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# Assessing circadian rhythms during prolonged midazolam infusion in the pediatric intensive care unit (PICU) children

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**Abstract:**
**Background:** This study evaluates possible circadian rhythms during prolonged midazolam infusion in 27 pediatric intensive care unit (PICU) children under mechanical ventilation.
**Methods:** Blood samples for midazolam and 1-OH-midazolam assay were collected throughout the infusion at different times of the day. The blood pressure, heart rate and body temperature were recorded every hour for the rhythms analysis. Population nonlinear mixed-effect modeling with NONMEM was used for data analysis.
**Results:** A two-compartment model for midazolam pharmacokinetics and a one-compartment model for midazolam metabolite adequately described the data. The 24 h profiles of all monitored physiological parameters were greatly disturbed/abolished in comparison with the well-known 24 h rhythmic patterns in healthy subjects. There was no significant circadian rhythm detected with respect to midazolam pharmacokinetics, its active metabolite pharmacokinetics and all monitored parameters.
**Conclusions:** We concluded that the light-dark cycle did not influence midazolam pharmacokinetics in intensive care units children. Also, endogenous rhythms in critically ill and sedated children are severely disturbed and desynchronized. Our results confirmed that it is necessary to adjust the dose of midazolam to the patient's body weight. The low value of midazolam clearances observed in our study was probably caused by mechanical ventilation, which was shown to decrease the cardiac output.

**Key words:**
midazolam, pharmacokinetics, children, critically ill, circadian rhythms, mechanical ventilation

## Introduction

Both undersedation and oversedation have an obvious effect on morbidity and mortality in critically ill patients [44]. Midazolam is one of the most commonly used sedative agents in the pediatric intensive care unit (PICU) [8]. Its pharmacokinetics (PK) and pharmacodynamics (PD) has been studied on intensive care unit (ICU) patients, including infants and children [8, 13, 37, 51, 58]. However, there are still many open questions regarding both disposition and pharmacological response of midazolam in the ICU. Com-

plications related to the use of analgesic and sedative agents in ICU patients under mechanical ventilation are common [25]. The active metabolites and prolonged effect of midazolam often delay awakening and weaning from mechanical ventilation [49, 53]. Therefore, knowledge about the PK of midazolam and its metabolites may serve as a valuable tool for developing optimal infusion regimens for pediatric intensive care patients. One of the open questions is the influence of circadian clock on midazolam sedation. The results of recent studies have suggested that time of the day may be an important factor influencing both PK and PD of anesthetics. However, for intravenous midazolam the data are equivocal [4, 10, 23, 24, 54]. Moreover, the data obtained from critically ill patients may differ from the results obtained from healthy subjects. The literature data obtained from adults suggest that the circadian profile of various physiological and biochemical parameters in critically ill patients are strongly disturbed and desynchronized [3, 35]. This may alter or abolish possible chronopharmacokinetic and chronopharmacodynamic properties of different drugs in this population. There are data for pediatrics in this field. Midazolam is an intermediate-to-high-extraction drug. Therefore, clearance is largely dependent on the hepatic blood flow. Any factor affecting the blood flow, including multiorgan failure, shock, or mechanical ventilation, can affect the pharmacokinetics of midazolam [52]. The first aim of this study was to propose a population pharmacokinetic model to describe the midazolam and 1-OH-midazolam concentration-time profiles. The developed model was next used to screen a large number of routinely monitored parameters as potential covariates in order to explain the usually high inter-individual variability in PK parameters observed in critically ill children. The second and main aim of this study was to examine the presence of circadian profile of various monitored physiological parameters in our children as well as to use the developed model to assess the influence of time of the day on the pharmacokinetics of midazolam and its active metabolite.

## Materials and Methods

### Patients

The study protocol was approved by the Ethics Committee of the Poznan University of Medical Sciences. The patients' parents gave their informed consent in writing. The patients were eligible for the study if they were aged between 0 and 18 years, needed midazolam for conscious sedation, and already had an indwelling catheter placed for the purposes of medical care. All the children underwent mechanical ventilation due to respiratory insufficiency under midazolam-sufentanil analgosedation. The patients were excluded if they: received concomitant neuromuscular blockade drugs, had a history of allergy to benzodiazepines or were exposed to midazolam before starting the investigation without detailed information on midazolam dosing. The reasons for admission to ICU were: postoperative course (n = 15), multiple trauma (n = 2), shock, multiple organ failure (MOF) (n = 7) and acute respiratory insufficiency (n = 3). The Pediatric Risk of Mortality (PRISM) [39], the Risk of Death as well as Pediatric Multiple Organ Dysfunction Score (P-MODS) [18, 29] were determined for each patient to assess mortality probability in the ICU setting. The patients, characteristics, laboratory data, and vital signs were recorded throughout the study (Tab. 1). All the collected data were taken to test whether they influence midazolam pharmacokinetics. They included the following categories: age, gender, height (HT), body weight (BW), body mass index (BMI), fat-free mass (FFM) [22], hemoglobin (Hb), albumin (Alb), protein fractions, pH, $pO_2$, $pCO_2$, aspartate transaminase (AST), alanine aminotransferase (ALT), serum creatinine (Cr), urea, blood pressure (BP), heart rate (HR), body temperature (TEMP), systolic (SBP) and diastolic (DBP) blood pressure. Additionally, we examined the time-of-day effects on midazolam pharmacokinetics. Therefore, blood samples were taken at different times of the day for each patient. Because midazolam is metabolized by cytochrome P450 3A enzymes, the patients were evaluated for exposure to the drugs known to affect CYP3A activity (e.g., phenobarbital, fluconazole, metronidazole, dexamethasone and erythromycin).

### Study design

Midazolam was administered as an intravenous infusion initiated at a rate of 0.09–0.50 mg/kg/h through microbore tubing into a central catheter. The sedation monitoring was provided with the Glasgow Coma Scale modified by Cook and Palma (GSCS) scale and the midazolam dosing was adjusted according to the clinical opinion of the attending physician and the ob-

served level of sedation. The GSCS is a previously validated sedation scale for use with mechanically ventilated patients [25, 44]. One aim of the study was to assess the data in the most naturalistic conditions possible. In order to do so, we attempted to record the environmental conditions present in the ICU, without altering the established workflow. All patients had their eyes closed throughout the 24 h sampling period. Artificial light was turned off during the night, except during therapeutic interventions or nursing rounds. Nutrition was mostly administered during the daytime hours. Blood pressure, heart rate and body temperature were assessed every hour during the study period with an IntelliVue MP 70 Anesthesia monitor (Philips, USA). An arterial blood sample (2 ml) was obtained before administration of the loading dose. Then, during the infusion of midazolam, serial arterial blood samples were obtained at the following time points: 1) 3 h after the initial bolus dose; 2) once a day at different times of the day (with a two-hour delay every next day of the infusion); 3) before and 5, 15, 30 min and 1, 2, 4, 6 and 12 h after discontinuation of the infusion.

The time of the day at the beginning of infusion was always precisely noted for proper chronopharmacokinetic and chronopharmacodynamic analysis. The blood samples were centrifuged immediately after collection and the plasma was stored at –70°C until assay.

### Drug assay

Plasma samples were analyzed for midazolam and 1-OH-midazolam by means of validated high-pressure liquid chromatography (Agilent 1200 series, Waldbronn, Germany) coupled with a triple quadrupole mass spectrometer, equipped with an electrospray ionization source (Agilent 6410B, Wilmington, Delaware, USA). The mass spectrometer was working in the MRM mode and three reactions for each compound were recorded. The column used was Zorbax Eclipse XDB C18 Rapid Resolution HT 4.6 × 50 mm, 1.8 μm (Agilent, USA). The mobile phase was: formate buffer pH 3.2 [A] and 0.1% formic acid in acetonitrile [B] (Merck, Darmstad, Germany) The flow rate was 0.5 ml/min. The gradient was programmed as follows: 90% [A] and 10% [B] for 1 min, followed by a linear change to 20% [A] and 80% [B] in 6 min, then 20% [A] and 80%[ B] was held for 1.5 min. Midazolam, 1-OH-midazolam and diazepam D5 (internal standard) were purchased from Crilliant (Round Rock, Texas USA). Abselut Nexus (Agilent, USA) solid phase extraction columns (60 mg/3 ml) were used for midazolam and metabolite extraction according to the manufacturer's procedure. Extraction recovery (% + SD) was 91.1 ± 3.5 and 86.8 ± 2.8 for midazolam and 1-OH-midazolam, respectively. Intraday precision (RSD, %) at 20 ng/ml standard was 5.3 and 7.2 for midazolam and its metabolite. Interday precision was 9.1 and 10.4 for midazolam and 1-OH-midazolam. The limit of quantification was 10 ng/ml for both analytes using 0.2 ml sample volume. The method was linear from 10 to 4000 ng/ml.

### Population pharmacokinetic modeling

Population nonlinear mixed-effect modeling was done using NONMEM (Version 7.2.0, Icon Development Solutions, Ellicott City, MD, USA) and the gfortran compiler 9.0. NONMEM runs were executed using Wings for NONMEM (WFN720, http://wfn. sourceforge.net). The first-order conditional estimation with interaction (FOCE) method was used. The Nonmem data processing and plots were done in Matlab® Software version 7.0 (The MathWorks, Inc., Natick, MA, USA).

### PK model of midazolam

The midazolam concentrations in the plasma ($C_P$) and peripheral compartment ($C_T$) was described by a two compartment model:

$$\begin{aligned} V_C \frac{dC_P}{dt} &= Input - Cl_C C_P - QC_P + QC_T \\ V_T \frac{dC_T}{dt} &= QC_P - QC_T \end{aligned} \quad (1)$$

where $t$ denotes time since the beginning of infusion, $V_C$ and $V_T$ denotes the volume of the central and peripheral compartment, and $Cl_C$ and $Q$ denotes the metabolic and inter-compartmental clearance of midazolam. The input denotes the infusion rate and all extra boluses that were given to the individual patient.

The midazolam metabolite (1-OH-midazolam) concentrations ($M$) in the plasma were described by a one-compartment model:

$$V_M \frac{dM}{dt} = Cl_C C_P - Cl_M M \quad (2)$$

where $V_M$ denotes the volume of the central compartment and $Cl_M$ denotes the metabolic clearance of 1-OH-midazolam. All the concentrations were in molar units.

The singular perturbation theory was used to simplify Eq. (2) [28]. Initially, Eqs. (1) and (2) were converted into the dimensionless form. Generally, variable $R$, unitless variable $\hat{R}$, and variable units $\left[\hat{R}\right]$ are related by the following relationship:

$$R = \hat{R}\left[\hat{R}\right] \qquad (3)$$

Thus, by introducing the following units for $C_P$, $M$, and $t$:

$$\left[\hat{C}_P\right] = \frac{Inf.Rate}{CL_C}; \left[\hat{M}\right] = \frac{Inf.Rate}{CL_M}; \left[\hat{t}\right] = \frac{V_C}{CL_C} \quad (4)$$

and combining with Eq. (2) one obtains its dimensionless form:

$$\frac{CL_C}{V_C}\frac{V_M}{CL_M}\frac{d\hat{M}}{d\hat{t}} = \hat{C} - \hat{M} \qquad (5)$$

By assuming $\frac{CL_C}{V_P}\frac{V_M}{CL_M}$ that is small, which holds when 1-OH-midazolam elimination rate constant (CL/V) is fast compared to midazolam rate constant, the left side of Eq. (5) can be approximated to zero. The solution after returning to the initial units becomes a direct relationship, implying proportionality between midazolam and its metabolite concentrations.

$$M = \frac{Cl_C}{Cl_M} C_P \qquad (6)$$

Inter-individual variability (IIV) for the PK parameters was modeled assuming log normal distribution:

$$P = \theta_P \exp(\eta_P) \quad (7)$$

where $P$ is the individual parameter, $\theta_P$ is the typical value of this parameter in the population, and $\eta_P$ is a random effect for that parameter with the mean 0 and variance $\omega_P^2$. The observed concentration of midazolam and its metabolite were defined by the following equations:

$$C_{P,obs} = C_P \bullet M_{Midazolam}(1 + \varepsilon_{prop,C}) \quad (8)$$

$$M_{obs} = M \bullet M_{1-OH-Midazolam}(1 + \varepsilon_{prop,M}) \quad (9)$$

where $C_P$, $M$ are defined by Eqs. (1) and (6) of the basic structural population model and $\varepsilon_{prop,C}$ and $\varepsilon_{prop,M,}$ represent the proportional residual random errors of midazolam and 1-OH-midazolam concentrations. It was assumed that ε is normally distributed with the mean of 0 and variances denoted by $\sigma^2$. $M_{Midazolam}$ and $M_{1\text{-}OH\text{-}Midazolam}$ are midazolam and 1-OH-midazolam molecular masses equal to 325.77 and 342.76 g/mol.

### Allometric scaling

The effect of body size on all the volume ($V_C$, $V_T$, $V_M$) and clearance ($Cl_C$, $Cl_M$) parameters was included based on allometric scaling as follows:

$$P_i = P_{pop}\left(\frac{BW_i}{16}\right)^K \qquad (10)$$

where $P_i$ denotes the individual value of volume and clearance term; $P_{pop}$ are the population estimates of volume and clearance terms, $BW_i$ is the individual body weight, 16 is a median body weight of all patients, and $K$ is the exponent equal to 0.75 for clearance and 1 for distribution volumes. The effect of body mass index and fat-free mass has been assessed in a similar way.

### Covariate search and rhythm assessment

Covariate search was performed by plotting individual estimates of the PK parameters against time independent covariates to identify their influence. For the time dependent covariates the conditional weighted residuals were plotted against the covariate to identify its influence. If the relationship was found by visual inspections it was included into the model by means of the following equation:

$$P = (\theta_{P1} + \theta_{P2}(COV - \overline{COV}))\exp(\eta_P) \quad (11)$$

where the $\theta_{P1}$ and $\theta_{P2}$ are the regression coefficients. The variables were centered around their median values, $\overline{COV}$, thus allowing $\theta_{P1}$ to represent the parameter estimate for the typical patient.

The categorical covariates (sex and co-administered drugs) were included into the model based on indicator variables:

$$P = \begin{cases} \theta_{P1}\exp(\eta_p) \; if \; IND = 0 \\ \theta_{P2}\exp(\eta_P) \; if \; IND = 1 \end{cases} \quad (12)$$

where *IND* is an indicator variable that has a value of 1 when the covariate is present and *0* otherwise. The $\theta_1$ and $\theta_2$ represents the value of parameter for each category of the covariate.

The main objective of this work was to demonstrate the presence of the diurnal changes in midazolam concentrations, and physiological parameters routinely monitored during the stay in ICU. For that purpose, the metabolic clearance and volume of distribution of midazolam and metabolic clearance of 1-OH-midazolam, HR, SBP, DBP and TEMP were sought to be time dependent according to the cosine function:

$$P = \theta_P \left( 1 + \theta_{A,P} \cos\left( \frac{2\pi}{24} \left( t - \theta_{S,P} + T \right) \right) \right) \exp\left( \eta_P \right) \quad (13)$$

where $P$ donates the studied parameter, $\theta_P$ is a messor (the average value around which the variable oscillates), $\theta_{A,P}$ is an amplitude, $\theta_{S,P}$ is an acrophase (the clock time at which the peak of a rhythm occurs) and $T$ is the clock time at the start of infusion. The equation assumes a 24 h fixed period.

The minimum value of the NONMEM objective function (*MOF*), typical goodness of fit diagnostic plots, and an evaluation of the precision of pharmacokinetic parameters and variability estimates were used to discriminate between various models during the model-building process. The difference in MOF obtained for the two hierarchical models (likelihood ratio) is approximately $\chi^2$ distributed. During the covariate and rhythm search the effect of each covariate was examined by adding Eqs. (11)–(13) to the base model. The likelihood ratio was determined as the difference in the objective function (MOF) of a full model (with covariate or with daily fluctuations) and reduced model (without covariate or without daily fluctuations) after refitting the data. The difference in MOF between models of 10.8 for one degree of freedom and of 13.8 for two degrees of freedom was considered to be statistically significant at $p < 0.001$ for the equation to be included into the base model.

## Results

The patients' characteristics are presented in Table 1. Sixteen male and 11 female patients were enrolled in the study. The studied group of patient was quite heterogeneous, as all patients entering the ICU and meeting relatively broad inclusion criteria were included in the analysis. The mean duration of the midazolam infusion

**Tab. 1.** The demographic data and physiological parameters of patients qualified for the study. For continuous variables (systolic blood pressure, diastolic blood pressure, body temperature and heart rate) the median value of all records throughout the entire infusion was used for calculations

| Parameter, unit | Mean ± SD (n = 27); range |
|---|---|
| Age, year | 6.7 ± 5.7; 0.17 – 17.3 |
| Body weight, kg | 26.4 ± 25.2; 5.8 – 90 |
| Height, m | 1.15 ± 0.39; 0.61 – 1.92 |
| Fat free mass, kg | 21.5 ± 19.2; 4.27 – 67.5 |
| Male/female | 16/11 |
| Total dose of midazolam, mg | 929 ± 1202; 75.9 – 5117 |
| Infusion duration, h | 136.3± 87.2; 43.5 – 403.5 |
| Systolic blood pressure, mmHg | 102.5 ± 10.0; 81.8 – 119.7 |
| Diastolic blood pressure, mmHg | 57.3 ± 6.9; 40.8 – 67.0 |
| Heart rate, beats/min | 111.4 ± 19.4; 58.8 – 145 |
| Body temperature, °C | 37.2 ± 0.29; 36.8 – 38.1 |
| Total Protein, g/dl | 5.33 ± 0.71;3.9 – 6.49 |
| Albumin, g/dl | 3.40 ± 0.51; 2.61 – 4.54 |
| α1, g/dl | 0.232 ± 0.85; 0.120 – 100 |
| α2, g/dl | 0.591 ± 0.150; 0.287 – 0.926 |
| β, g/dl | 0.332 ± 0.122; 0.010 – 0.601 |
| γ, g/dl | 0.534 ± 0.212; 0.219 – 0.982 |
| Cholesterol, mg/dl | 110.5 ± 45.4; 47 – 265 |
| Triglyceride, mg/dl | 101.9 ± 65.8; 23 – 317.5 |
| Hematocrit, % | 31.0 ± 3.71; 24.3 – 40.7 |
| pH | 7.42 ± 0.08; 7.1 – 7.5 |
| $pCO_2$, mmHg | 38.9 ± 5.3; 31.9 – 49.2 |
| $pO_2$, mmHg | 131.3 ± 26; 78.1 – 172.5 |
| ALT, U/l | 50.6 ± 49.9; 9.0 – 216 |
| AST, U/l | 80.82 ± 105.8; 16 – 431 |
| Urea, mg/dl | 32.5 ± 12.4; 7.0 – 75.3 |
| Creatinine, mg/dl | 0.48 ± 0.16; 0.30 – 0.82 |
| PRISM | 7.11 ± 7.41, 0 – 34 |
| Risk of Death | 7.32 ± 17.1, 0 – 86.1 |
| P-MODS | 3.55 ± 2.13, 0 – 8 |
| Use of phenobarbital (Yes/No) | 16/11 |
| Use of fluconazole (Yes/No) | 2/25 |
| Use of metronidazole (Yes/No) | 5/22 |
| Use of furosemide (Yes/No) | 23/4 |
| Use of omeprazole (Yes/No) | 22/5 |
| Use of spironolactone (Yes/No) | 23/4 |
| Use of thiopental (Yes/No) | 6/21 |
| Use of lidocaine (Yes/No) | 7/20 |
| Use of norepinephrine (Yes/No) | 10/17 |

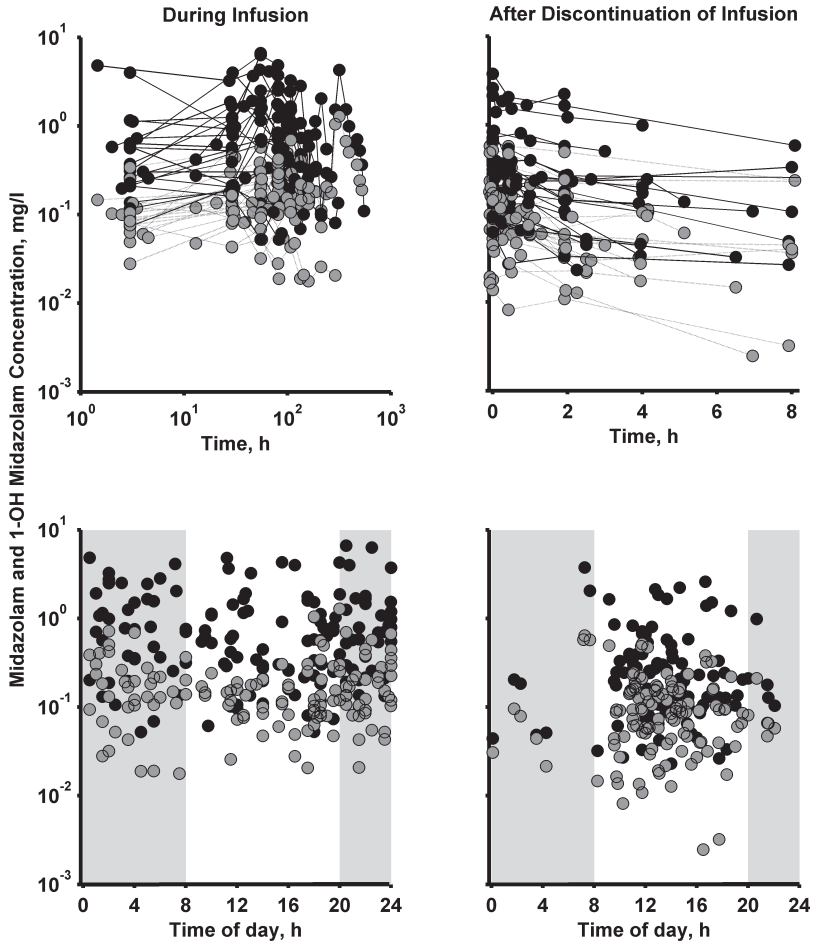


**Fig. 1.** The upper subplots present the individual midazolam (black symbols) and 1-OH midazolam (gray symbols) concentration *vs.* time profiles stratified with respect to the anesthesia phase (during or after infusion). The bottom subplots present the spread of the midazolam (black symbols) and 1-OH midazolam (gray symbols) concentrations over a 24 h period also stratified with respect to the anesthesia phase (during or after infusion)

was 136.3 ± 87.2 h; 249 and 250 (144 and 132 during infusion) concentrations were measured for midazolam and 1-OH midazolam. The row data and its spread over 24 h time period is presented in Figure 1.

The key steps of model building process are presented in Table 2. The pharmacokinetics of midazolam is fairly well understood. It has been studied in different patients groups of different health status [1, 6, 7, 11, 13, 19, 21, 28, 30, 32, 36–38, 42, 43, 46, 54, 57, 59]. We started with a two compartment model for midazolam and a one compartment model for its metabolite according to the literature findings [13, 37]. This model performed well, however, with large coefficients of variations for volume of distribution of midazolam and 1-OH-midazolam. The close observation of the data indicated that midazolam metabolite concentrations are proportionally lower to midazolam concentrations for all the subjects. It might suggest a rapid elimination of the metabolite when compared to midazolam. The terminal elimination rate constant for midazolam and its metabolite equaled 0.45 $h^{-1}$ and

**Tab. 2.** Decrease in the objective function value after key modeling steps

| Key modeling steps, Equation | Degree of freedom | MOF (ΔMOF) |
|---|---|---|
| Midazolam: 2 comp. model<br>1-OH midazolam: 1 comp. model | – | –1312.2 |
| Midazolam: 2 comp. model<br>1-OH midazolam: simplified model | – | –1312.6 |
| Midazolam: 2 comp. model<br>1-OH midazolam: simplified model<br>Allometric scaling of $CL_C$, $V_C$, $Q$, $CL_M$, $V_T$<br>Full covariance matrix | 0 | –1390.9 |
| + circadian rhythms on $CL_C$ | +2 | –1392.2 (1.3) |
| + circadian rhythms on $CL_M$ | +2 | –1400.6 (9.7) |
| + circadian rhythms on $V_C$ | +2 | –1402.1 (11.2) |
| + temperature on $CL_C$ | +1 | –1400.9 (10.0) |
| + γ globulin on $CL_M$ | +1 | –1403.9 (13.0) |

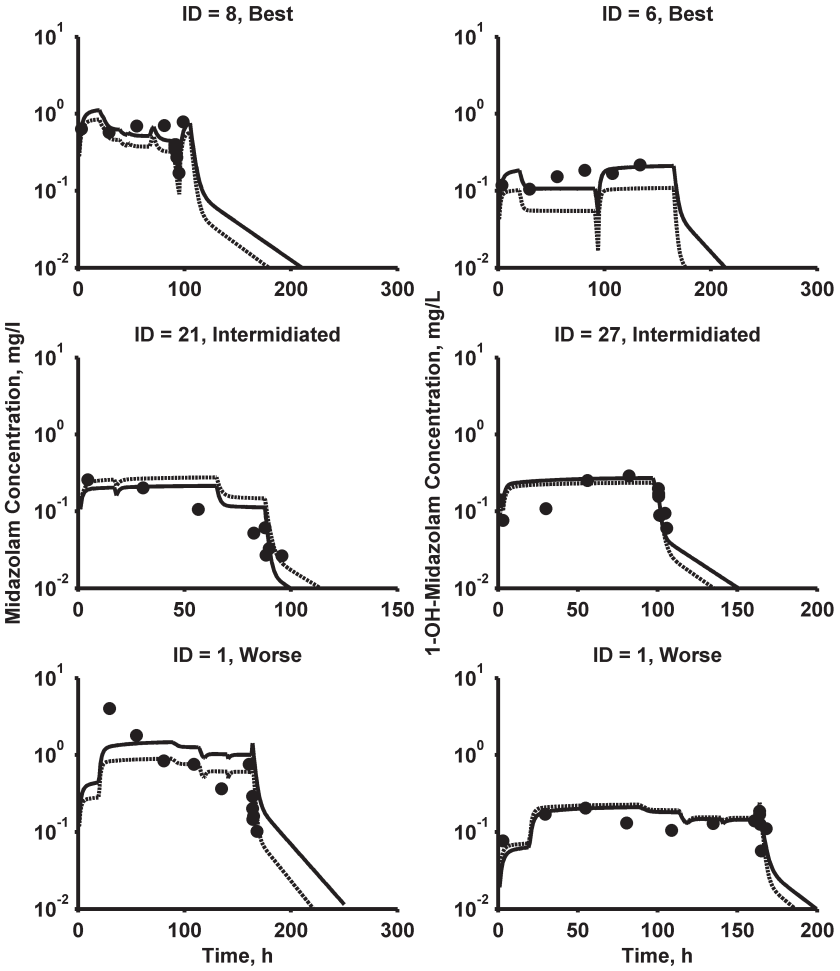


**Fig. 2.** The plot of observed (•), population predicted (--) and individual predicted (—) midazolam and 1-OH-midazolam concentrations *vs.* time for the best, modest and the worst performance of the final PK model

13.2 h-1 for the initial model. Thus the assumption that elimination rate constant of 1-OH midazolam is much faster than that of midazolam was justified and allowed to considerably simplifies the model with a negligible increase in MOF of 0.4 units. Similar findings that 1-OH-midazolam kinetics is formation rate-limited have already been reported in young healthy volunteers [20].

The patients had a great difference in the body mass ranging from 5.8 to 90 kg. It was accounted by the standard allometric scaling of all clearance and volume terms. The inclusion of allometric scaling and correlation between parameters led to substantial drop in MOF of 78.3. The body weight explained about 51, 13 and 32% of variability in $V_C$, $Cl_C$ and $Cl_M$, respectively. We have also investigated another measures of body mass, like body mass index and fat free mass, however, they were not advantageous over the usual body mass.

The covariate search comprised the assessment of age, gender, total dose, infusion duration, SBP, DBP, HR, TEMP, total protein and its fractions (albumin, $\alpha 1$, $\alpha 2$, $\beta$ and $\gamma$ globulin), cholesterol, triglyceride, hematocrit, pH, $pCO_2$, $pO_2$, ALT, AST, urea, creatinine, PRISM, Risk of Death, P-MODS and use of phenobarbital, fluconazole, metronidazole, furosemide, omeprazole, spironolactone, thiopental, lidocaine and norepinephrine on the individual PK parameter estimates. The only significant relationship ($p < 0.001$) between $Cl_M$ and $\gamma$ globulin concentrations was found. It explained 12% of variability in $Cl_M$.

The midazolam and 1-OH midazolam PK during and after prolonged infusion for selected (best, typical, worst) profiles is presented in Figure 2. This plot shows that the final PK model described the measured concentrations well. The typical goodness-of-fit plots of the final model are presented in Figure 3. The individual and population prediction *vs.* observed concentrations are relatively symmetrically distributed around the line of identity. The conditional weighted residuals *vs.* population predicted concentrations and

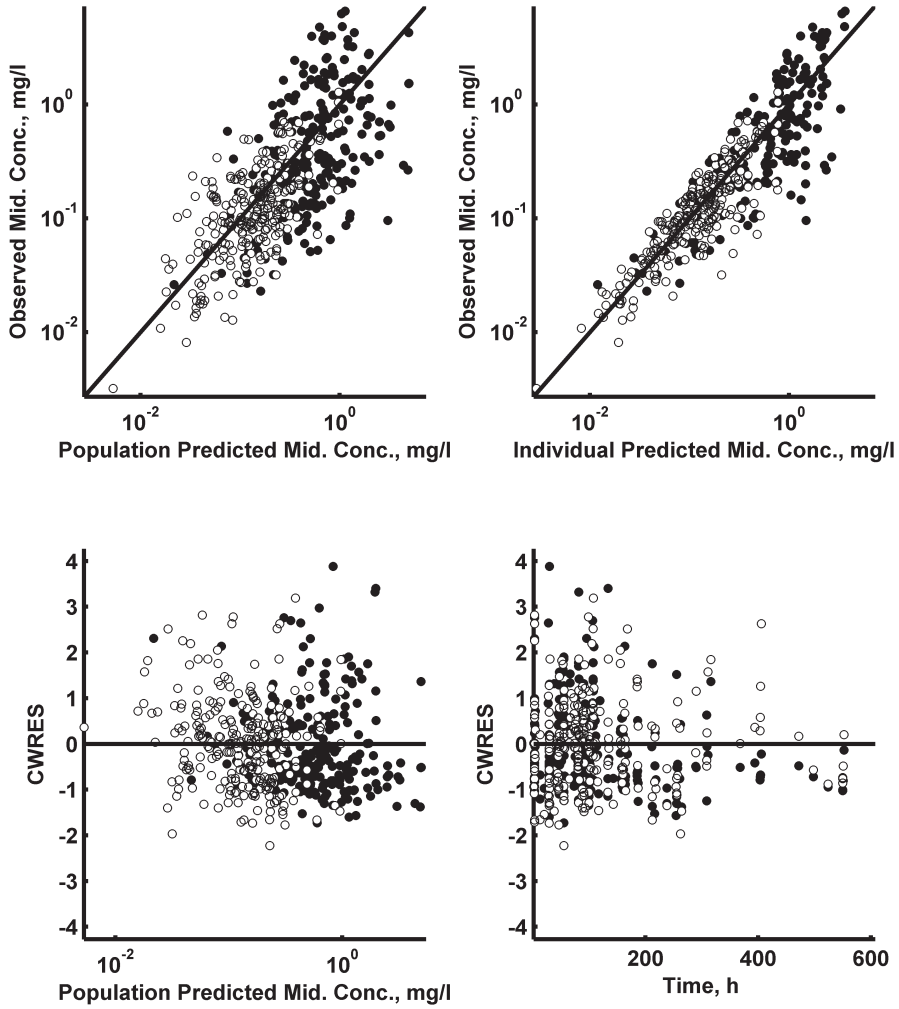


**Fig. 3.** Goodness of fit plots: the observed *vs.* the population predicted concentrations; the observed *vs.* the individual predicted concentrations; conditional weighted residuals *vs.* population predicted concentrations and *vs.* time. The closed circles represent midazolam, whereas the open circles represent the 1-OH midazolam concentrations in plasma

*vs.* time do not show any trend and are relatively uniformly distributed around the zero indicating good model performance in quantifying the PK data.

The parameter estimates obtained by the fitting of the PK data are listed in Table 3. All PK parameters, inter-subject and residual error variances were estimated well with CVs smaller than 30%. The volume of the central compartment for midazolam for a typical subject (16 kg) was 0.66 l/kg. The volume of the peripheral compartment was higher 1.05 l/kg. The midazolam metabolic clearance for typical subject equaled 4.93 ml/min/kg. The inter-compartmental clearance equaled 0.712 ml/min/kg. The 1-OH-midazolam clearance was higher than that of midazolam and equaled 20.7 ml/min/kg. It was found to be linearly dependent on γ globulin concentrations with 18.6% increase for every 0.1 g/dl difference from the median in γ globulin concentration (0.514 mg/dl). The relationship between the individual PK parameters and the individual values of the age, weight and γ globulin are presented in Figure 4.

The inter-individual unexplained variability for the $V_C$, $Cl_C$ and $Cl_M$, was high and equaled 137, 76.8 and 48.6%. The ETA shrinkage of the final model parameter estimates were 5.2, 0.34 and 1.5% for $V_C$, $Cl_C$ and $Cl_M$. This magnitude of shrinkage was small and generated reliable Bayesians estimates for covariate search.

The molar concentration ratio of midazolam and its metabolite is given by the ratio of its clearances and equals 0.29 with inter-individual variability 18.1%. It is a small value when compared to the inter-individual variability of other estimated parameters (53–138%).

The midazolam PK did not showed statistically significant circadian rhythms for CL, $CL_M$ and V. The MOF decreased by 1.3 for $CL_C$ and by 9.7 for $CL_M$ and by 11.2 for $V_C$, which was not statistically significant

**Tab. 3.** A summary of the final population PK parameters, inter-subject and residual error variance estimates of midazolam and its metabolite

| Parameter, unit | Basic PK Model | Influence of γ globulin |
|---|---|---|
| | Estimate (% CV) | Estimate (% CV) |
| Fixed effect (midazolam) | | |
| θ-$V_C$, [l] | 10.8 (26.7) · (BW/16) | 10.6 (30.9) · (BW/16) |
| θ-$CL_C$, l/h | 4.73 (15.8) · (BW/16) $^{0.75}$ | 4.73 (16.2) · (BW/16) $^{0.75}$ |
| θ-$Q$, l/h | 0.698 (17.3) · (BW/16) $^{0.75}$ | 0.684 (17.2) · (BW/16) $^{0.75}$ |
| θ-$V_T$, l | 16.7 (29.1) · (BW/16) | 16.8 (29.8) · (BW/16) |
| Fixed effect (1-OH-midazolam) | | |
| θ-$CL_M$, l/h | 19.1 (12.5) · (BW/16) $^{0.75}$ | 19.9 (12.0) · (BW/16)$^{0.75}$ (1 + 1.86 (14.1) · (GAM-0.514)) |
| Inter-individual variability | | |
| $\omega^2_{Vc}$, % | 137 (25.3) | 138 (25.4) |
| $\omega^2_{Clc}$, % | 82.8 (21.5) | 82.9 (21.5) |
| $\omega^2_{ClM}$, % | 64.8 (22.8) | 53.1 (29.3) |
| *Corr.* $V_C - CL_C$ | 0.601 | 0.615 |
| *Corr:* $Cl_C - CL_M$ | 0.714 | 0.745 |
| *Corr.* $Cl_M - V_c$ | 0.470 | 0.621 |
| Residual variability | | |
| $\sigma^2_{prop,C}$, % | 0.616 (10.0) | 0.614 (9.9) |
| $\sigma^2_{prop,M}$, % | 0.431 (6.70) | 0.433 (6.70) |

($p < 0.001$). Similarly, no significant ($p < 0.001$) circadian rhythms were found for monitored physiological parameters (heart rate, body temperature, systolic blood pressure, diastolic blood pressure). The corresponding 48 profiles are presented in Figure 5.

## Discussion

To our knowledge this is the first study describing population pharmacokinetics of midazolam in pediatric intensive care patients that includes the assessment of circadian variability of both pharmacokinetic parameters as well as monitored physiological parameters. Although the significance of chronobiology in anesthesia has been postulated, the problem is poorly recognized in the pediatric intensive care children [40].

Midazolam undergoes extensive metabolism in the liver, mainly by CYP3A4, CYP3A5 enzymes, and to a lesser extent by CYP3A7, to its main metabolite, 1-OH-midazolam. Midazolam is also a highly protein-binding (albumin) drug (96–98%), which undergoes significant first-pass oxidative metabolism in the liver and intestine and which has moderate to high hepatic extraction ratio [17, 34]. It is known that the P450 activities, protein level and also cardiac output fluctuate daily in rats [16, 33, 41, 50, 56]. Recently, Tomalik-Scharte et al. [55] noted circadian variability in the clearance of intravenous midazolam, given to healthy subjects. The authors concluded that liver enzyme activity demonstrates diurnal variations in humans. On the contrary, Klotz and Reimann [23] did not observe significant daily fluctuations in the clearance of midazolam given as continuous infusion to four healthy volunteers. Koopmans et al. [24] observed circadian changes in midazolam administrated orally to six healthy subjects. The elimination half-life was shortest at 14:00 and longest at 02:00 h.

In our study, we did not shown any circadian fluctuations in midazolam pharmacokinetics, concluding the time-of-day does not affect significantly midazolam requirements in the ICU children. For better in-

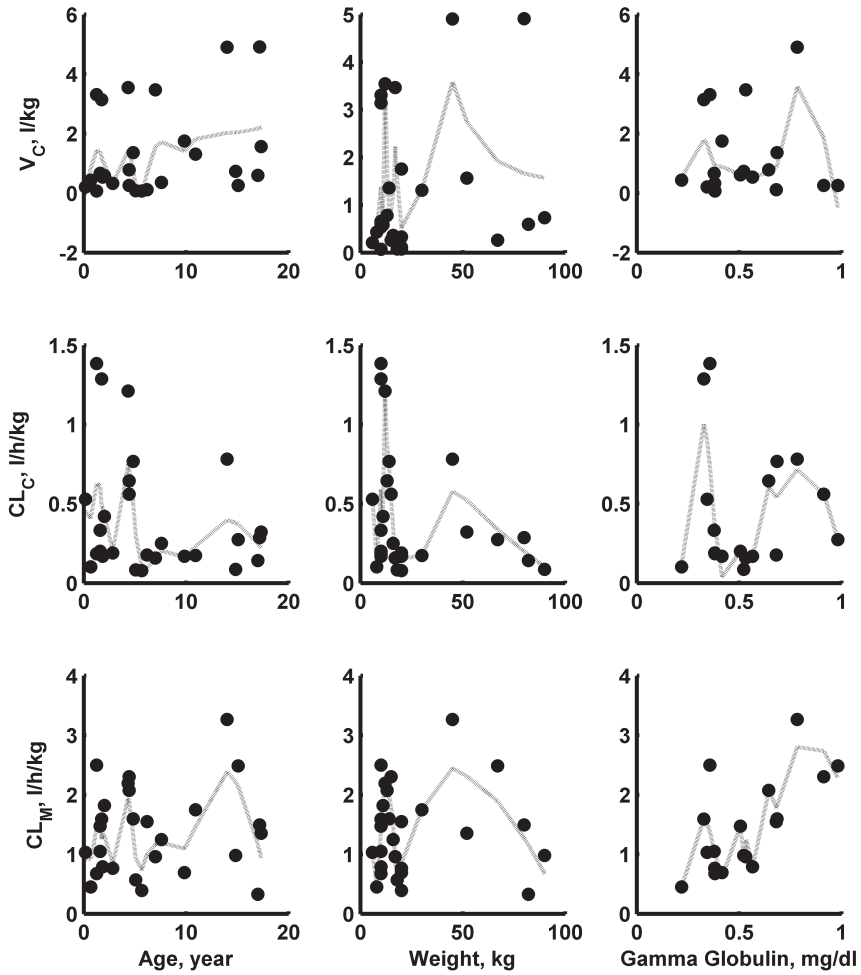


**Fig. 4.** The individual posterior Bayes estimates of the metabolic clearance of midazolam, the metabolic clearance of 1-OH-midazolam, and the volume of distribution from the midazolam population pharmacokinetic analysis in relation to the patient's age, weight and γ globulin concentration. The dotted line indicates the trend in the data (loess smooth)

terpretation of our results we also examined the circadian profile in various monitored physiological parameters, which normally are known to demonstrate circadian pattern, like blood pressure, heart rate and body temperature. We noted severe abnormalities from normal pattern in our children. In our opinion, observed disruption in these parameters may be at least partly responsible for the lack of rhythmicity in PK parameters. For highly extracted drugs, like propofol, but also midazolam the clearance is flow-dependent, thus the time-of-day effect on the pharmacokinetics or pharmacodynamics observed in animals may be due to daily fluctuation in cardiac output [4, 9, 45, 47]. However, in the ICU environment, the normal pattern in the physiological hemodynamic parameters seems to be disrupted [3, 35]. Similar results we recently obtained for adult patients sedated with prolonged infusion of propofol [3]. In contrary, Tomalik-Scharte et al. [55] noted circadian rhythmicity in the clearance of intravenous midazolam. However, the authors conducted their study in healthy subjects with probably normal healthy circadian pattern in physiological parameters. Midazolam is a very widely used sedative agent *via* both oral as well as intravenous route. We suppose that the time-of-day effect on the pharmacokinetics of this drug is dependent on the route of administration and population being examined, therefore, the results of our study may not be simply extrapolated to other populations and clinical scenarios. The other problem presented in our work is the physiological rhythm disruption *per se*. Development of many physiological processes, including circadian rhythms, occurs over the first age of life. Sleep show appearance of diurnal sleep patterns at 12–16 weeks of age. Thus, to interpret the results of chronobiological studies it should be taken into account that neonates are physiologically and behaviorally arrhythmic for several weeks or months after birth.

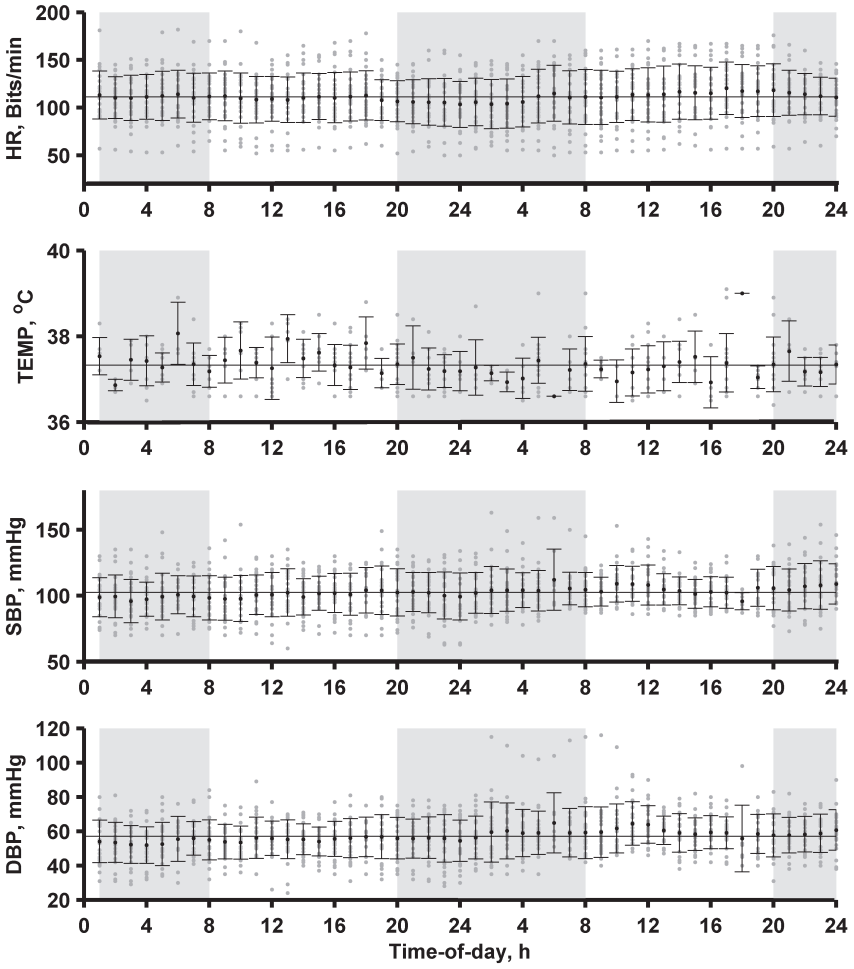


**Fig. 5.** The twenty-four-hour profiles of the heart rate, body temperature, systolic and diastolic blood pressure. The following items are shown: the row data (gray dots), the hourly means and SD, and the mean value (straight line). The shaded area represents the dark period (8 p.m. – 8 a.m)

Plasma melatonin and cortisol levels as well as heart rate have no discernable rhythm within the first days of life, but such daily variations were detected at approximately 3 months of age. As far as body temperature is concerned, infants show a nadir similar to adults [03:00 h] between 2 and 4 month of age [40]. In our study only two patients were below one year of age (patient 17 – two months of age and patient 18 – eight months of age) and for them the lack of normal circadian pattern should be expected. However, for the other ones, such deficiency should not be observed, and the lack of circadian rhythmicity shows the abnormalities. In conclusion, the present study provides evidence for a severe disturbance of the well-known circadian rhythms in cardiovascular functions as well as in body temperature in critically ill pediatric patients in an intensive care unit. However, it should be taken into account that they were under continuous analgosedation. The relative contribution of this medication to the observed rhythm disturbances is possible [31]. Also, the sedative agents may influence the sleep – activity cycle in humans. Recently, new benzodiazepine, cinazepam was synthetized. With cinazepam, the continuity of slow-wave sleep and paradoxical sleep are proportionally increased in contrast to many known hypnosedative drugs, such as diazepam, flunitrazepam and others [48]. The pharmacokinetics of midazolam has been shown to vary with age [54], especially in children over the first 6 months after birth. It is a consequence of the maturation of the hepatic microsomal oxidizing system that leads to an age dependent metabolic clearance [26]. Our data did not shown any age related changes, very likely due to the small number of very young patients. The comparison of individual estimates of clearance with literature values obtained for different patients groups of different health status is summarized in Figure 6. Despite the large variability of clearance, some trends can be distinguished. In general, children not hospitalized at ICU have slightly

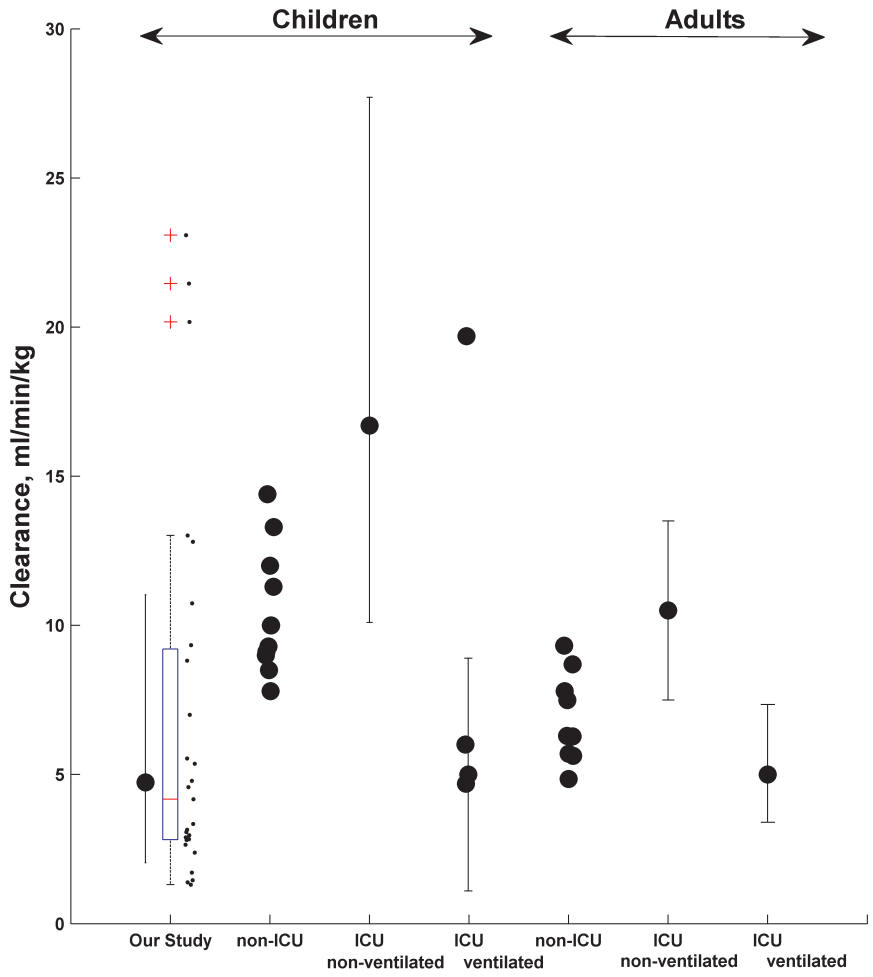


**Fig. 6.** The comparison of individual estimates of the metabolic clearance with literature average clearances obtained for various groups of patients [3, 6, 22–39] The data were stratified with respect to stay at ICU (ICU *vs.* non-ICU), age (children *vs.* adults), and use of mechanical ventilation (ventilated *vs.* non-ventilated). The error bars present the standard deviation of inter-individual variability. For clarity of presentation they were not provided for non-ICU patients

higher body weight normalized clearance than adults. Median clearance from all studies equaled 10.0 for children and 6.3 ml/min/kg for adults. It is very likely a consequence of a slower than proportional increase of clearance with body weight as already presented in the literature [2, 5]. For ICU patients, the literature data are not very rich, however, it can be found that the mechanically ventilated patients (including our study) have smaller clearance than non-ventilated ones. Peeters at al. [37] have determined the clearance in non-ventilated children as 16.7 ml/min/kg. It is about 3 fold higher than the clearance obtained in this study (4.93 ml/min/kg) and in the studies in ventilated ICU patients by de Wildt et al. [13] (5.0 ml/min/kg), Hughes et al. [21] (6.01 ml/min/kg for ages lower than 12 months, 4.69 ml/min/kg for 1–2 years old) and in adults by Zomorodi et al. [59], where it equaled 5.0 ml/min/kg. For spontaneously breathing adults, the clearance was 2 fold higher than in ventilated patients and equaled 10.5 ml/min/kg [14]. Only in one group of patients (> 3 years), in the study by Hughes et al. [21], the clearance was in a range of Peeters et al. (19.7 ml/min/kg). The clearance in non-ventilated ICU patients seems also to be lower than in the patients not hospitalized at ICU (non-ICU) where it ranged from 7.8 to 14.4 ml/min/kg in children and from 5.7 to 9.32 ml/min/kg in adults. Very likely the low value of clearance is caused by use of mechanical ventilation which is known to affect cardiac output. It has been shown in the literature that discontinuation of mechanical ventilation increases cardiac output by about 23% [15] and that an increase of the positive-pressure during mechanical ventilation decreases cardiac output [12].

The presented data on pharmacokinetics of young children and adolescents exhibits a considerable variability in all PK parameters. Despite the collection of a large number of covariates, only body weight and $\gamma$

globulin concentration were found to be statistically significant. The body weight is a usual covariate for volume of distribution and clearance, especially for patients considerably differing in weight. By inspecting the relationship between body weight normalized clearance of midazolam and body weight (Fig. 4) a transient increase in clearance from 0.2 to 1.2 l/h/kg can be observed in a range of body weights from 10 to 20 kg. We have tried to incorporate this relationship by means of spline function during the model building process, however, it turned out not to be statistically significant. We did not found any physiological explanation of the relationship between γ globulin and 1-OH-midazolam clearance. In the literature a decreased volume of distribution has been observed for an increased plasma albumin concentration in adult intensive care patients [58]. It has not been confirmed in our study, very likely due to the presence of patients with fairly narrow range of albumin levels.

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