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First-pass and systemic metabolism of cytochrome P450 3A substrates in neonates, infants, and children

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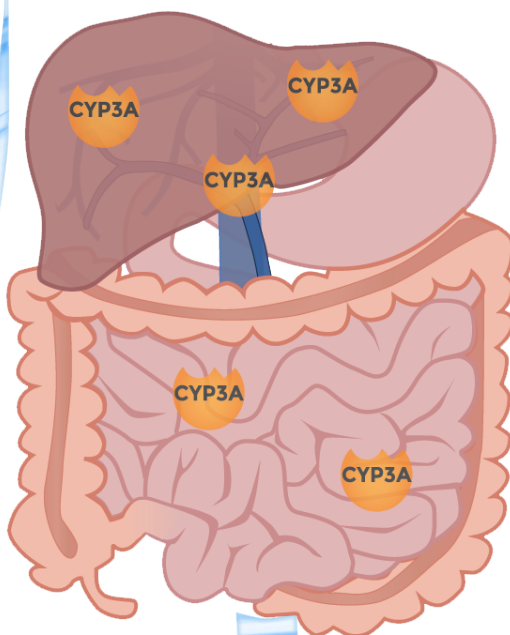
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Section V

Summary, Conclusions, and Perspectives



Chapter 8

**First-pass and systemic metabolism
of cytochrome P450 3A substrates
in neonates, infants, and children
– Summary, Conclusions, and
Perspectives**

Growth and development affect the pharmacokinetics (PK) of drugs administered to neonates, infants, and children (1, 2). Among these developmental changes is the maturation of drug metabolizing enzyme expression and activity, which impacts the rate of metabolic clearance of drugs (3). As described in **Chapter 1**, the research described in this thesis focused on the metabolism by cytochrome P450 (CYP) 3A enzymes, using midazolam as probe drug (4). The overall aim of this thesis was to predict CYP3A-mediated plasma clearance in neonates, infants, and children, by development of pediatric (physiological) population PK models. Accurate prediction of plasma clearance of drugs is essential to provide rational support for pediatric doses in first-in-child studies during drug development and to develop pediatric dose recommendations for clinical practice.

For this purpose, we presented in **Section I** our view on preferred approaches to estimate drug clearance to establish individualized dosing regimens for drugs in the pediatric population. **Section II** described the CYP3A-mediated systemic metabolism in critically ill pediatric patients. Within the developed population PK model, body weight, critical illness, and inflammation were identified as covariates to explain part of the inter- and intra-individual variability within this population. This model was next evaluated for its predictive performance for clearance in similar (postoperative or critically ill) patients, and in other populations including preterm neonates and adults. **Section III** focused on methods to distinguish between first-pass and systemic CYP3A-mediated metabolism to elucidate the role of intestinal and hepatic CYP3A in neonates and children covering the whole pediatric age range. Lastly, **Section IV** discussed how information on CYP3A-mediated clearance of the probe drug midazolam in children can be used for scaling of clearance of other CYP3A substrates, and described when a pediatric covariate function for CYP3A-mediated midazolam clearance can be applied to scale plasma clearance of other commonly used CYP3A substrates (including sildenafil) in the pediatric population.

I. Children in clinical trials: towards evidence-based pediatric pharmacotherapy using pharmacokinetic-pharmacodynamic modelling

Chapter 2 presented our view on model-based pediatric drug development. It discussed the lack of dedicated pharmacokinetic-pharmacodynamic (PKPD) studies in children compared to adults, and that therefore pediatric doses for many CYP3A substrates and other drugs are often linearly or allometrically scaled from adult values. However, predicting plasma clearance in especially young children is complicated, as each hepatic isoenzyme has a different maturation profile, and therefore maturation functions reflecting these changes to scale plasma clearance in the pediatric population are needed for each hepatic elimination pathway. These

functions are not always available, but studying the clearance of the CYP3A-substrate midazolam (5) in children will increase our understanding of the CYP3A enzyme maturation patterns.

General pediatric PK models, or physiologically-based models when sufficient system-specific and drug-specific information are available, describing drug clearance should be developed in order to develop evidence-based dosing regimens in children, and these models should be thoroughly evaluated and validated with external data, as also illustrated for instance in Chapter 4.

A proper study design is pivotal in answering the research questions of pharmacological studies and therefore a multidisciplinary team including clinicians, clinical pharmacologists, and pharmacometricians should be involved in the study design to discuss e.g. what (covariate) data should be collected at which time points. The covariate relationships with clearance in the developed PK models can be used to individualize drug regimens provided the therapeutic window and/or target exposure is known for the varying degrees of critical illness. Based on simulations with the model, dosing recommendations can be proposed and the optimized dosing schedule should be evaluated in clinical practice. For this approach, properly designed clinical PKPD studies will remain the backbone of pediatric research to develop and confirm model-based pediatric dose recommendations for drugs in children.

II. Systemic CYP3A-mediated metabolism in critically ill children

We previously observed large differences in the reported mean values of midazolam clearance in pediatric populations of similar ages. The most striking difference between these cohorts was the severity of illness, with healthier children showing higher midazolam clearance than critically ill children. We hypothesized that these differences could be due to severity of disease and/or inflammation.

In **Chapter 3** we described the systemic CYP3A-mediated clearance of midazolam in critically ill neonates, infants, and children after multiple intravenous administrations based on therapeutic need. Based on prospectively collected pharmacokinetic data from 83 patients between 0 and 17 years of age, a two-compartment PK model was developed that described the concentrations of midazolam well. In the model, body weight was found as most significant covariate for clearance, with an almost linear increase in clearance with increasing body weight. In line with our hypothesis, an increased concentration of the inflammatory marker C-reactive protein (CRP) was found to correlate to a decreased midazolam clearance, with a 51.2% lower clearance

when CRP increased from 10 to 100 mg/L. Disease severity was also related to midazolam clearance, as with an increased number of failing organs, e.g. from 1 to 2 or 3, the midazolam clearance decreased by e.g. 25.6% or 34.9%, respectively. As a result, CYP3A-mediated midazolam clearance is even up to 77.4% lower in patients with both increased CRP concentrations and an increased number of failing organs.

The decreased midazolam plasma clearance in critically ill children described in **Chapter 3**, may be due to decreased CYP3A enzyme activity when the inflammatory markers CRP and IL-6 concentrations increase (6-8), and multiple organ failure, in addition to inflammation, may lead to a further decreased midazolam clearance, although the underlying mechanisms of how cardiovascular, respiratory (9), hepatic, and renal failure (10) may affect the PK of midazolam are not well understood (6). This decreased midazolam clearance in critically ill children leads to increased plasma concentrations and exposure of midazolam in patients with inflammation, organ failure, or both (**Chapter 3**).

The developed population PK model was subsequently externally validated in **Chapter 4**, which evaluated the model's predictive performance in both critically ill children and other populations including preterm neonates receiving midazolam intravenously. The results showed that in critically ill term neonates, infants, children and adults, the model could adequately predict the midazolam clearance. Compared to reported values for midazolam clearance in literature, the clearance of the critically ill patients in our study was found to be generally lower, which may be due to their disease state, as most published clearance values come from PK studies in relatively healthy children (11-15).

In healthy adults, the observed and predicted clearance values were also higher compared to critically ill adults, which may also be explained by the fact that no inflammation and organ failure is present in this population. However, clearance was largely over-predicted in preterm neonates with a body weight below 3.5 kg and a gestational age of less than 37 weeks. This is most likely due to the fact that the model did not account for immaturity of CYP3A in preterm infants. In preterm neonates, we anticipate that the total CYP3A activity is much lower compared to term neonates and young infants, and also more immature than would be expected based on scaling based on body weight from term neonates (16-18). Hence, while the model developed in **Chapter 3** should not be used for extrapolations to very young neonates, this external validation in **Chapter 4** confirmed that the developed PK model could adequately predict pediatric CYP3A-mediated clearance in (pediatric) patients with varying levels of (critical) illness.

III. First-pass CYP3A-mediated metabolism in children after oral drug administration

A previous study showed that the oral bioavailability of midazolam is much higher in preterm infants (19) than in adults. This observation suggests immature intestinal and/or hepatic CYP3A activity in preterm infants, resulting in higher systemic midazolam exposure. However, to our knowledge, the relative contribution of intestinal and hepatic CYP3A metabolism and their relative changes with age have not been determined before.

The presystemic CYP3A-mediated clearance can only be described based on oral PK data, and preferably in combination with intravenous PK data. In **Chapters 5 and 6** we explored the role of gut wall and hepatic CYP3A enzymes in presystemic clearance of midazolam in preterm neonates and children from 1-18 years of age, respectively. For this, a novel approach called physiological population PK modelling was applied, utilizing both information on the biological system (physiology of the gastro-intestinal tract and liver) and population PK modelling of data of midazolam and its primary metabolite, 1-OH-midazolam, from children of varying ages.

The intrinsic clearance in the gut wall and liver both appeared to increase with increasing body weight in children of 1-18 years of age. Figure 1A shows that the intrinsic intestinal and hepatic CYP3A-mediated clearances do not increase in parallel, and that the intrinsic gut wall clearance increases faster with age. The intrinsic gut wall clearance is lower than the intrinsic hepatic clearance throughout the pediatric and adult age range, and the relative contribution of CYP3A enzymes in gut wall and liver to the presystemic metabolism of CYP3A substrates differs with age. In preterm neonates, the ratio of intrinsic hepatic over gut wall clearance is much larger than in children and healthy adults, with a ratio of approximately 340 in preterm neonates (**Chapter 5**) versus 153 in young infant up to 2 years of age, 87 for a typical 27-kg child, 48 in adolescents ≥ 16 years-of-age (**Chapter 6**), compared to a ratio of 60 in adults (20). This indicates a smaller contribution of intrinsic gut wall clearance to the presystemic clearance with decreasing age.

When we consider the intrinsic clearance of midazolam a surrogate marker of total intestinal and hepatic activity of CYP3A enzymes, this indicates that at 1 year of age the hepatic CYP3A activity is already close to the adult values, in contrast to the total intestinal CYP3A activity which needs to increase more with age (**Chapter 6**). Comparing these results with preterm neonates, the intestinal and hepatic CYP3A activity in preterm neonates both appear to be very immature (**Chapter 5**)(figure 1A),

and because of this very low CYP3A activity, preterm neonates should be regarded as a different population than other children and adults.

It has been reported that both the CYP3A enzyme content of the enterocytes and the total size of the small intestine increases with age (1, 21, 22), and together, this may lead to a higher intrinsic CYP3A-mediated clearance in the gut wall in adolescents compared to neonates and infants. For hepatic CYP3A activity, our analysis in **Chapter 6** suggests that liver growth mostly contributes to the increase in hepatic CYP3A-mediated intrinsic clearance in children, while the CYP3A abundance and the amount of microsomal protein in the liver may remain relatively constant with age (17, 23, 24).

Plasma clearance is mostly dependent on intrinsic hepatic CYP3A clearance, and can be calculated based on the well-stirred model using hepatic intrinsic clearance together with the hepatic blood flow, protein binding and the blood: plasma ratio. We found that the plasma clearance increases from 0.03-0.79 (median 0.18) L/h in preterm neonates and 2.5-8.7 (median 6.0) L/h in children of 1-2 years of age to 9.0-24.6 (median 17.5) L/h in children ≥ 16 years of age (**Chapters 5 and 6**)(figure 1B).

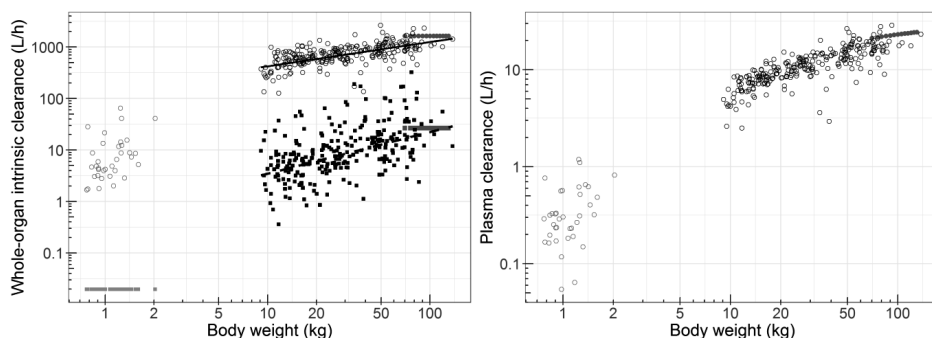


Figure 1. A) Whole-organ intrinsic clearance of midazolam in the gut wall (solid square) and the liver (open and solid circle) are plotted versus body weight, with values for preterm neonates (light grey solid squares and open circles)(Chapter 5) and children 1-18 years of age (black solid squares and open circles)(Chapter 6). Reported values from adults (20) of 26.7 (grey solid square) and 1640 L/h (grey solid circle), respectively, are shown as well. B) Total plasma clearance is shown versus body weight, with values for preterm neonates (light grey open circle)(Chapter 5), children 1-18 years of age (black open circle)(Chapter 6) and reported values for typical adults (grey closed circle)(20). Modified with permission from Brussee et al. (25).

Chapter 5 also described that the intestinal and hepatic extraction ratio of midazolam were very low (median of 0.04 each), leading to extremely low presystemic clearance in preterm neonates. The total bioavailability is the product of the fraction absorbed

(F_a), and the fractions escaping gut wall (F_g) and hepatic (F_h) metabolism, as per equation 1.

$$F_{total} = F_a \times F_g \times F_h \quad (\text{eq. 1})$$

The resulting bioavailability of 92.3% is therefore very high, but highly variable in the population (90%CI: 75.4-94.5%)(figure 2) and this may lead to large differences in drug exposure and drug effect after oral dosing of CYP3A substrates in preterm neonates.

We also report a large variability in bioavailability around the median of 20.8% in children of 1-18 years of age (90%CI: 4.6-44.6%)(Chapter 6). As figure 2 shows, the fraction escaping hepatic metabolism (i.e. F_h) appears to increase significantly with age, while the fraction escaping gut wall metabolism (i.e. F_g) decreases with age, resulting in an age-independent total bioavailability of midazolam (i.e. F_{total} , which is calculated per eq. 1).

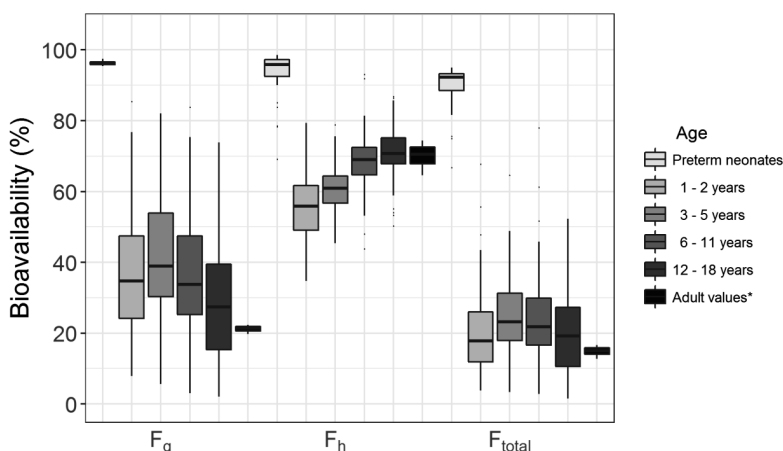


Figure 2. Midazolam bioavailability in the gut wall (F_g), in the liver (F_h) and total bioavailability (F_{total}) is much higher in preterm neonates (white)(Chapter 5), compared to children of four different age categories: 1-2 years, 3-5 years, 6-11 years, and 12-18 years of age (increasing grey scales)(Chapter 6) and compared to adult values (black)(20). *Adult bioavailability values are calculated based on their reported typical intrinsic hepatic clearance, hepatic blood flow for their body weight, the fraction unbound and blood: plasma ratio (20). Modified with permission from Brussee et al. (25).

IV. Midazolam as probe drug for other CYP3A substrates

Midazolam is a widely accepted probe drug for CYP3A activity (4, 5), and midazolam clearance in neonates, infants, and children has been used to reflect the ontogeny of CYP3A in the pediatric population. Therefore, in **section IV** of this thesis, it was assessed when pediatric PK information from midazolam can be used to predict pediatric clearance of other CYP3A substrates.

As it would require many resources to study the PK of all drugs (including all CYP3A substrates) in the pediatric population, other approaches besides clinical studies have been proposed including full physiologically-based PK (PBPK) models to predict pediatric clearance values. While PBPK models require extensive system-specific and drug-specific information which may not always be available, it has been hypothesized that PK information of drugs sharing the same elimination pathway may be used to predict plasma clearance of drugs in children (26). This between-drug extrapolation of clearance has been applied successfully in predicting clearance for individual antibiotics eliminated by glomerular filtration in neonates with amikacin as model drug (27, 28). Additionally, the clearance of the UGT2B7-substrate zidovudine in children could be accurately scaled using a pediatric covariate function for UGT2B7-mediated drug glucuronidation from a morphine PK model (26).

While these data are very reassuring, the question emerges whether this also applies to CYP3A-mediated metabolism. Calvier *et al.* (29) explored this approach in a systematic way for all hepatic isoenzymes and reported on the basis of their developed framework that accurate between-drug extrapolation of clearance on the basis of a shared elimination pathway, depends not only on the fraction metabolized by the specific hepatic isoenzyme, but also on other properties of the test drug, including the drugs extraction ratio in adults (ER), the type of binding plasma protein, and the unbound fraction in adults (f_u).

In **Chapter 7**, this framework was applied to scale pediatric clearance of various commonly used CYP3A substrates from adult clearance values using a pediatric covariate function for CYP3A-mediated midazolam clearance. According to the framework of Calvier *et al.* (29), clearance of CYP3A substrates can be systematically accurately scaled using the pediatric covariate function for CYP3A-mediated clearance from a midazolam PK model if they have an extraction ratio of 0.35-0.65 or 0.05-0.55 and bind <10% or >90% to albumin in adults, respectively. Less combinations of drug properties of AGP-bound CYP3A substrates lead to accurate scaling based on a midazolam pediatric covariate function, with no scenarios for drugs with low protein binding ($f_u \geq 0.9$), but clearance of drugs that are 90% bound to AGP and have an extraction ratio of 0.4-0.6 in adults can be accurately scaled. For alprazolam, atorvastatin, quinidine, sildenafil, solifenacin, sufentanil and tacrolimus, this means that scaling of pediatric clearance using the pediatric covariate function of pediatric

PK model for midazolam will be systematically accurate down to one day of age, based on their drug properties.

For CYP3A substrates for which pediatric and adult clearance values were available in literature, we could confirm the accurate pediatric clearance predictions for atorvastatin, quinidine, sildenafil, sufentanil, tacrolimus, and tamsulosin, down to various ages, and as low as one year of age, as the scaled clearance values were in agreement with the reported pediatric clearance values (prediction error <50%) (**Chapter 7**). For sirolimus and vincristine, a larger prediction error was observed, which may possibly be due to the known induction of CYP3A activity by sirolimus (30) impacting its plasma clearance, and to the larger contribution of CYP3A5 in the metabolism of vincristine (31), with a relative smaller role for CYP3A4 compared to midazolam.

Furthermore, based on PK data from 156 children receiving sildenafil, **Chapter 7** showed that clearance of the CYP3A substrate sildenafil in children varying in age between 1 and 17 years was accurately scaled by the pediatric covariate function for CYP3A-mediated midazolam clearance, as no large differences were observed (prediction error <50%) compared to the estimated clearance values from a PK model for sildenafil.

Between-drug extrapolation of clearance from midazolam to drugs which are mainly metabolized by CYP3A ($\geq 75\%$ metabolized by that pathway), with the above described drug properties (i.e. an ER of 0.35-0.65 or 0.05-0.55 for <10% or >90% HSA-bound drugs in adults, respectively), can therefore be a valuable tool to predict CYP3A-mediated clearance in children, especially for CYP3A substrates with no or limited available pediatric PK information.

Conclusions

CYP3A-mediated plasma clearance of midazolam in term neonates, infants, and children increases non-linearly with increasing body weight, and is strongly reduced in pediatric patients with inflammation and organ failure.

Pediatric midazolam PK models may be used to predict midazolam clearance in term neonates, infants, and children, but preterm neonates should be regarded as a different population due to their immature CYP3A activity.

To distinguish between metabolism by CYP3A enzymes in the gut wall and liver, and to quantify the fractions escaping gut wall (F_g) and hepatic (F_h) metabolism, a physiological population PK modelling approach has proven useful.

The first-pass effect by intestinal and hepatic CYP3A-mediated metabolism in preterm neonates is extremely low compared to infants, children and adults.

The maturation of gut wall and hepatic CYP3A activity is not parallel. While the gut wall CYP3A activity is lower than the hepatic CYP3A activity in neonates, infants, children, and adults, it contributes more to first-pass metabolism with increasing age.

Between-drug extrapolation of clearance of midazolam to other CYP3A substrates is possible: a pediatric covariate function for CYP3A-mediated midazolam clearance can accurately scale the clearance of various commonly used CYP3A substrates that are $\geq 75\%$ metabolized by CYP3A, with an $ER < 0.55$ or $0.35-0.65$ for low and highly albumin-bound drugs respectively, down to at least 1 year of age.

Perspectives

Translation to the clinic: the impact of disease on midazolam PK and PD

The developed PK model for midazolam in **Chapter 3** describes the CYP3A-mediated clearance in critically ill children and explains part of the inter-individual variability by taking into account the patient's body weight, their inflammation level (reflected by CRP concentrations) and organ failure. Based on these covariates for clearance, dosing recommendations can be derived when a therapeutic window or target concentration is known. For midazolam however, dosages are individually titrated to reach optimal sedation levels, and treatment starts with a maintenance dose between 0.05-0.2 mg/kg/h after a loading dose of 0.05-0.1 mg/kg (32, 33).

Based on the prediction of a lower clearance in critically ill patients (**Chapters 3 and 4**), also described in other patient populations (9, 10), this implies a lower dose would suffice to reach the same plasma concentration. However, studies have shown that interrupting or lowering the dose of midazolam in critically ill children does not improve the clinical outcome, which may suggest that higher midazolam plasma concentrations may be required to reach adequate sedation in children with inflammation and multiple organ failure (34). Also the contribution of the metabolites to the drug response is smaller in critically ill children, due to the decreased formation of the CYP3A-mediated metabolite 1-OH-midazolam, which is known to have half the activity of the parent drug (35), and the glucuronide metabolites who also have substantial pharmacological activity (36).

These results may be explained by differences in the PKPD or PD during inflammation. In rats, receptor binding to GABA_A and GABA_B-receptors is known to be affected by inflammation (37, 38), and also human intestinal GABA receptors appear to be affected by inflammation (39). In contrast to the possibly higher concentrations of midazolam required for adequate sedation in critically ill children (34), a deeper level of sedation has been reported in critically ill adults receiving propofol, which acts at least partly via GABA receptors (40). However, whether GABA receptors in the brain, the site of action for midazolam, are affected by inflammation and disease severity in critically ill patients is unknown.

In addition, it is important that irrespective of disease state, differences in drug response of midazolam at different ages can be anticipated, due to a different number, density, distribution and ligand affinity of the GABA receptors (41, 42). The GABA receptors need to mature in the first years of life (43), as an increasing activity is observed with increasing age. Therefore, further studies are needed to determine

the best variable to optimize drug effect (e.g. peak or trough concentration, steady-state concentration, or exposure), and to clarify the PD and the PKPD relationship of midazolam for varying levels of critical illness throughout the pediatric age range to come up with an optimized evidence-based dosing schedule for midazolam in neonates, infants, and children.

This dose optimization can for example be done by combining longitudinal PK measurements with time-to-event PD outcomes like the use of rescue medication for adequate sedation, and analyze these two types of data simultaneously (44). Repeated measurements of plasma concentrations (PK) and survival data (PD) are mostly analyzed separately or sequentially with different statistical methods, but together they have more informative value on the interplay between PK, PD, and the disease state (44). This is for example illustrated by Juul *et al.* in an analysis of postoperative analgesic requirements (45), and application of this joint modelling approach for sedation may improve pharmacotherapy in pediatric clinical practice as well.

PD endpoints

To evaluate and quantitatively measure drug effects, there is a need for validated, preferably non-invasive, biomarkers or PD endpoints in children that can be measured longitudinal to represent the dynamic changes of the system in healthy and diseased state (41). For midazolam, the COMFORT-B score can be used to assess sedation levels in children (46), but for many CYP3A substrates, the drug effect cannot be measured quantitatively. The emerging field of metabolomics may be useful for biomarker identification reflecting disease severity and/or drug effect in the pediatric population (47). Metabolomics applies a top-down systems biology approach (47, 48), in which a comprehensive analysis of compounds (metabolites) in body fluids is performed (48). In pediatrics, several matrices like urine, plasma and stool may be relevant for metabolomics analyses (47).

Because metabolomics is closer to the observed phenotype (e.g. disease, or treatment outcome) than for example genomics or proteomics, metabolic profiles are considered the most predictive for phenotypes (49), although standardization and validation of this new metabolomics methodology is still required (50, 51). Combining the fields of metabolomics, genomics, and proteomics, could be an even more powerful tool for the identification of biomarkers as early predictors of outcome (49).

The recent identification of several biomarkers for e.g. early detection and clinical diagnostics in the oncology field have shown that metabolomics has the potential

to be valuable in biomarker discovery (52-57), even though they cannot yet be used for clinical decision making, including diagnosis and monitoring of certain diseases and development of individualized pharmacotherapy (58). Identified biomarkers, after thorough validation, can be used for pediatric PKPD models in which drug exposure and PD biomarkers are related with clinical outcomes in neonates, infants, and children, and these models may provide rational support for pediatric dose finding to optimize pharmacotherapy in the pediatric population.

Physiological approach

In **Chapters 5 and 6**, we used PK data from preterm neonates and children 1-18 years of age to study first-pass and systemic metabolism after oral administration of midazolam, ultimately to gain insight in the ontogeny of gut wall and hepatic CYP3A activity. The combination of physiologically-based PK modelling and the population approach enabled us to estimate the intrinsic clearance parameters in the gut wall and liver using both PBPK principles and the available PK data for midazolam in neonates and children. The gut wall and hepatic CYP3A ontogeny profiles based on midazolam PK data may be informative for other CYP3A substrates as well, as demonstrated in **Chapter 7**.

In PBPK modelling, patient-specific parameters related to anatomy, physiology, and pathophysiology are combined with drug-specific properties like physicochemical characteristics (59). The main advantages of this ‘bottom-up’ approach include the possibility to integrate preclinical *in vitro* and *in vivo* information with clinical information (60), and the use of these PBPK models can speed up the drug development process, while putting less of a burden on patients (61).

PBPK modelling is an example of the systems approach, and fits within the emerging field of systems pharmacology (62, 63), which combines systems biology with PKPD modelling and simulation. Systems pharmacology focuses on the understanding of the behavior of a system as a whole by quantitative analysis of dynamic interactions between a drug and a biological system (62, 63). In **Chapters 5 and 6**, we used a model with less complexity than a full PBPK model, as the main disadvantage of these complex models is that they require a lot of system- and drug-specific information.

In order to predict drug clearance in the pediatric population using a more complex PBPK model, more physiological information on e.g. the anatomy of neonates, infants, and children is needed (59). Different values for tissue blood flows as well as for tissue volumes and intestinal surface area have been assumed throughout

literature. Harmonization of these values, based on reliable measurements throughout the pediatric range from preterm neonates up to adolescents, is of the utmost importance to reduce uncertainty in clearance predictions by PBPK models in a clinical setting or during drug development. More consistent physiological information will also lead to a better understanding of the mechanistic basis for the absorption, distribution, metabolism and excretion of drugs in the pediatric population (59). For all pediatric ages, information on tissue blood flows, especially hepatic blood flow, is essential, as the sensitivity analyses in **Chapters 5 and 6** revealed that assumptions on these flow rates may impact conclusions on intrinsic clearance and local bioavailability. The challenge for the next years will be collecting especially the hepatic blood flow and other physiological information in neonates, infants, and children, and this is especially urgently needed for preterm neonates, as in this specific population the least physiological information is available (22).

How to move forward for dosing of CYP3A substrates

To find the optimal first-in-child dose during drug development and to develop pediatric dose recommendations for clinical practice, accurate prediction of plasma clearance of drugs is essential. Based on the framework reported by Calvier *et al.* (29), we report in **Chapter 7** that, using midazolam as a probe drug, pediatric clearance of several CYP3A substrates could be accurately scaled down to 1 day of age from adult clearance values using the pediatric covariate function for CYP3A-mediated midazolam clearance. We anticipate that using this approach, clearance of other CYP3A substrates can be scaled in neonates, infants, and children (figure 3), provided they are either highly protein bound and have a low-intermediate extraction ratio or have an intermediate extraction ratio when low protein bound in adults.

As described in **Chapter 7**, the typical clearance (CL_i) of a CYP3A substrate administered to a pediatric subject $i > 1$ year of age with a body weight of WT_i (in kg) can be described by:

$$CL_i = CL_{adult} \times \left(\frac{WT_i}{70} \right)^{0.874} \quad (\text{eq. 2})$$

In which CL_{adult} is the reported adult clearance value, and in which both clearance values (CL_i and CL_{adult}) are expressed in volume per time. This pediatric covariate function for CYP3A-mediated clearance derived from midazolam may be especially useful for the prediction of clearance of CYP3A substrate drugs with a small therapeutic window or CYP3A substrates with high toxicity like some anticancer agents (64). Accurate clearance predictions for these substrates in children can lead to optimal exposure and thereby ultimately limit the toxicity. Furthermore, it may be useful in dose-finding of drugs in neglected (tropical) diseases, as for example

very limited PK information is available for anthelmintic CYP3A substrates like ivermectin and praziquantel in children (65).

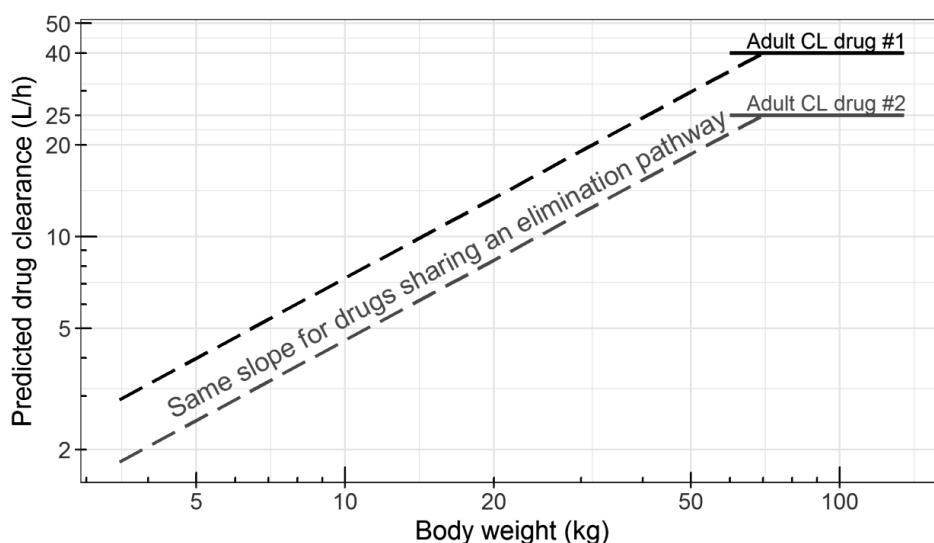


Figure 3. Example on how to scale pediatric clearance values from an adult clearance value. Using the framework developed by Calvier *et al.* (29), between-drug extrapolation of clearance can be assessed on the basis of drug properties (i.e. extraction ratio, type of plasma protein binding [e.g. HSA or AAG], fraction unbound, and elimination pathway). When accurate clearance predictions can be anticipated, the pediatric clearance of the new CYP3A substrates will follow the same pediatric covariate function as the model drug.

In this figure, the exponential function (Eq. 2) for CYP3A-mediated midazolam clearance is used to scale pediatric clearance of two hypothetical CYP3A substrates (#1 and #2, with an adult clearance of 40 and 25 L/h, respectively), which on a log-log scale shows as the same slope. The scaling of clearance should not be extrapolated beyond the studied population in which the pediatric covariate function is established, and also not to preterm neonates, which should be regarded as a different population due to their immature intestinal and hepatic CYP3A activity.

The use of pediatric covariate function for CYP3A-mediated clearance based on midazolam may significantly decrease the need for PK studies for each individual CYP3A substrate drug in the pediatric population, as information from one drug can be used to predict clearance of the next. This might also hold true for predictions of other pathways, with for example losartan, dextromethorphan, and omeprazole as probe drugs for CYP2C9, CYP2D6, and CYP2C19, respectively (66), as the same approach already has been shown to lead to accurate clearance predictions for e.g. UGT2B7-mediated drug metabolism and drug elimination by glomerular filtration in the pediatric population (26-28).

However, some limitations should be considered. In our analysis in **Chapter 7**, we assumed that the major elimination pathway is the same in children and adults, but the fraction metabolized by CYP3A activity may change with age for some drugs, and in that case this assumption may not hold true. For example for paracetamol, more sulfation and less glucuronidation is observed in neonates, infants and young children compared to children > 12 years of age and adults (67, 68). The ratio between the elimination pathways may change for some drugs (69), which may be resolved by taking into account the ontogeny of both pathways, rather than 1 major pathway. Moreover, for some drugs, the elimination route might change completely, for example caffeine is a CYP1A2-substrate in adults, while it is renally cleared in neonates (70). In addition, accurate clearance predictions may not be possible for all drugs across the entire pediatric age range, but only down to a certain age depending on its drug properties. Hence, more evaluations based on clinical data are needed, before this methodology can be applied for clearance predictions for all metabolic elimination pathways.

Despite these limitations for other pathways, the developed pediatric covariate function for CYP3A-mediated midazolam metabolism will aid in predicting pediatric clearance of various CYP3A substrates, and after evaluation, this function can be prospectively used for dose estimation of CYP3A substrates in the pediatric population.

Conclusion

To conclude, children are not just small adults, and therefore dose estimation of CYP3A substrates needs to be different in neonates, infants, and children compared to adults. For optimal treatment with CYP3A substrates, accurate predictions of CYP3A-mediated clearance throughout the pediatric age range are necessary. We found that body weight should be used in the pediatric covariate function for CYP3A-mediated clearance, as it best reflects the growth and maturation, except for preterm neonates which should be regarded as a different population due to their immature intestinal and hepatic CYP3A activity. Also, the effect of disease severity on the pharmacokinetics of CYP3A substrates should be taken into account, as midazolam clearance is significantly lower in patients with inflammation and multiple failing organs.

We confirmed that when midazolam clearance in children is used to reflect CYP3A-mediated metabolism in children ranging in age from 1-18 years, pediatric plasma clearance of various commonly used CYP3A substrates with varying drug properties can be accurately scaled from adult values. For CYP3A substrates with different properties, for example with a high extraction ratio, the pediatric

covariate function for CYP3A-mediated midazolam clearance may not lead to accurate prediction of plasma clearance throughout the pediatric age range, but may only be able to predict in children above a certain age. As the pediatric covariate function for CYP3A-mediated clearance from a midazolam PK model can predict the clearance of various CYP3A substrates, between-drug extrapolation of clearance of drugs sharing a metabolic elimination pathway is found to be possible. This function from a midazolam PK model will significantly improve CYP3A-mediated clearance predictions in neonates, infants, and children, and after evaluation of these model-based clearance predictions in pediatric PKPD studies, dosing recommendations for midazolam and many other CYP3A substrates can be applied in clinical practice.

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