Unraveling the drivers of antimicrobial pharmacokinetic variability in individuals with obesity and hospitalized patients with multimorbidity

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CHAPTER 6

6

Impact of mucositis on oral bioavailability and systemic exposure of ciprofloxacin gram-negative infection prophylaxis in patients with hematological malignancies

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Abstract

Introduction: Patients with hematological malignancies frequently endure neutropenia and gastro intestinal (GI)-mucositis after high-dose chemotherapy. In these patients, ciprofloxacin is used for gram-negative infection prophylaxis.

We investigate ciprofloxacin pharmacokinetics after oral administration in patients with hematological malignancies and explore the impact of GI-mucositis on oral bioavailability and clearance in order to assure adequate systemic exposure.

Methods: Adult hematological patients from two Dutch University Medical Centers received 500 mg twice daily oral ciprofloxacin for gram negative prophylaxis. The ciprofloxacin plasma concentrations were collected at various timepoints after oral ciprofloxacin administration and at various days after completion of chemotherapy. Data obtained after oral and intravenous ciprofloxacin administration in 28 healthy volunteers without mucositis served as a control group (391 samples). For hematological patients the degree of GI-mucositis was assessed using the Daily Gut Score (DGS), plasma citrulline and albumin. Data were analyzed by non-linear mixed-effects modelling.

Results: In total, 250 blood samples were collected in 47 patients with a wide variety of hematological malignancies between 0-30 days after start of chemotherapy. Mucositis was generally mild (DGS: 1 (1-1) and citrulline 16 µmol/L (12-23) (median, (IQR)). The time to C_{max} was slower in hematological patients compared to healthy volunteers although no association with the degree of mucositis (defined as DGS or citrulline) could be identified. Ciprofloxacin bioavailability and clearance were 60% and 33.2 L/h, respectively.

Conclusion: This study supports oral dosing of ciprofloxacin as gram negative infection prophylaxis in hematological patients with mild to moderate mucositis capable of oral intake.

INTRODUCTION

Fluoroquinolone prophylaxis reduces the relative risk of infection-related mortality in neutropenic patients with hematological malignancies by 68% while adverse effects and development of resistance are not significantly increased.[1-4] Of the fluoroquinolones, ciprofloxacin is the most frequently prescribed. It is typically administered orally in dosages of 500 mg twice daily and is rapidly and well absorbed from the gastro-intestinal tract in healthy volunteers with a bioavailability of approximately 60-80%. Ciprofloxacin is subject to glomerular filtration, tubular secretion, trans-epithelial intestinal secretion and hepatic metabolism.[5]

Patients with hematological malignancies treated with high dose chemotherapy often encounter mucosal disruption of the gastrointestinal (GI) tract (i.e. GI-mucositis).[6,7] Mucositis affects oral absorption unpredictably in patients with hematological malignancies; for example, posaconazole bioavailability is reduced while isavuconazole bioavailability remains unaltered.[8,9] The gold standard method to diagnose mucositis is a biopsy from the small intestine. As this procedure is invasive and therefore not clinically feasible, clinical scores and biomarkers are used to assess severity of mucositis.[10-13] Several mucositis scores are available although there are differences regarding the focus on oral- versus GI-mucositis. The Daily Gut Score (DGS) quantifies GI-mucositis by scoring frequency, consistency and incontinence of feces, nausea, vomiting, abdominal complaints and the ability of oral intake.[12] Nevertheless, the clinical assessment scale is subjective, based on symptoms that may not be specific for mucositis and could be influenced by analgesic agents.[14]

Both citrulline and albumin plasma concentration are also used as biomarkers for mucositis of which citrulline is the most potent.[14] Citrulline is a non-protein amino acid almost exclusively produced by enterocytes of the small intestine. As mucositis develops, mucosal barrier integrity deteriorates which is associated with a reduced citrulline plasma concentration. Consequently, citrulline serves as a biomarker for GI-mucositis. Plasma citrulline <10 µmol/L is associated with severe mucositis, 10-30 µmol/L is associated with mild mucositis, while >30 µmol/L is considered normal.[13,15,16] Citrulline plasma concentration starts declining shortly after initiation of chemotherapy. The lowest values are observed 7-10 days after start of high dose chemotherapy after which citrulline levels rise to normal values around 21 days after high dose chemotherapy.[13]

Besides mucositis, concomitant medication could also influence ciprofloxacin oral absorption. Prokinetic agents like metoclopramide or clarithromycin increase gastric motility and augment esophageal peristalsis which could accelerate oral absorption.[17] Proton-pump inhibitors and antacids could delay gastric emptying by increasing gastric

pH while opioids may delay oral absorption as a result of reduced intestinal motility.[17] Bioavailability may be reduced by co-ingestion with Al^{2+}, Ca^{2+}, Fe^{2+} or Mg^{2+}.[5]

Ciprofloxacin pharmacokinetics in patients with chemotherapy for hematological malignancies showed conflicting results in three case-series (participants n≤8 per series) as exposure was found to be unaltered or decreased.[18-20] Two studies did not report severity of mucositis at all while one study only used clinical markers to describe severity of oral mucositis. Consequently, a knowledge gap remains regarding the influence of GI-mucositis on oral absorption and clearance of ciprofloxacin, risking underexposure and possibly higher infection-related mortality.

In a cohort of neutropenic patients treated for a wide variety of hematological malignancies leading to a high risk of mucositis we investigated ciprofloxacin pharmacokinetics and evaluated if mucositis influences oral bioavailability and clearance, compared to healthy volunteers.

PATIENTS AND METHODS

Data

Data from three cohorts consisting of only hematological patients were collected in two Dutch academic hospitals. Cohort 1 was a prospective observational cohort at the Amsterdam UMC, location Academic Medical Centre (Amsterdam UMC, location AMC). Patients were recruited between March 2019 and December 2020 n=41 (NTR7520). Cohort 2 consisted of participants in a dense sampling study into micafungin pharmacokinetics (NCT02172768) who simultaneously received ciprofloxacin prophylaxis.[21] Cohort 3 consisted of participants in a dense sampling study after posaconazole pharmacokinetics (NCT02805946) who received ciprofloxacin prophylaxis.[8] Patients with concomitant use of ciprofloxacin on pharmacokinetic (PK)-sampling days of micafungin or posaconazole (n=2 and 4 patients, respectively) were selected. Finally, to compare the impact of mucositis caused by high dose chemotherapy on oral absorption, these data were compared to data from a previously performed dense-sampling pharmacokinetic study (n=28 with 391 samples) after oral and intravenous ciprofloxacin administration in healthy volunteers and obese patients (NTR6058).[22]

Hematological patients receiving reduced intensity conditioning regimens for allogeneic HSCT, first remission-induction chemotherapy for AML/myelodysplastic syndrome or CAR-T cell infusion for lymphoma and who received orally administered ciprofloxacin tablets (500 mg twice daily) for gram-negative prophylaxis were eligible for inclusion if they were legally competent and at least 18 years of age. Exclusion

criteria were admission to the intensive care unit, receiving renal replacement therapy or patients unable to take oral medication due to progression or worsening of mucositis and patients with a previous ciprofloxacin treatment course for whom discontinuation lasted less than 48 hours.

In cohort 1, two samples were collected around 1-2 hours after oral administration, one prior to administration and one random sample, all within 72 hours around 7 days after initiation of chemotherapy. Additionally, left-over material from routine sampling at >7 days after initiation of chemotherapy was collected. Blood samples were centrifuged immediately and plasma was stored at -80ºC at the clinical laboratory of the pharmacy department of the Amsterdam UMC, location AMC. In cohort 2 and 3 one trough concentration was collected daily with additional dense sampling (t= 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24) on two PK-days between day 1 and 15 after initiation of chemotherapy. Details on the sampling scheme and the schedule of the PK-days in relation to the start of chemotherapy are presented in table S1 and S2. Samples from cohort 2 and 3 were stored at -80ºC at the clinical laboratory of the department of pharmacy at the Radboud university medical center, Nijmegen, until analysis.

Data on patient characteristics (sex, age, weight, height, BMI, diagnosis, current and previous treatment details, comorbidity), ciprofloxacin treatment (dose, date and time of administration), factors leading to reduced ciprofloxacin exposure (vomiting within 2 hours after administration, interacting co-medication (ranitidine, Al^{2+}, Ca^{2+}, Fe^{2+} or Mg^{2+} containing drugs administered within 4 hours before or 2 hours after ciprofloxacin) and co-medication influencing the oral absorption process (prokinetic agents, Proton Pump Inhibitors, opioids); serum creatinine, mucositis biomarkers (albumin, citrulline) and DGS were recorded over time. To estimate glomerular filtration rate (eGFR), both indexed and de-indexed Chronic Kidney Disease Epidemiology collaboration (CKD-EPI) and Modification of Diet in Renal Diseases (MDRD) were calculated.[23,24] De-indexing was done by multiplying the respective eGFR by 1.73/body surface area (BSA) (BSA was calculated using du Bois-du Bois formula).[25]

A single citrulline plasma sample was drawn in cohort I, simultaneously with obtaining samples collected around 1-2 hours after oral administration. In cohort 3, citrulline plasma samples were collected daily. All citrulline samples were stored on ice and centrifuged within 2 hours. Plasma was stored at -80ºC until analysis. Plasma citrulline was not determined in cohort 3 and the control group.

DGS was retrospectively scored based on data available in the electronic patient registry on every day of PK-sampling. For all six components of the DGS, patients could score 0-3 points, with 0 indicating no complaints and 3 indicating severe complaints with

that component. GI mucositis was scored as mild (1-6 points), moderate (7-12 points) or severe (>12 points).[12]

Ethics

The research protocol for cohort 1 was approved by the certified Medical Ethics Committee of the Amsterdam UMC, location AMC (NL67783.018.18). All patients provided written informed consent. Participants in cohort 2, 3 and the control group provided written informed consent before inclusion in the respective studies (clinicaltrial.gov identifiers for cohort 2 and 3: NCT02172768, NCT02805946. Dutch trial registry number for the control group: NTR6058). The institutional review board permitted additional analysis on the previously collected samples from cohort 2 and 3 and waived informed consent. Additional clinical parameters were collected from the electronic patient registry if patients gave consent for inclusion in the Radboud UMC Biobank Hematology.

The study was conducted in accordance with the Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki.[26,27]

Laboratory analysis

Total ciprofloxacin plasma concentrations were analyzed using a validated LC-MS/MS assay at the Amsterdam UMC. The validated range of the analysis is 0.020 – 5.0 mg/L.[28] Citrulline samples were analyzed using a validated UPLC MS/MS assay at the Clinical chemistry laboratory of Canisius Wilhelmina Hospital, Nijmegen.[29]

Population pharmacokinetic analysis

Concentration-time data from hematological patients and healthy volunteers were analyzed simultaneously by non-linear mixed-effects modelling using FOCE with interaction and the ADVAN6 subroutine (NONMEM; v7.4.0 with PsN; v4.7.1 and Pirana v2.9.7).[30,31] Bioavailability (F) and clearance (Cl) are the pharmacokinetic parameters of primary interest as these drive systemic exposure (measured as AUC) after oral administration. Systemic exposure can be calculated using equation 1.

$$AUC = \frac{\mathrm{F} * \mathrm{Dose}}{\mathrm{Cl}} \quad (1)$$

A previously developed PK-model for ciprofloxacin in healthy volunteers and obese patients with a two-compartment structure and transit compartments for oral absorption was used as a starting point.[22] For the group of hematological patients (cohort 1-3), all PK-parameters were estimated relative to the control group using equation (2) with a correction factor significantly different from 1.0 indicating a difference in typical value between hematological patients and the control group for the respective parameter.

$$\theta_{haematological\ patient} = \theta_{healthy\ volunteer} * \ \theta_{correction\ factor} \quad (2)$$

The influence of covariates (age, sex, weight, BMI, CKD-EPI, MDRD, citrulline, albumin, DGS, days after chemotherapy) was tested for associations with model parameters. If multiple observations were available for one individual (creatinine, citrulline, albumin, DGS and days after chemotherapy) covariates were assessed as a time varying covariate with backward interpolation.

Continuous covariates were implemented in the model using exponential or linear relationships using equation (3) and (4), respectively. Pi and Pp represent individual and population parameter estimates, X represents the exponent for a power function and Z represents the slope for the linear covariate relationship. For dichotomous covariates different parameters were estimated for the respective subgroup.

$$P_i = P_p \times \left(\frac{COV}{COV_{standard}}\right)^X \quad (3)$$

$$P_i = P_p \times \left(1 + Z \times (COV - COV_{standard})\right) \quad (4)$$

In the model building process, a change in objective function value, goodness-of-fit, conditional weighted residual plots, reduction in interindividual variability and individual fit plots were used to compare models. A p-value of <0.05, representing a decrease of 3.84 in objective function value with one degree of freedom was considered statistically significant for structural parameters. In the covariate analysis a p-value of p<0.05 (objective function value decrease >3.84) was considered statistically significant in the forward inclusion step while p<0.001 (objective function value increase >10.8) was considered statistically significant in the backward elimination. Internal model evaluation and validation was done using GOF-plots, CWRES-plots split for cohort and time dependent covariates, visual predictive check (VPC) and sampling importance resampling (SIR).

Continuous data are presented as mean ± standard deviation (SD) and analyzed by t-test when normally distributed or as median ± IQR and analyzed by Mann-Whitney-U-test when not normally distributed.

After development and internal validation of the pharmacokinetic model, simulations were performed with 2500 virtual individuals per cohort with interindividual variability. The ciprofloxacin exposure, measured as AUC in hematological patients was compared to healthy volunteers after the standard of care dosing regimen of twice daily oral administration of 500mg.

RESULTS

Subject characteristics

Data of forty-seven patients (19 men and 28 women) with a wide variety of hematological malignancies were included in which 250 ciprofloxacin plasma samples were available. The oral absorption phase was captured in detail with 82 plasma samples (33%) collected in the first two hours after oral administration. Data of 28 healthy volunteers (14 men and 14 women) were included. 8 patients received semi-simultaneous oral and intravenous administration and 20 individuals received either oral or intravenous administration. In total 391 samples were collected in the control group, 178 after intravenous administration and 213 after oral administration. A detailed description of PK-data is provided in Table S2.

The majority of patients showed biochemically mild mucositis with the lowest observed citrulline (nadir) between 10-30 µmol/L (n=32, 68%) and a DGS indicating clinically mild mucositis (n=46, 98%). Clinically moderate mucositis (by DGS) was observed in 1 patient (2%) while biochemically severe mucositis (citrulline nadir ≤10 µmol/L) was observed in 9 patients (19%). Of these 9 patients, the DGS indicated clinically mild mucositis in 8 patients and moderate mucositis in 1 patient. Samples were collected median (IQR) 6 (3-11) days after start of high dose chemotherapy. In patients with multiple citrulline observations a declining plasma citrulline was observed until 10 days after start of conditioning. Concomitant medication that could influence the oral absorption process was used by 29 hematological patients (62%) at the time of PK-sampling. Drugs delaying oral absorption were used by 26 patients (89%) while drugs accelerating oral absorption were used by 14 patients (48%). Details of patient characteristics including the use of concomitant medication potentially influencing oral absorption of ciprofloxacin are presented in Table 1.

Pharmacokinetic analysis

The concentration-time profiles were best described by a two-compartment model with first order elimination and a transit compartment model for oral absorption with a correction factor of 2.07 (95%CI 1.4 – 2.5) on mean transit time for hematological patients, interindividual variability on clearance, volume of distribution and mean transit time and a proportional error model (for model structure, see Figure S1). Bioavailability and clearance were 60% and 33.2 L/h, respectively and were not significantly different between hematological patients and the control group. The degree of mucositis measured by DGS, citrulline and albumin plasma concentration as well as days after chemotherapy were investigated as potential drivers of the observed difference in the mean transit time between both groups, but evaluation of these parameters did not provide a better prediction of the observed data. Goodness-of-fit-plots show the model adequately describes the observed data and conditional weighted residual plots

Table 1: Population characteristics

	Hematological patients (n=47)	Control group (n=28)
Age	53 (47 – 64)	40 (27-52)
Sex, male (n (%))	19 (40)	14 (50)
Weight (kg)	78 (69 – 90)	123 (84-149)
Citrulline nadir (µmol/L)[a]	16 (12-23)	n.d.
Citrulline nadir ≤10 µmol/L (n)	9	n.d.
Citrulline nadir 10-30 µmol/L (n)	32	n.d.
Citrulline nadir ≥30 µmol/L (n)	4	n.d.
Albumin nadir (g/L)	42 (26 – 50)	n.d.
Daily Gut Score	1 (1-1)	n.d.
Diagnosis		n.a.
Acute leukemia	17	
Lymphoma	13	
Multipel myeloma	9	
Chronic leukemia	3	
Other	5	
Treatment (n)		n.a.
Remission-induction	19	
Autologous SCT	16	
Allogeneic HSCT	10	
Other	2	
Relevant co-medication		
Reduced bioavailability		
Magnesium hydroxide	1	0
Delayed absorption		
Proton pump inhibitor	24	0
Esomeprazole	19	
Pantoprazole	3	
Omeprazole	2	
Opioid	7	0
Oxycodone	5	
Morphine	1	
Fentanyl	1	
Tramadol	1	
Accelerated absorption		
Metoclopramide	13	0
Metoprolol	1	0
Clarithromycin	1	0
Serum creatinine (µmol/L)	74 (62 – 89)	72 (64 – 80)
CKD-EPI (ml/min/1,73 m^2)	97 (80 – 112)	134 (118 – 149)
$CKD\text{-}EPI_{de\text{-}indexed}$[b] (ml/min)	84 (68 – 105)	102 (95 – 109)
MDRD (ml/min/1,73 m^2)	96 (78 – 121)	126 (110 – 154)
$MDRD_{de\text{-}indexed}$[b] (ml/min)	82 (66 – 115)	98 (90 – 108)

Data are presented as median (IQR) unless stated otherwise. For citrulline and albumin the lowest observed value is reported if multiple observations were available per patient. For serum creatinine and corresponding estimators of GFR the observation at baseline is reported. Daily Gut Score (DGS) 1 represents mild mucositis (1-6 points on the DGS) and 2 represents moderate mucositis (7-12 points on the DGS). HSCT: Hematopoietic stem cell transplant, N.a.: Not applicable, nadir: lowest observed value for an individual, N.d.: Not determined, SCT: Stem cell transplant. [a] *Citrulline plasma concentration was determined in 45 hematological patients*
[b] *De-indexed by multiplying CKD-EPI or MDRD by BSA/1.73.*

indicate no model misspecification as residuals were randomly spread against time after start of ciprofloxacin, predicted plasma concentration, plasma albumin, plasma citrulline and days after chemotherapy (Figure S2). The use of concomitant medication in the hematological patients showed no significant association with altered ciprofloxacin pharmacokinetics in hematological patients. Also, the predictive performance of citrulline plasma concentration as a biomarker for impaired oral absorption was not significantly different for patients receiving stem cell transplant compared to patients receiving other therapy (Figure S3). Clearance, bioavailability and volume of distribution were unaffected by mucositis. Total body weight and renal function also did not provide a statistically significant improvement of the fit when tested as covariates on these parameters. Internal model validity was confirmed using VPC stratified for hematological group and control group (Figure S4). Model parameters and their uncertainty based on SIR are shown in Table 2, the mean percentage error is -5.4%.

Table 2: Pharmacokinetic parameter estimates for the final model

Parameter	Estimate (%RSE)	SIR 95% CI
CL (L/h)	33.2 (6.9)	29.9 – 37.0
V_c (L)	69.0 (19.1)	50.7 – 92.8
V_p (L)	140 (10.6)	120 – 160
Q (L/h)	71.4 (13.4)	57.3 – 83.5
F	0.603 (8.5)	0.535 – 0.669
K_a (h^{-1})	1.35 (14.5)	1.08 – 1.70
MTT Control group (h)	0.317 (13.1)	0.230 – 0.369
Relative MTT Hematological patients[a]	2.07 (14.8)	1.41 – 2.50
NN (n)	10.9 (40.2)	6.51 – 15.8
Interindividual variability (%)[b,c]		
CL	28.1 (12.8)	20.7 – 34.3
V_c	86.7 (23.7)	55.9 – 134
MTT	82.6 (13.2)	74.7 – 117
Residual error (%)[b]		
σ prop[d]	21.4 (5.9)	19.4 – 23.1

CL: Clearance from the central compartment, F: bioavailability, Ka: Absorption rate constant, MTT: Mean Transit Time, NN: Number of transit compartments, Q: Intercompartmental clearance, RSE: Relative Standard Error, SIR: sampling importance resampling based on 5000 samples and 1000 resamples, Vc: Volume of distribution of the central compartment, Vp: Volume of distribution of the peripheral compartment

*a absolute MTT for hematological patients is 0.656 h (according to Eq. 1: 0.317 h * 2.07 = 0.656 h)*

b Calculated by $\sqrt{(e^{\omega^2} - 1)}$

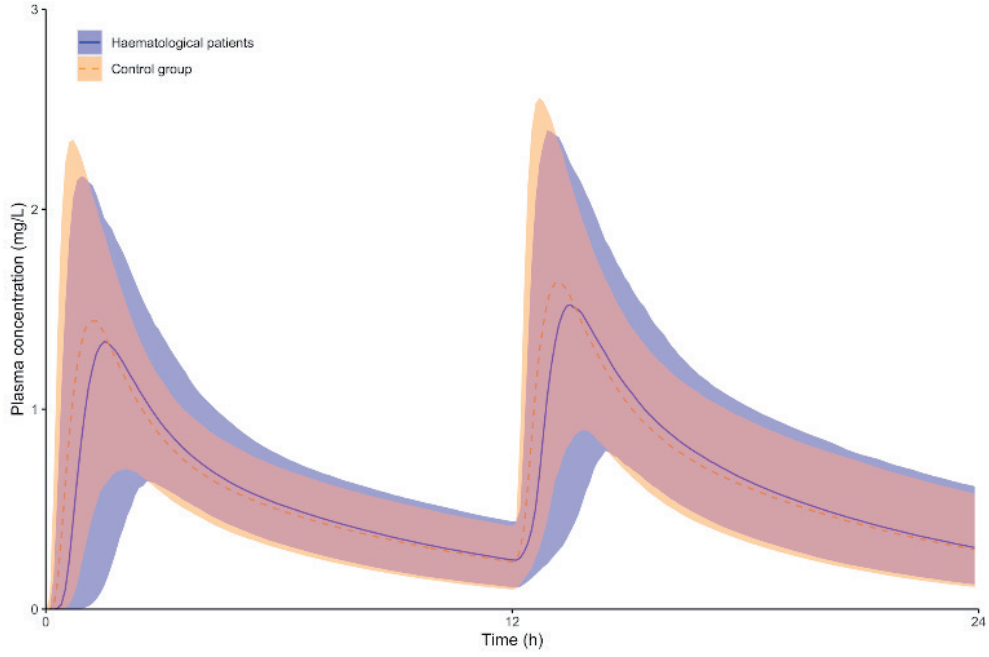
c η-shrinkage: CL 5%, V_c 26%, MTT 26%,

d ε-shrinkage: 11%

Model-based dose evaluations

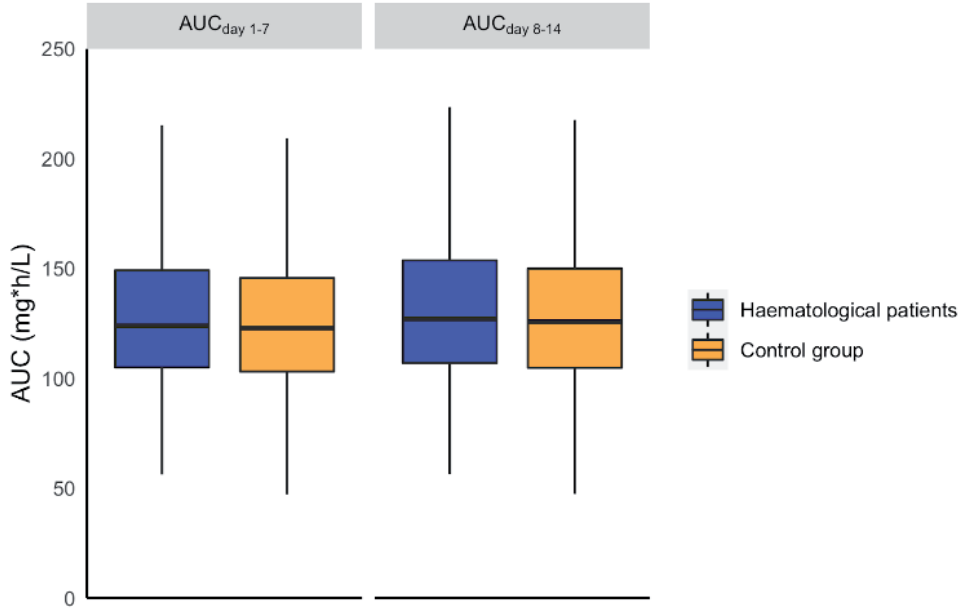
Using the final and internally validated model, exposure that can be expected upon the standard of care dosing regimen of a twice daily oral ciprofloxacin administration of 500 mg was evaluated. The median (IQR) time to ciprofloxacin C_{max} is 1.5 (1.2-2.1) hours for hematological patients and 1.2 (1.0-1.5) hours for the control group as shown in Figure 1. In the first week of treatment median (IQR) cumulative $AUC_{day\ 1\text{-}7}$ is 124 (105 – 149) mg*h/L for hematological patients and 123 (103 – 146) mg*h/L for healthy volunteers. In the second week of treatment median (IQR) cumulative $AUC_{day\ 8\text{-}14}$ is 127 (107 – 154) mg*h/L for hematological patients and 126 (105 – 151) mg*h/L for healthy volunteers as displayed in Figure 2.

Figure 1



Concentration-time curve (median (line) and 95% prediction interval (shaded area)) for hematological patients (pink) and healthy volunteers (teal) after twice daily oral ciprofloxacin 500mg. Data based on simulations with n=2500 per subgroup. The median simulated time to ciprofloxacin C_{max} is 1.5 hours for hematological patients and 1.2 hours for the control group

Figure 2



Boxplots illustrating similar cumulative systemic exposure in the first- and second week of therapy ($AUC_{day\ 1-7}$ left panel, $AUC_{day\ 8-14}$ right panel) for hematological patients (blue) and the control group (orange). Data are based on Monte Carlo simulations (n=2500 per subgroup) after the standard of care dosing regimen of 500mg PO twice daily.

DISCUSSION

Ciprofloxacin pharmacokinetics in patients with hematological malignancies and mild mucositis capable of oral intake and healthy volunteers is comparable. Oral absorption remains adequate during the whole chemotherapy treatment course, even in the second week after high-dose chemotherapy when mucositis is most severe. However, oral absorption was slower in hematological patients. Therefore, hematological patients with mild to moderate mucositis capable of oral intake can receive orally administered ciprofloxacin as gram negative infection prophylaxis.

We included 47 patients with a wide variety of hematological malignancies and a broad range in severity of underlying disease at different time points in their treatment course. The majority of participants capable of oral ciprofloxacin intake had clinically mild mucositis based on the DGS and as a result few data on oral absorption and clearance of ciprofloxacin could be collected in patients with clinically moderate mucositis and no

data was collected in patients with clinically severe mucositis. Patients unable to take oral medication due to progression or worsening of mucositis or developing fever were switched to intravenous therapy by their treating physician. There are no strict criteria for when to switch from oral to intravenous therapy. Yet, our results indicate that the clinical decision of the treating physician to switch patients from oral to intravenous therapy is at least not too late as no underexposure was observed. The majority of hematological patients used concomitant medication that could accelerate or delay the oral absorption process. Although the use of concomitant medication delaying oral absorption may have contributed to the observed increased time to C_{max} in hematological patients compared to healthy volunteers, the use of delaying concomitant medication was not a statistically significant covariate. Most importantly, only 1 patient used concomitant medication that could decrease bioavailability. Therefore, it is unlikely that the absence of a correlation of mucositis with ciprofloxacin exposure is attributable to the use of concomitant medication.

In our study the degree of mucositis was objectified using the most adequate and feasible clinical scores and biomarkers. Hematological patients suffered from mild mucositis in the opinion of the treating physician and were capable of oral ciprofloxacin intake. DGS corresponded with mild to moderate mucositis while citrulline plasma concentration ranged from values corresponding with normal values to severe mucositis. A mucositis scoring mismatch was observed in nine patients (19%) as citrulline plasma concentration showed severe mucositis (nadir <10 µmol/L) while DGS suggested mild (n=8) or moderate (n=1) mucositis. Four of these patients did not switch to intravenous therapy at any time during their treatment course, indicating no development of clinically severe mucositis in the opinion of the attending physician. Nevertheless, uncertainty remains if patients with mild mucositis according to the DGS actually showed mild mucositis in the small intestine. As patients were capable of oral intake and the physician judged mucositis to be generally mild we chose to draw conclusions regarding mild to moderate mucositis despite citrulline plasma concentrations corresponding with severe mucositis. Observations in patients treated with intravenous antibiotics whom were subsequently switched back to oral ciprofloxacin after clinical improvement were adequately described by our model. This may suggest ciprofloxacin oral absorption is not significantly altered in patients with a temporarily worsening clinical condition. The DGS was retrospectively scored based on data available in the electronic patient registry. Possibly, some components of the DGS may have been incompletely registered which could have led to an underestimation of mucositis severity using the DGS which is a limitation to our study. Also, citrulline was measured only once for participants in cohort 1. Although citrulline was observed when mucositis is expected to be worst mucosal damage may have worsened after, or was already improving at the time of monitoring. Therefore, the citrulline nadir may have been lower than the observed

value. An important strength of our study is the comparison of ciprofloxacin PK data in hematological patients with data from 28 healthy individuals as a PK reference standard, to compensate for the lack of a formal PK/PD target for gram negative prophylaxis using ciprofloxacin.

Three previous case-series reported contradictory results on exposure of orally administered ciprofloxacin in patients with hematological malignancies.[18-20] Two studies found no difference in drug concentration between hematological patients with mucositis and data from literature, although another study found a reduced drug concentration in patients with hematological malignancies.[18-20] Since these reports observed only 8, 6 and 5 patients, the external validity of the respective case-series is limited.[18-20] Moreover, two of the three case-series did not report severity of mucositis at all while one study focused on severity of oral mucositis and in contrast to our study the role of biomarkers was not evaluated before. In order to capture the severity of mucositis at the site of absorption GI-mucositis scores and biomarkers were analyzed concomitantly.

Ciprofloxacin is subject to OATP1 and OAT3 carrier mediated absorption.[32] Disruption of the mucosa could negatively impact OATP1 and OAT3 carrier capacity but at the same time mucosal barrier function may be impaired. As a result, both an increased and decreased rate of absorption and bioavailability could be anticipated. Hematological patients showed an increased time to C_{max} although this was not associated with a significant alteration in bioavailability. Both active and passive processes play a role in ciprofloxacin absorption. Therefore, the slightly longer time to C_{max} in hematological patients could theoretically be caused by a negative impact on active absorption as a result of mucositis while the net-influence on bioavailability remains negligible.

In patients with severe mucositis treated with IV ciprofloxacin, further research may be needed to clarify if destruction of the mucosa impacts the trans-epithelial intestinal secretion route of ciprofloxacin as ciprofloxacin clearance could still be altered.

In conclusion, we found no significant influence of mild mucositis on ciprofloxacin bioavailability or clearance. This study supports oral dosing of ciprofloxacin 500 mg twice daily as gram negative infection prophylaxis in hematological patients with mild to moderate mucositis capable of oral intake.

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Supplementary material

Table S1: Study procedures and handling of samples

	Cohort I	Cohort II	Cohort III	Control group
Study acronym		MATADOR	PIRANA	AMIGO
Site	Amsterdam University Medical Center, Amsterdam	Radboud University Medical Center, Nijmegen	Radboud University Medical Center, Nijmegen	Radboud university Medical Center and St Antonius hospital
Design	Prospective observational cohort	Randomized open-label multiple-dose intervention study (micafungin)	Randomized open-label, multiple-dose, multiple-dose level intervention study (posaconazole)	Open-label prospective pharmacokinetic intervention study (ciprofloxacin)
Sampling scheme	2 peak samples, 1 trough sample, 1 random sample. All collected within 72 hours >7 days after conditioning. Additional scavenged sampling from left-over material from routine monitoring	Daily trough sample at 8:00 AM. Rich sampling on day 4 or 5 and day 8 at: t= 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24 after micafungin administration.	Daily trough sample at 8:00 AM. Rich sampling on day 7, 12 and 16 at: t= 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24 after posaconazole administration.	Dense sampling until 12 hours after administration with 11 samples after IV administration, 15 samples after PO administration and 17 samples after semi-simultaneous administration.
Handling of samples	Ciprofloxacin: centrifuged the same day Citrulline: stored on ice and centrifuged within 2 hours after collection	Ciprofloxacin: centrifuged the same day Citrulline: not studied	Ciprofloxacin: Centrifuged the same day Citrulline: stored on ice and centrifuged within 2 hours after collection	Ciprofloxacin: Centrifuged the same day
Storage of samples	-80°C until analysis	-80°C until analysis	-80°C until analysis	-80°C until analysis
Albumin monitoring	Routine care, 8:00 AM	Routine care, 8:00 AM	Routine care, 8:00 AM	Not studied
Citrulline monitoring	Obtained once, simultaneously with the first peak sample	Not studied	Daily at 8:00 AM	Not studied
DGS monitoring	Every PK day. Retrospectively collected from EHR	Every PK day. Retrospectively collected from EHR	Every PK day. Retrospectively collected from EHR	Not studied

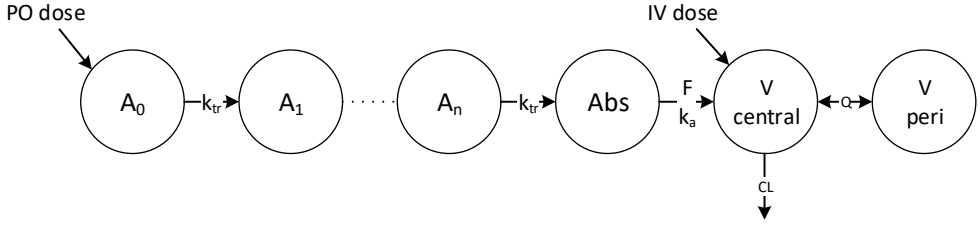
DGS: Daily Gut Score, EHR: Electronic Health Registry

Table S2: Description of pharmacokinetic data

	Cohort I	Cohort II	Cohort III	Control group	All hematological patients (Cohort 1,2,3)
Study acronym		MATADOR	PIRANA	AMIGO	
Patients (n)	41	2	4	28	47
Ciprofloxacin route of administration	PO	PO	PO	PO and IV	
Samples (n)	157	51	42	Total: 391 PO: 213 IV :178	250
Samples per patient (n)	3.8	25.5	10.3	PO: 7.6, IV: 6.4	5.3
Peak samples (n (%)) (0-2h after administration)	58 (37%)	15 (29%)	9 (21%)	PO: 92 (43%) IV: 45 (25%)	82 (33%)
Midway samples (n (%)) (2-10 h after administration)	41 (26%)	21 (41%)	10 (24%)	PO: 111 (52%) IV: 116 (65%)	72 (29%)
Trough samples (n (%)) (10-14h after administration)	54 (34%)	8 (16%)	10 (24%)	PO: 10 (5%) IV: 17 (10%)	72 (29%)
Wash-out samples (n (%)) (14-28h after administration)	4 (3%)	7 14%)	13 (31%)	PO: 0 (0%) IV: 0 (0%)	24 (10%)
Sampling at days after start of conditioning (median – IQR)	6 (3-12)	11 (7-15)	4 (1-7)	Not studied	6 (3-11)
Citrulline samples (n)	41	Not studied	24	Not studied	65
Albumin samples (n)	82	20	9	Not studied	111

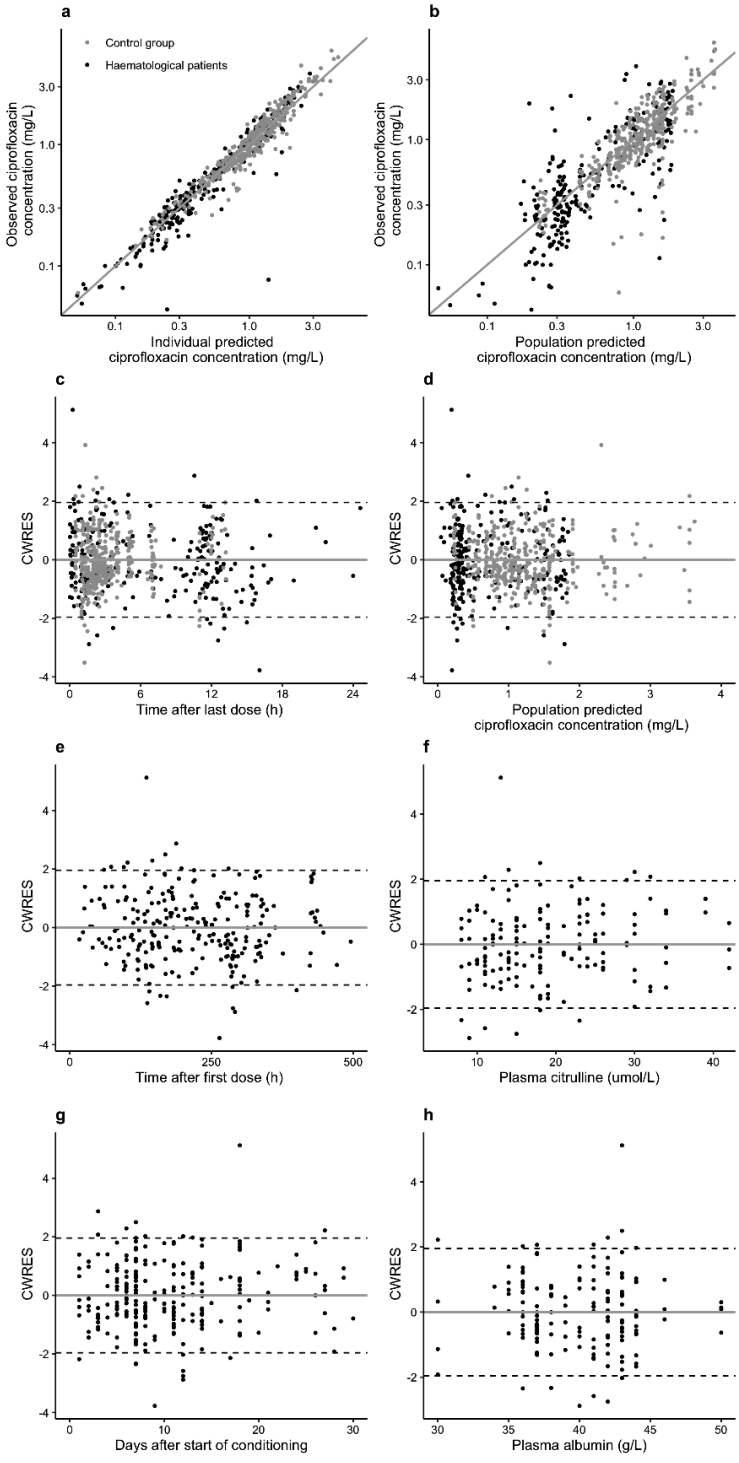
IQR: Inter-quartile range, IV: intravenous administration, PO: oral administration

Figure S1: Structure of the pharmacokinetic model



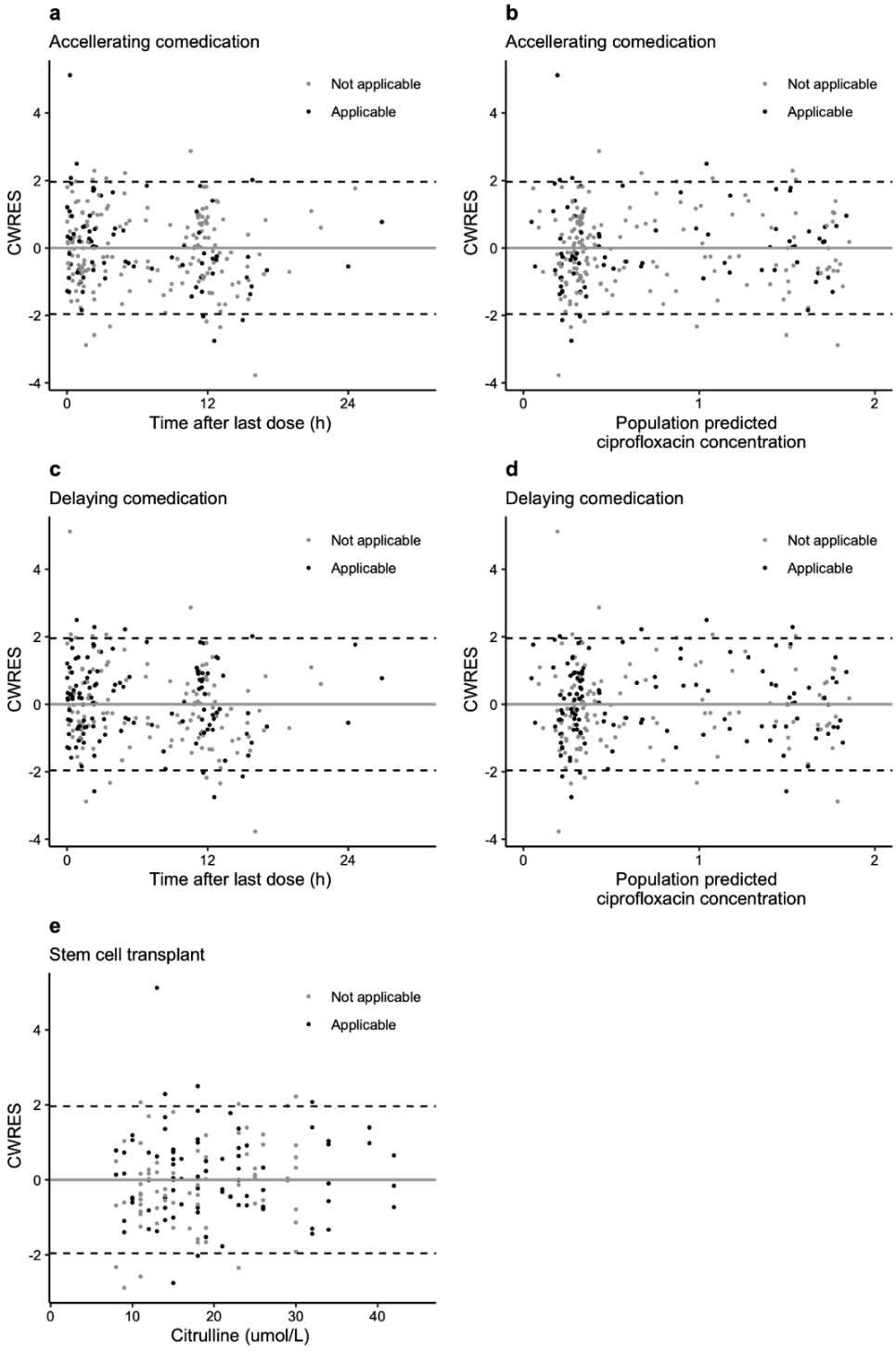
Abs: Absorption compartment, A_n: Transit compartment (n), CL: Clearance from the central compartment, F: Bioavailability, k_a: absorption rate constant, k_{tr}: transit rate constant, Q: Intercompartmental clearance, $V_{central}$: Central volume of distribution, V_{peri}: Peripheral volume of distribution.

Figure S2: Diagnostic plots of the final pharmacokinetic model



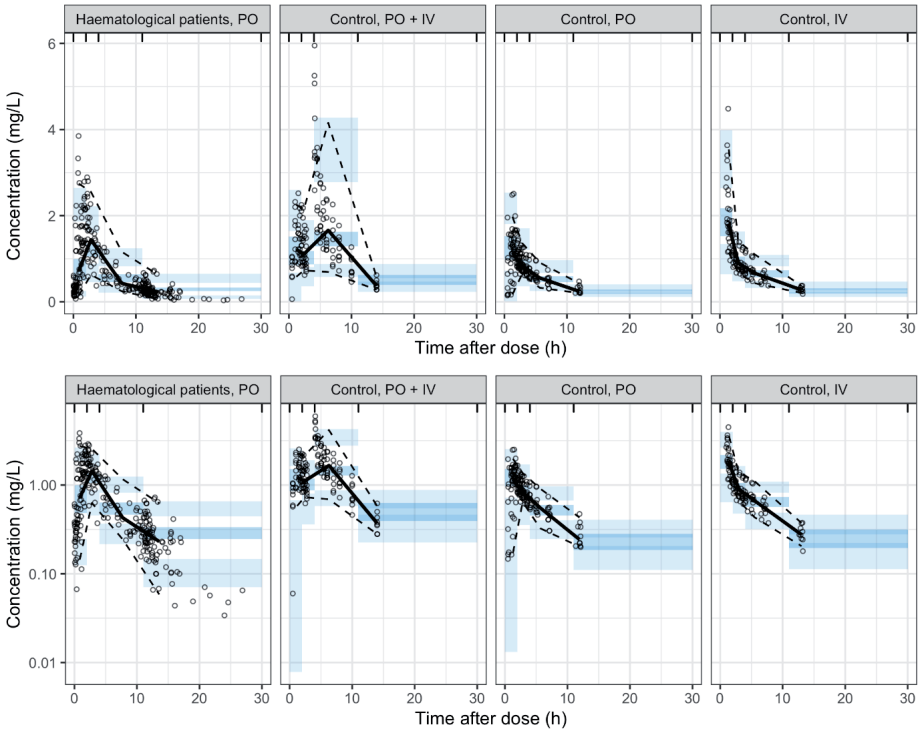
Diagnostic plots of the final model for hematological patients (black dots) and the control group (grey dots).
a: Individual predicted plasma concentrations versus observed plasma concentrations,
b: Population predicted plasma concentrations versus observed plasma concentrations.
c: CWRES versus time after last dose,
d: CWRES versus Population prediction
e: CWRES versus time after first dose,
f: CWRES versus Plasma citrulline,
g: CWRES versus days after start of conditioning,
h: CWRES versus plasma albumin.
The grey line indicates the line of identity and the dashed lines indicate the range within 95% of the observations are expected to fall. For plots e, f, g and h only hematological patient data is presented.

Figure S3: Diagnostic plots for concomitant medication use and stem cell transplant



Conditional weighted residuals (CWRES) plots of the final model for potential covariates in hematological patients showing trends versus time (a, c, e), predicted plasma concentration (b, d, f) or citrulline plasma concentration (g). Black dots represent observations where the potential covariate is applicable, grey dots represent observation where the potential covariate is not applicable.
a: Use of comedication potentially accelerating oral absorption, residuals versus time after dose,
b: Use of comedication potentially accelerating oral absorption, residuals versus PRED,
c: Use of comedication potentially delaying oral absorption, CWRES versus time after dose,
d: Use of comedication potentially delaying oral absorption, CWRES versus PRED,
e: CWRES versus citrulline plasma concentration for patients receiving stem cell transplant.
The grey line indicates the line of identity and the dashed lines indicate the range within 95% of the observations are expected to fall.

Figure S4: Visual Predictive Check of the final model



Visual predictive check of the final model stratified by subgroup (Hematological patients n=47, control PO+IV n=8, Control PO n=10, Control IV n=10) on linear scale at the top and on logarithmic scale at the bottom. The observed data are shown as circles with the median and 2.5th and 97.5th percentile of the observed data shown as a solid black line and the upper and lower dashed lines, respectively. The blue areas represent the 95% confidence interval of the simulated median (dark blue) and 5th and 95th percentile (light blue) of the simulated concentrations based on 1000 simulations of the original dataset. Vertical lines at the top of the panels represent the bins.
PO represents 500mg oral ciprofloxacin administration, PO+IV represents semi-simultaneous 500mg oral administration followed by 400mg IV infusion 3 hours after the oral administration, IV represents 400mg ciprofloxacin IV infusion.