6-17.9) were available for inclusion in this study, and were included between March 2019 and April 2021. A total of 28 children were excluded after receiving informed consent. From these 28 patients, 12 patients already received their last course of chemotherapy or no moderate/high emetogenic courses of chemotherapy were given anymore before study sampling was performed (e.g. due to switch of treatment protocol). Furthermore, two patients were defined as screening failures; one informed consent was withdrawn before study sampling and one patient passed away. In 12 children dexamethasone was stopped as an anti-emetic drug due to hypertension (n=3) behavioral problems (n=6) or a combination of those two (n=1); bradycardia (n=1) or no complaints of nausea (n=1). No patients were included in the study twice.

Of the 65 sampled patients, 18 patients were treated with dexamethasone without aprepitant (respectively 11, 2 and 5 patients across the three different age groups). In 94%

of the patients, samples were taken on day 1 of anti-emetic treatment. 47 patients used both dexamethasone and aprepitant (respectively 14, 14 and 19 patients across the three different age groups. Overall we experienced a lower median age in the dexamethasone group compared to the dexamethasone with aprepitant group). Samples were taken on day 1 of anti-emetic treatment for 87% of the patients. For trial profile see Figure 1. 62 of the patients (95%) were treated with intravenous dexamethasone. In total, 305 dexamethasone samples were available for analysis, of which 12 were below LLOQ (all were single below LLOQ samples post-dose). Supplementary Figure S1 displays the observed plasma concentrations over time. Detailed patient characteristics are shown in Table 2.

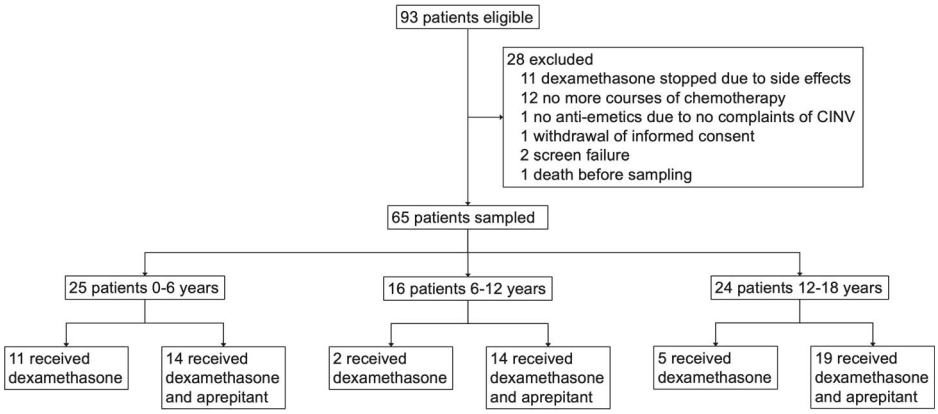

Figure 1. Trial profile

Table 2. Patient characteristics, median (range)

	Dexamethasone without aprepitant	**Dexamethasone with aprepitant**	**Total**
	N=18	**N=47**	**N=65**
Patient characteristics			
Female sex [n (%)]	8 (44%)	19 (40%)	27 (40%)
Age, years	3.9 (0.6-16.5)	10.0 (0.7-17.9)	8.8 (0.6-17.9)
Body weight, kg	16.3 (8.6-74.1)	32.5 (8.4-66.3)	29.5 (8.4-74.1)
Dexamethasone iv [n (%)]	18 (100%)	44 (94%)	62 (95%)
Available data			
Total no. of PK samples [n]	85	220	305
No of samples per patient	5 (1-6)	5 (1-7)	5 (1-7)

Dexamethasone model development and evaluation
A two-compartment model with first order absorption was appropriate to describe the PK of dexamethasone. The absorption rate constant (k_a) could not be estimated due to the small number of patients that were treated with dexamethasone orally, so k_a was fixed to 1.5 /h.[27,28] Interindividual variability (IIV) was included on CL, Q and Vc, interoccasion variability (IOV) was included on CL and Vc.

Inclusion of aprepitant as categorical covariate on CL led to an improved model fit (dOFV -7.2). The proportional factor by which CL changes was estimated at 0.74 (95% confidence intervals (CI) 0.56-1.01), signifying a 26% lower CL when aprepitant is administrated concomitantly.

Integrated dexamethasone and aprepitant model development and evaluation
The model was further optimized by integrating the dexamethasone and aprepitant models. A graphical representation of the combined model is showed in Supplementary Figure S3. The inhibition of dexamethasone CL was modelled by an aprepitant concentration-dependent reversible inhibition of enzymes. The total amount of enzymes consisted of an active and inactive part. Mass transport between $enzyme_{active}$ and $enzyme_{inactive}$ was modelled using an inhibition rate constant k_{inh} and a reactivation rate constant k_{reac}. No age-related effects on any of the PK parameters were found.

The final model consisted of a two-compartment model with first order absorption for dexamethasone, a one-compartment model with absorption transit compartments and two enzyme compartments describing the aprepitant concentration-dependent reversible inhibition of enzymes. The final estimates and 95% CI are shown in Table 3.

The goodness-of-fit (GOF) plots (Supplementary Figure S3A and B) showed accurate population and individual predictions, without any signs for over- or underprediction. CWRES are evenly distributed over the whole plasma concentration range (Supplementary Figure S3C) and time interval (Supplementary Figure S3D). The visual predictive checks (VPC) demonstrated that the median and the 95% CI of the observed data were in line with those from the simulation-based predictions from the model (Supplementary Figure S4).

Drug-drug interaction between dexamethasone and aprepitant
Simulations were carried out using 4000 individuals. Patients were hypothetically treated with 6 mg/m^2 dexamethasone (100%). Half of the patients were hypothetically treated with 3 mg/kg (max 125 mg) aprepitant. AUCs where calculated, see Supplementary Table S2 and Figure 2. Patients treated with 6 mg/m^2 dexamethasone without aprepitant achieved a 54% higher median exposure than patients treated with 6 mg/m^2 dexamethasone with aprepitant. When the patients with aprepitant were hypothetically treated with 3 mg/m^2 dexamethasone (reduction of 50%), we observed that the dexamethasone AUC of patients with aprepitant was 23% lower than the dexamethasone AUC of patients without aprepitant.

This again, shows that a dose reduction of dexamethasone of 50% is not accurate to achieve comparable exposure. A dexamethasone dose of 4 mg/m^2 (dose reduction of 33%) for patients receiving concurrent aprepitant was tested. This resulted in a comparable exposure between the two groups.

Table 3. Final dexamethasone population PK parameter estimates

PK parameter	Estimate	95% CI
$CL_{dex,70kg}$ (L/h)	23.3	20.4 - 26.0
$Vc_{dex,70kg}$ (L)	51.0	43.1 - 63.2
$Vp_{dex,70kg}$ (L)	42.5	36.2 - 47.4
$Q_{dex,70kg}$ (L/h)	47.6	40.1 - 57.9
k_{inh} (L/µg*h)	0.906	0.704 - 1.200
k_{react} (/h)	1.30	0.888 - 1.90
IIV CL_{dex} (%)	36.6	29.5 - 43.8
IIV Vc_{dex} (%)	59.0	42.5 - 72.4
IIV Q_{dex} (%)	57.8	38.0 - 77.2
IOV CL_{dex} (%)	17.9	13.4 - 27.5
IOV Vc_{dex} (%)	14.4	5.6 - 23.3
Proportional residual error (%)	28.1	25.1 - 32.0

PK pharmacokinetics, *CI* confidence interval obtained by sampling importance resampling, *CL* clearance, *Vc* volume of distribution of the central compartment, *Vp* volume of distribution of the peripheral compartment, *Q* intercompartment clearance between Vc and Vp, *IIV* interindividual variability, *IOV* interoccassion variability. Population estimates CL_{70kg}, Vc_{70kg}, Vp_{70kg}, Q_{70kg} correspond to a subject weighing 70 kg and are adjusted to an individual value, using allometric scaling.

DISCUSSION

This population PK model is the first to describe the PK of dexamethasone (with and without aprepitant) as anti-emetic agent in children. The PK of dexamethasone was best described using a two compartment model, which is consistent with previously published models.[29–32] Using an integrated dexamethasone and aprepitant model, the influence of aprepitant on dexamethasone CL was studied. We found that aprepitant reduced the dexamethasone CL by approximately 30%. This is not consistent with the results of studies in adults and current practice in children.[10–13] These studies all described a reduction of dexamethasone CL of approximately 50% or a doubling of the exposure to dexamethasone when combined with aprepitant.

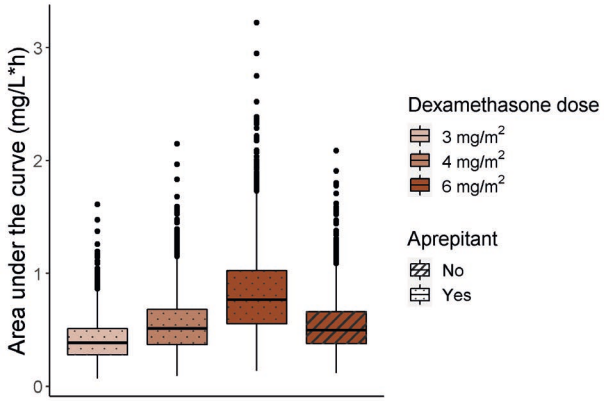

Figure 2. Dexamethasone area under the curve in simulated patients stratified for dose and aprepitant use.

At first, a possible explanation for the clinically relevant discrepancy with the results obtained in adults was thought to be related to age, as we considered that the rationale for dexamethasone dose reduction in the pivotal study by Kang et al.[9] was extrapolated from adults to children. However, in our model development, no reason for testing age as covariate on any of the PK parameters was found. For this, we concluded that age has no obvious effect on the PK of dexamethasone or aprepitant.

Another possible explanation for the difference between adults and children could be found in the route of administration of dexamethasone. In several of the previously published articles, dexamethasone was administered orally.[10,12] It was hypothesized that the presence of aprepitant reduces the dexamethasone metabolism by inhibiting CYP3A4 enzymes in the gastro-intestinal (GI) tract, when dexamethasone is administered orally.[14] The inhibition of CYP3A4 enzymes in the GI-tract will lead to reduction of the first pass effect and thus bioavailability, which leads to a higher systemic exposure. When dexamethasone is administered intravenously, as in 95% of our pediatric patients, this effect will not play a role and only the systemic elimination of dexamethasone is influenced by concurrent use of aprepitant. However, two studies in adults did study the effect of aprepitant on intravenously administered dexamethasone, and also did find a reduction of the dexamethasone CL of approximately 50%.[11,13] Since the drug-drug interaction with either oral or intravenous dexamethasone was never compared within one study, we initiated a new study to examine this. Within this study, patients will receive dexamethasone orally and intravenously in a cross-over design. We will include patients with and without aprepitant in a 1:1 ratio. The difference in PK of dexamethasone and the influence of aprepitant will be studied in these cohorts (https://www.trialregister.nl/trial/8981).

In the current study, 11 (12%) children were excluded, even after informed consent was given, due to stopping dexamethasone as part of the anti-emetic regimen because

of off-target side effects of e.g. hypertension and/or behavioral problems. Although addition of dexamethasone as anti-emetic drug is strongly recommended in current clinical guidelines[1–3,33], the side effects are a major issue in daily practice. Moreover, the appropriate dose to reduce CINV is intensively debated among the different hospitals treating pediatric oncology patients. For example, in a survey among 36 children's oncology institutions, two groups never administered dexamethasone. In the other 34 institutions, 29 different dexamethasone dosing schedules were used for children receiving HEC.[34] A recent systematic review aimed to describe all different dexamethasone doses studied for the prevention of chemotherapy induced vomiting (CIV) in pediatric patients and their effects on achieving complete acute CIV control.[35] However, due to the heterogeneity of the studies and the wide variety in dosing schedules, no optimal dexamethasone dose to control acute CIV was found. Dosing regimens varied between 6-27 mg/m^2/day in patients receiving HEC and between 0.6-24 mg/m^2/day in patients receiving moderate emetogenic chemotherapy (MEC).[35]

Translating the results of this study into clinical practice is challenging. On one hand, this current study shows that a reduction of the intravenous dexamethasone dose with 50% in children might be too high, since we showed that aprepitant inhibits dexamethasone CL by approximately 30%. Based on the results of the current study, we advise to reduce the intravenous dexamethasone dose with 30% in children, when concurrently used with aprepitant. However, as we already see many side effects of dexamethasone in our current dose schedule it is not very appealing to upgrade the current dexamethasone dose of 4dd 3 mg/m^2 in presence of aprepitant. As triple therapy is the cornerstone of anti-emetic treatment, we hypothesize that it is possible to decrease the dexamethasone dose to prevent side effects, and therefore prevent omitting this important anti-emetic agent. We suggest to dose dexamethasone intravenously 4dd 2 mg/m^2 in presence of aprepitant and 4dd 3 mg/m^2 without aprepitant during HEC treatment in children. With this, a dose reduction of 30% in presence of aprepitant is accomplished, and the dosing regimen is within the dexamethasone dosing range (6-27 mg/m^2/day) as described in a recent systematic review about the variation of different dexamethasone dosing schedules worldwide in patients receiving HEC.[35] Future studies should address the starting dose and the duration of anti-emetic therapy in children, as many pediatric chemotherapy courses last much longer than the typical 3 day aprepitant regimen.

Furthermore, our study underlines that extrapolating results from adults to children can be extremely difficult and should be done with caution. In our example, we found a clinically relevant discrepancy with the results within adults. That extrapolations from adults to children should be done with caution was previously described by Cella et al.[38] They underlined that assuming a linear relationship between body weight and dose is not right for all drugs and that it can lead to either under- of overdosing. They suggested that dose recommendations for children should be derived from an integrated (model-based) analysis

of pharmacokinetic data rather than from empiricism. This is fully in line with the results from our current study.

This study has several strengths. To start with, this is the first study that assessed the PK of dexamethasone as anti-emetic agent and the influence of aprepitant on the PK of dexamethasone in children. Secondly, we used a rich sampling scheme and were able to collect at least 5 blood samples in 40 patients (62%). Furthermore, we developed an integrated dexamethasone and aprepitant model, where the inhibition of dexamethasone CL was dependent on the plasma concentration of aprepitant. This is closest to the real life situation in patients. To our knowledge, this was not tested before.

However, this study had some limitations too. Dexamethasone was administered intravenously in almost all patients, and therefore the difference of the effect of aprepitant on oral versus intravenous dexamethasone CL could not be identified. Because of this limitation, based on the current study, we can only give recommendations for intravenously administered dexamethasone in children.

Our second limitation refers to our planned sample size. We included 65 of the defined 90 patients. This was due to inclusion difficulties because of Covid-19 restrictions and the loss of eligible patients due to dexamethasone cancellation. The sample size of 90 patients was suggested in order to achieve a diverse population to study the effect of aprepitant on dexamethasone PK. However, we have shown in this analysis that we were able to accurately study the effect with 65 patients. Moreover, we included our planned 5 children across all age groups except for the dexamethasone monotherapy age group of 6-11 year, in which only 2 children were sampled. The lower median age in the dexamethasone group compared to the dexamethasone with aprepitant group was thought to be explained by the experience that older patients were treated with triple therapy more frequently than younger patients.

Future studies are needed to optimize anti-emetic control in pediatric oncology treatment. From this study we have learned the effect of aprepitant on dexamethasone CL in the pediatric oncology population. This important knowledge on the difference in CL compared to the adult population will be proceeded to the next phase in the optimisation of anti-emetic control. We are also planning to conduct a randomized controlled trial in which we will study prolonged use of aprepitant.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. CYP3A4 substrates and/or –inhibitors. Patients using these CYP3A4 substrates and/or -inhibitors within seven days or CYP3A4 inducers within 30 days before the start of anti-emetic therapy were excluded

CYP3A inhibitor (strong)	CYP3A inducer (strong)
Boceprevir	Avasimibe
Clarithromycin	Carbamazepine
Conivaptan	Phenytoin
Grapefruit juice	Rifampin
Indinavir	St John's wort
Itraconazole	
Ketoconazole	
Lopinavir	
Mibefradil	
Nefazodone	
Nelfinavir	
Posaconazole	
Ritonavir	
Saquinavir	
Telaprevir	
Telithromycin	
Voriconazole	

Supplementary Table S2. Sample scheme

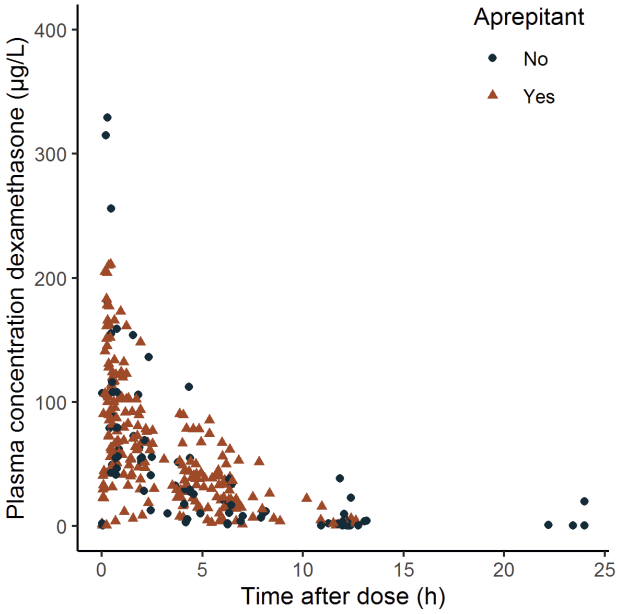
Time after first dexamethasone/ (fos)aprepitant administration	t=0	t=0.5h	t=1-2h	t=4h	t=6h	t=12h	t=24h
Sample	X	X	X	X	X	X	X

Sample scheme:
Sample 1 (1 mL): t = 0 hour after first administration of dexamethasone and (fos)aprepitant
Sample 2 (1 mL): t = 0.5 hour after first administration of dexamethasone and (fos)aprepitant
Sample 3 (1 mL): t = 1-2 hours after first administration of dexamethasone and (fos)aprepitant
Sample 4 (1 mL): t = 4 hours after first administration of dexamethasone and (fos)aprepitant
Sample 5 (1 mL): t = 6 hours, just before the second administration of dexamethasone
Sample 6 (1 mL): t = 12 hours, just before the third administration of dexamethasone
Sample 7 (1 mL): t = 24 hours, just before the fifth administration of dexamethasone and second administration of (fos)aprepitant

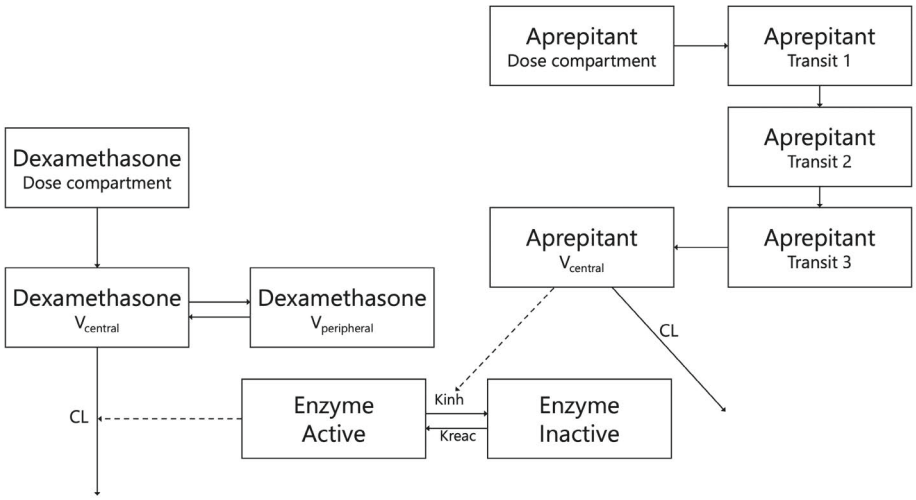
Supplementary Table S3. Dexamethasone area under the curve in mg/L*h in simulated patients stratified for dose and aprepitant use.

	With aprepitant		Without aprepitant	
	Median	*Range*	*Median*	*Range*
Dexamethasone 6 mg/m²	0.768	0.135 – 3.311	0.498	0.117 – 2.090
Dexamethasone 4 mg/m²	0.512	0.090 – 2.207	NA	
Dexamethasone 3 mg/m²	0.384	0.068 – 1.656	NA	

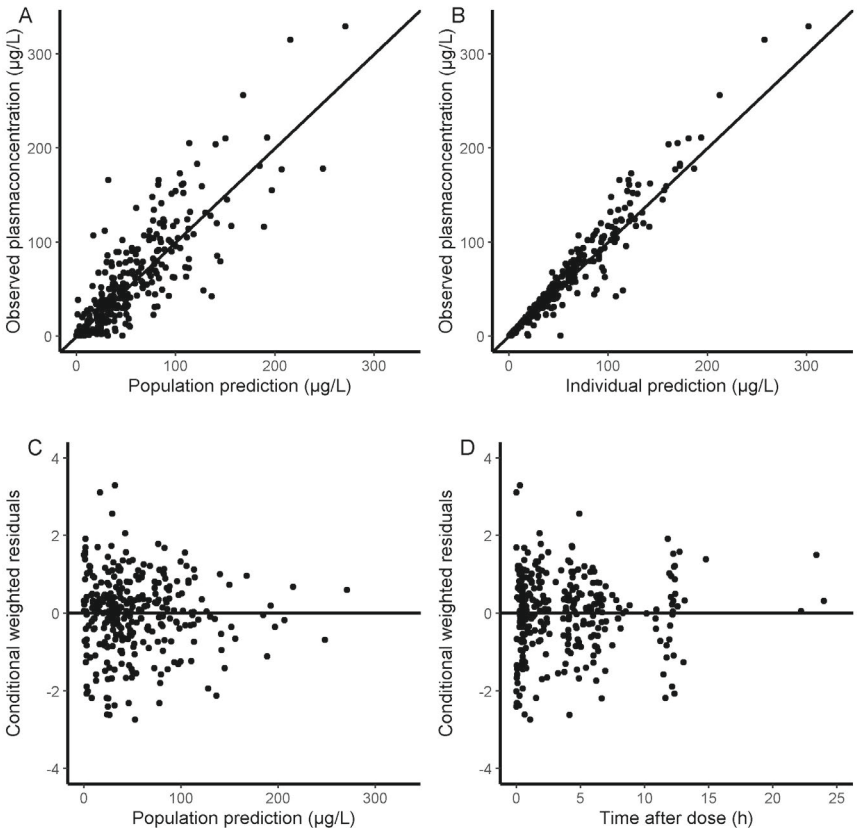
NA not applicable



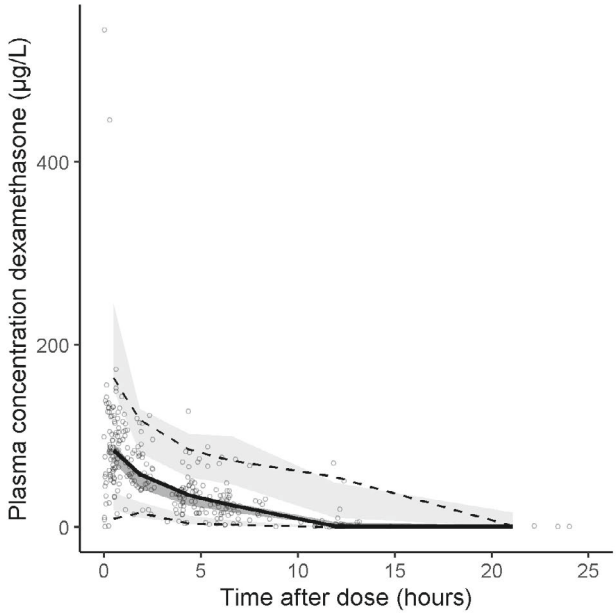
Supplementary Figure S1. Dexamethasone plasma concentrations versus time after dose



Supplementary Figure S2. Graphical representation of the final integrated dexamethasone and aprepitant model



Supplementary Figure S3. Goodness-of-fit plots dexamethasone



Supplementary Figure S4. Prediction-corrected visual predictive check. Black lines depict the observed median (solid) and 2.5% and 97.5% percentile (dashed) concentrations. Dark- and light-grey areas represent 95% prediction intervals of the simulated mean and the 2.5 and 97.5% percentiles, respectively. Round dots represent observations.

Conclusions and perspectives

CONCLUSIONS AND PERSPECTIVES

This thesis focuses on pharmacological dose optimization of chemotherapeutic and anti-emetic agents in (young) children with cancer. Various modelling methods were used to describe and predict the pharmacokinetics (PK) of these drugs. Several key points, displayed in bold in **Table 1**, were extracted from this thesis. These key points will be further discussed in this chapter.

Table 1. Key points (in bold) that can be extracted from this thesis

Also in pediatric oncology, children are not small adults, and this should structurally be considered in precision dosing. Clinical pharmacological knowledge should be included in clinical practice to optimise the dose of (chemotherapeutic) agents in pediatric oncology. Moreover, **collection of (real life) data from young children is possible** and can be used for **in depth pharmacokinetic modelling, which is essential to translate pharmacokinetic data into practical (dose) recommendations**.

Also in pediatric oncology, children are not small adults

Though this lesson has been described numerous times many years ago (articles citing this sentence date back to the late 90s)[1–3], this thesis points out that, also in pediatric oncology, children are not small adults. To emphasize this even more, one could say that an infant is not just a small child.[4] Due to developmental changes during the first years of life, differences in PK, i.e. drug absorption, distribution, metabolism and excretion (ADME)[5–9], occur, and this should structurally be considered in pediatric dosing.

Precision medicine is not only about finding the right drug for every patient, but also about finding the right dose for every patient (precision dosing). For many drugs, empirical, adult based, dosing regimens are standard practice for neonates and infants. The usual body surface area (BSA) dose (in mg/m^2) is divided by 30 (the typical body weight for a child with a BSA of 1 m^2) in order to generate a mg/kg dose. In some cases, this dose is reduced even further for very young children to correct for maturational changes, even though evidence for these approaches is often lacking. Since all drugs behave differently in the human body, a single generic dose recommendation for all drugs for neonates and infants cannot be derived. This was shown in **Chapter 2, 3, 4, 5 and 6**. These chapters pointed out that empirical dosing of neonates and infants is not the right approach. In addition, **Chapter 9** showed that empirical extrapolation of results from adults to children does not

result in optimal exposure either. Taking these observations together, we strongly advocate against empirical dose regimens, especially since better dose derivations can be made by taking prior clinical pharmacological knowledge into account.

Knowledge on the PK properties of a drug is usually available, even though pediatric PK data of long-used (chemotherapeutic) drugs are lacking in numerous cases. Clinical pharmacological knowledge is generated during the drug development phase, either in adults and/or in children, as part of the marketing authorisation. Existing information on ADME can be extrapolated to the population of interest and so, age-related differences in PK can be approached. The PK properties (e.g. route of elimination) of a drug should be investigated when searching for an appropriate dose for treatment of, for example, an infant. By combining these PK properties with our knowledge on developmental changes, dose recommendations for young children can be derived. Therefore, this approach is recommended instead of empirical dose regimens. In addition, this clinical pharmacological approach can be used for patients with impaired renal- or hepatic function, like in **Chapter 1**, where carboplatin and cisplatin doses for a 3 year old boy with hepatoblastoma receiving peritoneal dialysis were individualised, and **Chapter 3**, where a clofarabine dosing algorithm was proposed that takes renal function into account.

Besides the described problems with empirical dosing regimens, the commonly used cut-off points for age or body size in dosing regimens for neonates and infants have their limitations as well. These dosing regimens often lead to stepwise increases in the dose as soon as a defined cut-off point in body weight or age is reached (**Chapter 2 and 4**). Growth and maturation is a gradual process, making this approach irrational. This problem was previously assigned by the Children's Oncology Group's Chemotherapy Standardization Task Force.[10] Using a theoretical framework, they suggested gradual transitions from body weight-based to BSA-based dosing by composing BSA dose banding dosing tables for infants and children with a BSA <0.6 m^2. It is recommended to think about this gradual process of growth and maturation while developing dosing guidelines for neonates and infants. Alternatives for these rough cut-off points could be development of dosing algorithms (**Chapter 3**) or dose banding based on multiple age or body size cut-off points.

Collection of (real life) data from young children is possible

In order to study the age-related differences in PK, it is preferred to use PK data of children of various ages. Inclusion of infants in PK studies can be challenging due to practical and ethical issues. To limit the invasiveness of procedures carried out to infants, blood volume limits for sampling have been established.[11] In order to minimise the number of samples per patient, population PK analyses can be performed, which allow the use of flexible and limited sampling schedules.[12] **Chapter 7** describes the development of a sensitive assay for the simultaneous quantification of aprepitant and dexamethasone. This assay only requires small sample volumes and is therefore especially suitable for PK studies in pediatric patients, because it minimises the amount of blood that needs to be sampled even further.

Since these sensitive assays are now available and limited sampling strategies can be used, there are no major practical obstacles for the inclusion of infants in PK studies anymore. Moreover, pediatric oncology patients usually have a central line in situ, that facilitates sampling and minimizes the patient burden. For this reason, a large prospective observational PK study, the PINOCCHIO-study (acronym for Pharmacokinetics of chemotherapeutic agents in children's oncology), was initiated in the Princess Máxima Center for Pediatric Oncology. This study aims to characterize age-related differences in PK of various chemotherapeutic agents in children and aims to include 10 infants per compound using a limited sampling schedule. Formal sample size calculations for explorative PK studies cannot be performed, therefore the sample size of 10 infants for the PINOCCHIO-study was based on previous experience with vulnerable patient populations. We have observed that inclusion of 10 infants for commonly used drugs is feasible in a national center for pediatric oncology like the Princess Máxima Center. To date, this PK-study continues to include patients. It was experienced that parents of (young) children are very willing to participate in this study, another demonstration that inclusion of young children in PK studies is possible. Moreover, the fact that it is possible to include infants is emphasized in **Chapter 4**, where 25 patients under the age of 1 year were included. This large number of infants could be reached due to collaboration with other hospitals and the use of historical, previously published data.

When studying the PK of (marketed) drugs in young children (more specifically, infants), it is recommended to develop sensitive quantification assays that require small samples volumes and to use limited sampling strategies.

In depth pharmacokinetic modelling is essential to translate pharmacokinetic data into practical (dose) recommendations

In order to obtain a complete description of the PK of a drug, in depth PK modelling is essential. The first option for in depth modelling is to incorporate prior clinical pharmacological knowledge in empirical population PK models, as shown in **Chapter 3, 4 and 9**, leading to so called semi-mechanistic PK models. These models try to approach the real life situation as well as possible and give, therefore, better results than reached with empirical modelling. For example, prior knowledge on drug absorption (**Chapter 8**), distribution (**Chapter 4**), metabolism (**Chapter 9**) and elimination (**Chapter 3**) was incorporated in PK models in this thesis. Adequate results were achieved using this approach, so this is therefore recommended for all future studies where sufficient data is available.

The second option for in depth modelling, is to use physiologically based (PB) PK models (**Chapter 5**). Where population PK models are data driven and describe the PK of the included population, mechanistic PBPK models are based on a broader understanding of the human body and its mechanisms and can predict the PK in a population based on extrapolation. Prior information on for example protein binding partners, transport proteins, metabolizing enzymes and renal clearance can be specified. Another advantage of PBPK

modelling is that it can be done without or with limited PK data. A PBPK study can be used to mechanistically investigate the PK of a drug. It can therefore be conducted either before start of a PK trial, where PBPK models can for example support in deciding the first-in-child dose of a new drug or the sample size of a population PK study. Furthermore, it can be conducted when (limited) data are available, to assist in finding age-related differences in PK and verification of developed PBPK models in children. The added value of PBPK models has been acknowledged by regulatory authorities like European Medicines Agency (EMA) and Food and Drug Administration (FDA).[13,14] Therefore, these models are more and more used in the marketing authorization process of new drugs and in drug development in pediatric oncology.[15–17]

A synergic effect can be reached by combining population PK models with PBPK models (**Chapter 4 and 5**). By merging the information that both types of models generate, a most accurate representation of the PK of a drug can be achieved. This is especially recommended for populations in which PK studies are difficult to conduct, like very young children.

That in depth PK modelling transcends empirical PK modelling is clear. It can, however, be very hard to apply model-based PK parameters to clinical practice. Therefore, we recommend to translate these PK parameters into practical (dose) recommendations. The feasibility of this approach was shown in **Chapter 3, 4, 6, 8 and 9**. Practical dose recommendations, a simple dosing algorithm and support for an alternative drug formulation were developed using in depth PK modelling approaches. By providing practical (dose) recommendations, PK studies will impact clinical practice and improve multidisciplinary collaboration between (hospital) pharmacists and pediatric oncologists.

Recommended work-flow for studying the PK of marketed drugs in neonates and infants

As pointed out in this thesis, it is very important to use evidence-based dosing regimens rather than empirical dosing regimens for young children. This is especially important in pediatric oncology, because of the delicate balance between efficacy and toxicity. In order to derive practical dosing recommendations for marketed drugs, for which empirical dosing regimens are common practice, the PK can (and should) be studied.

Based on the key points of this thesis, a work-flow for studying the PK of marketed drugs in young children is suggested. This work-flow is inspired by the learn-confirm cycle by Sheiner.[18] The goal of the learn phase is a mechanistic understanding of dose-exposure relationships and the goal of the confirm phase is to demonstrate this dose-exposure relationship in a representative cohort. The purpose of the learn-confirm cycle is to inform dosing decisions, with the aim of safe and effective therapy, in order to increase the survival rates. This learn-confirm cycle was made applicable for studying the PK of marketed drugs in young children and is displayed in **Figure 1**. It is recommended to firstly mechanistically 'learn' about the

PK of a drug, using prior clinical pharmacological knowledge, developmental changes in young children and PBPK modelling techniques ❶. Subsequently, an assay for quantification of the drug, suitable for pediatric studies, should be developed ❷, whereafter a PK study can be conducted. The results of the learn phase can be 'confirmed' by advanced population PK analyses that also include prior PK knowledge ❸, in order to derive practical dose recommendations for the specific population ❹. These recommendations are dependent on the drug, and could represent precision dosing per patient (for example by adjusting the dose according to therapeutic drug monitoring or biomarkers) or dosing algorithms according to, for example, body size. The recommendations can subsequently be evaluated in clinical practice in order to continuously improve treatment of patients. Furthermore, if necessary, due to new information or unexpected findings in the confirm phase, the cycle can be gone through again by going back to the learn step ❺. In exceptional situations, the learning phase can be sufficient. Examples of such situations are drugs with very little variation in PK, drugs for which the PK is easy to predict (like monoclonal antibodies) or drugs for which biomarkers or pharmacodynamic parameters can be used to adjust the therapy.

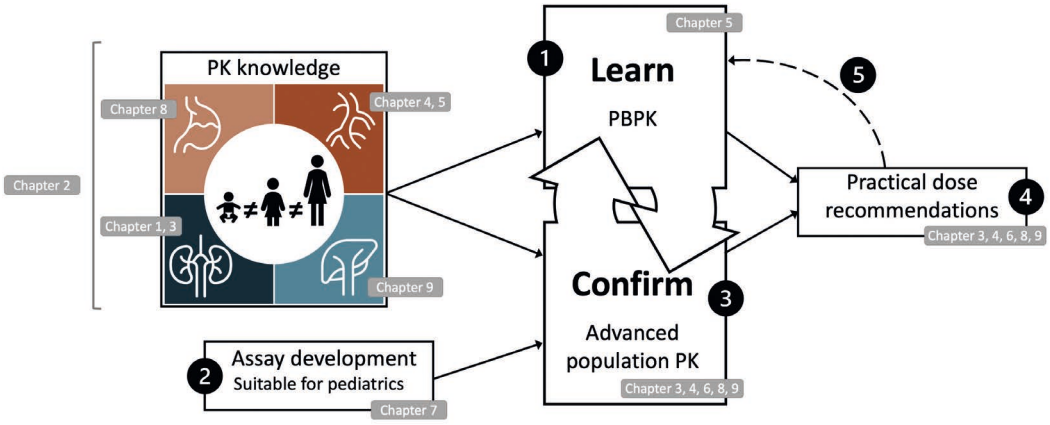


Figure 1. Recommended 'learn-and-confirm' work flow for studying the pharmacokinetics (PK) of marketed drugs in infants. *PBPK* physiologically based pharmacokinetics

CONCLUDING REMARKS

This thesis describes the pharmacological dose optimization in children with cancer. By combining various PK modelling approaches and including prior PK knowledge in the models, practical (dose) recommendations that can be used in clinical practice were given. In addition, a recommended work flow for studying the PK of marketed drugs in young children is suggested in **Figure 1**.

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Summary

SUMMARY

Dosing of (chemotherapeutic) agents in pediatric patients with cancer can be challenging. An optimal exposure to chemotherapeutic and anti-emetic agents is needed, either to be effective, and on the other hand, to minimize toxicity. Developmental changes can influence drug absorption, distribution, metabolism and excretion (ADME), and should, therefore, structurally be considered in pediatric dosing. This thesis focused on age-related differences in pharmacokinetics (PK) and pharmacological dose optimization of chemotherapeutic (**Part I**) and anti-emetic (**Part II**) agents in (young) children with cancer. Several PK modelling techniques were used to study the PK of five chemotherapeutic and two anti-emetic agents in children.

PART I - Pharmacokinetics of chemotherapeutic agents in pediatric patients
In **Chapter 1** the case of a pediatric patient with hepatoblastoma is described. A three year old boy with pre-existing end-stage renal disease on peritoneal dialysis, required treatment with cisplatin and carboplatin. The area under the concentration time curve (AUC) of both agents was measured and doses were optimized for the subsequent cycles. This approach led to adequate exposures, acceptable clinical tolerance and complete remission after 6 months. This chapter shows that it is feasible to measure PK exposure for cisplatin and carboplatin and to individualize the dose of these drugs according to the PK results and clinical parameters. Furthermore, it underlines the relevance of PK research in pediatric patients, which is the overarching theme of this thesis.

Clinical pharmacological evidence for the use of chemotherapeutic agents in neonates and infants is summarized in **Chapter 2**. A systematic review was conducted and available data was ranked by a level of evidence. A grade of recommendation for each agent was provided on a consensus basis, together with recommended dosing approaches indicated as appropriate. Practical, evidence based dosing guidance for neonates and infants was provided for 17 chemotherapeutic agents. For 12 agents, evidence based dosing guidance could not be provided due to lacking evidence.

Chapter 3 discusses the population PK of clofarabine in 80 pediatric patients. Pediatric patients were treated with clofarabine as part of the conditioning regimen for allogeneic hematopoietic stem cell transplantation (HCT). Previously, it has been shown that PK exposure of different agents commonly used in conditioning regimens is strongly related to HCT outcome. Therefore, an attempt to minimize the variability in clofarabine exposure was made. Body weight and estimated glomerular filtration rate (eGFR) appeared as important covariates influencing clofarabine clearance and were included in a clofarabine dosing algorithm. Dosing with this algorithm resulted in a more predictable exposure than the current body surface area (BSA) based dosing.

In **Chapter 4**, a semi-mechanistic population PK approach was used to study the PK of vincristine in children. Vincristine is known for its dose-dependent peripheral neuropathy, which might be more prevalent in older patients. Vincristine's saturable binding to β-tubulin was incorporated in the PK model and age-related differences in vincristine binding to β-tubulin were studied. Body weight and age were covariates significantly influencing the maximal binding capacity to β-tubulin, which increased with increasing body weight and decreased with increasing age. This suggests that young children might tolerate higher doses of vincristine as compared to adolescents and adults. An association between thrombocyte levels and the vincristine binding capacity to β-tubulin could not be found.

In follow-up to Chapter 4, a physiologically based PK model of vincristine is presented in **Chapter 5**. In this chapter, the hypothesis of vincristine binding to blood cells was evaluated. The model, including vincristine binding to blood cells, adequately described the PK of vincristine. A higher binding capacity of blood- and tissue-β-tubulin for infants, children and adolescents (2.5-, 2.0- and 1.5-fold, respectively) as compared to adults was necessary in order to adequately describe the PK in these populations. A higher binding capacity to β-tubulin leads to a more pronounced initial distribution phase of vincristine. Reduced amounts of free vincristine in the central compartment will potentially lead to a lower risk of peripheral neuropathy, since it is assumed that free vincristine is able to distribute to peripheral tissue, where it causes peripheral neuropathy. The higher binding capacity of vincristine to β-tubulin in (young) children might explain the fact that children are able to tolerate higher relative doses of vincristine and the need for vincristine dose capping in adults.

Chapter 6 evaluated the doxorubicin exposure in young children using a population PK modelling approach. Several doxorubicin dosing regimens for young children were compared and doxorubicin AUCs were calculated. Doxorubicin exposure using the current mg/kg regimen leads to a slightly lower exposure in young patients than achieved with the corresponding dose in older patients, but recent attempts for optimizing the dose did not give better results. Age-related differences in the PK of doxorubicin could not be found, but younger children might be more susceptible for cardiotoxicity, so increasing the current dose for younger children is not recommended, unless a clinical-safety study shows that this is possible.

PART II - Pharmacokinetics of anti-emetic agents in pediatric patients

In **Chapter 7**, the development of a combined liquid chromatography tandem-mass spectrometry assay for the simultaneous quantification of aprepitant and dexamethasone is presented. The method was validated according to the EMA and FDA guidelines on bioanalytical method validation over a linear range of 0.1-50 ng/mL for aprepitant and 1-500 ng/mL for dexamethasone. Samples exceeding the upper limit of quantification could be diluted 100 times. Stability experiments showed that the compounds are stable in the biomatrix for 25h at room temperatures and 89 days at -20°C. This assay was considered

suitable for PK studies and was used to study the PK of aprepitant and dexamethasone in pediatric patients in Chapter 8 and 9.

Chapter 8 presents the PK parameters of a simple extemporaneous oral suspension of aprepitant. The commercial (powder for) suspension was not available worldwide for a prolonged period of time and, therefore, a 10 mg/mL aprepitant oral suspension was extemporarily prepared to prevent suboptimal anti-emetic treatment. The bioavailability of the extemporaneous oral suspension was not significantly different to that of the capsules. The absorption of the extemporaneous oral suspension was slightly faster than that of capsules, but was comparable to that of the commercial oral suspension. An adequate exposure to aprepitant in children was yielded and, therefore, this extemporaneous oral suspension was considered an adequate alternative for the commercially (un)available oral suspension in young children.

The effect of aprepitant on dexamethasone PK in children, was investigated in **Chapter 9**. An integrated dexamethasone and aprepitant PK model was developed in order to investigate the drug-drug interaction between these agents. Aprepitant reduced dexamethasone clearance by approximately 30% in children receiving intravenously dexamethasone. This result is not consistent with the results of adult studies, where a 50% reduction was observed. This difference was not age-dependent, but might be related to the route of administration of dexamethasone. Therefore, it is advised to reduce the dexamethasone dose by 30% instead of 50%, when dexamethasone is given intravenously as a component of triple therapy to prevent chemotherapy induced nausea and vomiting in children.

In summary, this thesis describes the pharmacological dose optimization of chemotherapeutic agents and anti-emetics in pediatric patients. Practical dosing recommendations for multiple agents were formulated. Moreover, this thesis points out that, also in pediatric oncology, children are not small adults, so clinical pharmacological knowledge should be included in clinical practice to optimise the dose of (chemotherapeutic) agents. Furthermore, collection of (real life) data from young children is possible and can be used for in depth PK modelling, which is essential to translate PK data into practical (dose) recommendations. A recommended work-flow for studying the PK of marketed drugs in neonates and infants was proposed, which forms the basis for further optimization of dosing guidelines of (chemotherapeutic) agents in pediatric oncology.

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Het juist doseren van (chemotherapeutische) middelen bij kinderen met kanker kan een uitdaging zijn. Een optimale blootstelling aan chemotherapeutica en anti-emetica is nodig, enerzijds om effectief te zijn, anderzijds om het risico op toxiciteit zo laag mogelijk te houden. Ontwikkeling van het lichaam in de eerste levensjaren van een kind kan absorptie, distributie, metabolisme en excretie (ADME) van een geneesmiddel beïnvloeden en moet daarom structureel in overweging genomen worden bij het doseren van kinderen. Dit proefschrift focust op leeftijdsafhankelijke verschillen in farmacokinetiek (PK) en farmacologische dosisoptimalisatie van chemotherapeutica (**Deel I**) en anti-emetica (**Deel II**) bij (jonge) kinderen met kanker. Verschillende PK modelleer technieken zijn gebruikt om de PK van vijf chemotherapeutica en twee anti-emetica bij kinderen te bestuderen.

DEEL I – Farmacokinetiek van chemotherapeutica bij kinderen

In **Hoofdstuk 1** wordt de casus van een kind met hepatoblastoom beschreven. Een driejarige jongen met pre-existent eindstadium nierfalen met peritoneaal dialyse, moest behandeld worden met cisplatin en carboplatin. De blootstelling aan beide middelen is gemeten en de doseringen zijn geoptimaliseerd voor de opeenvolgende cycli. Deze aanpak leidde tot adequate blootstelling, acceptabele klinische tolerantie en complete remissie na zes maanden. Dit hoofdstuk laat zien dat het haalbaar is om de PK blootstelling aan cisplatin en carboplatin te meten en dat het mogelijk is om de doseringen te individualiseren op basis van de PK resultaten en klinische parameters. Bovendien onderstreept het de relevantie van PK onderzoek bij pediatrische patiënten, het overkoepelende thema van dit proefschrift.

Klinisch farmacologisch bewijs voor het gebruik van chemotherapeutica bij neonaten en zuigelingen is samengevat in **Hoofdstuk 2**. Een systematische review werd uitgevoerd en de beschikbare data werd gerangschikt op basis van het niveau van bewijskracht. Op consensusbasis werd elk middel van een aanbevelingsgraad voorzien, samen met de aanbevolen doseringsadviezen, wanneer deze gegenereerd konden worden. Voor 17 chemotherapeutica werden praktische, *evidence-based* doseringsrichtlijnen voor neonaten en zuigelingen gegeven. Voor 12 middelen konden geen doseringsrichtlijnen worden verstrekt vanwege gebrek aan bewijs.

Hoofdstuk 3 beschrijft de populatie PK van clofarabine bij 80 kinderen. Deze kinderen werden behandeld met clofarabine als onderdeel van het conditioneringsregime voor allogene hematopoëtische stamceltransplantatie (HCT). Eerder is aangetoond dat PK blootstelling van verschillende middelen, gebruikt in conditioneringsregimes, sterk gerelateerd is aan HCT uitkomst. Om die reden werd er een poging gedaan om de variabiliteit in clofarabine blootstelling te verminderen. Lichaamsgewicht en nierfunctie bleken belangrijke covariaten die de clofarabine klaring beïnvloedden en werden opgenomen in een clofarabine doseringsalgoritme. Doseren volgens dit algoritme resulteerde in een

meer voorspelbare blootstelling dan de huidige, op het lichaamsoppervlak gebaseerde, dosering.

In **Hoofdstuk 4** werd een semi-mechanistisch populatie PK model ontwikkeld om de PK van vincristine bij kinderen te bestuderen. Vincristine staat bekend om dosisafhankelijke perifere neuropathie, die mogelijk vaker voorkomt bij oudere patiënten. De verzadigbare binding van vincristine aan β-tubuline werd opgenomen in het PK model en leeftijdsafhankelijke verschillen in vincristine binding aan β-tubuline werden bestudeerd. Lichaamsgewicht en leeftijd waren covariaten die de maximale bindingscapaciteit aan β-tubuline significant beïnvloedden. De maximale bindingscapaciteit nam toe met toenemend lichaamsgewicht en af met toenemende leeftijd. Dit suggereert dat jonge kinderen hogere doses vincristine kunnen verdragen in vergelijking met adolescenten en volwassenen. Een associatie tussen trombocytengetal en de bindingscapaciteit aan β-tubuline kon niet worden gevonden.

In vervolg op Hoofdstuk 4 werd een fysiologisch gebaseerd PK model van vincristine beschreven in **Hoofdstuk 5**. In dit hoofdstuk werd de hypothese van de binding van vincristine aan bloedcellen geëvalueerd. Toevoeging van binding van vincristine aan bloedcellen verbeterde het model. Een hogere bindingscapaciteit van bloed- en weefsel-β-tubuline voor zuigelingen, kinderen en adolescenten (respectievelijk 2.5, 2.0 en 1.5 keer zo hoog) in vergelijking met volwassenen was nodig om de PK in deze populaties adequaat te beschrijven. Een hogere bindingscapaciteit aan β-tubuline leidt tot een snellere daling van de vincristine plasmaconcentratie. Verminderde hoeveelheden vrij vincristine in het centrale compartiment kunnen leiden tot een lager risico op perifere neuropathie, aangezien wordt aangenomen dat vrij vincristine zich kan verspreiden naar perifeer weefsel, waar het perifere neuropathie veroorzaakt. De hogere bindingscapaciteit van vincristine aan β-tubuline bij (jonge) kinderen kan een verklaring zijn voor het feit dat kinderen hogere relatieve doses vincristine kunnen verdragen en voor de noodzaak van het afkappen van de vincristine dosis bij volwassenen.

Hoofdstuk 6 evalueerde de blootstelling aan doxorubicine bij jonge kinderen met behulp van een populatie PK model. Verschillende doseringsschema's voor doxorubicine voor jonge kinderen werden vergeleken en de blootstelling aan doxorubicine werd berekend. Blootstelling aan doxorubicine met het huidige mg/kg-regime leidt bij jonge patiënten tot een iets lagere blootstelling dan bij oudere patiënten met een corresponderende dosis, maar recente pogingen om de dosis voor jonge kinderen te optimaliseren gaven geen betere resultaten. Er konden geen leeftijdsafhankelijke verschillen in de PK van doxorubicine worden gevonden, maar jongere kinderen zijn mogelijk vatbaarder voor cardiotoxiciteit, dus het verhogen van de huidige dosis voor jongere kinderen wordt niet aanbevolen, tenzij uit een klinisch veiligheidsonderzoek blijkt dat dit mogelijk is.

DEEL II - Farmacokinetiek van anti-emetica bij kinderen

In **Hoofdstuk 7** wordt de ontwikkeling van een vloeistofchromatografie tandem-massaspectrometrie (LC-MS/MS) assay voor de gelijktijdige bepaling van aprepitant en dexamethason plasmaconcentraties beschreven. De methode werd gevalideerd volgens de EMA- en FDA-richtlijnen voor de validatie van bioanalytische methoden over een lineair bereik van 0.1-50 ng/mL voor aprepitant en 1-500 ng/mL voor dexamethason. Monsters die de bovengrens van het bereik overschrijden, kunnen 100 keer worden verdund. Stabiliteitsexperimenten toonden aan dat beide middelen stabiel zijn in de biomatrix gedurende 25 uur bij kamertemperatuur en 89 dagen bij -20°C. Deze assay werd geschikt geacht voor PK onderzoek en werd gebruikt om de PK van aprepitant en dexamethason bij kinderen te bestuderen in Hoofdstuk 8 en 9.

Hoofdstuk 8 beschrijft de PK parameters van een eenvoudige geïmproviseerde orale suspensie van aprepitant. De commerciële (poeder voor) suspensie was gedurende lange tijd wereldwijd niet verkrijgbaar, dus daarom werd een 10 mg/mL aprepitant suspensie voor oraal gebruik geïmproviseerd om suboptimale anti-emetische behandeling te voorkomen. De biologische beschikbaarheid van de geïmproviseerde orale suspensie was niet significant anders dan die van de capsules. De absorptie van de geïmproviseerde suspensie was iets sneller dan die van capsules, maar was vergelijkbaar met die van de commerciële suspensie. Een adequate blootstelling aan aprepitant in kinderen werd bereikt en daarom werd deze geïmproviseerde orale suspensie beschouwd als een geschikt alternatief voor de niet beschikbare commerciële orale suspensie bij jonge kinderen.

Het effect van aprepitant op dexamethason PK bij kinderen is onderzocht in **Hoofdstuk 9**. Een geïntegreerd dexamethason en aprepitant PK model werd ontwikkeld om de geneesmiddel interactie tussen deze middelen te onderzoeken. Aprepitant verminderde de klaring van dexamethason met ongeveer 30% bij kinderen die intraveneus dexamethason kregen. Dit resultaat komt niet overeen met de resultaten bij volwassenen, waar een vermindering van 50% werd waargenomen. Dit verschil was niet leeftijdsafhankelijk, maar kan gerelateerd zijn aan de toedieningsweg van dexamethason. Daarom wordt geadviseerd om de dosis dexamethason met 30% te verlagen in plaats van 50%, wanneer dexamethason intraveneus wordt toegediend als onderdeel van drievoudige therapie om misselijkheid en braken veroorzaakt door chemotherapie bij kinderen te voorkomen.

Samenvattend beschrijft dit proefschrift de farmacologische dosisoptimalisatie van chemotherapeutica en anti-emetica bij kinderen. Er werden praktische doseringsaanbevelingen gegeven voor meerdere middelen. Daarnaast wijst dit proefschrift erop dat, ook in de kinderoncologie, kinderen geen kleine volwassenen zijn, en dat klinisch farmacologische kennis gebruikt moet worden in de klinische praktijk om de dosering van (chemotherapeutische) middelen te optimaliseren. Bovendien is het

verzamelen van (*real-life*) data van jonge kinderen mogelijk en kan deze worden gebruikt voor geavanceerde PK modellering, wat essentieel is om PK resultaten te vertalen naar praktische (dosis)aanbevelingen. Een aanbevolen stroomschema voor het bestuderen van de PK van reeds op de markt gebrachte geneesmiddelen bij neonaten en zuigelingen werd voorgesteld, welke de basis vormt voor verdere optimalisatie van de doseringsrichtlijnen van (chemotherapeutische) middelen in de kinderoncologie.

Appendices

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CURRICULUM VITAE

Laura Nijstad was born on 21 May 1991 in 's-Gravenhage, the Netherlands. After graduating from secondary school (gymnasium) at the Dalton Den Haag, she started studying Pharmacy at Rijksuniversiteit Groningen in 2009. As part of her master program, she performed a research internship titled 'Effect of acute and chronic amphetamine administration on D2-receptors, μ-opioid receptors and TSPO in rat brain: an autoradiography study' at Karolinska Institutet in Stockholm, Sweden. During her study, Laura was active in several student organizations and acted as a board member of A.S.V. Dizkartes for a full year.

After graduating as a pharmacist in 2016, Laura started her career in hospital pharmacy at University Medical Center Utrecht, where she was accepted into the hospital pharmacy residency program, in combination with a PhD research project. After completing her residency in 2020, she continued with her PhD research project at the Department of Clinical Pharmacy (prof. dr. Alwin Huitema and dr. Arief Lalmohamed), in close collaboration with the Princess Máxima Center for Pediatric Oncology (prof. dr. Michel Zwaan). During her PhD, she worked on the prospective observational PINOCCHIO-study and the aprepitant PK pilot study, investigating the pharmacokinetics of chemotherapeutic and anti-emetic agents in pediatric patients. During the hospital pharmacy residency and PhD research, she also trained as a clinical pharmacologist.