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

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Chapter 4

Predicting CYP3A-mediated midazolam metabolism in critically ill neonates, infants, children, and adults with inflammation and organ failure

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ABSTRACT

Aims: Inflammation and organ failure have been reported to impact cytochrome P450 (CYP) 3A-mediated clearance of midazolam in critically ill children. Our aim was to evaluate a previously developed population pharmacokinetic model in both critically ill children and other populations in order to allow the model to be used to guide dosing in clinical practice.

Methods: The model was externally evaluated in 136 individuals, including (pre) term neonates, infants, children, and adults (body weight 0.77-90 kg, CRP 0.1-341 mg/L and 0-4 failing organs) using graphical and numerical diagnostics.

Results: The pharmacokinetic model predicted midazolam clearance and plasma concentrations without bias in post-operative or critically ill paediatric patients and term neonates (median prediction error (MPE) <30%). Using the model for extrapolation resulted in well-predicted clearance values in critically ill and healthy adults (MPE <30%), while clearance in preterm neonates was over predicted (MPE >180%).

Conclusion: The recently published pharmacokinetic model for midazolam, quantifying the influence of maturation, inflammation, and organ failure in children yields unbiased clearance predictions and can therefore be used for dosing instructions in term neonates, children, and adults with varying levels of critical illness including healthy adults, but not for extrapolation to preterm neonates.

Key words
Children, drug metabolism, cytochrome P450

What is known about the subject:
- Recently, the impact of inflammation and organ failure on cytochrome P450 (CYP) 3A-mediated midazolam metabolism has been quantified in critically ill children
- Before population pharmacokinetic models can be used for clinical decision making and deriving dosing recommendations, they should be thoroughly evaluated in external datasets

What this study adds:
- Metabolic midazolam clearance can be accurately predicted in critically ill term neonates, infants, and children using C-Reactive Protein and number of failing organs
- While extrapolation to preterm neonates on the basis of this model leads to considerable over-prediction of metabolic midazolam clearance, extrapolation to adults and patients beyond the studied disease severity levels yielded unbiased midazolam clearance predictions

INTRODUCTION

Various studies suggest that cytochrome P450 3A (CYP3A)-mediated drug metabolism may be reduced by inflammation and disease (1-5). Decreases in CYP3A-mediated clearance may result in overdosing and side effects in certain patient populations. A previous study in critically ill children showed substantially reduced CYP3A-mediated clearance of midazolam in patients with inflammation and organ failure (6). A population pharmacokinetic (PK) model for midazolam was developed based on data from both critically ill term neonates and children between 0 and 17 years of age who were on mechanical ventilator support (6). Increased inflammation, reflected by a ten-fold increase in C-reactive protein (CRP) concentrations from 32 to 300 mg/L, was found to correlate with a 50% reduction in midazolam clearance in this population. Furthermore, an increase in disease severity, reflected by the number of failing organs (cardiovascular, renal, respiratory, haematological and/or hepatic failure) e.g. increasing from 1 to 3 or >3, correlates with 35 or 47% reduced midazolam clearance, respectively (figure 1).

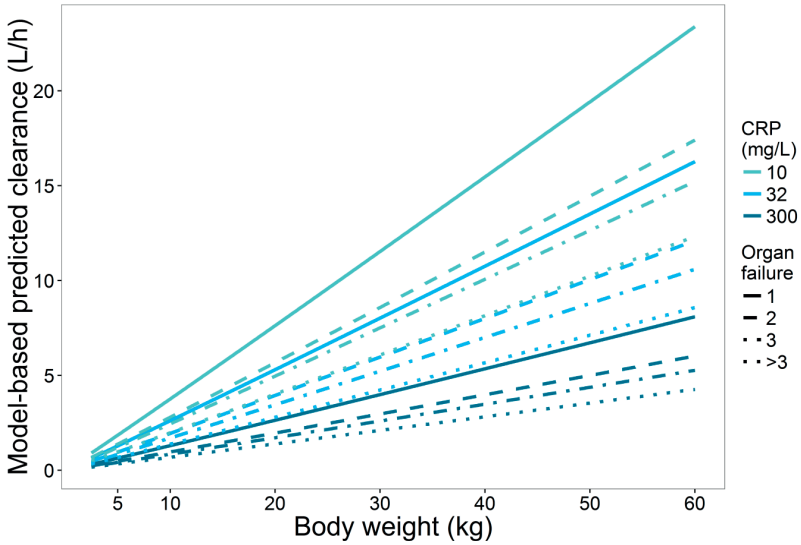

Figure 1. Model-predicted pediatric midazolam clearance for different levels of inflammation, as reflected by CRP concentrations of 10, 32, 300 mg/L (top to bottom), and disease severity scenarios, reflected by number of organ failures.

To allow population models to be used for clinical decision making, for instance for deriving dosing recommendations, they should be thoroughly evaluated (7-9). Without proper validation and evaluation, models can only be regarded as descriptive rather than predictive, thereby limiting their safe use for clinical and

Table I. Patient and study characteristics of datasets used for external validation (13, 14), and extrapolation (15-18), compared to model building data (6).

	Model building	New data for external validation		New data for extrapolation			
Study	**Vet et al (6)**	**De Wildt et al (13)**	**Valkenburg et al (14)**	**De Wildt et al (15)**	**Jacqz-Aigrain et al (16)**	**Van Gerven et al (17)**	**Swart et al (18)**
Patient population	Critically ill children	Critically ill children	Infants after cardiac bypass surgery	Preterm neonates	Preterm neonates	Healthy male adults	Critically ill adults
Indication for midazolam	Mechanical ventilation in intensive care	Conscious sedation in intensive care	Post-operative sedation	Sedation for invasive procedure	Mechanical ventilation in intensive care	Healthy volunteers	Sedation in intensive care
Number of patients	83	18	26	31	24	20	17
Samples (n (median/ patient))	523 (6)	262 (11)	153 (6)	231 (8)	63 (3)	336 (16)	238 (13)
Postnatal age (median (range))	5.1 months (1 d – 17 yr)	30 months (1 d – 17 yr)	6 months (3-31 mo)	5 days (3-44 d)	3 days (1-7 d)	24 years (20-31 yr)	56 years (21-84 yr)
Body weight (kg) (median (range))	5.6 (2.5 – 63)	13 (2.8 – 60)	6.7 (3.6 – 12.9)	1.1 (0.77 – 1.6)	1.6 (0.96 – 3.7)	72.5 (64 – 89)	75 (63 – 90)
C-reactive protein (mg/L) (median (range))	32.0 (0.3-385)	40.2 (0.1-341)	47.5 (0.1-259)	NA	NA	NA	NA
Number of organ failure (median (range))	2 (0-5)	2 (0-4)	1 (1-2)	1 (0-2)	1 (1)	0 (0)	1 (1-3)
Administered dose during study	0.15 (0.02-0.6) mg/kg /h infusion with 0.1 (0.02-0.6) mg/kg loading dose	0.05-0.4 mg/kg/h infusion with 0.1 (0.05-0.35) mg/kg loading dose	0.12 (0.02-0.3) mg/kg/h infusion with 0.05 (0.025-0.5) mg/kg loading dose titrated for effect	0.1 mg/kg (0.05-0.25) 30-min infusion	0.06 mg/kg/h infusion. If GA<33wk, after 24h 0.03 mg/kg/h	0.1 mg/kg 20-min infusion	0.2 (0.03-0.85) mg/kg/h infusion with 0.1 (0.04-0.4) mg/kg loading dose titrated for effect

NA = not available

research applications (8). Three categories in model evaluation with increasing order of quality have been described (9-11): basic internal methods, advanced internal methods and external model evaluation. Marsot *et al.* found that only 10% of the population models in paediatric subjects from neonates to 2 years of age developed up to 2010 were externally evaluated (10), even though this step is essential if the model is to be used to predict adequate dosing regimens in routine clinical practice. An external validation is based on new data that were not used for model development. A valid population model should at least be able to accurately predict data from patients with a comparable distribution of characteristics (e.g. weight/age range or disease severity) as the patient population included in model development (8). When a model is applied to predict pharmacokinetics in individuals with characteristics outside the range of the population used in model development, this not an external validation, but a form of extrapolation and this may affect the model's predictions in the new population (12).

The previously developed PK model for midazolam quantifying CYP3A-mediated clearance in critically ill children (6) has the potential to define midazolam dosing regimens that reliably achieve target plasma concentrations. The aim of this study is to evaluate the predictive performance of the population pharmacokinetic model in external data from patients with the same patient characteristics as in the original model (i.e. critically ill children, infants and term neonates). Moreover, the extrapolation potential of the model was investigated by evaluating its predictive performance in populations beyond the studied age range (i.e. preterm neonates or adults) and disease severity (healthy state).

METHODS

Patients and Data

From the literature, data from six studies were available that could be used for this external validation and extrapolation study (13-18). These studies covered different patient populations, ranging largely in age from preterm neonates to adults with different disease severity levels. All studies had been approved by ethics committees and informed (parental) consent was obtained. Table I gives an overview of the patient and study characteristics of the available data for external validation (13, 14) and extrapolation (15-18) as well as of the internal data from the original model development (6) as comparison. The new data included 136 preterm neonates, infants, children, and adults, who all received intravenous midazolam. Organ failure, counted from 0-5, was defined based on a maximum sub score for cardiovascular,

renal, respiratory, haematological and hepatic failure on the paediatric logistic organ dysfunction (PELOD) score (19) for the paediatric subjects or on the Sequential Organ Failure Assessment (SOFA) score (20) for the adult subjects. For all study participants, information on respiratory function was known, while information on the other organ functions or CRP was not always reported. For missing CRP data in preterm neonates and healthy adult volunteers, values for an healthy individual were assumed, i.e. a CRP concentration of 10 mg/L. In case of missing sub scores on organ dysfunction, no organ failure was assumed. For missing CRP values in critically ill adults (18), a CRP concentration of 32 mg/L was assumed, which was the median CRP value in the previously reported model (6) (table I), see under Original model. In total ten observations from two individuals were discarded, because of a substantial increase in midazolam plasma concentration without a recorded prior dosing event, of which for at least one individual this was known to be due to flushing of the intravenous line in the contralateral arm before sampling.

Original Model

The original population PK model consisted of a two compartment model in which the effect of body weight, inflammation, and organ failure on midazolam clearance in critically ill term neonates and children up to the age of 17 years was quantified (6). For a median patient of 5 kg with a concentration of the inflammation marker CRP of 32 mg/L and 1 failing organ, clearance was 1.29 L/h (6). Individual clearance was quantified as follows:

$$CL_i = CL_{5\ kg} \cdot (WT_i/5)^{1.02} \cdot (CRP_i/32)^{-0.312} \quad \text{(eq. 1)}$$

in which $CL_{5\ kg}$ varies for different number of organs failing with 1.29, 0.96, 0.83 or 0.67 L/h for 1, 2, 3 or >3 failing organs respectively, WT_i is the body weight (in kg) of individual *i* and CRP_i is the C-reactive protein concentration (in mg/L) of this individual. This corresponds with a clearance of 19.0 L/h for a 70 kg-individual with a CRP concentration of 32 mg/L and 1 failing organ. Volume of distribution in the central compartment for an individual *i* was

$$V1_i\,(L) = 3.28 \cdot (WT_i/5)^{1.34} \quad \text{(eq. 2)}$$

corresponding to 113 L for a 70 kg individual, and the peripheral distribution volume and the inter-compartmental clearance were 5.44 L and 1.52 L/h respectively.

Model Evaluation

The predictive performance of the PK model was evaluated using several tools. First, we obtained population and individual concentration predictions using the model and its published model parameters in NONMEM (version 7.3, ICON, Ellicott City, MD, USA). Using R (version 3.2.2) and R-studio (version 0.98.1078), goodness-of-fit plots were constructed. Concentration predictions were visually compared to the

observed concentrations and the distribution of conditionally weighted residuals (CWRES) versus the population prediction of the plasma concentrations and versus time were visually assessed. When model predictions are unbiased, CWRES are randomly distributed around zero. Additionally, we plotted individual and population clearance predictions versus the most dominant covariate (i.e. body weight) to assess the accuracy of the covariate model. Furthermore, bias was calculated per dataset by taking the median of the prediction error (PE):

$$PE = \frac{(pred - obs) \times 100}{obs} \qquad \text{(eq. 3)}$$

in which *pred* are the predicted concentration and the individually predicted clearance, and *obs* the observed concentration and the population predicted clearance to evaluate PE in concentration and clearance respectively. An MPE of <30% was considered to be an accurate prediction. Moreover, a normalized prediction distribution error (NPDE) analysis was performed using the NPDE package in R (21). For each observed concentration in the external datasets, 1000 midazolam concentrations were simulated. The simulations were based on the dosing regimen, body weight, CRP concentrations and level of organ failure of the patients, and on the parameter values including inter-individual and residual variability that were obtained for the original model (table II). These 1000 predicted concentrations were compared with the observed concentrations in the external datasets. For accurate concentration predictions, the mean of the NPDE is expected to be 0 and an adequate description of the variability in the model is expected to yield a variance in the NPDE distribution of 1.

RESULTS

Overall, 1045 plasma concentrations from 136 subjects, aged 1 day - 84 years with a body weight ranging from 0.77-90 kg, were available for the external validation and extrapolation (table I) (13-18). To compare, table I also shows the data used for development of the original model which were collected in 83 critically ill term neonates and children, ranging in age from 1 day up to 17 years (6). The subjects for the external validation included 18 critically ill children (13) and 26 children after cardiac bypass surgery (14), who were within the age and body weight range of the model building dataset and had comparable CRP concentrations and levels of organ failure. Furthermore, 55 preterm neonates ranging in age between 1-44 days (15, 16), 17 critically ill adults without alcohol abuse (18) and 20 healthy adults (17) were included to evaluate the extrapolation potential of the model predictions to patients outside the studied age and weight range and with different levels of disease severity.

Model building data	Critically ill children (6)	
External validation data	Critically ill children (13)	
	Patients after cardiac bypass surgery (14)	
Extra-polation data	Preterm neonates (15, 16)	
	Healthy adults (17)	
	Critically ill adults (18)	

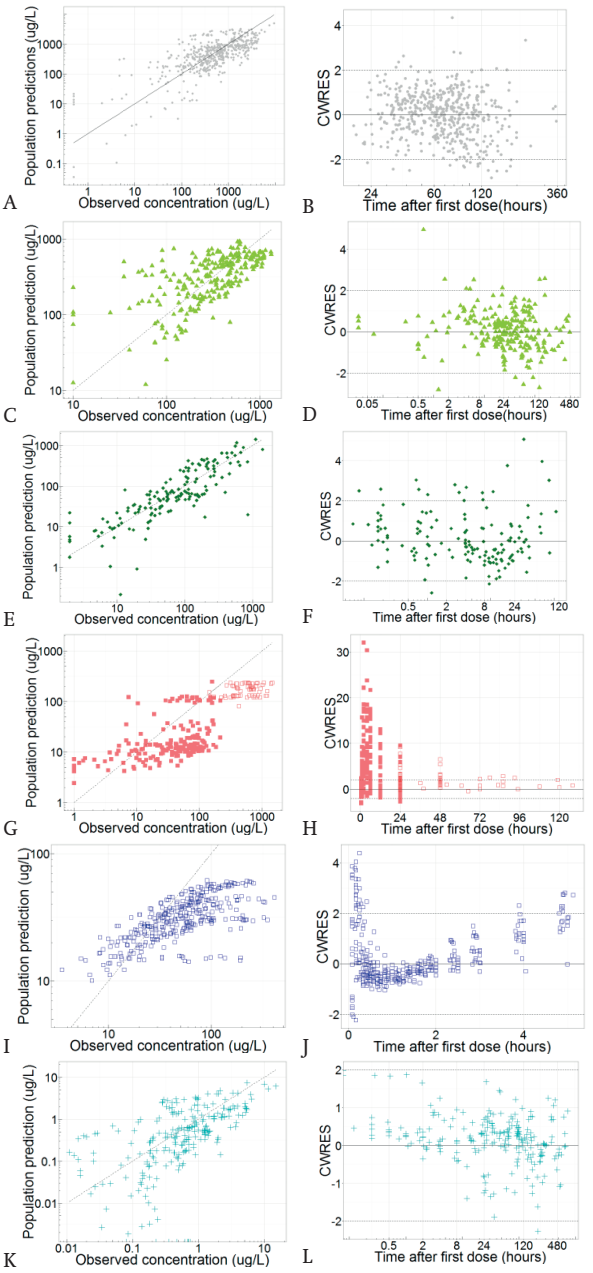

Figure 2. Goodness-of-fit plots, stratified per study. First column shows the population predicted concentrations versus the observed concentrations and the second column shows the conditionally weighted residuals (CWRES) over time for the different indicated patient populations. For panels G-H, closed squares (▪) represent predictions from preterm neonates from the study of De Wildt et al (15), while open squares (□) are data from preterm neonates from the study of Jacqz-Aigrain et al (16).

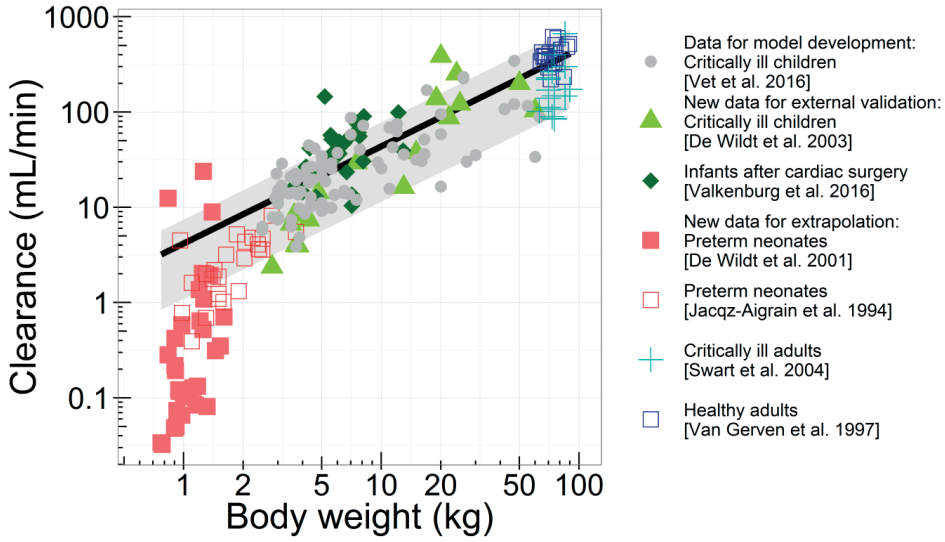

Figure 3. Population (line) and individual (symbols) predicted clearance versus the patient's body weight. The population prediction assumes an individual with a CRP concentration of 32 mg/L and 1 organ with organ failure. The grey shaded area indicates the range of population predicted clearance values from the healthiest patients (without inflammation [CRP 10mg/L] and without organ failure) (upper boundary) to the patient with most severe disease state (CRP 300 mg/L and >3 organ failures) (lower boundary).

The model described midazolam concentrations in the original dataset (figure 2, panels A-B) and was able to predict midazolam clearance and plasma concentrations without bias in critically ill children (13) and children after cardiac bypass surgery (14) (figure 2, panels C-F). Also, no trends were observed in the CWRES versus predicted plasma concentrations (plot not shown), confirming there was no bias in the peak and trough concentration predictions. In addition, the MPE was <30% for both concentrations and clearances (table II). The NPDE results indicated that model predictions are accurate without trends over time or concentration range (supplemental material). The mean of the NPDE for both populations was not significantly different from 0 (0.034 and -0.062 respectively), while the variance of the variability in the external data was statistically significantly larger than predicted by the model (2.24 and 1.95 respectively). This indicates that the concentrations in the population were accurately predicted, but that more variability is observed in the new data than is predicted by the model. Figure 3 shows that the individual clearance predictions (data points) which are based on the patient's level of inflammation and organ failure, are scattered around the population clearance predictions for patients with varying body weight and a CRP concentration of 32 mg/L and 1 organ with organ failure (black line).

Table II. Median Prediction Error (MPE) for predicted concentrations vs. observed concentrations and individual predicted clearance vs. population predicted clearance

	Study	MPE (%)*	
		Plasma concentrations	Clearance
Model building	Vet et al (6)	-13.7	5.27
New data for external validation	De Wildt et al (13)	-14.1	25.4
	Valkenburg et al (14)	3.1	22.0
New data for extrapolation	De Wildt et al (15)	-63.5	1746
	Jacqz-Aigrain et al (16)	-68.3	186
	Van Gerven et al (17)	-35.6	1.48
	Swart et al (18)	-40.6	-1.67

*The MPE is the median of the prediction error which reflects for plasma concentrations the difference in observed and predicted concentration (see Methods, eq. 3). For clearance, the difference in individual predicted and population predicted clearance is calculated.

The model building dataset included term neonates, but no preterm neonates (22). Extrapolation of model predictions to preterm neonates without inflammation or organ failure, resulted in under prediction of the high plasma concentrations at early time points (figure 2G, H) with an MPE > 60% for both datasets (table II). The NPDE results also indicated biased model predictions and an under prediction of the variability (figure S1, panels J-L). Figure 3 shows that clearance was generally over predicted for this population.

When the model was used for extrapolation to healthy adults without organ failure and an assumed CRP concentration of 10 mg/L, midazolam clearances were within the predicted range (MPE < 30%), albeit at the upper range, which may be expected given their normal CRP concentrations and lack of organ failure (figure 3). However, in the population predicted versus observed plot (figure 2I), in the CWRES over time plot (figure 2J) and the CWRES *versus* population predictions plot (not shown), a bi-phasic trend was observed, causing a large over prediction of peak and trough concentrations and under prediction for other plasma concentrations, which is suggestive of misspecification of drug distribution. The NPDE (figure S1M-O) also indicated this model misspecification for healthy adults. For critically ill adults with varying levels of organ failure and an assumed CRP concentration of 32 mg/L, midazolam clearances were also predicted within the range (MPE < 30%), although in the lower range (figure 3). Furthermore, the plasma concentrations were predicted with reasonable accuracy (figure 2 K,L). However, the NPDE also showed some model misspecification (mean distribution error is significantly different from 0,

see supplemental material), which may result from inappropriate information upon drug distribution.

DISCUSSION

In this analysis, the predictive performance and extrapolation potential of a recently developed population PK model for midazolam, quantifying CYP3A-mediated clearance in critically ill children (6), was evaluated. According to the applied model evaluation methods, midazolam clearance and plasma concentrations are well-predicted in external data from critically ill children, infants and term neonates and children after cardiac bypass surgery who are in the same age range and have similar levels of inflammation and organ failure. Extrapolation to subjects outside the age range and with different levels of disease severity, resulted in biased clearance for preterm neonates and biased concentration predictions in healthy adults. Extrapolation to subjects outside the age range with similar levels of disease severity, e.g. critically ill adults, resulted in adequate clearance predictions (figure 3).

To our knowledge, the evaluated PK model (6) in this study is the first model to describe and quantify the relationship between inflammation and organ failure on midazolam clearance in children. As in the model, besides maturation, both inflammation and organ failure proved of relevance, these factors could be relevant for dosing of CYP3A substrates. Model evaluation is however essential before a model can be used for clinical decisions, like developing dosing recommendations (7-10). Ideally, a prospective study with more subjects for external evaluation would be undertaken, to ensure that patient characteristics and covariate information would be recorded in a standard way. However, with literature data available (13-18), it is unethical and unnecessary to put additional burden on these vulnerable paediatric critically ill patients by performing another PK study (49).

The external validation of this model in cohorts of critically ill paediatric patients and infants after cardiac bypass surgery confirms the accuracy of the obtained relationships also in patients not included in model building. The PK model predicts a 30% decrease in midazolam clearance when CRP concentrations reflecting inflammation increases 3-fold from 32 to 100 mg/L, irrespective of the cause of elevated CRP concentrations, which could be e.g. respiratory disorders, cardiac disorders, sepsis or (non-)cardiac surgery (6). Clearance decreases 26% when disease severity, expressed as number of organs failing, increases from 1 to 2 (figure 1). Cardiovascular, renal, respiratory, haematological and hepatic failure

each contributed to number of failing organs, in which e.g. cardiac failure and mechanical ventilation may cause changes in cardiac output, thereby impacting midazolam clearance. As during ICU stay, the number of organs failing and CRP concentrations may change over time thereby influencing midazolam clearance, so drug dosing in clinical practice may require adjustments over time, assuming the same effective concentration. In any case, it seems advisable to monitor drug effects of midazolam during ICU stay in patients with major inflammation and/or organ failure. This may especially be relevant in preterm neonates, as there are known risks for adverse neurological effects due to the immaturity of GABA receptors. By evaluation of the effects in both children and adults, also the fact that target plasma concentrations may be influenced by inflammation or organ failure is taken into account. Whether these results for midazolam also apply to other CYP3A substrates needs further study, and therapeutic drug monitoring may be required in case of a small therapeutic window of the CYP3A substrate involved.

Many PK studies on the CYP3A substrate drug midazolam (23-25) have been performed in children over the last two decades (26-36), ranging from critically ill children to relatively healthy children undergoing elective surgery, and we compared our clearance predictions to the reported clearance values in the literature from studies up to 25 years ago (figure 4). The clearance values predicted by our model are within the reported ranges, albeit in the lower range and with high variability. High inter-individual variability in clearance in children is partly due to maturation of CYP3A-mediated metabolism (37), but CYP3A activity is also known to be down-regulated by inflammatory cytokines *in vitro* (3). The reason for our model predictions being generally lower than the paediatric values reported in literature (figure 4) might have been due to differences in disease states, as the 'healthiest' children in our study still have 1 organ failure and are still admitted to the ICU, while reported values in the literature mainly originate from non-ICU children. This suggests that within a certain age and weight range, the disease state is relevant for drug dosing. For example, for paediatric oncology patients with acute-phase inflammatory disease, a decreased midazolam clearance has been reported (38), while for relatively healthy children undergoing elective craniofacial surgery, a much higher midazolam clearance has been reported (27).

A limitation of comparing results to literature values is that some studies only report summarized clearance data, which is less informative than individual data. Moreover, the clearance values described in literature are mostly reported per kg body weight (28-36), without reporting the individual body weight values or body weight ranges (31, 32, 34, 36). Furthermore, detailed information on inflammation and/or organ failure is missing in those studies.

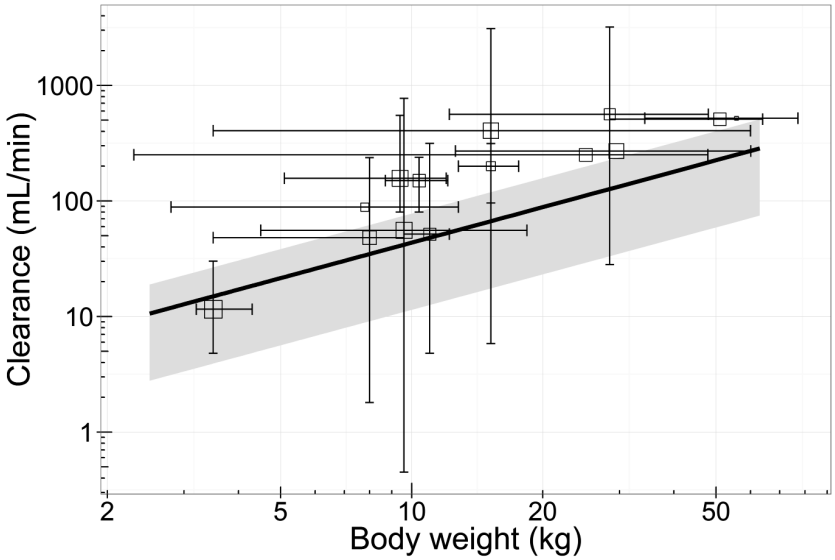

Figure 4. Population (line) predicted clearance versus the patient's body weight compared to literature reported clearance values (open squares with error bars) (27-36). The population prediction assumes an individual with a CRP concentration of 32 mg/L and 1 organ with organ failure. The grey shaded area indicates the range of population predicted clearance values from the healthiest patients (without inflammation [CRP 10mg/L] and without organ failure) (upper boundary) to the patient with most severe disease state (CRP 300 mg/L and >3 organ failures) (lower boundary). The squares represent the reported clearance for a mean body weight. The hortizontal error bars represent the included body weight range or the body weight range derived from the patient's age (40-42). Vertical errorbars represent the total range of reported clearances, or in case of 1 study the 95% confidence interval. Literature data was obtained after a literature search in PubMed with keywords including midzolam, clearance, pediatric, children and pharmacokinetics (PK) and additional studies were identified from reviews and summarizing studies (26, 43-48). Studies published up to 25 years ago were included if pediatric subjects in the study received intravenously administered midazolam. Studies with only preterm neonates, and patients receiving extracorporeal membrane oxygenation (ECMO) treatment were excluded.

The external validation of our model confirmed accurate predictions of the pharmacokinetics of midazolam in critically ill children. However, the results also show that the model should not be used for extrapolations to younger populations or populations with different levels of disease severity. 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, which is likely due to biased maturation predictions of CYP3A activity and/or abundance in these young patients, or possibly the lower level of inflammation and organ failure in this patient group. Due to rapid maturation after birth, which is not accounted for in our model, CYP3A capacity is likely over predicted in our model. Based on the current data, it is however not possible to discriminate between maturation and disease severity in this population. Disease state in the neonatal ICU population is known to be very diverse, and unfortunately,

information on CRP was not available from most of the preterm infants. Assuming lower CRP values than 10 mg/L would result in higher predictions of clearance, rather than lower clearance and with more failing organs (e.g. 1 to 4), the predicted clearance would be up to 47.3% lower, while the observed clearances in this population are even lower. Therefore, until the additional maturation processes in preterm neonates are accounted for in the model, it should not be used for extrapolation to this very young and critically ill population.

The extrapolation to adults included both healthy and critically ill subjects alike. In the healthy adult population, clearance was well predicted assuming normal CRP and the lack of organ failure that would be expected (figure 3); however the drug distribution was misspecified, resulting in a clear trend in the CWRES over time plot (figure 2J). This bi-phasic trend suggests that an additional peripheral compartment is required to describe the distribution after a single (semi-) bolus dose applied in adults. In the healthy volunteer study, more samples were taken directly after dosing, possibly allowing for the identification of relatively fast equilibrating peripheral compartments (figure 2J). In critically ill adults who were less densely sampled, this trend in CWRES was indeed not observed (figure 2L). Other possible explanations for these observations could be altered plasma protein binding in critically ill patients, which may alter drug distribution or capillary leaking in critically ill patients (39), which may result in ultra-fast equilibration of central and peripheral compartments. This ultra-fast equilibration in these patients prevents the identification of peripheral compartments that can be identified in healthy patients without capillary leaking.

In critically ill adults, midazolam clearance values were predicted in the lower range of the expected clearance values (figure 3). This indicates that despite the different age and weight range in the external validation data, the model was able to predict clearance in the critically ill adults with similar levels of inflammation and organ failure.

Some limitations of this extrapolation study should be acknowledged. As CRP concentrations were not available for all studies, the assumption was made that in healthy adults and preterm neonates, CRP was 10 mg/L and that in critically ill adults the inflammation marker had the median value of the internal dataset of 32 mg/L. To assess the impact of different CRP concentrations on clearance predictions, model-based simulations were performed with CRP values of 10 mg/L and 300 mg/L in figure 3 (outer boundaries of the grey area). For preterm neonates at the neonatal intensive care unit, assuming CRP values of 1 or 300 mg/L would both result in over prediction of midazolam clearance (likely due to immature CYP3A in this

population), while for critically ill adults, assuming a value of 10 mg/L or 300 mg/L yields an prediction of their clearance values within the predicted range (figure 3, grey area). The model should therefore not be extrapolated to preterm infants. In our study, we could not account for genetic variation in CYP3A4/5 activity, since in the original dataset the variability in genotype was too low to identify a statistically significant impact of CYP3A4/5 genotype on midazolam clearance and in the datasets of the current study information on genotype was not available. In literature, it has however been suggested that patients with expression of functional CYP3A5, metabolize midazolam faster and may compensate for the suppression of CYP3A4 activity due to inflammation or organ failure (50). These genotypes may also be of relevance in the different populations studied here, but, given the data obtained in this study, this could neither be confirmed nor rejected.

Finally, despite the adequate extrapolation potential of our model to the patient populations included in the current study, it should be noted that extrapolation to (special) populations not included in the current analysis (e.g. obese patients, pregnant women etc.) is not warranted without first formally evaluating the extrapolation potential to these populations.

CONCLUSION

The recently published paediatric pharmacokinetic model for midazolam quantifying the influence of maturation, inflammation, and organ failure can be used for predictions of CYP3A-mediated midazolam clearance in term neonates, children, and adults with varying levels of critical illness, including infants after cardiac bypass surgery and healthy adults. Extrapolations with this model resulted in biased predictions of clearance in preterm neonates. The predictive performance of our model and its value for the development of paediatric dosing regimens for midazolam and potentially other CYP3A substrates is therefore confirmed for term neonates, infants, children, and adults with varying levels of critical illness.

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SUPPLEMENTAL MATERIAL (CHAPTER 4)

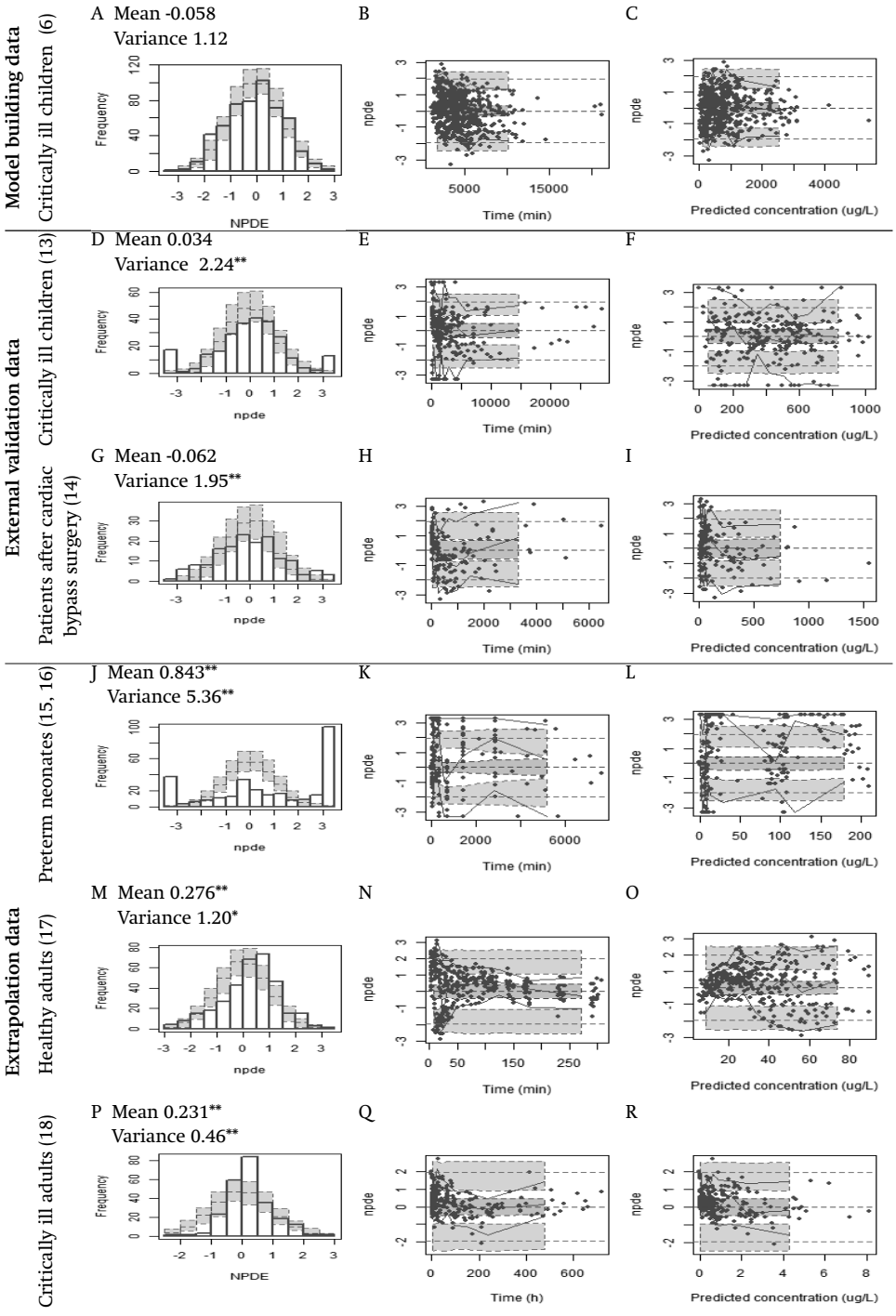

Figure S1. NPDE results, stratified per subpopulation. The histograms in the first column show the overall distribution of the normalized prediction distribution error (NPDE), and the second and third column show the NPDE versus time and the population predicted plasma concentrations respectively. Mean and variance are tested for signifcant difference from 0 and 1 respectively (** $p<0.001$, * $p<0.05$).

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