The role of glomerular filtration and active tubular secretion in predicting renal clearance of drugs in children using population pharmacokinetic and physiology-based pharmacokinetic modeling approaches: unspinning the yarn

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Section V. Summary, conclusion, and perspectives

Chapter 7

Summary, conclusion, and perspectives

7.1 Exploring renal clearance in children

Differences in size and physiological development between adults and children are known to influence several aspects of drug disposition. This thesis focuses on the disposition of renally excreted drugs, which, among others, relates to changes in kidney size, number of glomeruli and of proximal tubule cells and transporter expression. As introduced in **chapter 1**, this thesis explores how the changes in size and physiology throughout the pediatric age-range influence the contribution of glomerular filtration (GF) and active tubular secretion (ATS) to renal clearance (CL_R) using both population pharmacokinetic (popPK) and physiologically-based pharmacokinetic (PBPK) approaches. The extent to which these developmental changes impact CL_R and, subsequently, drug dosing, was explored in pediatric populations either for existing drugs using clinical data or for hypothetical drugs with an array of different properties excreted by either GF or both GF and ATS to meet the following research objectives:

1. Further development of population pharmacokinetic models by characterizing the maturation in CL_R for antibiotics (i.e. amikacin, vancomycin) mainly excreted by GF in (pre)term neonates and quantify the influence of disease and co-therapy on CL_R. These models are subsequently used to propose dosing recommendations for the antibiotics administrated to these special populations (**section II**).
2. Establish a general scaling method for CL_R from adults to children for drugs eliminated by GF and systematically investigate how maturation of plasma protein concentrations influence the unbound fraction of drugs, and subsequently, scaling of pediatric CL_R and drug doses (**section III**).
3. Use a pediatric PBPK-based model for CL_R to systematically investigate the influence of transporter ontogeny on the contribution of ATS to CL_R and illustrate how a combined population PBPK approach could be used to derive in vivo ontogeny functions for renal transporters involved in ATS (**section IV**).

7.2 Population PK modelling to guide dosing of renally excreted drugs in preterm neonates

To meet our first objective, in **section II** (**chapters 2** and **3**) PK data were used to build covariate models that explain inter-individual variability and capture changes in PK parameters related to development, co-therapy, and disease status.

In **chapter 2**, amikacin PK was studied in neonates with perinatal asphyxia treated with therapeutic hypothermia (TH). Perinatal asphyxia is expected to have an impact on amikacin PK. Therefore, we quantified the differences in amikacin PK between neonates with and without perinatal asphyxia using popPK modelling, to propose suitable dosing recommendations. To this end, PK data for amikacin collected retrospectively from routine therapeutic drug monitoring of neonates with perinatal asphyxia during TH was combined with a previously published amikacin PK dataset in (pre)term neonates without other co-therapy to assess the impact of perinatal asphyxia with TH on amikacin PK. Subsequently, model simulations were performed to establish amikacin exposures in neonates with perinatal asphyxia during TH after dosing according to the current guidelines and according to proposed model-derived dosing guidelines. Peak and trough plasma concentrations were used as a measure of efficacy and safety. Peak levels within 24-35 mg/L and trough levels strictly under 5 mg/L were aimed for to ensure a safe and effective treatment. Amikacin clearance was found to be decreased by 40% in neonates with perinatal asphyxia with TH, with no changes in volume of distribution. Simulations showed that, increasing the dosing interval with 12 hours results in a decrease in percentage of neonates reaching toxic trough levels (> 5 mg/L) from 40–76% to 14–25%, while still reaching target peak concentrations, as compared to current dosing regimens. The range in percentage represents the maximum percentage of patients reaching toxic levels obtained with different dosing regimen and for different weight groups.

In **chapter 3** dose adjustments were proposed for vancomycin when administrated together with either

ibuprofen or indomethacin. Both are used to induce patent ductus arteriosus (PDA) closure in (pre)term neonates. Previously, a popPK model for vancomycin co-administrated with ibuprofen was developed for (pre)term neonates (suspected) of sepsis and PDA. In that analysis, co-administration of ibuprofen for PDA was found to reduce vancomycin clearance by 16%. PK data of vancomycin administered with indomethacin was collected and added to the existing modelling dataset. In the current analysis, co-administration of indomethacin was found to decrease vancomycin clearance by 55%. The updated vancomycin popPK model was used to revise and propose dose adjustments that yield effective vancomycin exposure (i.e. $AUC_{0\text{-}24h}$ between 300-550 mg·h/L) in preterm neonates with PDA treated with ibuprofen or indomethacin. Model simulations showed that, as compared to a dosing regimen for vancomycin in neonates without co-administration of ibuprofen or indomethacin, a 20% and 60% decrease of the loading and maintenance dose of vancomycin, respectively, is required when aiming for optimized exposure in the neonatal population with PDA treated with either ibuprofen or indomethacin, respectively.

Both amikacin and vancomycin are eliminated mainly by GF. Previously, a covariate model for amikacin has been used to describe the maturation of CL_R in neonates for other antibiotics mainly cleared by GF (i.e. gentamycin, tobramycin) [1]. Therefore, it is likely that the CL_R of other drugs cleared by GF could be impaired by perinatal asphyxia during TH or co-administration of NSAIDs.

7.2.1 Key messages

- Population PK models can be used to describe the maturation of CLR resulting from all changes in underlying physiological processes.
- Based on CLR and the combined effect of disease and/or co-therapy on CLR, dose adjustments were derived for (pre)term neonates with perinatal asphyxia during TH or PDA treated with NSAIDs either by extending the dosing interval or reducing the dose, respectively.

7.3 PBPK-based dosing of GFR cleared drugs in children

In literature, the maturation of GFR throughout the entire pediatric age-range has been characterized by different functions. However, it has not been established yet which GFR maturation function predicts CL_R most accurately throughout the whole pediatric age-range. Therefore, in **section III** (**chapter 4**), different published GFR maturation functions were compared to measured levels of GFR markers (i.e. inulin and mannitol). For drugs eliminated by GF, CL_R is not only determined by GFR but also by unbound fraction of the drug in plasma. Therefore, the accuracy of pediatric CL_R scaling using the best GFR maturation function was assessed and compared to PBPK CL_R predictions for hypothetical drugs binding to varying extends to serum albumin or α-acid glycoprotein. Additionally, the accuracy of empiric bodyweight-based scaling methods was also assessed.

The published GFR maturation functions yielded comparable maturation profiles, with the function of Salem ***et al.*** [2] leading to the most accurate predictions across all ages. This function was used for PBPK-based predictions of pediatric PBPK CL_R values and it was directly used for simplified GFR-based scaling. This GFR-based scaling was found to systematically yield reasonably accurate (percentage prediction error ≤ 50%) pediatric CL_R values for all drugs cleared by this route, except in neonates for some drugs highly bound to AGP. Since the difference between pediatric PBPK CL_R values and CL_R obtained with GFR-based scaling is directly related to the maturation of f_u, these results also imply that after the neonatal period, the maturational changes in plasma protein concentrations have a minimal impact on CL_R scaling of GF excreted drugs. This means that a reliable measure of unbound drug fraction obtained in adults is enough to perform GFR-based scaling from adults to children for CL_R and dose. GFR-based scaling was overall more accurate than linear or 0.75 allometric bodyweight-based scaling.

As proposed in **chapter 3**, Table 3.2, CL_R predictions could be used to inform dosing for drugs eliminated by GF for typical pediatric patients of different ages as a percentage of the established adult dose. Nonetheless, these pediatric dose approximations should be carefully validated. Moreover, the limitations of the scaling method used to derive pediatric CL_R and the afferent dose should be well understood and clearly stated.

7.3.1 Key messages

- The most accurate maturation function for GFR throughout the whole pediatric age-range is quantified by Salem et al.[2].
- Knowing unbound drug fractions in adults is sufficient to use the GFR maturation function to scale CLR and dose from adults to children for drugs that are mainly cleared through GF.

7.4 Ontogeny of renal transporters and its impact on renal clearance in children

In addition to GF, other processes such as ATS, renal metabolism, and reabsorption, may also contribute to CL_R. In **section IV** (**chapter 5**) we focused on ATS, as this process remains understudied across the pediatric age-range. It has been reported before that the expression of renal transporters changes in children due to development [3]. Therefore, we systematically analyzed the influence of transporter ontogeny in children on the relative contribution of GF and ATS to CL_R for drugs with different properties. To do so, a PBPK-based model developed to obtain adult CL_R was extrapolated to the pediatric population by including maturation functions for the system-specific parameters. This model was used to predict GF and ATS for hypothetical drugs with a range of drug-specific properties, including transporter-mediated intrinsic clearance ($CL_{int,T}$) values, that are substrates for renal secretion transporters with different ontogeny patterns. The impact of transporter ontogeny on ATS and total CL_R was assessed using a % prediction difference calculated between the predicted CL_R in the presence and absence of transporter ontogeny. Transporter ontogeny was included as either a hypothetical fraction of adult activity or as a fraction of adult activity derived from reported pediatric expression profiles as measured for a few transporters (i.e. OAT1, OAT3, OCT2, Pgp) in pediatric kidney samples. Our analysis showed that the contribution of ATS to CL_R ranges between 41% and 90% in children, depending on f_u and $CL_{int,T}$ values. Predictions of CL_R are inaccurate for the majority of drugs that undergo ATS in the absence of transporter ontogeny, regardless of the pediatric age, if the real ontogeny of renal transporters is <0.2 of adult values. Ignoring ontogeny patterns for secretion transporters results in CL_R predictions that are not systematically accurate for all hypothetical drugs (% prediction discrepancy > 50% for some drugs) in children younger than 2 years.

Recently, Cheung **et al.** [3] published ontogeny functions for 8 renal transporters following direct measurements of protein expression specific for each transporter. According to this report, BCRP, MATE1, MATE2-K, and GLUT2 have expression levels similar to the adult throughout the studied pediatric age-range, whereas, the ontogeny of OAT1, OAT3, OCT2 and Pgp increases with increasing age [3]. As the ontogeny profiles of individual transporters are different, the contribution of these transporters to the ATS of a drug changes differently with age as well. OAT1 and OAT3 have a slow rate of ontogeny, reaching adult levels around 2 years of age, whereas expression of OCT2 and Pgp is fully developed after 3 and 6 months, respectively [3]. Disregarding ontogeny of transporters leads to over-predictions of CL_R in young patients. If these predictions were used as the basis for pediatric dose adjustments, they could lead to over exposure to drugs and to an increase in risk of toxic events.

Drugs that lead to high % prediction discrepancy could potentially be used as sensitive in vivo probes to derive transporter ontogeny and complement research similar to what has been performed by Cheung et al.. Following the proposed framework in **chapter 5**, the best probe drugs should have a $CL_{int,T}$ of 5-50 µL/min/mg protein and medium to high fraction unbound in adults (f_u = 0.55 – 0.95). Drugs for which GF is the main elimination pathway or drugs with a high enough $CL_{int,T}$ to have their elimination

limited by renal blood flow, will have a limited use in characterizing ontogeny profiles.

The ontogeny of different renal transporters has been quantified based on specific protein expression levels measured in a limited number of kidney samples. However, it remains unknown whether the ontogeny of protein levels reflects the ontogeny of ***in vivo*** transporter activity. Hence, in **section IV**, **chapter 6**, a combined population and physiologically-based PK modelling approach (popPBPK) was proposed to derive the transporters ontogeny ***in vivo***. To obtain the ontogeny function for OAT3, PK data on two probe drugs administrated simultaneously - clavulanic acid, which is mainly cleared by GF, and amoxicillin, which is mainly cleared by a combination of GF and ATS by OAT3- were used to differentiate between clearance through GF and OAT3-mediated ATS. First, individual post-hoc values for pediatric CL_R values for clavulanic acid and amoxicillin were obtained with a previously published popPK model developed for those data and these were used as dependent variables for the popPBPK approach. Then, CL_R was re-parameterized according to PBPK principles, using known maturation profiles for all system-specific parameters, while only leaving the OAT3-mediated intrinsic clearance ($CL_{int,OAT3,in\ vivo}$) and its ontogeny profile to be estimated. The estimated ontogeny function for OAT3 was included in a pediatric PBPK-based model for CL_R and used to scale CL_R of other OAT3 substrates (i.e. cefazolin, piperacillin). ***In vivo*** $CL_{int,OAT3}$ for these drugs in adults, was obtained following ***in vitro–in vivo*** extrapolation and adjusted by comparing adult PBPK CL_R predictions to literature values. Subsequently, pediatric PBPK CL_R values were compared to typical CL_R estimates, as obtained with published popPK models for each drug. As described by a sigmoidal Emax function based on PNA in weeks, $CL_{int,OAT3,in\ vivo}$ reached half of adult levels around 7 months of age. Estimating the ***in vivo*** ontogeny of OAT3 lead to similar results to those measured by Cheung **et al.** [3] as protein expression levels. The ontogeny of OAT3 between 1 month and 15 years was characterized, with a minimal quantified ontogeny of 0.1 of the adult value at 1 month and reaching adult values at 15 years. Adding the OAT3 ontogeny to the PBPK-based model yielded accurate CL_R predictions for cefazolin and piperacillin (%RMSPE of 21% and 12%).

With this popPBPK approach, $CL_{int,OAT3,in\ vivo}$ for amoxicillin in adults was estimated at 4.4 µl/min/mg protein which is in line with the published value of 4.3 µl/min/mg protein [5]. Judging by the $CL_{int,OAT3}$ value and f_u of 0.826, amoxicillin has the potential of quantifying OAT3 ontogeny in a popPBPK approach because according to **chapter 5** the best probe drugs should have a $CL_{int,T}$ of 5-50 µL/min/mg protein and medium to high fraction unbound in adults (f_u = 0.55 – 0.95). Here, since amoxicillin was given together with clavulanic acid, we were able to disentangle the two routes that contribute to CL_R (i.e. GF and ATS) and quantify the ontogeny of OAT3 activity for a broad pediatric age-range between 1 month and 15 years. With this methodology the ontogeny of renal transporters can be derived ***in vivo***, also for other transporter substrates for which data that allows the differentiation between GF and ATS is available. In addition, this methodology does not require direct kidney samples to quantify transporter ontogeny of remaining transporters.

7.4.1 Key messages

- Realistic combinations of f_u and $CL_{int,T}$ values lead to contributions of ATS to CL_R between 41% and 90% in children.
- If ontogeny of renal transporters is <0.2 of adult values, predictions of CL_R in the absence of transporter ontogeny are inaccurate for the majority of drugs, regardless of the pediatric age.
- The ***in vivo*** ontogeny of OAT3, estimated using population PBPK modelling on amoxicillin data in children, reaches half of adult levels of activity around 7 months of age.
- PBPK-based predictions based on this ***in vivo*** OAT3 ontogeny function were accurate for other OAT3 substrates such as cefazolin and piperacillin throughout the pediatric age-range.
- The proposed popPBPK approach could be used to derive ***in vivo*** transporter ontogeny of other renal transporters.

7.5 Perspectives

Based on the models and approaches developed in this thesis we have explored how these could be used to answer additional clinically relevant research questions. We focus on three specific topics in this perspectives section:

1. How predictive are trough concentrations as surrogates for vancomycin exposure, considering the correlation between vancomycin trough concentrations and exposure (i.e. AUC_{24h}) for different pediatric age groups and dosing regimens.
2. How well do empirical scaling methods based on bodyweight and GFR-based function perform for scaling CL_R of drugs that are not only cleared through GF (as described in **chapter 5**), but are also actively secreted by renal transporters.
3. How to estimate with the use of a combined population PK and PBPK approach parameters with high impact that are difficult to measure in children.

7.5.1 How predictive are vancomycin trough samples as a surrogate for exposure across age?

In **chapters 2** and **3**, we show how peak and trough levels (amikacin) or only trough levels (vancomycin) collected in clinical practice for therapeutic drug monitoring (TDM) are used as to ensure a safe and effective exposure to these antibiotics during treatment for (suspected) septicemia. In the case of vancomycin, trough levels are surrogates for AUC_{24h} which is the most predictive index for safe and/or effective exposures. However, the correlation between vancomycin trough levels and AUC_{24h} is assumed to remain constant across dosing regimens and all ages. In this analysis, this assumption is challenged by assessing how the correlation between trough levels and vancomycin exposure at 24 h (i.e. AUC_{24h}) changes for different pediatric patients treated with vancomycin following dosing guidelines that include different dosing frequencies and/or a loading dose.

The model presented in **chapter 3** is suitable for such an analysis and was used to simulate typical vancomycin PK profiles for 6 representative pediatric individuals: neonates of 14 days and gestational ages of 24, 34 and 40 weeks, and children of 6 months, 4 and 12 years of age following treatment with a recently validated dosing regimen [7] (Table 7.1). The relationships between the simulated vancomycin trough concentrations and corresponding AUC_{24h} were compared between dosing regimens with and without a loading dose (see Table 7.1) and for regimens with different dosing frequencies (i.e. for the dosing regimen in Table 7.1 the number of doses per day of the maintenance dose was changed to 4, 3 and 2, corresponding to dosing intervals of 6h, 8h and 12h, respectively). These relationships were compared at the end of the first day of treatment and at steady-state (after 7 days of treatment).

Table 7.1 Vancomycin dosing regimen for neonates and children aiming for a target AUC_{24h} of 300 - 500 mg·h/liter

Clinical characteristics			**Vancomycin Dosing [7]**	
PNA (days)	**BW (g)**	**CW (kg)**	**Loading Dose**	**Maintenance Dose***
0-7	≤700		16 mg/kg	15 mg/kg/day in 3 doses
	700-1000			21 mg/kg/day in 3 doses
	1000-1500			27 mg/kg/day in 3 doses
	1500-2500			30 mg/kg/day in 4 doses
8-14	≤700		20 mg/kg	21 mg/kg/day in 3 doses
	700-1000			27 mg/kg/day in 3 doses
	1000-1500			36 mg/kg/day in 3 doses
	1500-2500			40 mg/kg/day in 4 doses

Clinical characteristics			**Vancomycin Dosing [7]**	
PNA (days)	**BW (g)**	**CW (kg)**	**Loading Dose**	**Maintenance Dose***
14-28	≤700		23 mg/kg	24 mg/kg/day in 3 doses
	700-1000			42 mg/kg/day in 3 doses
	1000-1500			45 mg/kg/day in 3 doses
	1500-2500			52 mg/kg/day in 4 doses
21-28	≤700		26 mg/kg	24 mg/kg/day in 3 doses
	700-1000			42 mg/kg/day in 3 doses
	1000-1500			45 mg/kg/day in 3 doses
	1500-2500			52 mg/kg/day in 4 doses
>28		< 2.5	18 mg/kg	32 mg/kg/day in 4 doses
		2.5-5	24 mg/kg	40 mg/kg/day in 4 doses
		5-10	27 mg/kg	52 mg/kg/day in 4 doses
		> 10	30 mg/kg	60 mg/kg/day in 4 doses

*the maintenance dose was adapted for dosing frequencies in 2, 3, 4 doses in a day for frequencies of 12h, 8h, 6h, respectively

In addition, to assess the influence of inter-individual variability (IIV) in CL_R on the trough concentrations and corresponding AUC_{24h}, we performed stochastic simulations for the representative individuals treated with the vancomycin dosing regimen of Table 7.1 (more details on stochastic simulations see **chapter 3**) [7]. Briefly, for each representative individual we performed 1000 stochastic simulations with the model taking into account the IIV in CL_R. The simulated vancomycin concentration-time profiles were used to calculate the AUC_{24h}. The results are summarized for each representative individual for AUC_{24h} target intervals between 350-550 mg·h/L as well as for the commonly used > 400 mg·h/L (with a toxic level of 700 mg·h/L) [8] in Table 7.2.

Finally, to assess the influence of variability in demographic characteristics within the pediatric population on CL_R, Monte Carlo simulations were performed for the entire pediatric age-range using demographic characteristics from patients included in a previous study [9]. The probability of target attainment between 350-550 mg·h/L was calculated for each age-group (i.e. neonates, infants, children, adolescents). Briefly, a virtual pediatric population was created by resampling with replacement 1000 patients demographics from a previous study [9]. The model with IIV on CL_R was used to simulate vancomycin concentration-time profiles which served as basis to calculate the 24 h exposure (i.e. $AUC_{0\text{-}24h}$).

Table 7.2 - Results of stochastic simulations upon dosing according to table 1 (with loading dose) to understand the impact of inter-individual variability in CL on the 24h exposure and corresponding trough concentration at the end of day 1.

	Representative individuals	**Vancomycin $AUC_{0\text{-}24h}$ % within 300 – 550 mg·h/L**	**Vancomycin trough (mg/L) corresponding to $AUC_{0\text{-}24h}$ within 300 – 550 mg·h/L (median [min-max])**	**Vancomycin $AUC_{0\text{-}24h}$ % within 400 – 700 mg·h/L**	**Vancomycin trough (mg/L) corresponding to $AUC_{0\text{-}24h}$ within 400 – 700 mg·h/L (median [min-max])**
1	GA=24 weeks PNA=14 days	87%	11.2 [6.9 – 17.2]	55%	13.2 [10.7- 23.3]
2	GA=34 weeks PNA=14 days	84%	12.4 [7.3 – 18.1]	68%	14.2 [11.0- 25.9]
3	GA=40 weeks PNA=14 days	78%	12.1 [6.6 – 17.7]	60%	14.4 [10.8- 23.9]
4	PNA=6 months	70%	11.2 [6.1 – 16.5]	67%	14.3 [10.0- 23.7]
5	PNA=4 years	62.5%	10.3 [5.6 – 15.5]	66%	13.8 [9.31- 22.5]
6	PNA=12 years	65%	10.8 [5.6 – 15.6]	65%	13.2 [9.19- 21.2]

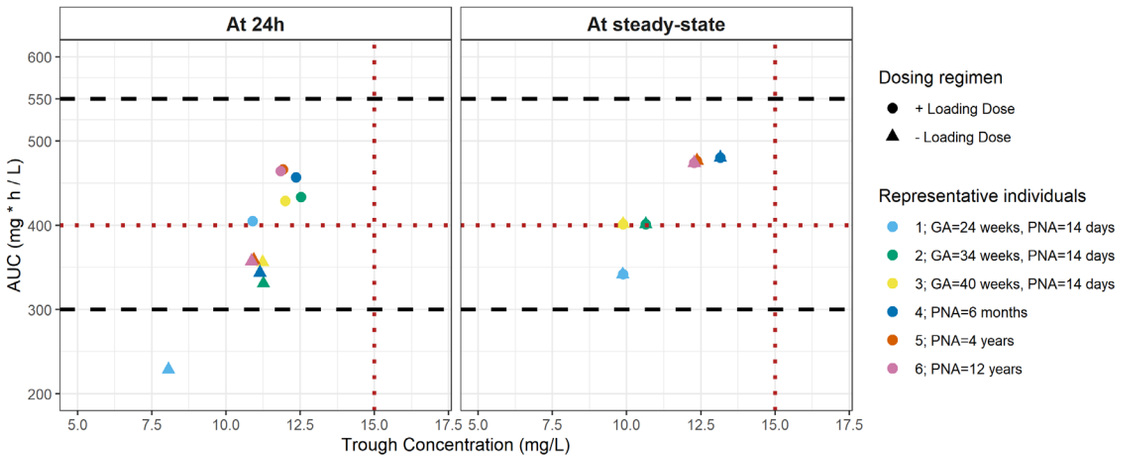


Figure 7.1 - AUC_{24h} vs. trough concentrations of vancomycin in the first day of dosing (left panel) and at steady-state (right panel) upon dosages according to Table 7.1. Different colors correspond to different ages. Symbols distinguish between the dosing regimen with (circles) and without (triangles) a loading dose (Table 7.1). Horizontal interrupted lines show the desired exposure thresholds, i.e. between 300 – 550 mg·h/L with black dashed lines, and 400 mg·h/L with a red dotted line. The vertical red dotted line marks the 15 mg/L trough concentration as previously suggested to correlate with an AUC_{24h} of >400 mg·h/L in adults.

Figure 7.1 shows the relationship between vancomycin trough and corresponding AUC_{24h} at the end of the first day of treatment following the dosing regimen in (Table 7.1) with or without administrating a loading dose. Trough levels are comparable between regimens with and without a loading dose, i.e. they vary between 11 – 12.5 mg/L and 8 – 11.3, respectively, with the lowest level attributed to the smallest preterm individual. Nonetheless, the corresponding AUC_{24h} is systematically higher for the regimen with a loading dose (AUC_{24h} > 400 mg·h/L for all representative individuals). Once steady-state is reached, there is no difference in the two exposure measures between the regimens with and without a loading dose. Regarding safety and efficacy, Figure 7.1 shows that all patients reach AUC_{24h} within the 300 – 550 mg·h/L in the first 24 h of vancomycin treatment except for the smallest preterm neonate (i.e. GA of 24 weeks) that did not receive a loading dose. Therefore, the efficacy of vancomycin treatment in preterm neonates could be improved by using the dosing regimen with a loading dose, particularly for small preterm neonates.

Figure 7.1 shows that following the vancomycin dosing in Table 7.1, trough levels between 8 and 13 mg/L correspond to effective AUC_{24h} levels in the representative individuals. By decreasing dosing frequency of the regimen without a loading dose, in Figure 7.2 we show that trough levels also decrease, while yielding similar AUC_{24h} for all typical pediatric individuals after the first day of vancomycin treatment. In this example the daily dose remains unchanged while dosing frequencies change to every 6h, 8h and 12h. At the end of the first day of treatment, while corresponding to similar AUC_{24h}, trough levels decrease from 9-11.3 mg/L to 7.5-8 mg/L by decreasing the dosing frequency from every 6 h (4 times

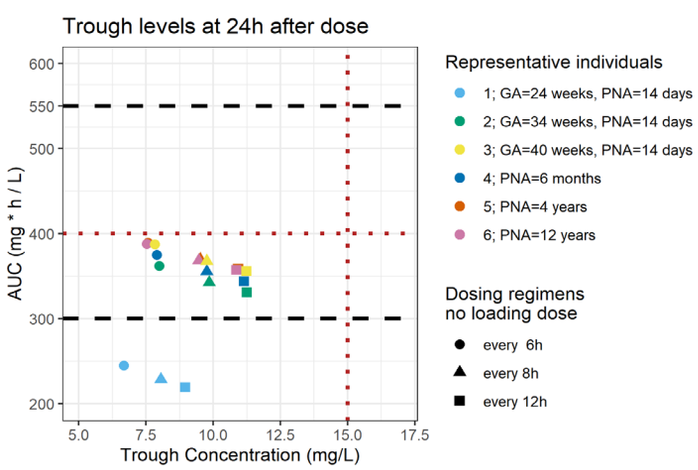


Figure 7.2 - AUC_{24h} vs. trough concentrations of vancomycin on the first day of dosing upon dosages according to Table 7.1 (without a loading dose) for different dosing frequencies represented by different symbols: squares for every 12 h, circles for every 8 h and triangles for every 6 hours. Different colors correspond to different ages. Horizontal interrupted lines show the desired exposure thresholds. i.e. between 300 – 550 mg·h/L with black dashed lines, and 400 mg·h/L with a red dotted line. The vertical red dotted line marks the 15 mg/L trough concentration as previously suggested to correlate with an AUC_{24h} of >400 mg·h/L in adults.

daily) to every 12 h (2 times daily). Regarding safety and efficacy of treatment, all patients achieve AUC_{24h} within 300 – 550 mg·h/L in the first 24 h except the smallest preterm neonate that does not reach effective AUC_{24h} levels (Figure 7.2, results given for dosing regimen without a loading dose).

After including IIV on CL_R for the same representative individuals, the trough concentrations and corresponding AUC_{24h} resulting after treatment with the dosing regimen with loading dose (Table 7.5.1.1) were explored and compared to literature values. In literature, vancomycin trough levels of at least 15 mg/L [8] for adults and between 7-11 mg/L [10] for neonates were reported to be associated with an exposure above 400 mg·h/L. The results in Table 7.5.1.2 following stochastic simulations with the model developed in **chapter 3** show that, when aiming for an exposure between 400 and 700 mg·h/L, the median trough concentrations for both neonates and children is between 13.2 and 14.4 mg/L, which is higher than the interval recommended for neonates and closer to the recommended trough value for adults. Even when aiming for a lower AUC_{24h} (i.e. 300- 550 mg·h/L), representative neonates have a median trough concentration above 11 mg/L (between 11.2 – 12.1 mg/L). Moreover, the unexplained IIV on CL_R alone leads to very broad intervals for trough levels that correspond to an effective AUC_{24h} for each representative individual.

In addition to the IIV in CL_R, Monte Carlo simulations include also the variability coming from the distribution of patient demographics for different age groups. Figure 7.3 illustrates the probability of attaining an AUC_{24h} within 300 – 550 mg·h/L based on the trough levels in the end of day 1 of vancomycin treatment following the dosing regimen in Table 7.1 with a loading dose for 4 different age groups. In clinical practice, the trough concentration guides dose adjustments while aiming for a certain target AUC_{24h}. The figure shows that for all age groups, when trough levels are between 7.5 and 12.5 mg/L, the probability of reaching the target exposure is above 0.8. For all age groups, the probability of target attainment decreases when trough levels are above 15 mg/L (< 0.75 for all age-groups except neonates) because the probability of reaching AUC_{24h} values above 550 mg·h/L increases. This indicates an increased risk of adverse events. On a population level, neonates appear to have a high chance of reaching the target exposure for a broader range of trough levels (5 – 15 mg/L), as compared to the other age groups. Even though the range of possible trough levels that would results in an effective AUC_{24h} is broader, this does not imply that effective levels will always be achieved at the individual level. With increasing age, the trough levels indicating an effective exposure become narrower (7.5- 12.5 mg/L for adolescents). This could be explained by the decrease in IIV for CL_R in older children as compared to neonates. As compared to literature values, the trough levels obtained for neonates are similar to the published 7-11 mg/L, however, levels obtained for adolescents are below the reported value in adults of 15 mg/L.

As shown by the results of the stochastic and Monte Carlo simulations, a large range of trough concentrations corresponds to an effective AUC_{24h}. Therefore, in order to know whether a measured trough concentration indeed corresponds to the target AUC_{24h}, TDM samples should be obtained and analyzed with Bayesian software in order to obtain individual PK parameters and AUC_{24h}. To ensure safe and effective treatment despite the high inter-individual variability, TDM samples could be taken earlier (e.g. samples after the first dose) instead of the last trough of the first day of treatment. Furthermore, with the new data the Bayesian model can be refined over time. By individualizing therapy both bacterial resistance and risk for adverse events could potentially be reduced.

To summarize, vancomycin trough concentrations corresponding to effective exposure change with age and with dosing regimens (i.e. with and without loading dose or with different dosing frequencies). This aspect should be taken into account when performing TDM on trough levels to guide dosing. Correlating a trough level with an effective AUC_{24h} is challenging as it is difficult to establish cut-off values that would always correlate with a successful outcome. However, when dosing using the guidelines in Table 7.1 with loading dose a probability above 0.8 of reaching an effective AUC_{24h} between 300 – 550 mg·h/L

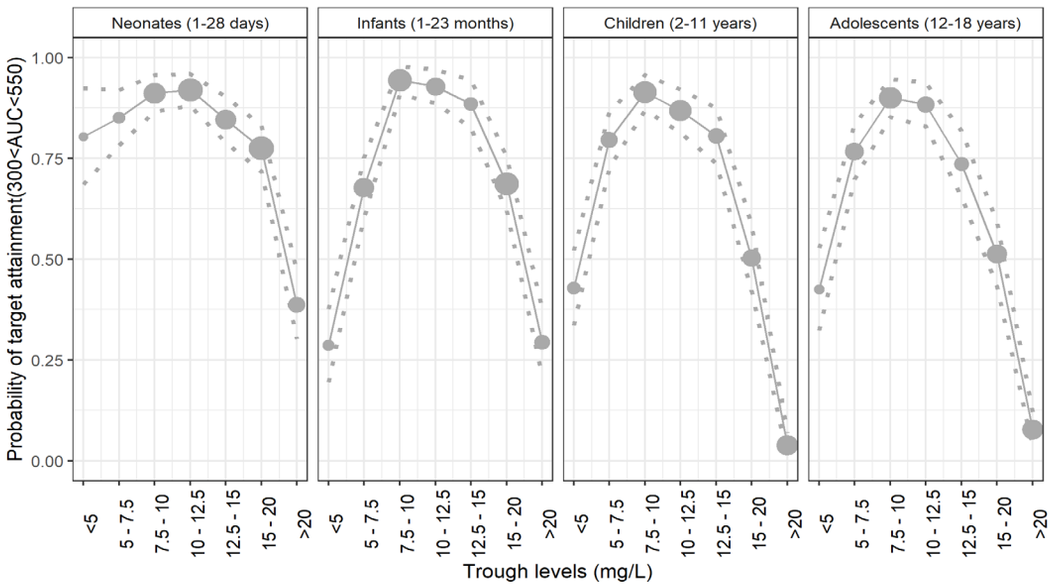


Figure 7.3 - Results of a Monte Carlo simulation for which the vancomycin dosing with loading dose was administrated according to Table 7.1. The circles show the probability of AUC_{24h} target attainment (between 300 – 550 mg·h/L) for each vancomycin trough concentration interval in different age groups on the first day of treatment. The 90% confidence interval of the probabilities is shown by the dotted line. The size of the circles is correlated to the number of individuals in each trough interval on the x-axis for which the probability is calculated.

corresponds to a trough range between 7.5 and 12.5 mg/L.

7.5.2 How can we scale CLR of drugs eliminated by GF and ATS?

In **section III, chapter 4**, we showed that CL_R of drugs exclusively eliminated by GF can be scaled accurately by using a GFR based function throughout the whole pediatric age range, except in neonates for drugs highly bound to AGP. Here, we use a similar pediatric PBPK framework as in **chapter 4** to determine whether scaling based on a GFR maturation function can be used for drugs eliminated by GF and ATS. For comparative purposes, the accuracy of this GFR-based scaling was evaluated together with linear bodyweight-based scaling and bodyweight-based allometric scaling with a fixed exponent of 0.75, two commonly used empirical pediatric CL_R scaling methods. Age-appropriate body surface area (BSA), height, and weight values were derived from the NHANES database [10] and used for pediatric PBPK CL_R predictions with ages ranging from term newborn to 15 years.

Since information about the ontogeny profiles of transporters is scarce in literature, different hypothetical ontogeny fractions and hypothetical drugs with different properties were used here to obtain the PBPK-based CL_R predictions, as described in detail in **section IV**, **chapter 5**. Briefly, pediatric PBPK CL_R predictions for 3800 hypothetical drugs which differ in type of binding plasma protein, fraction unbound, blood to plasma ratio, and transporter-mediated intrinsic clearance. Ontogeny levels were explored as relative ontogeny fractions to adult levels that remained constant throughout the pediatric age with the following values: 0.05, 0.2, 0.5, 0.7 and 1.

$$CL_R = CL_{GF} + CL_{ATS} = f_u \times GFR + \frac{(Q_R - GFR) \times f_u \times CL_{int,sec}}{Q_R + f_u \times \frac{CL_{int,sec}}{BP}} \quad [1]$$

where CL_{GF} and CL_{ATS} represent the clearance by GF and ATS, respectively and f_u is the fraction unbound, GFR is the glomerular filtration rate, QR is renal blood flow, BP is the blood to plasma ratio of the drug, and $CL_{int,sec}$ is the intrinsic secretion clearance of the active transporters.

$$CL_{int,sec} = CL_{int,T} \times ont_T \times PTCPGK \times KW \quad [2]$$

$CL_{int,sec}$ was obtained as the product of transporter-mediated intrinsic clearance ($CL_{int,T}$), transporter

ontogeny levels (ont_T), the number of proximal tubule cells per gram kidney (PTCPGK), and kidney weight (KW), as shown in equation [2].

The pediatric CL_R obtained using equations 1-2 were compared to scaled CL_R values by GFR function (equation [3]), and the two empirical bodyweight-based relations (i.e. linear and allometric equations [5][6]). The 'true' adult CL_R predictions required for equations 3-5 were obtained with equations [1] and [2] by using the adult values for system-specific parameters as input.

$$GFR\ scaled\ CL_{ped} = 'true' CL_{adult} \times \left(\frac{GFR_{ped}}{GFR_{adult}}\right) \quad [3]$$

$$Linear\ scaled\ CL_{ped} = 'true' CL_{adult} \times \left(\frac{WT_{ped}}{WT_{adult}}\right) \quad [4]$$

$$Allometric\ scaled\ CL_{ped} = 'true' CL_{adult} \times \left(\frac{WT_{ped}}{WT_{adult}}\right)^{0.75} \quad [5]$$

The criteria for accurate scaling was consistent between the previous analysis (**section II**, **chapter 4**) and the current analysis. By calculating a percentage prediction error (%PE- equation [6]) relative to PBPK CL_R predictions, the scaled CL_R values were considered accurate when %PE was within the range of ±30%, inaccurate when %PE outside the range of ±50% and reasonably accurate in-between (i.e.-50%--30%-30%- 50%).

$$\%PE_{CL} = \frac{scaled\ CL_{ped} - 'true' CL_{ped}}{'true' CL_{ped}} \times 100 \quad [6]$$

Figure 7.4 summarizes the performance of GFR-based scaling for CL_R as proposed in **chapter 4** of **section II** of drugs eliminated by both GF and ATS and provides general guidance for applying GFR-based scaling throughout the pediatric age-range. GFR-based scaling leads to accurate CL_R values down to 5 years of age and reasonably accurate CL_R values down to 1 year of age when transporter ontogeny is mature (> 0.7 of the adult values) and for drugs with fu in adults higher than 0.15. Down to 1 year of age and at ontogeny levels of 0.7 GFR-based scaling performs similarly to linear scaling (Appendix, Figure S7.1). However, scaling by GFR maturation has a worse performance than linear and allometric scaling (Appendix, Figures S7.1. and S7.2) when ontogeny is as low as 0.5 of adult values, with no more than 20 - 48% of drugs leading to accurate CL_R scaling down to 1 year of age. These drugs have in general low to medium $CL_{int,T}$ (8-100 µL/min/g kidney). For drugs with a lower f_u in adults, the percent of inaccurately scaled drugs is higher. For children younger than 3 months and ontogeny of 0.5 of adult values, GFR-based scaling leads to accurate predictions in all typical individuals with the exception of newborns. In newborns GFR-based scaling led to inaccurate predictions for 13% and 17% of drugs bound to HSA and AGP, respectively. Similar results are obtained for linear scaling (Appendix, Figure S7.1). When transporter ontogeny is ≤ 0.2, for newborns up to 1-month GFR-based scaling leads to accurate CL_R values for 8-46% of drugs, which is more than either linear (2 – 28%) or allometric scaling (0%). However, these are drugs mainly eliminated by GF. For drugs for which ontogeny of transporters plays a major role, GFR-based scaling becomes highly inaccurate for all ages. This is the case for allometric and linear scaling as well.

In the appendix, Figures S7.1. and S7.2 summarize the performance of linear and allometric scaling for drugs cleared by GF and ATS in a similar manner as presented in Figure 7.1. This work was added to the results published by our group for scaling clearance of drugs undergoing hepatic elimination [11,12]. It has to be noted that these three scaling methods are mostly useful for scaling CL_R when no PK data is available for the pediatric population for the drug of interest. As shown here and previously by our group [11,12], drug clearance through different routes has to be scaled differently, depending on the pathway(s) involved.

To summarize, CL_R of drugs eliminated by GF and ATS can be reasonably accurately scaled using the GFR

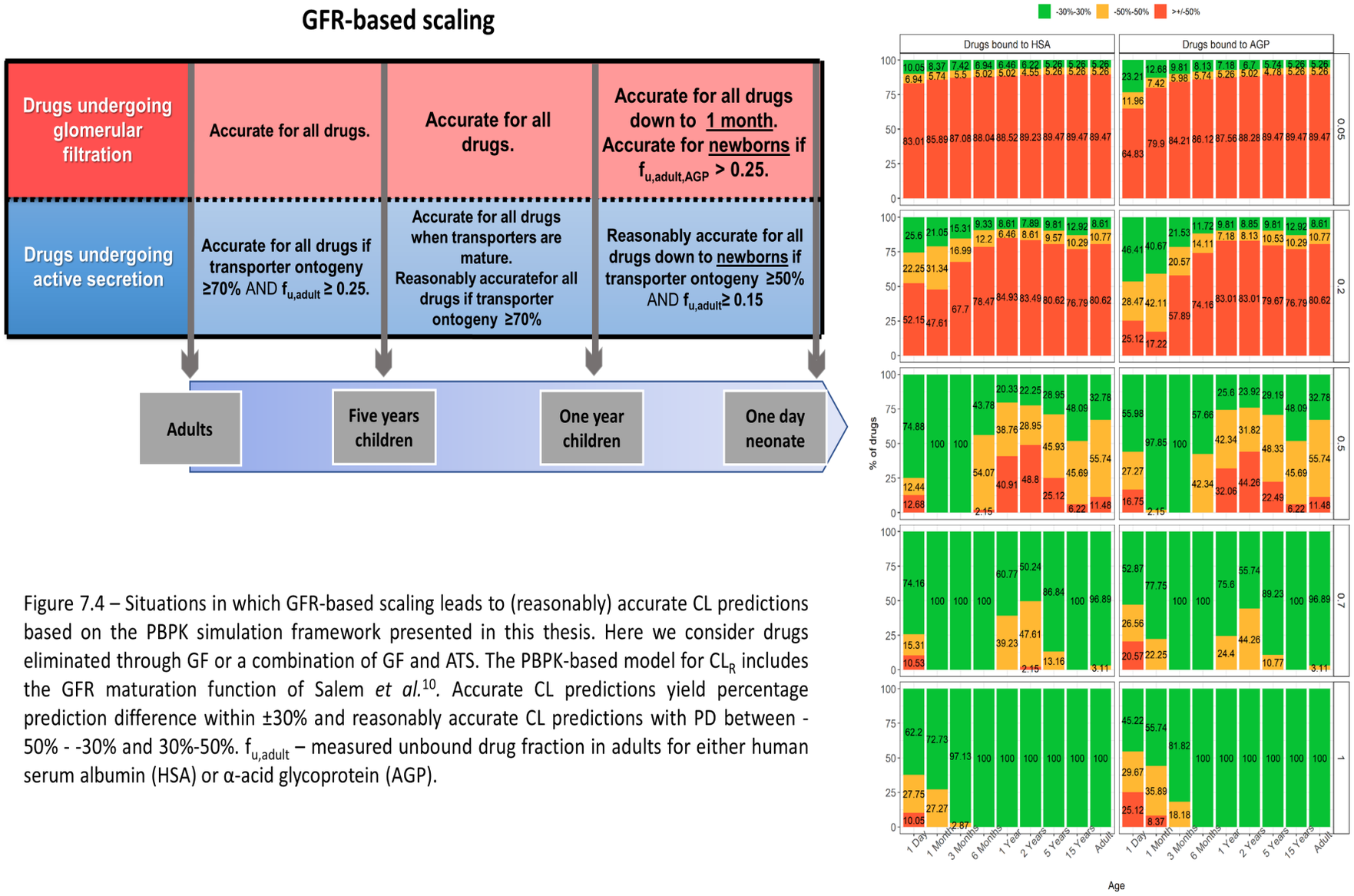


Figure 7.4 – Situations in which GFR-based scaling leads to (reasonably) accurate CL predictions based on the PBPK simulation framework presented in this thesis. Here we consider drugs eliminated through GF or a combination of GF and ATS. The PBPK-based model for CL_R includes the GFR maturation function of Salem *et al.*[10]. Accurate CL predictions yield percentage prediction difference within ±30% and reasonably accurate CL predictions with PD between -50% - -30% and 30%-50%. $f_{u,adult}$ – measured unbound drug fraction in adults for either human serum albumin (HSA) or α-acid glycoprotein (AGP).

maturation function of Salem **et al.** down to 1 year of age when transporter ontogeny is > 0.7 of the adult levels for drugs with a f_u in adults > 0.15. For children younger than 1 year, GFR-based scaling can be applied if transporter ontogeny is > 0.5 and f_u in adults > 0.15. By using the guidelines presented in Figure 7.1 (reasonably) accurate initial estimates can be obtained for pediatric CL_R. These guidelines could be extended further with renal metabolism and reabsorption.

7.5.3. How to use popPBPK to derive parameters with high impact but difficult to measure?

In **section III**, **chapter 4** we proposed a general dosing table for drugs eliminated exclusively by GF (Table 7.2) in which the dose was expressed as a percentage of the adult dose and resulted from scaling by a GFR maturation function [2]. Developing this dosing guideline was possible because all the impactful parameters and their maturation profiles involved in predicting CL_R of drugs eliminated by GF have been extensively researched throughout the pediatric age-range. Nonetheless, in addition to GF there could be other processes involved in CL_R such as active renal tubular transport which remains understudied, especially in the pediatric population. To be able to predict this component of CL_R more information on the ontogeny of renal transporters throughout the pediatric age range is needed. This kind of information is based on protein expression levels measured in renal tissue from children.

Table 7.2 - Pediatric doses presented as % of adult dose for drugs eliminated through GFR with varying fu values. The 'true' doses predicted based on 'true' pediatric CL values are dependent on fu whereas the scaled doses derived from CL values scaled with the three different scaling methods (i.e. GFR scaling, linear scaling and allometric scaling) are not.

Demographic Characteristics of Typical Individuals			**'True' dose (% of adult dose) obtained based on 'true' CL**				**Scaled dose (% of adult dose) obtained using three CL scaling methods**		
Age	Weight (kg)	GFR (ml/min)	Drugs binding to HSA		Drugs binding to AGP		GFR scaling	Linear scaling	Allo-metric scaling
			f_u=0.1	f_u=0.9	f_u=0.1	f_u=0.9			
1 Day	3.4	4.3	5%	4.1 %	10.1 %	4.2 %	4 %	5.2%	11 %
1 Month	4.3	6.2	6.6 %	5.8 %	8.3 %	5.9 %	5.7 %	6.5 %	13 %
3 Months	5.8	10.7	11.1 %	10 %	12.7 %	10.1 %	9.9 %	8.6 %	16 %
6 Months	7.5	17.6	17.9 %	16.4 %	19.6 %	16.5 %	16.2 %	11.4 %	20 %
9 Months	8.9	23.2	23.5 %	21.6 %	25.1 %	21.8 %	21.4 %	13.4 %	22 %
1 Year	9.9	27.4	27.5 %	25.5 %	29.1 %	25.6 %	25.3 %	14.9 %	24 %
2 Years	12.3	35.9	35.4 %	33.3 %	36.5 %	33.4 %	33.1 %	18.6 %	28 %
5 Years	18.2	47.7	46 %	44.2 %	46.6 %	44.3 %	44 %	27.4 %	38 %
10 Years	32.5	68.9	65.4 %	63.8 %	65.6 %	63.8 %	63.6 %	48.9 %	58 %
15 Years	54.2	95.3	89.7 %	88.1 %	89.7 %	88.1 %	87.9 %	81.6 %	86 %
Adult	66.5	108.4	100 %	100 %	100 %	100 %	100 %	100 %	100 %

In **section IV**, **chapter 6** we show that a combined population PK and PBPK approach can be used to derive values for parameters that cannot be measured ***in vivo*** by leveraging the knowledge included in PBPK models on underlying physiological processes and information that can be derived from concentration-time profiles in patients with a population approach. By applying this methodology on informative clinical data, we were able to derive the renal OAT3 transporter ontogeny ***in vivo***. The clinical data used for this case-study included both a descriptor of GF - clavulanic acid – and a descriptor of GF and ATS through OAT3- amoxicillin – obtained after the administration of both drugs simultaneously, in the same formulation, to each patient. Having PK data of both drugs administrated to the same patient facilitated the separation between GF and ATS for each individual and allowed the estimation of ontogeny for the OAT3. The resulting ontogeny function for OAT3 was included in the pediatric PBPK-based model for CL_R (equations [1] and [2]) for two new OAT3 substrates that lead to accurate predictions of CL_R throughout the entire pediatric age-range (% root mean square prediction error of 21% and 12% for cefazoline and piperacillin).

Even though having clinical data that includes probe drugs for both GF and ATS in the same individual is desirable, the combined population PK and PBPK approach can also be applied for only one probe drug for a specific renal transporter. In **section IV**, **chapter 5** we propose basic selection guidelines for drugs with relevant properties to serve as *in vivo* probes for quantifying the ontogeny of transporters underlying ATS. From the results in **chapter 5** we concluded that the best probe drugs should have a $CL_{int,T}$ of 5-50 µL/min/mg protein and medium to high fraction unbound in adults ($f_{u,adults}$ = 0.55 – 0.95). Drugs for which GF is the main elimination pathway or drugs with extremely high $CL_{int,T}$ that cause renal blood flow to be limiting for elimination, will have a limited use in characterizing ontogeny profiles of renal transporters.

Quantifying the individual transporters would be of great value, as it would improve the PBPK predictions of drugs for which ATS plays an important role, especially when ontogeny is immature (<0.2 of the adult value) or for children younger than 2 years. The combined population PK and PBPK approach could be used with existing clinical data on other substrates of renal transporters to characterize the *in vivo* ontogeny of the remaining renal transporters. In addition, more studies as the one presented in **chapter 6**, including specific drug probes for more than one underlying transporters would provide information about the IIV of CL_R through ATS. These results would complement and confirm existing findings, such as the *in vitro* results of Cheung et al. [3]. Once ontogeny of individual transporters becomes available, generalized dosing tables such as the one presented in **chapter 3** could be developed not only for drugs substrates specific for one transporter pathway but also for combinations of transporters working in tandem.

This type of research should not be limited to active transport only. In addition, renal reabsorption and metabolism together with their dependencies on physiological properties like pH at the tubule side, ionization, enzyme abundance, affinity, and maturation, could be explored in a similar manner in subsequent analyses.

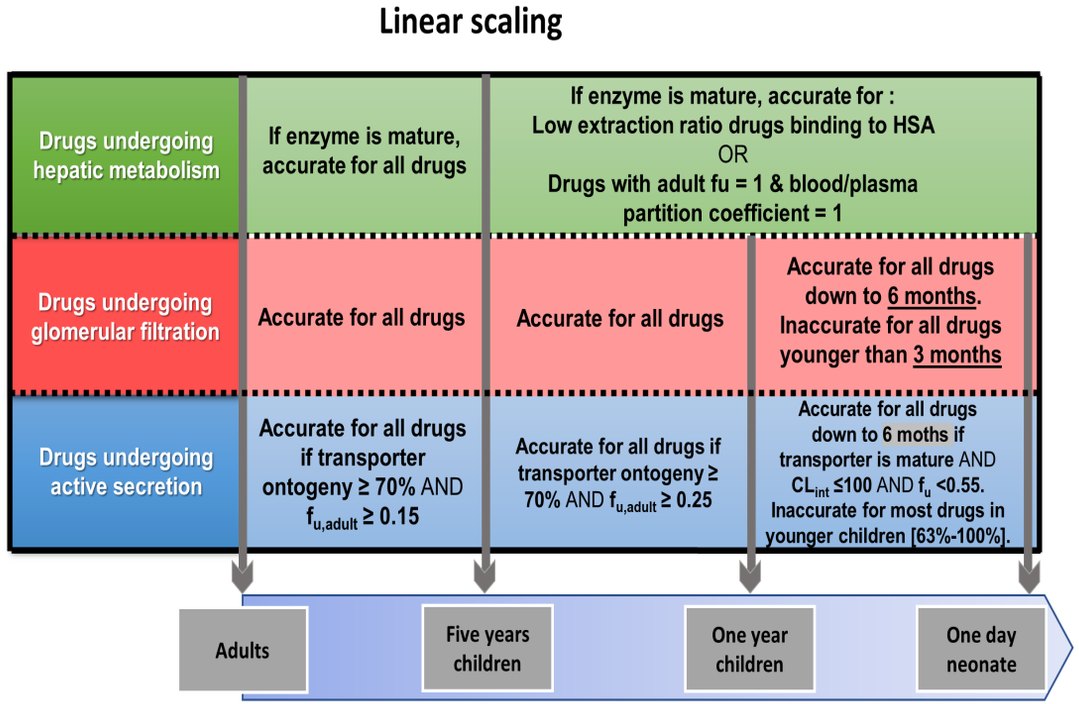
7.6 Conclusions

Predicting PK in children, especially in children under the age of 5 years who are still developing, remains challenging. Throughout this thesis we used different modeling and simulation techniques to guide pediatric dosing when clinical data is available (population PK models) or in the absence of such data (PBPK methods). Population PK methods (i.e. covariate analyses) were used to characterize the changes in CL_R as a function of developmental changes. Obtained population PK models could be used to guide dose adjustments in vulnerable pediatric sub-populations to ensure a safe and effective treatment from the start of therapy. When PK data is scarce or unavailable, pediatric CL_R can be obtained using the information already included in PBPK models and used to derive pediatric doses. Furthermore, performing sensitivity analyses on established PBPK models for CL_R allowed the study of active tubular secretion and how this process is influenced by underlying physiological development (e.g. ontogeny of renal secretion transporters) for a broad array of drugs with different properties. Finally, the information included in the PBPK model for CL_R was further extended by integration with relevant clinical data (popPBPK). With this approach poorly characterized parameters and/or ontogeny/maturation functions were derived from data collected *in vivo*. The results presented in this thesis can serve as a basis for similar explorations to disentangle the remaining relevant processes involved in CL_R and translate relevant findings into guides for safe and effective pediatric dosing.

7.7 References

1. De Cock RFW, Allegaert K, Sherwin CMT, Nielsen EI, De Hoog M, Van Den Anker JN, Danhof M, Knibbe C a J (2014) A Neonatal amikacin covariate model can be used to predict ontogeny of other drugs eliminated through glomerular filtration in neonates. Pharm Res 31:754–767 . https://doi.org/10.1007/s11095-013-1197-y
2. Salem F, Johnson TN, Abduljalil K, Tucker GT, Rostami-Hodjegan A (2014) A re-evaluation and validation of ontogeny functions for cytochrome P450 1A2 and 3A4 based on in vivo data. Clin Pharmacokinet. https://doi.org/10.1007/s40262-014-0140-7
3. Cheung KWK, van Groen BD, Spaans E, van Borselen MD, de Bruijn ACJM, Simons-Oosterhuis Y, Tibboel D, Samsom JN, Verdijk RM, Smeets B, Zhang L, Huang SM, Giacomini KM, de Wildt SN (2019) A Comprehensive Analysis of Ontogeny of Renal Drug Transporters: mRNA Analyses, Quantitative Proteomics, and Localization. Clin Pharmacol Ther. https://doi.org/10.1002/cpt.1516
4. Mathialagan S, Piotrowski MA, Tess DA, Feng B, Litchfield J, Varma M V. (2017) Quantitative prediction of human renal clearance and drug-drug interactions of organic anion transporter substrates using in vitro transport data: A relative activity factor approach. Drug Metab Dispos. https://doi.org/10.1124/dmd.116.074294
5. FDA (2008) AUGMENTIN® (amoxicillin/clavulanate potassium) Powder for Oral Suspension and Chewable Tablets. https://www.accessdata.fda.gov/drugsatfda_docs/label/2008/050575s037550597s044050725s025050726s019lbl.pdf
6. Janssen EJH, Välitalo P a. J, Allegaert K, de Cock RFW, Simons SHP, Sherwin CMT, Mouton JW, van den Anker JN, Knibbe C a. J (2015) Towards rational dosing algorithms for vancomycin in neonates and infants based on population pharmacokinetic modeling. Antimicrob Agents Chemother AAC.01968-15 . https://doi.org/10.1128/AAC.01968-15
7. Neely MN, Youn G, Jones B, Jelliffe RW, Drusano GL, Rodvold KA, Lodise P (2014) Are Vancomycin Trough Concentrations Adequate for Optimal Dosing ? 58:309–316 . https://doi.org/10.1128/AAC.01653-13
8. De Cock RFW, Allegaert K, Brussee JM, Sherwin CMT, Mulla H, De Hoog M, Van Den Anker JN, Danhof M, Knibbe C a J (2014) Simultaneous pharmacokinetic modeling of gentamicin, tobramycin and vancomycin clearance from neonates to adults: Towards a semi-physiological function for maturation in glomerular filtration. Pharm Res 31:2643–2654 . https://doi.org/10.1007/s11095-014-1361-z
9. Frymoyer A, Hersh AL, El-Komy MH, Gaskari S, Su F, Drover DR, Van Meurs K (2014) Association between vancomycin trough concentration and area under the concentration-time curve in neonates. Antimicrob Agents Chemother 58:6454–6461 . https://doi.org/10.1128/AAC.03620-14
10. National Health and Nutrition Examination Survey www.cdc.gov/growthcharts/index.htm
11. Calvier E, Krekels E, Valitalo P, Rostami-Hodjegan A, Tibboel D, Danhof M, Knibbe CAJ (2017) Allometric scaling of clearance in paediatrics: when does the magic of 0.75 fade? Clin Pharmacokinet 56:273–285 . https://doi.org/doi: 10.1007/s40262-016-0436-x.
12. Krekels EHJ, Calvier EAM, van der Graaf PH, Knibbe CAJ (2019) Children Are Not Small Adults, but Can We Treat Them As Such? CPT Pharmacometrics Syst Pharmacol. https://doi.org/10.1002/psp4.12366

7.8 Supplementary material



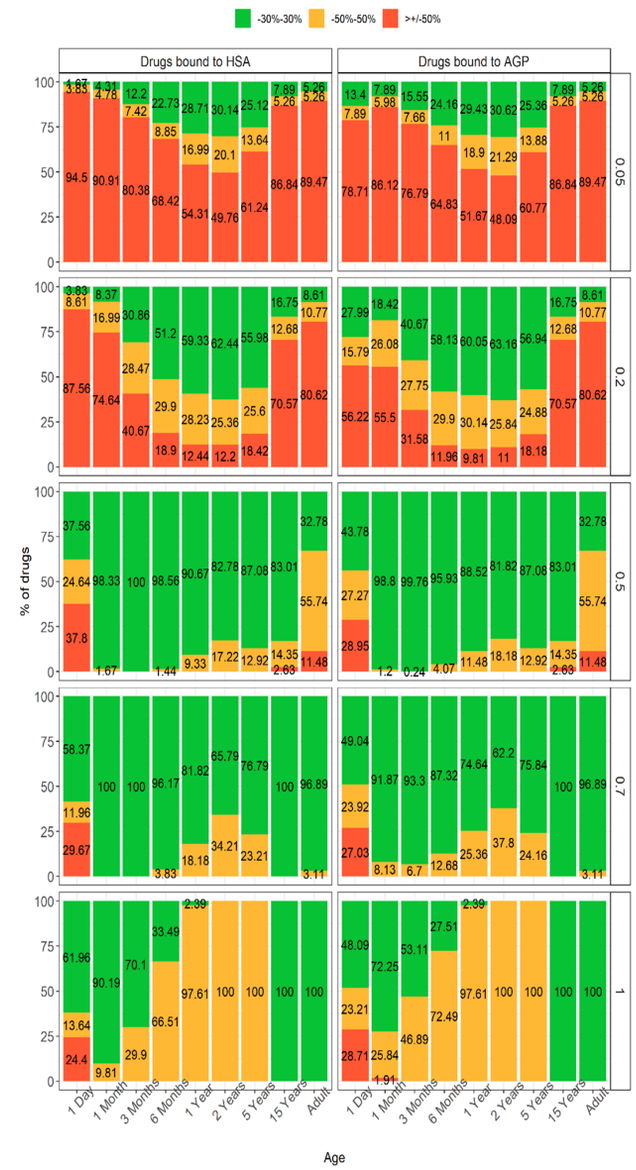
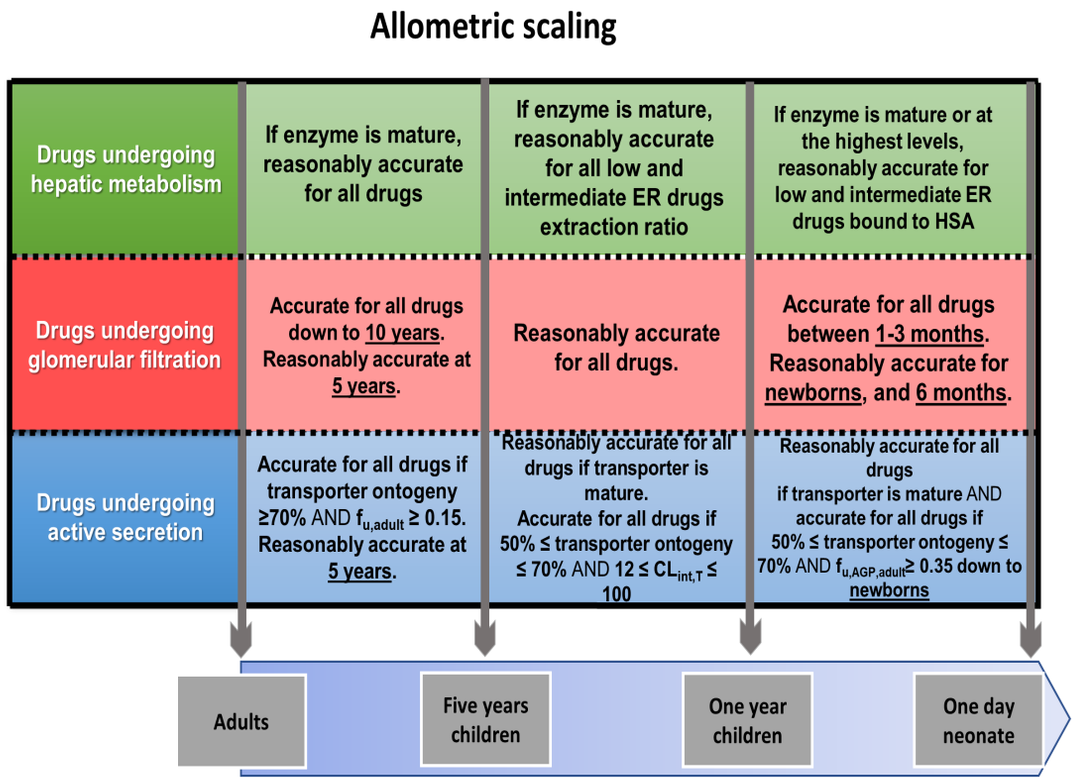


Figure S7.1 – Situations in which linear scaling leads to (reasonably) accurate CL predictions based on the PBPK simulation framework presented in this thesis. Here we consider drugs eliminated through GF or a combination of GF and ATS. Drugs eliminated by hepatic pathways have been discussed elsewhere[9]. The PBPK-based model for CLR includes the GFR maturation function of Salem et al.[10]. Accurate CL predictions yield percentage prediction difference within ±30% and reasonably accurate CL predictions with PD between -50% - -30% and 30% - 50%. CL_{int} - transporter-mediated intrinsic clearance (µl/min/g kidney), $f_{u,adult}$ – measured unbound drug fraction in adults for either human serum albumin (HSA) or α-acid glycoprotein (AGP).



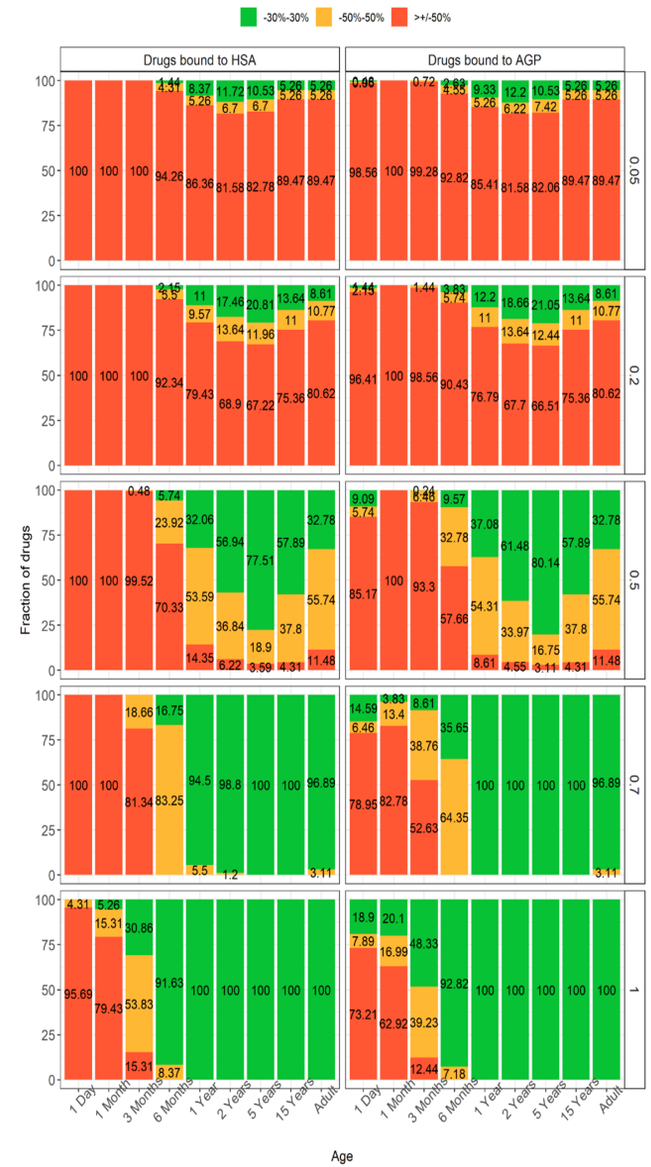


Figure S7.2 – Situations in which allometric scaling leads to (reasonably) accurate CL predictions based on the PBPK simulation framework presented in this thesis. Here we consider drugs eliminated through GF or a combination of GF and ATS. Drugs eliminated by hepatic pathways have been discussed elsewhere[9]. The PBPK-based model for CL_R includes the GFR maturation function of Salem *et al.*[10]. Accurate CL predictions yield percentage prediction difference within ±30% and reasonably accurate CL predictions with PE% between -50% - -30% and 30% - 50%. CL_{int} - transporter-mediated intrinsic clearance (µl/min/g kidney), $f_{u,adult}$ – measured unbound drug fraction in adults for either human serum albumin (HSA) or α-acid glycoprotein (AGP).