Appendix II

A novel maturation function for clearance of the CYP3A substrate midazolam from preterm neonates to adults

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Abstract

Background and objective: Major changes in Cytochrome P450 (CYP) 3A may be expected in the first months of life with later on relatively limited changes. In this analysis we studied the maturation of *in vivo* CYP3A mediated clearance using midazolam as model drug, from preterm neonates of 26 weeks gestational age (GA) onwards to adults. To investigate the maturation of *in vivo* CYP3A mediated clearance of drugs from prematurely born neonates with gestational ages (GAs) of 26 weeks throughout adulthood using midazolam as a model drug.

Methods: Pharmacokinetic data after intravenous midazolam were obtained from 6 previously reported studies. Subjects were prematurely born neonates (n=24; GA 26-33.5 weeks, PNA 3-11 days, and n=24; GA 26-37 weeks GA, PNA 0-1 days), 23 children after elective major craniofacial surgery (3-23 months), 18 pediatric intensive care patients (2 days to 17 years), 18 pediatric oncology patients (3-16 years) and 20 healthy male adults (20-31 years). Population pharmacokinetic modeling with a systematic covariate analysis was performed using NONMEM v6.2.

Results: Across the entire lifespan from prematurely born neonates to adults bodyweight was a significant covariate for midazolam clearance. The influence of body weight was best described using an allometric equation with an exponent changing with bodyweight in an exponential manner from 0.84 in preterm neonates (0.77kg) to 0.44 in adults (89kg) showing that the most rapid maturation occurs during the youngest age range.

Conclusions: An *in vivo* maturation function for midazolam clearance from prematurely born neonates to adults has been developed. This function can be used to derive evidence-based doses for children, and to simulate exposure to midazolam and possibly other CYP3A substrates across the pediatric age range in population pharmacokinetic or physiologically based pharmacokinetic (PBPK) models.

II.1. Background

Cytochrome P4503A (CYP3A) is the most abundant CYP enzyme in the human liver. [1] It is involved in the metabolism of over half of all metabolized drugs. [2] Large inter-individual and intra-individual variation has been shown for CYP3A, resulting in inter-individual differences in the clearance of CYP3A substrate drugs. An important factor explaining variation in CYP3A activity is age. However, the exact ontogenetic pattern of the CYP3A isoforms CYP3A4 and CYP3A5 *in vivo* is still unclear. [3-7] This information gap hampers the development of individualized dosing guidelines of CYP3A4/5 substrate drugs in children. To define the *in vivo* activity of CYP3A4/5, surrogate probes that correlate with actual enzyme activity can be used. [8] The best validated CYP3A4/5 probe is midazolam, which has been used extensively in adults and children. [9, 10]

For CYP3A4/5 mediated clearance of midazolam in children between one month and 17 years of age, critical illness proved a more significant covariate than bodyweight. [11] However, particularly in the first days and weeks of life, CYP3A4/5 activity may be expected to be low on the basis of *in vitro* data and *in vivo* reports on reduced clearance of CYP3A substrates in neonates [6, 7, 12-15]. Therefore, we report here on the maturation of *in vivo* CYP3A mediated clearance of midazolam across the entire human life span, using a dataset consisting of 6 midazolam studies in which the pharmacokinetics were studied in populations ranging in age from prematurely born neonates to adults. The resulting maturation function may be used as a basis for evidence based dosing of midazolam and potentially other CYP3A substrates in population pharmacokinetic or physiology based pharmacokinetic (PBPK) models.

II.2. Methods

Patients and data

Individual data from six previous studies were included in the analysis. [3, 6, 16-19] Individuals were prematurely born neonates (two datasets: n=32; gestational age (GA) 26-33.5 weeks, postnatal age (PNA) 3-11 days, and n=24; GA 26-37 weeks, PNA 0-1 days), 23 children after elective major craniofacial

surgery (3-23 months), 18 pediatric intensive care patients (2 days to 17 years), 18 pediatric oncology patients (3-17 years) and 20 healthy male adults (20-31 years). Details on the datasets are provided in table 1.

Pharmacokinetic data analysis

Population pharmacokinetic analysis was performed using the first-order conditional estimation (FOCE) with η-ε interaction in NONMEM version 6.2, release 1.1 (GloboMax LLC, Hanover, MD, USA) [20]. S-plus version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands) was used to visualize the data. Model development was performed in four steps: (1) choice of the structural model, (2) choice of the error model, (3) covariate analysis, and (4) validation of the model. Discrimination between different models was done by comparison of the objective function. A decrease in the objective function of 3.8, corresponding to a value of $p < 0.05$, was considered statistically significant. Goodness-of-fit plots (observed *versus* individually predicted concentration, observed *versus* population predicted concentration, conditional weighted residuals *versus* time, and conditional weighted residuals *versus* population predictions) of all data and stratified per dataset, were used for diagnostic purposes. In addition, the confidence interval of the parameter estimates, the correlation matrix, and visual improvement of the individual plots were used to evaluate the model. Furthermore, η-shrinkage as defined by Karlsson *et al*. [21] was calculated for all model parameters for which inter-individual variability was estimated, and over-parameterization (ill-conditioning) of the model was tested by calculating the condition number. [22]

Model development

For the structural model of midazolam, a one, two and three compartment model was tested. In the model, the previously reported influence of critical illness on CYP3A mediated clearance of midazolam [11] was incorporated, implying a reduction in midazolam clearance in critically ill patients (datasets of prematurely born neonates and pediatric intensive care patients) compared to non-critically ill children and adults (datasets of children after major craniofacial surgery, pediatric oncology patients, and adults). The individual value (*post hoc* value) of the parameters of the *i*th subject was modeled using equation 1, where P_i equals the individual or *post hoc* value of the parameters

of the *i*th subject, and P_{pop} is the population value of the parameter. Random variable (RV) is assumed to be a gaussian random variable with mean zero and variance of ω^2, assuming log-normal distribution.

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

The intra-individual variability was described with a proportional error model for all data, assuming a constant coefficient of variation over the entire concentration range, shown in equation 2, where *j* is the observed midazolam concentration (Y) of the *i*th individual, C_{pred} is the predicted midazolam concentration and RV_{ij} is the random variable for midazolam, with mean zero and variance σ^2.

$$Y_{ij} = C_{pred,ij} \times (1 + RV_{ij}) \quad (2)$$

Covariate analysis

Individual post hoc parameter estimates were plotted independently against the covariates to visualize potential covariate relationships. The following covariates were tested for all parameters: bodyweight, postnatal age, gestational age, study population, and mechanical ventilation (yes/no). Continuous covariates were separately entered into the model using a linear function (equation 3), or an allometric equation (equation 4), where P_i equals the individual or post hoc value of the parameters of the ith subject, P_{pop} is the population value of the parameter, COV is the concerned covariate, K is the exponent which may be estimated or fixed to 0.75.

$$P_i = P_{pop} \times (COV_i / COV_{median}) \quad (3)$$

$$P_i = P_{pop} \times (COV_i / COV_{median})^K \quad (4)$$

Alternatively, an allometric equation with an exponent that changes with bodyweight [23] was tested (equation 5) in which *BDE* represents the body weight dependent exponent. For *BDE*, a sigmoidal function [23] and an exponential function (equation 6) with *Coeff* as a coefficient, *BW* as body weight and exp1 as an exponent) [24] were tested.

$$P_{pop} \times (COV_i / COV_{median})^{BDE} \quad (5)$$

$$BDE = Coeff \times BW^{\exp 1} \quad (6)$$

Categorical covariates (e.g. mechanical ventilation (yes/no) or study population (critically ill / healthy)) were tested using 'IF' statements, in which the parameter for one subgroup was estimated as a multiple of the parameter estimate for the other subgroup, shown in equations 7 and 8, in which *factor* is the multiplication factor, and y equals one of the two subgroups.

$$P_i = P_{pop} \quad (7)$$

$$IF \text{ var} iable = y, Pi = Ppop \times (factor) \quad (8)$$

The influence of a covariate was statistically tested for significance by use of the objective function. P-values < 0.005 (decrease of objective function of 7.8 points) were applied to evaluate the covariates in the forward inclusion, while a more stringent p value of <0.001 (decrease of objective function of 10.83 points) was used in the backwards deletion step. When two or more covariates were found to significantly improve the model, the covariate that showed the largest reduction of the objective function was included in the model. Additional covariates had to reduce this objective function further to be retained in the model. The choice of the model was further evaluated as discussed under 'pharmacokinetic data analysis' and 'model development'. This included also individual and population parameter estimates versus the most predictive covariate in the model. [25]

Validation of the model

For the internal validation of the final model, a bootstrap analysis was performed in S-plus, version 6.2.1 (Insightful software, Seattle, WA) with NM.SP. interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands). Two hundred datasets were resampled from the combined original datasets, and refitted to the model. [26]

Secondly, Normalized Prediction Distribution Errors (NPDE) were calculated [27] using the NPDE package in R. [28] For this method, the dataset used for building the model was simulated 1000 times with inclusion of the inter-individual variability and residual error.

Simulation dosing regimen

Simulations were performed in S-plus, version 6.2.1 (Insightful software, Seattle, WA) with NONMEM SP.interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands). The parameter estimates of the final Pharmacokinetic model were used to simulate midazolam concentrations in critically ill children ranging from prematurely born neonates to adolescents varying in body weight between 0.75 and 60 kg (0.75, 1, 2, 3, 5, 10, 20, 45, and 60 kg) upon a bolus injection and an infusion duration of 24 hrs. Both dosing schedules used in clinical practice (traditional dosing schemes) and model based dosing schedules were simulated. The traditional dosing scheme for prematurely born neonates was an iv bolus of 0.05 mg/kg and an infusion of 0.06 mg/kg/hr [29-31] while for children and adolescents an iv bolus of 0.1 mg/kg and an infusion of 0.1 mg/kg/hr (conscious sedation in critically ill patients [32]) was chosen. The model based dosing regimen consisted of a dosing regimen that would result in therapeutic midazolam concentrations 0.25-0.37 µg/mL for conscious sedation in children in pediatric intensive care (PICU) patients. [32] As therapeutic midazolam concentrations for prematurely born neonates are unknown, these were set at 0.2 µg/ml being the lowest target concentration aimed for in a prematurely born neonate study. [18]

II.3. Results

Patients and data

1105 midazolam concentrations from 136 individuals of six different datasets, [3, 6, 16-19] with a postnatal age varying between 0 days and 31 years of age, and a body weight between 0.77 kg and 89 kg were included for analysis. None of the patients received CYP3A inhibitors or inducers. A summary of the patient and data characteristics of the six studies is shown in table 1.

Table 1 Overview of datasets.

Parameter	de Wildt SN *et al.*[6] (2001)	Jacqz-Aigrain E. *et al.*[18] (1994)	Peeters, M.Y. *et al.*[3] (2006)	de Wildt SN *et al.*[16] (2003)	de Wildt SN *et al.*[17] (2000)	van Gerven J.M.A. *et al.*[19] (1997)
Patient Population	Preterm neonates	Preterm neonates with respiratory distress syndrome	Children after elective major craniofacial surgery	Pediatric intensive care patients	Oncology patients	Male adults
Indication for midazolam sedation	Sedation for invasive procedure in Intensive Care	Mechanical ventilation in Intensive Care	Postoperative sedation	Conscious sedation in Intensive Care	Sedation for invasive procedure	Healthy volunteers
Number of Patients	24	24	23	18	18	20
Postnatal Age *median (range)*	5 days (2.9-11)	0 days (0-1)	11.5 months (3.2 - 24.7)	38.5 months (0.03-203.5)	6.1 years (3.2 – 16.2)	24 years (20-31)
Gestational Age *median (range)*	28.3 weeks (26-33.6)	32 weeks (26-37)	-	-	-	-
Bodyweight *median (range)*	1.07 kg (0.77-1.6)	1.64 kg (0.96-3.7)	9.6 kg (5.1– 12)	14 kg (2.8-60)	22.5 kg (12.6– 60.1)	72.5 kg (64-89)
Mechanical ventilation N/N_{TOTAL}	13 / 24	24 / 24	2 / 23	15 / 18	0 / 18	0 / 20
Midazolam Dose *median (range)*	0.1 mg/kg iv infusion in 30 minutes	60 µg/kg/hr iv infusion If GA < 33w → after t > 24hr 30 µg/kg/hr	0.1 mg/kg iv loading dose, 0.05-0.2 mg/kg/hr infusion	0.1 mg/kg loading dose, 0.05-0.4 mg/kg/hr infusion	0.1 (0.03-0.53) mg/kg iv bolus dose	0.1 mg/kg iv infusion in 20 minutes
Number of Samples	155	63	198	233	82	336

Model development and covariate analysis

The pharmacokinetics of midazolam was best described with a two-compartment model with a proportional error model. In the model, the lower midazolam clearance due to critical illness [13] was incorporated for the datasets with prematurely born neonates [6, 18] and the dataset obtained in children admitted to an intensive care [16]. Introducing inter-individual variance for CL, Q, V_1, and V_2, significantly improved model performance with a total decrease in objective function (-2LL) of 3276.7 ($p < 0.001$).

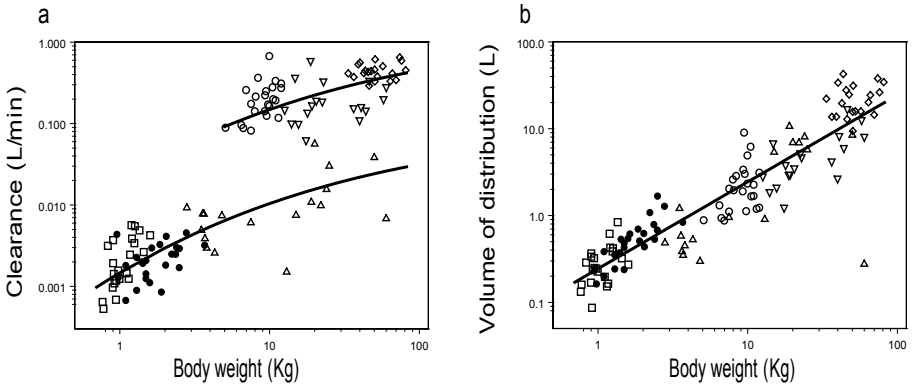

Figure 1 Posthoc estimates of CYP3A4 mediated clearance of midazolam *versus* body weight (a), and central volume of distribution *versus* body weight (b) in the final model. □ = preterm neonates, ● = preterm neonates with RDS syndrome, ○ = children after elective major craniofacial surgery, △ = pediatric intensive care patients, ▽ = pediatric oncology patients, and ◇ = male adults, with a black line as their post-hoc population predicted values, respectively.

In the covariate analysis, body weight was the most significant covariate for CL resulting in a decrease in objective function of 149.7 points ($p < 0.001$) and explained 65.4% of the inter-individual variability for CL (a decrease from 202.58% to 70.13%). The relation between bodyweight and CL was best described using an allometric function with an exponent that varies with bodyweight (body weight dependent exponent (BDE) [23]), as shown in figure 1 (*left*). The BDE was defined as Coeff x BW^{exp1}, in which BW represents body weight, Coeff proved 0.81 (CV of 8.2%) and exp1– 0.135 (CV of 31.4%) (table 2), resulting in a BDE which decreased from 0.84 in a preterm neonate of 0.77kg to 0.44 in adult of 89 kg in an exponential manner. The covariate analysis also revealed that bodyweight was of significant influence on V_1 (delta -2LL of -52.8, $p < 0.001$). The nature of this influence was best described using a linear function (figure 1, *right*), because the exponent was found to not differ significantly from 1 when using an allometric function. For V_2, in the postoperative craniofacial surgery patients [3] a 6-fold higher peripheral volume of distribution compared to the five other datasets was found. A stable model could only be achieved when estimating this peripheral volume of distribution for the craniofacial surgery patients simultaneously with the addition of bodyweight in an allometric function to V_2. This resulted in a significantly improved model (-2LL of -184.6, $p < 0.001$). A separate addition resulted in model termination, with a significant decrease in objective function for each covariate. For Q, there was no significant influence of age, bodyweight or population. None of the other tested covariates were of influence on any of the pharmacokinetic parameters.

The pharmacokinetic parameters of the final model along with their confidence intervals, inter-individual variability, and the results of the bootstrap validation are shown in table 2. This table also shows that after the covariate analysis, the inter-individual variance for Q could be fixed to 0 in the backwards deletion step without deterioration of the model while a successful covariance step could be achieved.

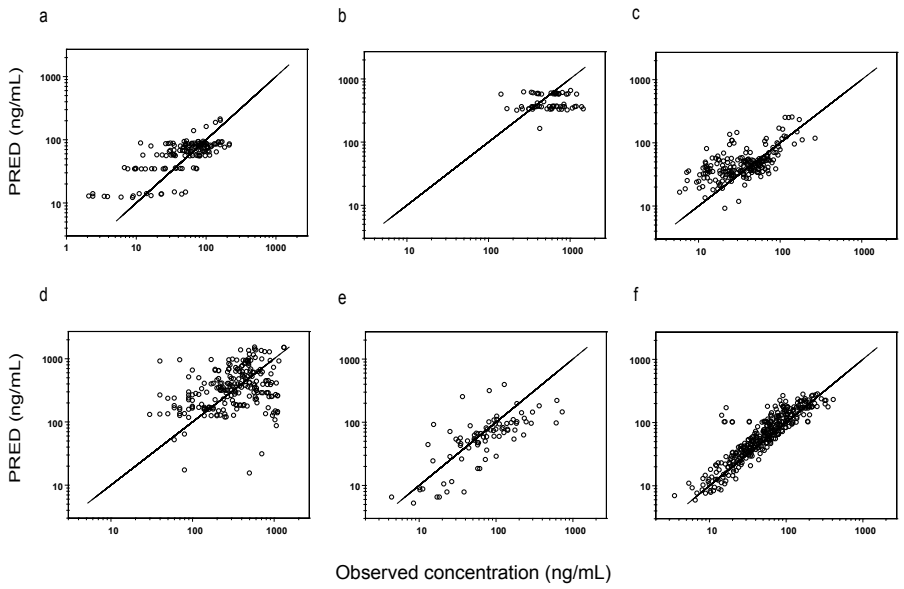

Figure 2 Visual diagnostics of the final model per dataset. Population predicted concentrations by the model versus the observed concentrations of midazolam. (a) de Wildt SN *et al*. [6]: preterm neonates; (b) Jacqz-Aigrain E. *et al*. [18]: preterm neonates with respiratory distress syndrome; (c) Peeters, M.Y. *et al*. [3]: children after elective major craniofacial surgery; (d) de Wildt SN *et al*. [16]: pediatric intensive care patients; (e) de Wildt SN *et al*. [17]: oncology patients; and (f) van Gerven J.M.A. *et al*. [19]: Male adults. PRED predicted concentration. The solid line indicates the line of unity.

Figure 2a-f illustrates the population predicted concentrations *versus* observed concentrations for each dataset separately. These diagnostic plots (in log-scale) indicate that overall the concentrations are predicted by the developed pharmacokinetic model without bias, as the concentrations are evenly distributed around the line of unity, except for the lower concentrations from the craniofacial surgery patients (Figure 2c), for which a slight under-prediction was observed.

Validation of the final model using NPDE simulations (figure 3) illustrate the distribution of the NPDEs, with a mean close to zero (-0.04 for the mean, and 0.98 for the variances, respectively), and with no observed trends in time or concentration. In the final model, all values of η-shrinkage were below 20% (CL: 5.1%; $V_{1:}$:17.7% ; V_2 14.3%), which indicates that the individual parameter estimates are reliable. [21] The calculated condition number of 195 of the final model was well below the critical value for the indication of ill-conditioning of 1000. [22]

Table 2 Population parameter estimates obtained for the final midazolam pharmacokinetic model

Parameter	Model fit		Bootstrap results		Explanation
	Value	*(CV%)*	*Value*	*(CV%)*	
$CL_i = f \cdot CL_{13kg} \cdot (BW/13)^{BDE}$					*CYP3A4/5 mediated clearance of midazolam*
$BDE = Coeff \cdot BW^{exp1}$					*BW dependent exponent of allometric exponent function on CL*
CL_{13kg} (L/min)	0.17	(8.4)	0.17	(8.2)	*CL for a median individual of the population with a BW of 13 kg*
Coeff	0.81	(8.2)	0.81	(8.0)	*Coefficient of the BDE function*
exp1	- 0.135	(31.4)	- 0.138	(31.6)	*Exponent of the BDE function*
Q (L/min)	0.69	(18.1)	0.71	(20.4)	*Inter-compartmental clearance between central and peripheral compartment*
$V_{1i} = V_{1,13kg} \cdot (BW/13)$	2.7	(18.3)	2.7	(18.4)	*Distribution volume of central compartment for a median individual of the population with a BW of 13 kg*
$V_{1,13kg}$ (L)					
$V_{2i} = g \cdot V_{2,13kg} \cdot (BW/13)^{exp2}$					*Distribution volume of peripheral compartment (V_2)*
$V_{2,13kg}$ (L)	6.38	(5.9)	6.38	(6.2)	*V_2 for a median individual of the population with a BW of 13 kg*
exp2	0.76	(7.6)	0.76	(6.1)	*Allometric exponent of V_2 in: $V_{2i} : V_2 \cdot (BW/13)^{exp2}$)*
g	6.0	(18)	6.2	(19.4)	*Multiple of V_2 for children after elective major craniofacial surgery[3]; g=6 children after elective major craniofacial surgery, g=1 all other patients*
ω^2 CL	0.37	(17.6)	0.35	(16.8)	*Variance in CL*
ω^2 V_1	0.58	(32.4)	0.38	(46.3)	*Variance in V_1*
ω^2 V_2	0.37	(37.5)	0.36	(37.7)	*Variance in V_2*
ω^2 (V_2 / V_1)	0.41	(41.3)	0.56	(41.4)	*Correlation between the Variance in V_2 and V_1*
$\sigma^2_{proportional}$	0.12	(14.5)	0.12	(11.7)	*Residual error (proportional error)*

f : fraction to estimate CL in intensive care patients compared to CL (f=1 healthy patients, f=0.07 intensive care patients), **BW** : body weight, **BDE** : bodyweight dependent exponent. Coeff : coefficient of the BDE function, g : multiple of distribution volume of peripheral compartment for children after elective major craniofacial surgery, CL_{13kg} : midazolam clearance for a median individual of the population with a body weight of 13 kg, $V_{1,13kg}$: distribution volume of central compartment for a median individual of the population with a body weight of 13 kg, $V_{2,13kg}$: distribution volume of peripheral compartment for a median individual of the population with a body weight of 13 kg. [11]

Simulations to derive a model based dosing regimen

Simulations have been performed using the typical value of the parameters with the aim to show the exact influence of the covariates on the overall concentration-time relationships for different typical individuals. Figure 4 (left panels) illustrates that based on the traditional dosing scheme for conscious sedation in prematurely born neonates and critically ill children large differences in midazolam concentrations between individuals can be anticipated, particularly in the pediatric subpopulation. This results from the fact that the traditional dosing regimen is expressed in mg/kg/h while the results of this study show that clearance relates nonlinearly to body weight. Upon a model based dosing regimen aiming for predefined midazolam concentration ranges over a 24 hour period (table 3) similar midazolam profiles are anticipated across the human life span (Figure 4, right panels). This model-based dosing regimen that resulted from the simulations consists for preterm

neonates of 0.05 mg/kg bolus and 0.06mg/kg$^{0.85}$/h for 7 hours followed by 0.03mg/kg$^{0.85}$/h for 17 hours (table 3). For children in the Intensive Care (>5 kg) requiring conscious sedation, the model based dosing regimen consists of 0.1 mg/kg bolus and 0.1 mg/kg/h for 3 hours followed by 0.05 mg/kg$^{0.65}$/h for 21 hours (table 3). In table 3, both traditional and model-based guidelines are presented for conscious sedation with midazolam in preterm neonates and children in the Intensive Care ranging in bodyweight between 0.75 and 60 kg.

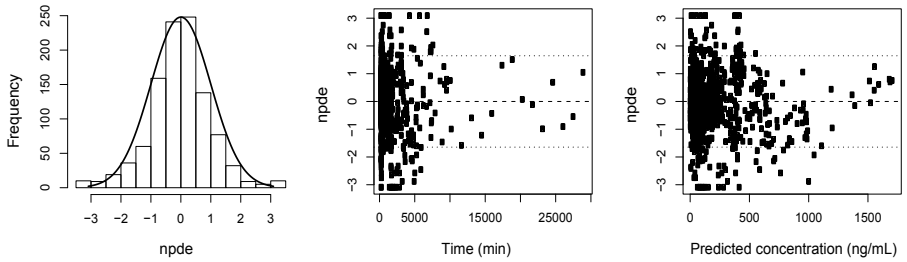

Figure 2 Internal validation, Normalised Prediction Distribution Errors (NPDE) of the final model. The histogram illustrates the distribution of the NPDE's for midazolam in which the solid line represents a normal distribution. In addition, the distribution of NPDE's in time after first dose and against the observed concentrations is illustrated.

II.4. Discussion

We have developed a novel maturation function for midazolam clearance based on a dataset consisting of data obtained in six different clinical studies performed with midazolam in (prematurely born) neonates, infants, toddlers, children, adolescents, and adults. This model provides a quantitative insight in the developmental pattern of *in vivo* CYP3A activity across the pediatric age range, including prematurely born neonates. This model may provide guidance to dosing of midazolam in children across all age ranges and potentially other CYP3A substrates, i.e. by applying this function that describes the increase in midazolam clearance over weight, in population pharmacokinetic and physiologically based models, as *a priori* information.

Table 3 Traditional and model-based dosing schemes for conscious sedation with midazolam in preterm neonates and children in the intensive care

Patient group	Weight	Infusion duration	Traditional dose$		Model-based dose*$	
	kg	*over 24 hrs*	*mg/hr*	*mg/kg/hr*	*mg/hr*	*mg/kg/hr*
Preterm Neonates	0.75	0 – 7 hrs	0.045	0.06	0.047	0.063
		7 – 24 hrs	0.045	0.06	0.023	0.031
	1	0 – 7 hrs	0.06	0.06	0.06	0.06
		7 - 24 hrs	0.06	0.06	0.03	0.03
	2	0 - 7 hrs	0.12	0.06	0.11	0.054
		7 - 24 hrs	0.12	0.06	0.054	0.027
	3	0 - 7 hrs	0.18	0.06	0.15	0.051
		7- 24 hrs	0.18	0.06	0.076	0.025
Children	5	0 – 3 hrs	0.5	0.1	0.5	0.1
		3– 24 hrs	0.5	0.1	0.14	0.028
	10	0 – 3 hrs	1	0.1	1	0.1
		3- 24 hrs	1	0.1	0.22	0.022
	20	0 -3 hrs	2	0.1	2	0.1
		3- 24 hrs	2	0.1	0.35	0.018
	45	0 - 3 hrs	4.5	0.1	4.5	0.1
		3- 24 hrs	4.5	0.1	0.59	0.013
	60	0 – 3 hrs	6	0.1	6	0.1
		3– 24 hrs	6	0.1	0.72	0.012

*****Model-based doses aim for reported therapeutic midazolam concentrations of 0.25-0.37 µg/mL [32] for children and 0.2 µg/mL [29-31] for preterm neonates, and are 0.06mg/kg$^{0.85}$/h for 7 hours followed by 0.03mg/kg$^{0.85}$/h for 17 hours for preterm neonates and 0.1 mg/kg/h for 3 hours and 0.05 mg/kg$^{0.65}$/h for 21 hours for intensive care children. **$** Depicted doses are maintenance doses after a bolus dose of 0.05 mg/kg for preterm neonates and 0.1 mg/kg for intensive care children.

Previously, we have shown in children between 1 month and 17 years of age that critical illness was a more important covariate for CYP3A mediated clearance of midazolam than bodyweight. [11] As CYP3A is known to mature particularly in the first days and weeks of life, we included in the current analysis midazolam clearance values spanning the age range from prematurely born neonates to adults. This allowed to describe the change in CYP3A activity from birth onwards to adults. The current analysis suggests that the major change in midazolam clearance occurs in the first year of age. (figure 1). More specifically, in prematurely born neonates between 0.5 and 4 kg, midazolam clearance was estimated to be only 2.6% to 21.8% of adult values. These results confirm a very low CYP3A4/5 activity following birth [14] as midazolam is only slightly metabolized by CYP3A7. [33] Our finding that the largest change in clearance occurs in the first weeks of life is in line with CYP3A4/5 *in vitro* and *in vivo* phenotyping data. CYP3A4 mRNA levels in human fetal liver microsomes (gestational age 11-30 weeks) were reported to be on average 10% of that in

adults. [14, 34] At the age of one to three months, Lacroix *et al.* reported an increase in CYP3A4 activity *in vitro* reaching 30-40% of adult levels [14], which approximates our *in vivo* findings of 25-35% of adult activity.

Data on CYP3A4 activity after the first year of life, as derived from *in vitro* and different *in vivo* studies are highly discrepant [35-38]. The *in vitro* finding by Stevens *et al.* reporting CYP3A4 protein content at age 5–15 years to be only about 20% of adult levels [15] is not in agreement with the *in vivo* data on total clearance of midazolam. Based on reported average midazolam clearance data from the literature, Johnson *et al.* summarized that CYP3A4/5 mediated midazolam clearance expressed in L/hr reaches approximately 18% and 40% of the clearance in adults at the age of 1 year, [39] and 5 years, [39] respectively. Interestingly, Anderson *et al.* using average *in vivo* clearance values from literature data estimated midazolam clearance at birth in a term neonate to be 14% of an adult, reaching 64% of that in adults at one year of age and 90% at two years of age. [40] Additionally, using the urinary ratio of dextromethorphan and its metabolite 3-hydroxymorpinan, Johnson *et al.* simulated an increase in CYP3A4 activity that reaches 72% of that in adults at the age of 1 year based on a phenotyping study by Blake *et al*. [37] An explanation for the large variation reported in CYP3A activity in *in vivo* and *in vitro* studies may relate to the sub-optimal quality of post-mortem tissue used for the *in vitro* studies, resulting in erroneously low CYP3A activity levels. In addition, the studies by Johnson *et al.* [39] and Anderson *et al.* [40] both used average clearance values instead of individual doses and concentrations to model the maturation of midazolam clearance. Furthermore, the number of underlying individual data of children between 0 and 1 year of age in these pooled studies was very low. which could have resulted in erroneous estimations for this age range [40] For our analysis we had access to all raw data on demographics, doses, concentrations, and covariates of 136 individuals varying in age between prematurely born neonates and adults. We therefore suggest that our analysis was less prone to the mentioned drawbacks, and increases the validity of the value of the estimated maturation function for *in vivo* CYP3A4/5 activity. However, as the estimated CYP3A4/5 abundance by Johnson *et al.,* and Anderson *et al.* [39, 40] was related to age, and not to body weight, the exact difference with our model based on body weight, is difficult to derive, and should be kept in mind when comparing the results.

The maturation of CYP3A as assessed by the clearance of midazolam was found to be highly nonlinear (figure 1), and could be described best by an allometric function with a body weight dependent exponent (BDE). This conforms with the report of Wang *et al.* who first reported that an exponent changing with bodyweight that is accounted for with an allometric function, best described the changes in propofol clearance occurring between neonatal to adult age. [23] In their model, the allometric exponent changed in a sigmoidal manner (E_{max} model) with bodyweight, and had four parameters to be estimated.[23] For propofol clearance, this exponent was found to change from 1.43 for a hypothetical bodyweight of 0 kg, to 0.55 for 10 kg upwards.[23] In our model, we used a simplified version of the model of Wang *et al.* [23] by allowing the exponent to change in an exponential manner, thereby reducing the degrees of freedom from four to two, which was previously applied for busulphan in children. [24] This approach allowed us to adequately estimate all pharmacokinetic parameters without overparameterization of the model, and to describe the midazolam clearance from prematurely born neonates to adults without bias over the entire range of bodyweight. This contrasts to an allometric function with an estimated exponent or a fixed exponent of 0.75, as this resulted in failure to minimize successfully or in inadequate description of clearance throughout the entire weight range of the population, without adding more age related information in the model. Using those models, especially in the lowest weight range, midazolam clearances were overestimated. While the addition of an age-based function to capture maturation in the youngest age ranges correcting for this overestimation could be considered, we decided to use a bodyweight-dependent exponent model instead. With the BDE model, the use of two different functions of covariates that are correlated (i.e. age and bodyweight) which may potentially lead to inappropriate functions, is prevented. [41] Interestingly, using the BDE model in which the exponent was found to vary between 0.84 in neonates to 0.44 in adults, midazolam clearance, was well estimated across the entire age range. The fact that maturation of CYP3A activity appears less steep than reported for propofol (highest exponent of 0.91 versus 1.44, respectively) may reflect differences in pathways involved in metabolism of midazolam and propofol, being CYP3A oxidation and glucuronidation, respectively. [23] As such it seems that pathway-specific functions need to be developed across the age range for different common metabolic and elimination pathways that can be used to

predict changes in drug metabolism and elimination in children. Particularly, in view of the growing interest in pediatric PBPK models, [42] it is important to quantify the maturational *in vivo* behavior of all metabolic enzyme systems that are clinically relevant.

In this analysis, the results of the current function for CYP3A mediated clearance of midazolam have been used to derive a model based dosing scheme for conscious sedation in prematurely born neonates and children in the Intensive Care. Table 3 shows that instead of empiric dosing in mg/kg/h, it is proposed to dose the maintenance dose in a nonlinear manner. In accordance with the exponent that was found for changes in clearance within the pediatric range, an exponent of 0.85 is proposed for prematurely born neonates while an exponent of 0.65 is used for older children. In order to prevent dosing errors, a table is provided to guide dosing according to these non-linear functions. Figure 4 shows that upon this nonlinear dosing scheme, similar concentrations can be expected across a wide range in bodyweight, while in mg/kg/h dosing schedule large variations may be expected due to the nonlinear nature of clearance across bodyweight. The target midazolam concentration chosen from literature [18, 32] may, however, be arbitrary, as it is conceivable that some children may require higher concentrations than others. Therefore the dosing table (table 3) is only a guide for dose adjustments between children, while the absolute value (e.g. 0.06 in 0.06 mg/ $kg^{0.85}$/hr for prematurely born neonates) may be adjusted at the discretion of the attending physician. A previous study of our group has shown that it is indeed feasible to apply a nonlinear dosing scheme in the context of a clinical trial. [43] In that study, morphine was dosed according to a function with an exponent of 1.44, implying that neonates require less and older children require more than what is currently administered based on mg/kg/h dosing schedule.

No dosing implications have been derived on the basis of the results of this study for non-critically ill patients. We have reported previously that absolute midazolam clearance in critically ill patients is much lower (figure 1 left) compared to relatively healthy children [11]. We hypothesized this phenomenon to be due to critical illness related inflammation response causing CYP3A4 gene repression. [44] Due to the absence of midazolam data in non-critically ill neonates it was not possible to quantify the maturational behavior of CYP3A

in healthy neonates, using midazolam clearance as surrogate. However, as healthy neonates and children most often receive bolus doses of midazolam for a diagnostic or therapeutic intervention, changes in volume of distribution as a result of age may be more important than changes in clearance for this age group. While volume of distribution was found to scale linearly with bodyweight, there are no clear reasons to change the current practice of dosing a bolus in mg/kg across the pediatric age range when midazolam is given for procedural sedation. For V2, a 6-fold higher peripheral volume of distribution was found in the postoperative craniofacial surgery patients [3] compared to the five other datasets. As blood loss during craniofacial surgery may increase up to average values of 24% of the total volume of blood, [45] blood transfusion is often required to maintain the blood cell-, thrombocyte-, and plasma levels at normal values. This may have eventually have led to an increase of peripheral volume of distribution of midazolam in these patients, compared to the other patients.

As we described in this study, the maturation of hepatic CYP3A activity using midazolam as an *in vivo* probe, the impact of ontogeny on intestinal CYP3A activity is still unknown. It has become clear over the years that CYP3A activity in the intestine is of considerable importance, thereby necessity the investigation of the maturation of CYP3A mediated first pass-elimination of midazolam in children across different ages. Another limitation is that external validation of a pharmacokinetic model using independent data, and ultimately prospective clinical evaluation, which are important steps following internal validation of model still needs to be performed. [46] An important issue in the validation process of pediatric modeling, is the lack of sufficient data to perform the external validation, and the ethical constraints for performing prospective clinical evaluation of a drug in children. However, although only 17% of published pediatric pharmacokinetic and pharmacodynamic models are validated, [47] we believe that all models should be at least fully internally validated. [46]

In addition, extrapolation of the maturation model for the CYP3A substrate midazolam, will test the ability of the model to predict the system specific properties of other drugs that share, at least partly, the similar pharmacokinetic pathway. [48] Another CYP3A substrate from the literature that has been

studied quite adequately during the first months of life is cisapride. [49] Using the maturation model for midazolam to describe cisapride pharmacokinetics in the youngest infants would be another step in the validation process of the semi-physiological pharmacokinetic model.

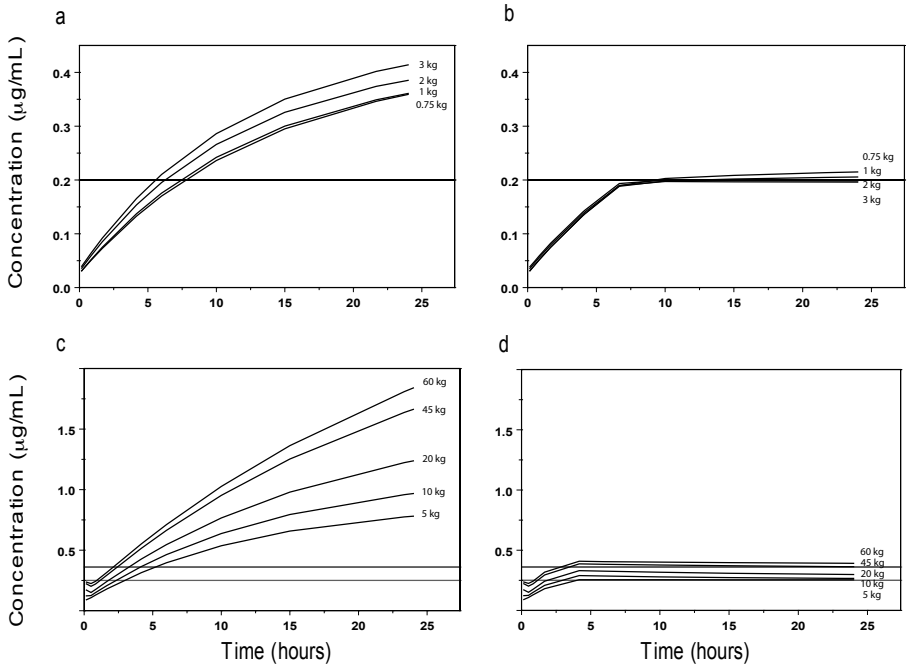

Figure 4 Simulated midazolam concentrations versus time in preterm neonates (a, b) and children (c, d) in intensive care with traditional dosing regimen (a, c) and model-based dosing regimen (b, d) aiming for reported therapeutic midazolam concentrations (horizontal grey lines). (a, b): midazolam concentrations in preterm neonates (0.75, 1, 2, 3 kg body weight) after traditional dosing regimen [bolus dose of 0.05 mg/kg followed by 0.06 mg/kg/h for 24 hours (a)], and after model-based dosing regimen as shown in table 3 (b). (c, d): midazolam concentrations in critically ill children (5, 10, 20, 45, and 60 kg body weight) after traditional dosing regimen [0.1 mg/kg bolus dose followed by 0.1 mg/kg/h for 24 hours (c)], and after model-based dosing regimen as shown in table 3 (d).

II.5. Conclusion

In conclusion, an *in vivo* maturation function for CYP3A mediated clearance of midazolam from prematurely born neonates to adults has been developed. This maturation function can be used to derive evidence-based doses for prematurely born neonates, infants and children, and to simulate exposure to midazolam and possibly other CYP3A substrates across the pediatric age range in population pharmacokinetic or physiologically based pharmacokinetic (PBPK) models.

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