ORIGINAL RESEARCH ARTICLE

Hyperinflammation Reduces Midazolam Metabolism in Critically Ill Adults with COVID-19

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Abstract

Background and Objective Many patients treated for COVID-19 related acute respiratory distress syndrome in the intensive care unit are sedated with the benzodiazepine midazolam. Midazolam undergoes extensive metabolism by CYP3A enzymes, which may be inhibited by hyperinflammation. Therefore, an exaggerated proinflammatory response, as often observed in COVID-19, may decrease midazolam clearance. To develop a population pharmacokinetic model for midazolam in adult intensive care unit patients infected with COVID-19 and to assess the effect of inflammation, reflected by IL-6, on the pharmacokinetics of midazolam.

Methods Midazolam blood samples were collected once a week between March 31 and April 30 2020. Patients were excluded if they concomitantly received CYP3A4 inhibitors, CYP3A4 inducers and/or continuous renal replacement therapy. Midazolam and metabolites were analyzed with an ultra-performance liquid chromatography–tandem mass spectrometry method. A population pharmacokinetic model was developed, using nonlinear mixed effects modelling. IL-6 and CRP, markers of inflammation, were analyzed as covariates.

Results The data were described by a one-compartment model for midazolam and the metabolites 1-OH-midazolam and 1-OH-midazolam-glucuronide. The population mean estimate for midazolam clearance was 6.7 L/h (4.8–8.5 L/h). Midazolam clearance was reduced by increased IL-6 and IL-6 explained more of the variability within our patients than CRP. The midazolam clearance was reduced by 24% (6.7–5.1 L/h) when IL-6 increases from population median 116 to 300 pg/mL.

Conclusions Inflammation, reflected by high IL-6, reduces midazolam clearance in critically ill patients with COVID-19. This knowledge may help avoid oversedation, but further research is warranted.

Key Points

Inflammation, reflected by high IL-6, reduces midazolam clearance in critically ill patients with COVID-19.

Renal failure, reflected by high creatinine, reduces 1-OH-midazolam-glucuronide clearance in critically ill patients with COVID-19.

This knowledge may help to identify critically ill patients with COVID-19 with a reduced midazolam clearance and therefore at risk for oversedation.

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1 Introduction

Current guidelines for the management of pain, agitation, delirium, immobility and sleep (PADIS) in critically ill patients are widely endorsed and applied at Intensive Care Units (ICUs) worldwide. They include aiming for a comfortably awake state, avoiding benzodiazepine-based continuous sedation and other measures in order to avoid or mitigate delirium [1]. However, titrating analgesia and sedation towards wakefulness has been challenging in acute respiratory distress syndrome (ARDS) related to coronavirus disease 2019 (COVID-19) due to frequent application of neuromuscular blockade and prone positioning, requiring higher levels of sedation [2]. This has translated into high incidence of benzodiazepine-related delirium and an increased number of days spent in coma [3]. It seems likely that benzodiazepine drug dosing may have been too high in many patients in circumstances where deep sedation was perceived as essential to avoid patient-ventilator dyssynchrony and ensure patient comfort during proning.

Midazolam, the most used benzodiazepine for continuous intravenous (iv) sedation, undergoes extensive metabolism by hydroxylation via CYP3A enzymes to form mainly the metabolite 1-OH-midazolam and a very small amount of 4-OH-midazolam. After hydroxylation, hydroxymidazolam is subsequently metabolized to chiefly 1-OH-midazolam-glucuronide as its major metabolite and renally excreted. Both an in vitro study and a study in healthy volunteers showed that the metabolites 1-OH-midazolam and 1-OH-midazolam-glucuronide have a sedative potency compared to midazolam of 60-80% and 10%, respectively [4, 5]. Due to the CYP3A-mediated metabolism of midazolam to 1-OH-midazolam, midazolam is a known indicator for human CYP3A4 activity. CYP3A can be down regulated by cytokine release during inflammation resulting in decreased drug clearance [6–9]. In an in vitro study, it was found that CYP3A4 activity is reduced by 50% (EC_{50}) at an interleukin 6 (IL-6) concentration of 17.1 pg/mL [10]. Furthermore, Vet et al. demonstrated reduced CYP3A-mediated clearance of midazolam in critically ill children with inflammation and organ failure [11].

Since hyperinflammation plays an important role in COVID-19, altered midazolam pharmacokinetics seem likely. Therefore, we aimed to develop a population pharmacokinetic model for midazolam and to assess the effect of inflammation, reflected by IL-6 and C-reactive protein (CRP), on the pharmacokinetics of midazolam administered as continuous infusion to adult intensive care patients with COVID-19 infection. We hypothesized that a strong proinflammatory response reduces midazolam clearance.

2 Methods

2.1 Setting, Patient Selection and Ethics

We performed a prospective observational study between March 31 and April 30 2020 at the tertiary ICU of Erasmus MC University Medical Center (Rotterdam, the Netherlands). At the end of April 2020, we reached the end of the first COVID-19 wave in the Netherlands. As a result, the study population decreased and no more midazolam samples were obtained. Mechanically ventilated adult patients with COVID-19, receiving intravenous midazolam were eligible for participation in the study. Patients were excluded if they concomitantly received strong CYP3A4 inducers (like e.g. rifampicin) and/or inhibitors (like e.g. erythromycin) or continuous renal replacement therapy (CRRT). Patients receiving CRRT were excluded from analysis, since CRRT may interfere with the clearance of the metabolite 1-OH-midazolam-glucuronide and insufficient information (e.g. sieving coefficient) was present to calculate accurately the clearance. The medical research ethics committee approved the study and waived informed consent requirement, given that all data acquired for this study were part of usual care (MEC 2020-0381).

Data were extracted from the electronic medical records for the duration of the midazolam treatment until end of study, including, Richmond Agitation-Sedation Scale (RASS), Acute Physiology and Chronic Health Evaluation (APACHE) IV score, Kidney Disease: Improving Global Outcomes (KDIGO) stage, Sequential Organ Failure Assessment (SOFA) score, serum creatinine, C-reactive protein (CRP) and interleukin (IL)-6, aspartate aminotransferase (ASAT), alanine transaminase (ALAT), bilirubin, albumin and the use of sedative co-medication.

2.2 Sampling and Measurements

Midazolam samples were obtained and analyzed weekly as part of routine clinical care. Blood samples were collected during continuous midazolam infusions or in some cases after withdrawal of the continuous infusion. Blood samples were centrifuged and plasma was refrigerated until analysis. Midazolam, 1-OH-midazolam and 1-OH-midazolam-glucuronide plasma samples were analyzed by means of an FDA-validated method using ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) with electrospray ionization in the positive ionization mode on a Waters Xevo TQ-S micro 1. The limits of quantification of the method were 2-2400 μg/L for midazolam, 3-2300 μg/L for 1-OH-midazolam and 10-3000 μg/L for 1-OH-midazolam-glucuronide. If concentrations exceeded

the upper limit of quantification, the calibration curve was used to extrapolate those concentrations.

2.3 Population Pharmacokinetic Analysis

2.3.1 Structural Model and Choice of Statistical Model

The concentrations time profiles of midazolam and the metabolites 1-OH-midazolam and 1-OH-midazolam-glucuronide were analyzed using the nonlinear mixed effects modeling (NONMEM®) approach with first-order conditional estimates (FOCE) with interaction [version 7.44, ICON, Development Solutions, MD, USA]. Pirana [version 2.9.9, Certara, NJ, USA] was used as modeling environment and data were further analyzed and visualized in R [version 3.6.1, R Foundation for statistical computing, Vienna, Austria]. All concentrations of 1-OH-midazolam and 1-OH-midazolam-glucuronide were corrected to their midazolam equivalent using molecular weight. Initially, one-, two- and three-compartment models were tested for midazolam and its metabolites. Subsequently, the metabolites were added to the model in series.

Throughout the process of pharmacokinetic modeling, different mathematical models were fitted to the data to determine which model best described the data. The models were evaluated both numerically, using precision, objective function values (OFV), shrinkage and condition number, and visually using goodness-of-fit plots and visual predictive checks (VPC). A decrease of 3.84 points in OFV for one degree of freedom was considered statistically significant ($p < 0.05$). Inter-occasion variability (IOV) was evaluated. Constant, proportional and combined error models were evaluated to describe the residual error for midazolam and metabolites (both combined and separately). The inter-individual variability (IIV) on clearance was estimated for midazolam and both metabolites.

2.3.2 Covariate Analysis

The correlation with PK parameters was evaluated for the following covariates: age, gender, weight, body surface area (BSA), APACHE IV score, SOFA score, creatinine, CRP, IL-6, ASAT, ALAT, bilirubin and albumin. Covariate values closest to the time of observation were used for model predictions except for baseline data. Continuous covariates were centered on the median and missing values were carried forward or backward from last observation. When a covariate was entirely missing, it was replaced by the median. The evaluation of covariates was performed using stepwise covariate model building where a decrease of ≥ 3.84 in OFV was considered significant with P-values of ≤ 0.05. After forward inclusion, a backward exclusion procedure was then performed where a change in OFV of > 10.82 was

considered significant with P-values of ≤ 0.001. A covariate was retained in the model if its inclusion resulted in a significant decrease in OFV and decrease in unexplained variability or improved goodness-of-fit plots.

2.3.3 Model Evaluation

The robustness of the population pharmacokinetic model was evaluated using a non-parametric bootstrap procedure. Variables obtained with the bootstrap replicates ($n = 500$) were compared to the estimates obtained from the original dataset. Goodness-of-fit plots and VPCs were performed for internal validation of the model.

2.3.4 Simulations

A simulation was performed to compare the effect of different concentrations of IL-6 on the midazolam plasma concentrations. In addition to the IL-6 median concentration (116 pg/mL), a simulation was also performed with a normal Il-6 value (10 pg/mL) and an increased IL-6 value (300 pg/mL). Moreover, another simulation was performed to compare 1-OH-midazolam-glucuronide plasma concentrations with different serum concentrations of creatinine of 100 μmol/L, 200 μmol/L and 300 μmol/L. The creatinine values 100 μmol/L an a two and three fold increase to 200 μmol/L and 300 μmol/L represent respectively a normal renal function and stage 2 and 3 for acute kidney injury staging. Finally, to compare our pharmacokinetic results with another pharmacokinetic midazolam model in critically ill adult patients, a simulation was performed using our final model and a previously published model from Swart et al. [12]. The midazolam pharmacokinetics studied by Swart et al. were described by a two-compartment model with alcohol abuses, APAPCHE II score and age as significant covariates. The simulations were performed for a median age of 57 years and median APACHE II score of 26 from the study by Swart et al., with and without alcohol abuse. The plasma concentrations of midazolam and 1-OH-midazolam-glucuronide were simulated over a time course of 200 h after the administration of a 5 mg midazolam loading dose followed by our median infusion rate of midazolam continuous intravenous infusion.

2.4 Intensive Care Management

All patients fulfilling the criteria of moderate to severe ARDS were ventilated with a tidal volume of 6 ml/kg ideal bodyweight, limiting driving pressure to 12 cm H2O when possible. PEEP setting was according to the high-PEEP table of the ARDS-network studies and adjusted by using electrical impedance tomography [13]. Patients admitted with a P/F-ratio of less than 150 mmHg were ventilated in prone

position during the first 24 h after admission, and for at least 16 h/day the following 3 days [13]. In case of ventilation in prone position, patients were deeply sedated as per protocol with midazolam (0.05–0.2 mg/kg/h) and sufentanil (0.3–0.7 µg/kg/h) infusion and treated with neuromuscular blockade if needed. Continuous infusion of clonidine (0.2–0.6 µg/kg/h) could be added when deemed necessary. A RASS of at least − 4 was aimed for in case of controlled mechanical ventilation and prone positioning.

3 Results

3.1 Patients and Samples

During the study period, midazolam blood samples were obtained from 38 patients. Of these patients, 7 were excluded for the following reasons: non-COVID-19 ($n = 1$), concomitant use of erythromycin ($n = 1$) and use of CRRT ($n = 5$). The remaining 31 patients were included for population pharmacokinetic modelling. Patients' characteristics are summarized in Table 1. The median duration of infusion was 166 h (range 11–611 h), with a median infusion rate of 25 mg/h (range 3–80 mg/h) and a median dose of 0.28 mg/kg/h (range 0.03–1.00 mg/kg/h).

From 31 patients, 201 blood samples were in steady state collected (67 midazolam, 67 1-OH-midazolam and 67-OH midazolam-glucuronide). Exclusion of the samples with concentrations below the limit of quantification (3 midazolam and 9 1-OH-midazolam) resulted in, 189 samples eligible for pharmacokinetic analysis. Measured plasma concentrations ranged from 2 to 9400 µg/L for midazolam, 4–1416 µg/L for 1-OH-midazolam and 11–39,000 µg/L for 1-OH midazolam-glucuronide.

3.2 Pharmacokinetics of Midazolam

A one-compartment model was developed for midazolam and the metabolites 1-OH-midazolam and 1-OH midazolam-glucuronide with a proportional error model to describe the residual standard error of midazolam and both metabolites. IIV was included on clearance of midazolam, 1-OH-midazolam and 1-OH-midazolam-glucuronide. Finally, the IIV of 1-OH-midazolam and 1-OH-midazolam-glucuronide clearance were set to be similar. Due to many patients with only 1 or 2 observations, addition of IOV was not possible. Table 2 presents the pharmacokinetic variable estimates of the final model. The population mean estimates for clearance were 6.7 L/h (RSE 14.2%) for midazolam, 132.0 L/h (RSE 14.8%) for 1-OH-midazolam and 8.7 L/h (RSE 11.1%) for 1-OH-midazolam-glucuronide. The population mean estimate for volume of distribution was 135 L (RSE 19.9%) for midazolam, 443 L (RSE 23.0%) for 1-OH-midazolam and

Table 1 Patient characteristics

Patients (*n*)	31
Sex, m/f (%)	25/6 (80/20)
Age, y	65 (25–76)
Weight, kg	90 (64–143)
BSA, m²	2.10 (1.73–2.67)
KDIGO stage at ICU admission, n (%)	
Stage 0	21 (68)
Stage 1	6 (19)
Stage 2	3 (10)
Stage 3	1 (3)
APACHE IV, %	17.3 (2.80–79.0)
SOFA	9 (0–16)
Concomitant analgesic/sedative agents	
Sufentanil, *n* (%)	29 (94)
Remifentanil, *n* (%)	4 (13)
Clonidine, *n* (%)	13 (42)
Propofol, *n* (%)	1 (3)
CRP, mg/L	201 (5–692)
IL-6, pg/mL	109 (2–5987)
Creatinine, µmol/L	96 (33–593)
ASAT, U/L	64 (14–22581)
ALAT, U/L	47 (6–10164)
Albumin, g/L	18 (10–32)
Bilirubin, µmol/L	7 (3–100)
Midazolam	
Samples (*n*)	201
Midazolam	67
1-OH-midazolam	67
1-OH-midazolam-glucuronide	67
Plasma concentration, µg/L	
Midazolam	1446 (2–9400)
1-OH-midazolam	115 (4–1416)
1-OH-midazolam-glucuronide	2570 (11–39000)
Duration of administration (h)	166 (11–611)
Infusion rate (mg/h)	25 (3–80)
Dose (mg/kg/h)	0.28 (0.03–1.00)

Data are presented as median (range), or other when specified

y, years; kg, kilograms; BSA, body surface area; ICU, intensive care unit; KDIGO, Kidney Disease: Improving Global Outcomes; RASS, Richmond Agitation-Sedation Scale; APACHE IV, Acute Physiology and Chronic Health Evaluation IV; SOFA, Sequential Organ Failure Assessment; CRP, C-reactive protein; IL-6, interleukin (IL)-6; ASAT, aspartate aminotransferase; ALAT, alanine transaminase; h, hours; mg, milligram; µg, microgram.

100 L (RSE 16.6%) for 1-OH-midazolam-glucuronide. As a small unknown amount of midazolam is metabolized into 4-OH-midazolam, which is not described in our model, the subsequent compartments after the parent midazolam are expressed per available fraction (F). See Fig. 1 for the schematic representation of the structural model for midazolam and its two main metabolites.

Table 2 Pharmacokinetic parameter estimates of final model and bootstrap analysis

NONMEM parameters	Estimates (95% CI)	RSE (%)	Shrinkage (%)	Bootstrap average (95% CI)
Proportional residual error (%)	47.0 (40.6 to 53.4)	6.9		46.1 (40.4 to 52.0)
Midazolam				
CL (L/h)	6.7 (4.8 to 8.5)	14.2		6.7 (5.2 to 8.5)
V_d (l)	135 (82.4 to 187.5)	19.9		139.7 (98.1 to 203.7)
1-OH-midazolam				
CL/F (L/h)	132.0 (93.6 to 170.4)	14.8		132.6 (99.7 to 172.7)
V_d/F (L)	443 (243.0 to 642.9)	23.0		436.4 (176.8 to 940.1)
1-OH-midazolam-glucuronide				
CL/F (L/h)	8.7 (6.8 to 10.6)	11.1		8.7 (7.3 to 10.3)
V_d/F (L)	100.0 (67.5 to 132.5)	16.6		103.0 (68.9 to 145.0)
Covariate effect midazolam CL				
$(\text{IL-6}_{ng/L}/116)^{-0.29}$	-0.29 (-0.39 to -0.20)	16.6		-0.29 (-0.42 to -0.16)
Covariate effect 1-OH-midazolam-glucuronide				
CL $(\text{Creatinine}_{\mu mol/L}/96)^{-1.24}$	-1.24 (-1.60 to -0.88)	15.0		-1.23 (-1.71 to -0.87)
IIV (%)				
CL midazolam	57.9 (18.6 to 85.0)	45.0	6.1	54.9 (28.8 to 81.3)
CL 1-OH-midazolam	69.7 (45.2 to 91.3)	27.0	16.0	66.4 (40.6 to 85.9)
CL 1-OH-midazolam-glucuronide	69.7 (45.2 to 91.3)	27.0	0.7	66.4 (40.6 to 85.9)

RSE, relative standard error; CL, clearance; 95% CI, 95% confidence interval; V_d, distribution volume; F, fraction of previous concentration; IL-6, interleukin 6; IIV, inter-individual variability

Fig. 1 Schematic representation of the structural model for midazolam and its two main metabolites. CL1/F: fractional midazolam clearance and formation of 1-OH-midazolam; CL2/F: fractional 1-OH-midazolam clearance and formtion of 1-OH-midazolam-glucuronide; fractional clearance of 1-OH-midazolam-glucuronide
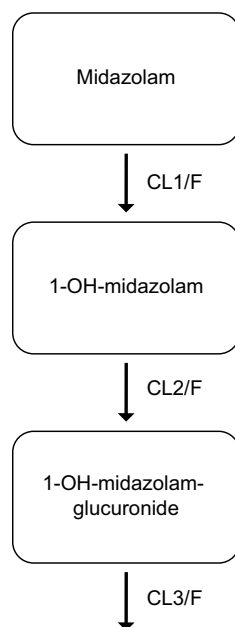
The covariates were tested once at the time for improvement of the structural model.

In a univariate analysis IL-6 (dOFV − 40.0), CRP (dOFV − 38.1), weight (dOFV − 4.0), albumin (dOFV − 4.4), ALAT (dOFV − 18.5), gender (dOFV − 4.9) and BSA (dOFV − 6.2) on midazolam clearance and age (dOFV − 4.4), ALAT (dOFV − 8.7) and creatinine (dOFV − 38.1) on OH-midazolam-glucuronide clearance were found to provide a significant improvement of the model ($p < 0.05$). Subsequent iterations resulted in creatinine and ALAT on OH-midazolam-glucuronide clearance as significant improvement of the model (dOFV − 5.9).

The other covariates did not show a significant improvement of the model ($P \geq 0.05$). After backward elimination with a lower threshold $P \leq 0.001$, only two covariates remained in the final model, namely IL-6 for midazolam clearance (31.8 OFV difference) and creatinine (29.0 OFV difference) for 1-OH-midazolam-glucuronide clearance. Both IL-6 and creatinine were negatively correlated with clearance of midazolam and 1-OH-midazolam-glucuronide respectively, with a covariate effect of − 0.29 (− 0.39 to − 0.20) and − 1.24 (− 1.61 to − 0.88). The addition of IL-6 on midazolam clearance increased the IIV from 42.2 to 57.9%. However, since the proportional error decreased from 54.7 to 47.0 the increase in unexplained IIV was accepted.

Incorporation of creatinine as covariates on 1-OH-midazolam-glucuronide reduced the IIV from 107.4 to 69.7%.

With a condition number of 20.1 (< 1000) the model showed no signs of over-parameterization. Testing the robustness of the model with the bootstrap procedure showed that the estimates of the final model are in accordance with the results of the 500 bootstrap replicates. A total 485 of the 500 runs were successful. Results of the bootstrap

are shown in Table 2. The goodness-of-fit plots of the final model also indicated a successful characterization of the data (Fig. 2). The individual predictions for midazolam and metabolites were evenly distributed around the line of unity when plotted against the observations, which indicates that the model is able to properly estimate the individual plasma concentrations. The population predictions for midazolam and metabolites tend to lower concentrations and higher clearance, resulting in an under prediction of the lower concentrations. This were mainly patients with samples after withdrawing of continuous infusion. Other goodness-of-fit plots are presented in the supplementary information. Evaluation of the predictive performance by VPCs showed accurate predictive ability. The VPCs show the median and 95% confidence interval of the observed midazolam, 1-OH-midazolam and 1-OH-midazolam-glucuronide concentrations (Fig. 3).

3.3 Simulations

Simulated plasma profiles of midazolam and the effect of various IL-6 levels on midazolam clearance and the comparison to the model previously published by Swart et al. are shown in Fig. 4 [12]. The midazolam clearance was reduced by 24% (from 6.7 to 5.1 L/h) when IL-6 increased from population median 116–300 pg/mL.

The effect of serum creatinine on the simulated plasma profiles of 1-OH-midazolam-glucuronide are shown in Fig. 5. The effect of serum creatinine concentration on 1-OH-midazolam-glucuronide clearance resulted in a reduction in clearance of 60% (from 8.7 to 3.5 L/h) when creatinine increased from 100 to 200 µmol/L. A further increase in creatinine to 300 µmol/L reduced the 1-OH-midazolam-glucuronide clearance by another 16% to 2.1 L/h.

4 Discussion

We developed a population pharmacokinetic model of intravenous midazolam provided as a continuous infusion in critically ill COVID-19 patients. The aim of our study was to determine the effect of inflammation on the pharmacokinetics of midazolam. We found that incremental levels of IL-6, an important mediator of inflammation in COVID-19, were significantly associated with decreased midazolam clearance and predicted decreased clearance more significant than CRP. Our finding of a negative correlation between IL-6 levels and midazolam clearance is in line with the study of Vet and colleagues in a pediatric ICU population [11].

We also showed that decreased renal function was significantly associated with decreased 1-OH-midazolam-glucuronide clearance. Similarly, a previous study observed accumulation of 1-OH-midazolam-glucuronide in patients with renal failure [5].

In contrast to previous studies albumin was not found to be correlated to midazolam clearance (midazolam is 94–97% protein bound) [14–16]. Although midazolam is highly protein bound, it seems unlikely that midazolam clearance is reduced during hypoalbuminemia. You would expect that a decrease in albumin would increase the unbound midazolam concentration and therefore result in increased clearance. Given the found reduced midazolam clearance, other proteins may also play a role in protein binding of midazolam or as suggested by the authors of Franken et al. the reduced clearance may be due to an inflammatory response and that hypoalbuminemia can be an expression of inflammation and critical illness [14]. However, there may be a difference in patients between our population and the studied terminally ill patients by Franken et al. (43–93 years; median 71 years, albumin 13–39 g/L; median 25 g/L and CRP 1 to 625 mg/L; median 92 mg/L).

The pharmacokinetics of midazolam established in the current study differ from those reported by Swart et al [12]. Swart and colleagues reported midazolam pharmacokinetics in critically ill adults (21–84 years; mean 57 years and APACHE II 6 to 34; median 26) with and without alcohol abuse. Patients with alcohol abuse showed a lower midazolam clearance, which probably is the result of reduced CYP3A activity due to alcoholic cirrhosis. The simulated midazolam pharmacokinetics with normal IL-6 concentration established in the current study are similar to the study of Swart et al. in the patient group without alcohol abuse and so without possible reduced CYP3A4 activity [12]. However, when increased IL-6 levels are taken into account a clearly reduced midazolam clearance was observed.

We found a median midazolam plasma concentration of 1446 µg/L. This appears to be high compared to previous investigations [17–19]. Our median plasma concentration is comparable to the finding by Nies et al. They observed a median midazolam concentration of roughly 1500 µg/L for RASS -5 [20]. The difference in median concentrations are probably due to the fact that on the one hand, the study by Bremer et al. included post-operative ICU patients and the study by Oldenhof et al. included only 17 patients, and on the other hand that the study by Nies et al. included more long term severe ICU patients.

There appears to be a good correlation between both median midazolam and 1-OH-midazolam plasma concentrations with the degree of sedation [19]. However, since there is a large interpatient variability in plasma concentrations of midazolam and metabolites, it is difficult to formulate a target attainment for a particular degree of sedation [19]. Nonetheless, when we simulated an IL-6 concentration of 116 pg/mL in our model, a steady-state midazolam concentration of almost 3500 µg/L was reached. Similar to our

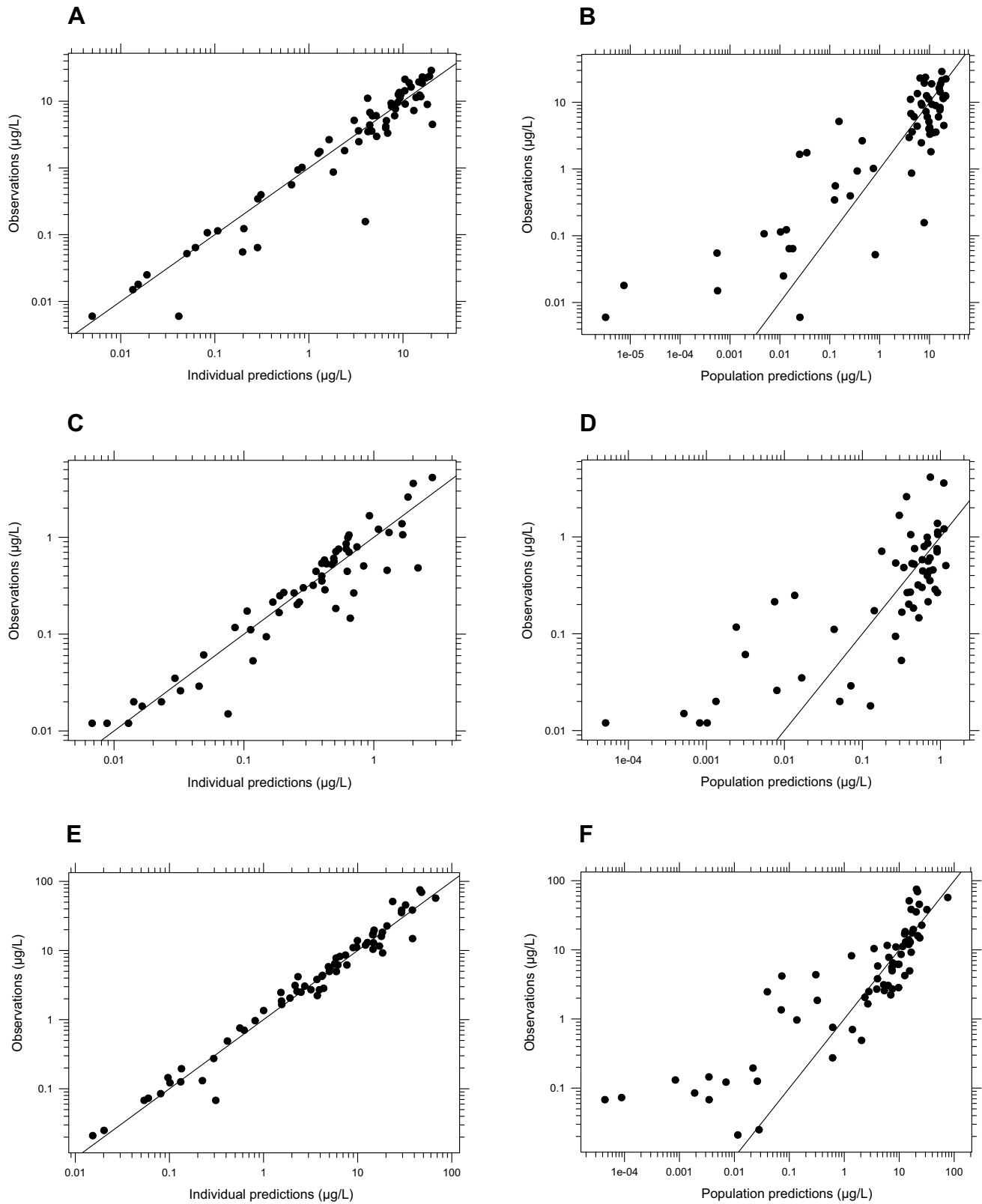**Fig. 2** Goodness-of-fit plots of the final model. Individual (**A**) and population predictions (**B**) vs. observations of midazolam, individual (**C**) and population predictions (**D**) vs. observations of 1-OH-mida-zolam and individual (**E**) and population (**F**) predictions vs. observations of 1-OH-midazolam-glucuronide
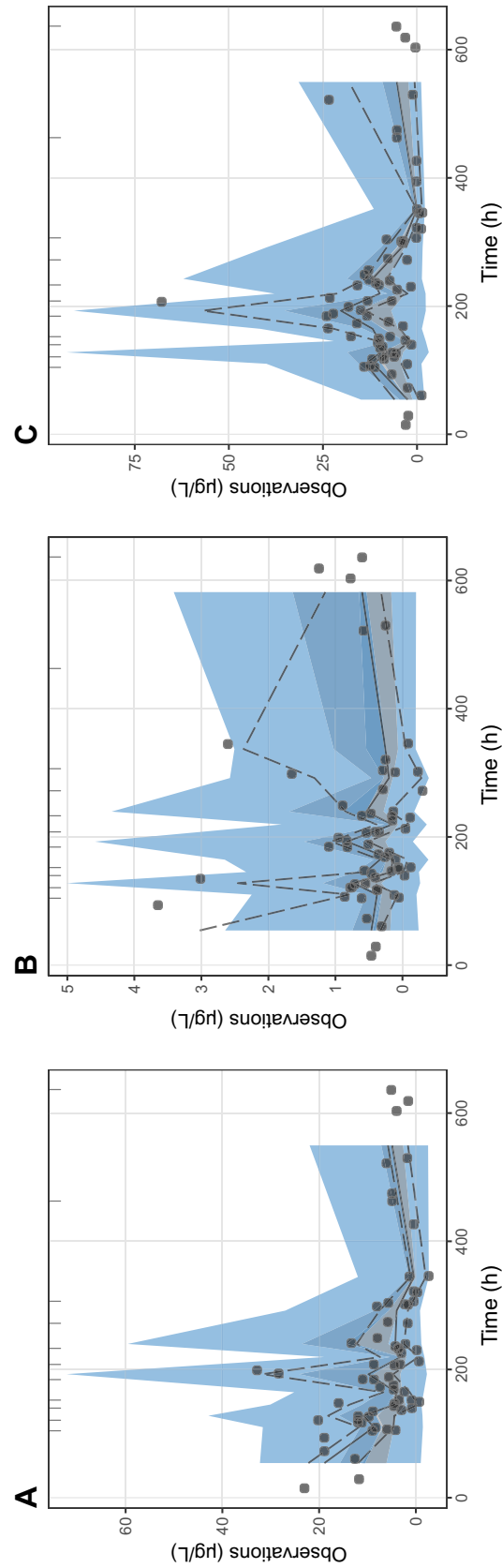
Fig. 3 Prediction-corrected (PC) visual predictive check. PC-visual predictive check for midazolam (**A**), 1-OH-midazolam (**B**) and 1-OH-midazolam-glucuronide (**C**). The grey solid line indicates the median observed concentrations and the surrounding grey area the simulation based 95% confidence interval for the median. The grey dashed lines indicates the observed 5% and 95% percentiles, and the surrounding opaque blue areas show the simulated 95% confidence intervals for the corresponding predicted percentiles

Fig. 4 Simulated plasma profiles of midazolam after a 5 mg midazolam loading dose followed by 25 mg/h continuous infusion for patients with an estimated IL-6 of 10 pg/mL (black line), 116 pg/mL (black small lines) and 300 pg/mL (black big lines) and compared to critically ill patients with (red line) and without (red line with dots) alcohol abuse as reported by Swart et al.
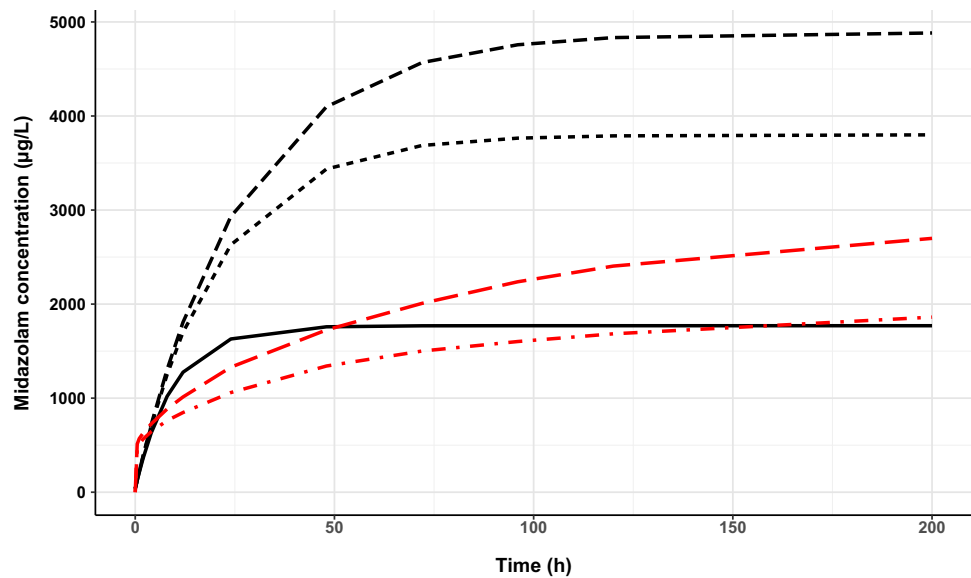
Fig. 5 Simulated plasma profiles of 1-OH-midazolam-glucuronide after a 5 mg midazolam loading dose followed by 25 mg/h continuous infusion for patients with an estimated serum creatinine of 100 μmol/L (black line), 200 μmol/L (black small lines) and 300 μmol/L (black big lines)
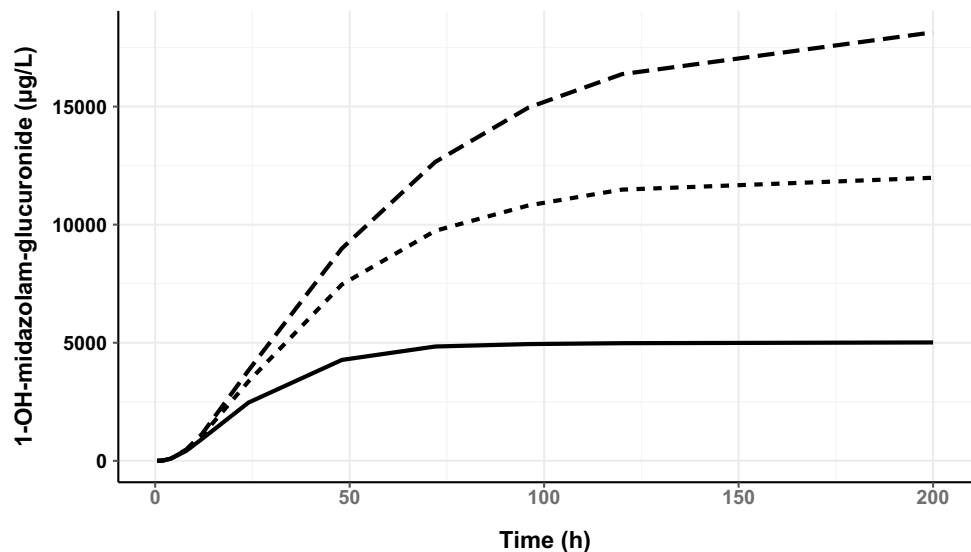
study, other studies observed also relatively high midazolam dose requirements in patients with COVID-19-related ARDS [21, 22]. Interestingly, Tapaskar et al. found a higher total median dose of midazolam in COVID-19 ARDS patients compared to non-COVID-19 ARDS patients. However, no difference in median RASS scores were found between their COVID-19 ARDS group and non-COVID-19 ARDS group, suggesting the higher midazolam dose in COVID-19 ARDS patients was required to achieve equivalent levels of sedation targeted in non-COVID-19 ARDS patients. [22] The high sedation requirements in COVID-19 patients are possibly related to patient factors such as agitation, increased patient-ventilator dyssynchrony, challenging mechanical ventilation in prone position and ARDS, and hyperinflammation, which has previously been linked to opioid tolerance [2, 3, 23]. Additionally, the binding of midazolam to

the GABA receptor is saturable [24]. Therefore, a so-called 'ceiling effect' may occur, due to which greater doses will not produce greater pharmacological effects. However, limited information is known about the effect of COVID-19 and hyperinflammation on the functioning of GABA receptors and more research is needed on the possible saturation of the receptors.

Our results may have implications for more personalized titration of midazolam in COVID-19, mainly to avoid oversedation and subsequently increased length of ICU stay. Our findings indicate that IL-6 is an important biomarker indicative of reduced midazolam clearance. This theoretically contributes to prolonged coma, increased delirium rates and longer ICU stay, which seems to be supported by recent work [3]. Additionally, serum creatinine is an important biomarker for decreased 1-OH-midazolam-glucuronide

elimination. Despite the potency of only 10% for 1-OH-midazolam-glucuronide, plasma concentration up to 39,000 µg/L as found in our study can contribute significantly to prolonged sedation effect in patients with kidney injury.

Therefore, increasing levels of IL-6 and renal failure should lead to incentives to decrease midazolam dosage as much as feasible to mitigate adverse consequences of oversedation.

Gordon et al. recently showed that in critically ill patients with COVID-19, an IL-6 receptor antagonist improved outcomes, including survival and becoming standard care of treatment [25]. In our cohort, none of the patients received an IL-6 receptor antagonist. Theoretically, IL-6 inhibition could neutralize the downregulation of CYP3A enzymes and hence also the reduced clearance of midazolam. Therefore, we suggest that the effect of IL-6 receptor antagonists on midazolam clearance should be further investigated.

In this study, we did not relate the pharmacokinetics to the pharmacodynamics of midazolam. Model-based Therapeutic Drug Monitoring (TDM) might be a valuable tool to relate the pharmacokinetics to the pharmacodynamics of midazolam and to identify patients at risk. Model-informed precision dosing has already been used for antibiotics in critically ill patients, but for sedative agents such models are scarce [26, 27]. For the individual patient, model-based TDM may help earlier dose reduction of midazolam or a switch to other sedative drugs, which may contribute to shorter duration of mechanical ventilation and mitigate days in coma and with delirium.

Several limitations of the study should be noted. The data was limited with respect to the number of patients and samples. The pharmacokinetic model showed a tendency towards under prediction of midazolam concentrations in the first week after the start of treatment, which could not be described in the pharmacokinetic model due to the limited number of available samples within this interval. In addition, especially for observations after withdrawing infusion, the model tends to an under prediction of the lower concentrations of midazolam and metabolites. Furthermore, depending on how concentrations of midazolam and metabolites behave above the upper limit of quantification (ULQ), the extrapolated samples above the ULQ may influence the model performance with more variability or bias.

We did not correlate the effect of the reduced midazolam clearance with the previous reported risk of delirium with midazolam and other risks, such as prolonged coma and increased ICU length of stay [3]. We also did not elucidate the reasons for the observed high-dose midazolam administration. Therefore, further studies on the pharmacodynamics in this population are needed before any firm conclusions can be drawn on dose adjustments. For future research, we propose a larger study population with more observations and development of a population pharmacodynamic model

based on RASS scores. Another possible limitation of our study was that we did not identify patients' genetic polymorphisms of CYP3A4/5. When expressed, it could potentially affect the metabolism of midazolam [28].

5 Conclusions

This study shows that inflammation, reflected by high IL-6, significantly reduces midazolam clearance in critically ill patients with COVID-19. This knowledge may help to identify COVID-19 patients at increased risk for prolonged sedation due to oversedation. Further studies are warranted to relate the pharmacokinetics to the pharmacodynamics of midazolam.

Declarations

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