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High unbound flucloxacillin fraction in critically ill patients

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Objectives: To describe the unbound and total flucloxacillin pharmacokinetics in critically ill patients and to define optimal dosing strategies.

Patients and methods: Observational multicentre study including a total of 33 adult ICU patients receiving flucloxacillin, given as intermittent or continuous infusion. Pharmacokinetic sampling was performed on two occasions on two different days. Total and unbound flucloxacillin concentrations were measured and analysed using non-linear mixed-effects modelling. Serum albumin was added as covariate on the maximum binding capacity and endogenous creatinine clearance (CL_{CR}) as covariate for renal function. Monte Carlo simulations were performed to predict the unbound flucloxacillin concentrations for different dosing strategies and different categories of endogenous CL_{CR}.

Results: The measured unbound concentrations ranged from 0.2 to 110 mg/L and the observed unbound fraction varied between 7.0% and 71.7%. An integral two-compartmental linear pharmacokinetic model based on total and unbound concentrations was developed. A dose of 12 g/24 h was sufficient for 99.9% of the population to achieve a concentration of >2.5 mg/L (100% $fT_{>5\times MIC}$, MIC = 0.5 mg/L).

Conclusions: Critically ill patients show higher unbound flucloxacillin fractions and concentrations than previously thought. Consequently, the risk of subtherapeutic exposure is low.

Introduction

Flucloxacillin is the preferred treatment for MSSA infections.[1,2] Flucloxacillin is typically dosed 6–12 g daily as continuous infusion or as intermittent infusion.[1,3,4] Over the past decades it has become clear that critically ill patients demonstrate highly variable pharmacokinetics, which makes optimal dosing of antibiotics such as flucloxacillin challenging.[4,5] This is of paramount clinical relevance, as inadequate antimicrobial dosing may increase the risk of clinical failure or result in toxicity.[6,7]

Flucloxacillin is mainly excreted unchanged in the urine by glomerular filtration and tubular secretion, and, to a limited extent, metabolized in the liver.[8] Therefore, renal function should be taken into account when dosing. The lack of knowledge on prediction of renal flucloxacillin clearance is likely complicated by the difficulties of estimating renal function in critically ill patients, due to declined muscle mass, sepsis and fluid retention, among other factors.[9] Estimation of glomerular filtration rate (eGFR) based on plasma creatinine is currently the most used method. As creatinine is dependent on muscle mass, it does not accurately reflect GFR in critically ill patients. Cystatin C is known to provide better estimations of GFR.[9,10] eGFR with cystatin C combined with creatinine is found to describe variability in flucloxacillin clearance best in non-critically ill patients.[11] It is still unknown whether eGFR based on cystatin C is a better predictor of flucloxacillin clearance in critically ill patients.

Flucloxacillin is approximately 95%–97% protein bound in healthy volunteers, mainly to albumin.[1,12] In hospitalized patients, protein binding varies between 63% and 97%.[4,11] Hypoalbuminaemia, frequently observed in critically ill patients, is known to alter flucloxacillin protein binding.[3,4,13] It is important to fully characterize the extent of protein binding in this patient population.

The efficacy of flucloxacillin is driven by the time that the unbound drug concentration remains above the MIC of the targeted pathogen (fT>MIC).[14] A target of 100% $fT_{>1-5\times MIC}$ is often used in the critically ill population.[15,16] Although β-lactam antibiotics are thought to have a wide therapeutic window, there is increasing evidence that critically ill patients are at risk for both subtherapeutic exposure, as well as toxicity.[4,7,17] Flucloxacillin has been associated with nephrotoxicity and neurotoxicity. Only neurotoxicity has been described to be concentration dependent.[7,18] A total flucloxacillin concentration of 125 mg/L and an unbound concentration of 20 mg/L have been associated with an increased likelihood of neurotoxicity, probably mediated by interference with the gamma-aminobutyric acid.[7,18]

In view of the expected changes in pharmacokinetics of flucloxacillin in critically ill patients and the necessity of dose individualization, it is pivotal to study its pharmacokinetics in this specific group. The objective of the present study was to describe the unbound and total flucloxacillin pharmacokinetics in critically ill patients and to evaluate different dosing regimens in critically ill patients.

Patients and methods

Study design

A prospective observational pharmacokinetic study was performed in patients treated with flucloxacillin IV therapy as part of routine clinical care in three ICUs. The research was conducted in accordance with the Declaration of Helsinki, and national and institutional standards. The study was registered at ClinicalTrials.gov (identifier NCT02993575).

Study design justification

A stochastic simulation and estimation of 500 virtual pharmacokinetic studies, based on a previously developed pharmacokinetic model for unbound concentrations of flucloxacillin in ICU patients,[3] was performed to evaluate the study design a priori. Pharmacokinetic sampling was performed on two occasions with an interval of 48 h. Patients could enter the study on any given moment after start of treatment. For patients on an intermittent dosing regimen, samples were drawn on Day 1 of the study at $t = 0$ h (pre-dose) and $t = 0.25, 0.5, 1, 1.5, 2$ and 3 h after completion of a 30 min infusion, and on Day 3 at $t = 0$ h (pre-dose) and $t = 0.5$ and 2 h after infusion. For patients on a continuous dosing regimen, three samples (every 8 h) were drawn on both Day 1 and Day 3 of the study. A sample size of 30 patients, of which 20 patients (with a total of 200 samples) were on an intermittent dosing and 10 patients (60 samples) on a continuous dosing regimen, resulted in an accurate and precise estimation of the pharmacokinetic parameters (relative bias and imprecision <15%).

Study population

Patients admitted to the ICU who were treated with flucloxacillin IV therapy were eligible for inclusion if they were ≥18 years of age and if they were managed with a central venous or arterial catheter to facilitate blood sampling. Dose, duration and administration mode of flucloxacillin therapy were determined by the attending physician as a part of routine clinical care.

Data collection

Demographic, biochemical and microbiology data were collected from the medical charts of patients, including age, sex, total body weight, height, Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA), renal replacement therapy, indication for flucloxacillin use, identified pathogen and drug dose history. Biochemical data included serum albumin, creatinine, cystatin C and urine creatinine (collected from urine over a 24 h interval).

Bio-analysis of total and unbound flucloxacillin plasma concentrations

Total and unbound flucloxacillin plasma concentrations were analysed using a validated ultra-performance liquid chromatography coupled with tandem mass spectrometry (XEVO TQ-S, Waters). The unbound fraction of flucloxacillin was obtained by ultrafiltration at 37°C. The assay was validated in the range of 0.4–125 mg/L and 0.1–50 mg/L for total and unbound concentrations, respectively. The accuracy range was 100.5%–107.6% for total flucloxacillin concentrations and 99.8%–105.2% for unbound concentrations. Within-day precision varied between 2.7% and 5.4% for total flucloxacillin and between 1.9% and 7.1% for unbound flucloxacillin. Between-day precision varied between 0.6% and 1.3% for total flucloxacillin and between 0.8% and 2.9% for unbound flucloxacillin.

Pharmacokinetic analysis

Population pharmacokinetic analysis of flucloxacillin was performed with non-linear mixed-effects modelling using the software package NONMEM® (version 7.4.1). The first-order conditional estimation method, with interaction between random effects and residual variability, was used throughout model building. All clearance and volume parameters were allometrically scaled to a standardized fat-free mass of 58.2 kg, corresponding to a 1.80 m adult male of 70 kg, a priori, with allometric exponents of 0.75 and 1, respectively.[19] The inter- and intra-individual variability was assumed to be log-normally distributed. Parameter precision was calculated using the sampling importance resampling procedure as described previously.[20]

An integral pharmacokinetic model for total and unbound flucloxacillin pharmacokinetics was developed. The pharmacokinetic parameters were estimated from the unbound flucloxacillin concentrations. The relationship between unbound and bound flucloxacillin concentrations was described by the following equations, as described previously:[11]

$$C_{total} = C_{unbound} + C_{bound} \qquad (1)$$

$$C_{bound} = (C_{unbound} \times B_{max}) / (K_d + C_{unbound}) \qquad (2)$$

In Equations 1 and 2, C_{total} is the total flucloxacillin concentration, $C_{unbound}$ is the unbound flucloxacillin concentration, C_{bound} is the protein bound concentration, B_{max} is the maximum binding capacity and K_d is the equilibrium dissociation constant.

Continuous renal replacement therapy (CRRT) and various glomerular filtration estimation (eGFR) equations were tested as covariates for clearance: Modification of Diet in Renal Disease (MDRD),[21] Chronic Kidney Disease Epidemiology Collaboration based on serum creatinine (CKD-EPI$_{creat}$),[22] based on serum cystatin C (CKD-EPI$_{cysC}$),[10] and on both serum creatinine and serum cystatin C (CKD-EPI$_{creat-cysC}$),[10] the Cockcroft and Gault formula,[23] and 24 h urine creatinine clearance (CL$_{CR}$). Serum albumin, blood urea nitrogen and bilirubin were tested as covariates on B_{max}.

Structural model selection and covariate analysis were guided by physiological plausibility and objective function value (OFV). A decrease of >3.84 points in OFV, corresponding to a significance level of $P<0.05$ for nested models, was considered statistically significant in univariate testing.

Dose optimization studies

The final model was used to perform Monte Carlo simulations to predict the unbound flucloxacillin concentration. We simulated with anonymized data of 5000 ICU patients. Three different dosing regimens were predicted on Day 4 after start of therapy with 6, 8 and 12 g daily as continuous infusion. The unbound steady-state flucloxacillin concentration was predicted for different categories of CL_{CR}: <10, 10–30, 30–50, 50–90, 90–130, and >130 mL/min. The target MIC was based on the epidemiological cut-off value from the MIC distribution of cloxacillin for MSSA according to EUCAST: 0.5 mg/L.[24] The MIC distribution of flucloxacillin for MSSA is lacking, but is suggested to be similar to cloxacillin.[25] In addition, an MIC of 2 mg/L was evaluated.[24] A target flucloxacillin concentration of 100% $fT_{>MIC}$ and 100% $fT_{>5×MIC}$ was used.

An upper limit of 20 mg/L, which is 10× an MIC of 2 mg/L, was chosen.[17,18] A concentration of more than 20 mg/L is not likely to result in extra therapeutic value.

Results

Patient characteristics

Thirty-three patients were enrolled in the pharmacokinetic study (Table 1). Median age was 59 years old (range 30–83 years old). Median APACHE II score was 19 (range 7–42). A total of 85% (28 out of 33) of patients had a serum albumin <25 g/L. The majority of the patients was treated with flucloxacillin for bloodstream, respiratory or skin infections caused by *Staphylococcus aureus*, at doses ranging from 4 to 12 g daily. The dosing interval for patients receiving intermittent infusion was 4–6 h and the infusion duration was <60 min. A total of 238 total and 235 unbound flucloxacillin plasma concentrations were collected. The median observed unbound fraction was 18.9% (range 7.0%–71.7%). The unbound fraction increased when the unbound flucloxacillin concentration increased (Spearman's correlation r=0.85, P<0.001, Figure 1).

Flucloxacillin pharmacokinetics

A two-compartmental linear pharmacokinetic model fitted the data best. Separate proportional error models for both the unbound and total concentrations were used. Residual error correlation between unbound and total concentrations could not be estimated. Inter-individual variability could be identified for clearance, central volume of distribution and B_{max}. Intra-individual variability could be identified for clearance. Parameter estimates of the model are shown in Table 2.

In line with previous findings[4,11] and the observations in Figure 1, protein binding showed to be saturable. As flucloxacillin is predominantly bound to albumin, serum albumin was added as a linear covariate for B_{max} (Equation 3), in agreement with the observed inverse relationship in Figure 2:

$$B_{max} = \theta B_{max} + \text{serum albumin} \times \theta \text{alb} \qquad (3)$$

Introduction of serum albumin as a covariate for B_{max} decreased the objective function with 8 points. The inter-individual variability in B_{max} decreased from 28.2% to 25.5%.

Various equations for eGFR were tested as continuous covariates for renal clearance, assuming a linear relationship:

Table 1. Patient and clinical characteristics at baseline

	Evaluable patients (n = 33), median (range)
Demographics	
sex, *n*	
male	24
female	9
age (years)	59 (30–83)
total body weight (kg)	79 (53–135)
height (cm)	176 (159–192)
Clinical characteristics	
APACHE II score	19 (7–42)
SOFA score	9 (2–24)
creatinine (μmol/L)	106 (38–352)
cystatin C (mg/L)	1.46 (0.79–8.24)
24 h urine CL_{CR} (mL/min)	65 (0–273)
CRRT, *n*	7
albumin (g/L)	18 (8–30)
blood urea nitrogen (mmol/L)	12 (3–34)
total bilirubin (μmol/L)	5 (2–81)
administration mode, *n*	
intermittent infusion	23
continuous infusion	10
flucloxacillin daily dose, *n*	
4000 mg	4
6000 mg	10
8000 mg	1
9000 mg	1
12 000 mg	17
duration of flucloxacillin before start of study (days)	1 (0–26)
Microbiological characteristics	
indication, *n*	
bacteraemia	12
skin infection	6
pneumonia	6
endocarditis	5
other	4
Pathogen, *n*	
Staphylococcus aureus	21
other	3
unknown	9

$$CL = \theta CL_{non-renal} + eGFR \times \theta CL_{renal} \qquad (4)$$

The introduction of eGFR as a covariate for clearance of unbound flucloxacillin improved the model fit. Except for CKD-EPI$_{creat}$, all eGFR algorithms improved the model significantly. Although the relative differences in model fit for the different eGFR algorithms was marginal, the model using the 24 h urine CL_{CR} was chosen as the final model, because this equation resulted in the greatest reduction in objective function and explained most inter-individual variability in clearance as this variability decreased from 117% to 64.4%. Blood urea nitrogen and bilirubin could not be identified as
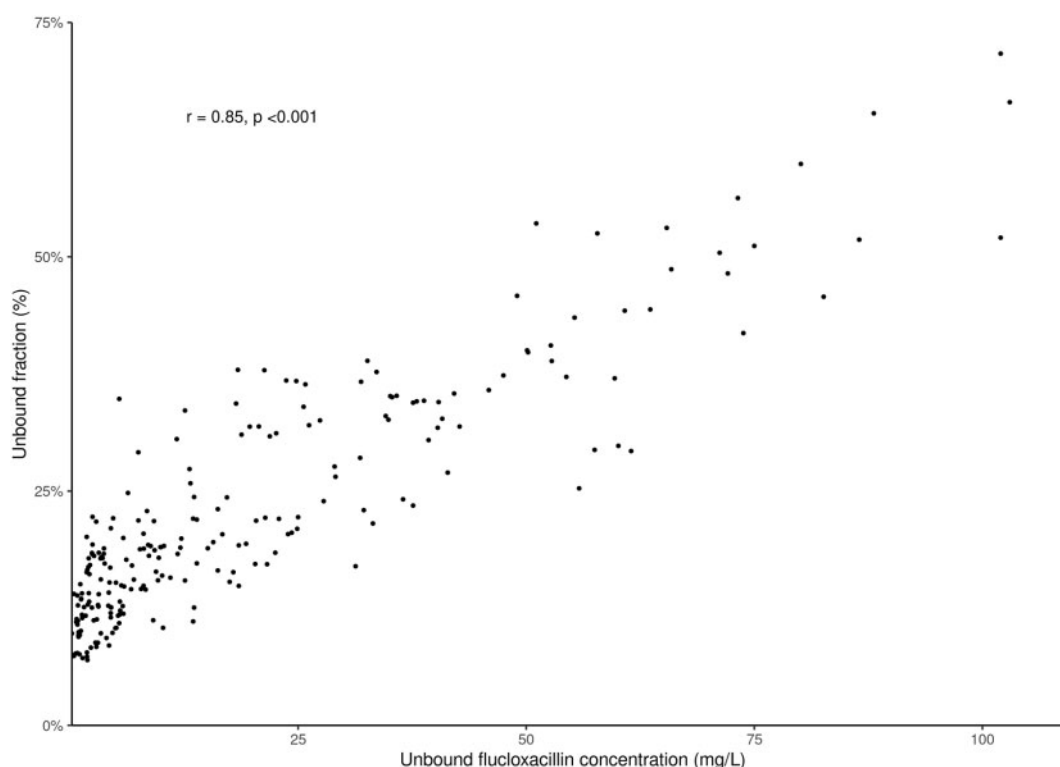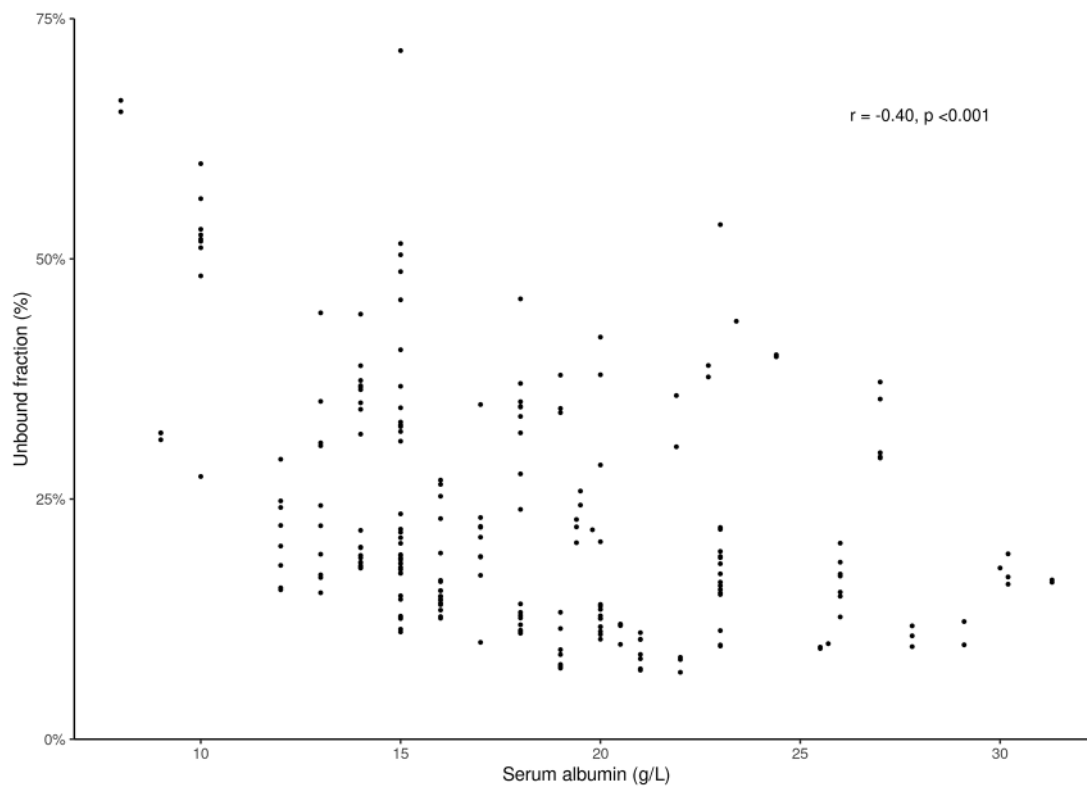
Figure 1. Unbound fraction flucloxacillin versus unbound flucloxacillin concentrations.

Table 2. Pharmacokinetic parameter estimates of the base and final model

	Parameter estimates (95% CI)						
	base model	MDRD	Cockroft–Gault	CKD-EPI$_{creat}$	CKD-EPI$_{cysC}$	CKD-EPI$_{creat-cysC}$	urinary CL$_{CR}$ (final model)
CL (L/h)	33.8 (24.9–45.2)						
θ CL$_{renal}$ (L/h/mL/min)	–	0.26 (0.12–0.42)	0.30 (0.13–0.48)	0.30 (0.13–0.47)	0.18 (0.03–0.43)	0.30 (0.10–0.47)	0.32 (0.20–0.46)
θ CL$_{non-renal}$ (L/h)	–	13.4 (5.45–24.7)	12.5 (3.38–24.3)	12.0 (3.25–24.8)	23.2 (11.7–35.9)	14.5 (6.32–28.3)	16.7 (11.7–23.5)
V_1 (L)	69.1 (45.6–101)	65.5 (47.6–89.5)	62.7 (43.6–82.5)	63.4 (46.3–83.5)	63.6 (45.8–88.9)	63.0 (46.7–90.6)	64.3 (47.8–92.9)
Q (L/h)	29.5 (21.0–38.0)	29.8 (21.1–41.4)	29.9 (20.1–42.7)	29.8 (19.9–40.4)	29.6 (20.5–39.4)	29.7 (20.8–39.1)	29.7 (21.2–40.2)
V_2 (L)	33.1 (24.5–46.0)	33.4 (24.8–47.5)	33.3 (23.9–46.2)	33.3 (23.9–46.7)	33.8 (25.0–46.7)	33.7 (24.5–46.9)	33.2 (25.1–44.7)
K_d (mg/L)	20.5 (15.3–28.0)	20.2 (15.0–26.5)	19.9 (14.9–27.1)	19.9 (15.2–27.1)	20.1 (15.0–27.7)	20.0 (15.2–26.9)	20.3 (15.1–28.3)
θ B$_{max}$ (mg/L)	74.2 (35.5–113)	74.5 (37.9–124)	74.1 (38.4–114)	74.2 (39.1–119)	74.1 (35.7–115)	74.4 (35.6–116)	72.8 (38.1–114)
θ albumin	3.44 (1.19–6.07)	3.34 (1.09–5.64)	3.30 (1.37–5.82)	3.29 (1.08–5.86)	3.35 (1.28–5.86)	3.31 (1.06–6.05)	3.47 (1.30–5.83)
Inter-individual variability (IIV)							
IIV$_{CL}$ (%)	117 (86.7–164)	77.9 (53.7–107)	80.8 (60.2–109)	79.4 (56.3–108)	105 (80.0–137)	89.1 (67.1–120)	64.4 (45.6–90.3)
IIV$_{V1}$ (%)	174 (97.5–328.4)	145 (86.4–272)	128 (83.5–214)	132 (82.2–216)	136 (88.8–218)	135 (87.4–216)	153 (95.5–256)
IIV$_{Bmax}$ (%)	25.8 (19.1–33.5)	25.4 (19.1–32.7)	25.1 (18.3–33.0)	25.2 (18.5–33.9)	25.4 (18.5–35.2)	25.3 (18.7–34.5)	25.5 (18.9–33.0)
Inter-occasion variability (IOV)							
IOV$_{CL}$ (%)	28.7 (14.9–47.7)	29.6 (13.8–47.9)	29.8 (16.8–49.4)	30.4 (16.5–50.6)	27.6 (12.6–46.9)	28.5 (13.9–47.9)	29.0 (15.0–49.4)
Residual error total (%)	20.4 (18.3–23.1)	20.6 (18.6–23.1)	20.8 (18.6–23.1)	20.7 (18.6–22.8)	20.7 (18.7–23.3)	20.7 (18.4–23.3)	20.5 (18.5–22.9)
Residual error unbound (%)	21.7 (19.4–24.6)	21.9 (19.7–24.3)	22.2 (19.9–24.8)	22.1 (19.8–25.0)	22.2 (19.9–25.1)	22.2 (19.9–25.1)	21.7 (19.4–24.6)
Difference in objective function	Reference	–12.38	–10.51	–9.75	–1.98	–6.77	–16.54

V_1, central volume of distribution; V_2, peripheral volume of distribution.

Figure 2. Unbound fraction flucloxacillin versus serum albumin.

covariates on B_{max}, nor could CRRT be identified as covariate on clearance.

The goodness-of-fit plots are depicted in Figure 3. The population and individual predicted concentrations are in concordance with the observed concentrations. Furthermore, the conditional weighted residuals indicate no model misspecification, as the distribution of these residuals was homogeneous and the majority of the data lie within the (-2 to $+2$) interval. The visual predictive checks for the final pharmacokinetic model are shown in Figure 4. The distribution of observed concentrations was consistent with the distribution of the predicted concentrations, suggesting a good internal validity of the model.

Dose optimization studies

The simulated unbound flucloxacillin concentrations for patients with a continuous dose of 6, 8 and 12 g/24 h are shown in Figure 5. The median unbound flucloxacillin concentration was 11.7 mg/L (IQR 7.3–19.0 mg/L) for patients receiving 6 g/24 h, 15.5 mg/L (IQR 9.8–25.4 mg/L) for patients receiving 8 g/24 h and 23.3 mg/L (IQR 14.6–38.0 mg/L) for patients receiving 12 g/24 h.

A dose of 6 g/24 h is sufficient in 100% of the population to reach a target of 0.5 mg/L, and in 98.5% of the population to reach a target of 2.5 mg/L. A concentration of 10 mg/L is reached in 88.1% of the population with a dose of 12 g/24 h. The proportion of patients with a CL_{CR} of >130 mL/min who reached a concentration level of >10 mg/L was 72.5%.

A total of 59.0% of the patients reached the upper limit of 20 mg/L unbound flucloxacillin with a dose of 12 g/24 h.

This increased to 91.5% for patients with a CL_{CR} less than 30 mL/min.

Discussion

In this study we describe the unbound and total flucloxacillin pharmacokinetics in critically ill patients. It contains the most richly sampled pharmacokinetic dataset of flucloxacillin in critically ill patients so far. The results of the current study relate to a heterogenous group of critically ill patients containing both patients with severe renal insufficiency and patients with augmented renal clearance. We found unbound flucloxacillin concentrations that were notably higher than previously reported in critically ill patients. Furthermore, we identified a large inter-individual variability of unbound flucloxacillin pharmacokinetics, and, to our knowledge, we are the first to identify intra-individual variability. Albumin and renal function only partly explained this variability. We showed that a dose of 6 g/24 h, given as a continuous infusion, is sufficient to reach an unbound flucloxacillin concentration of 0.5 mg/L in 100% of the critically ill population. When aiming for a higher target or in the case of infections caused by less susceptible pathogens, higher doses are required.

Unbound fractions of up to 72% were observed. In an earlier study in critically ill patients, unbound fractions up to 36.6% were reported.[4] Also, the unbound flucloxacillin concentrations were markedly high, ranging from 0.2 to 110 mg/L compared with a maximum of 30 mg/L reported in previous studies.[3,4] Our pharmacokinetic parameters estimates for volume of distribution were
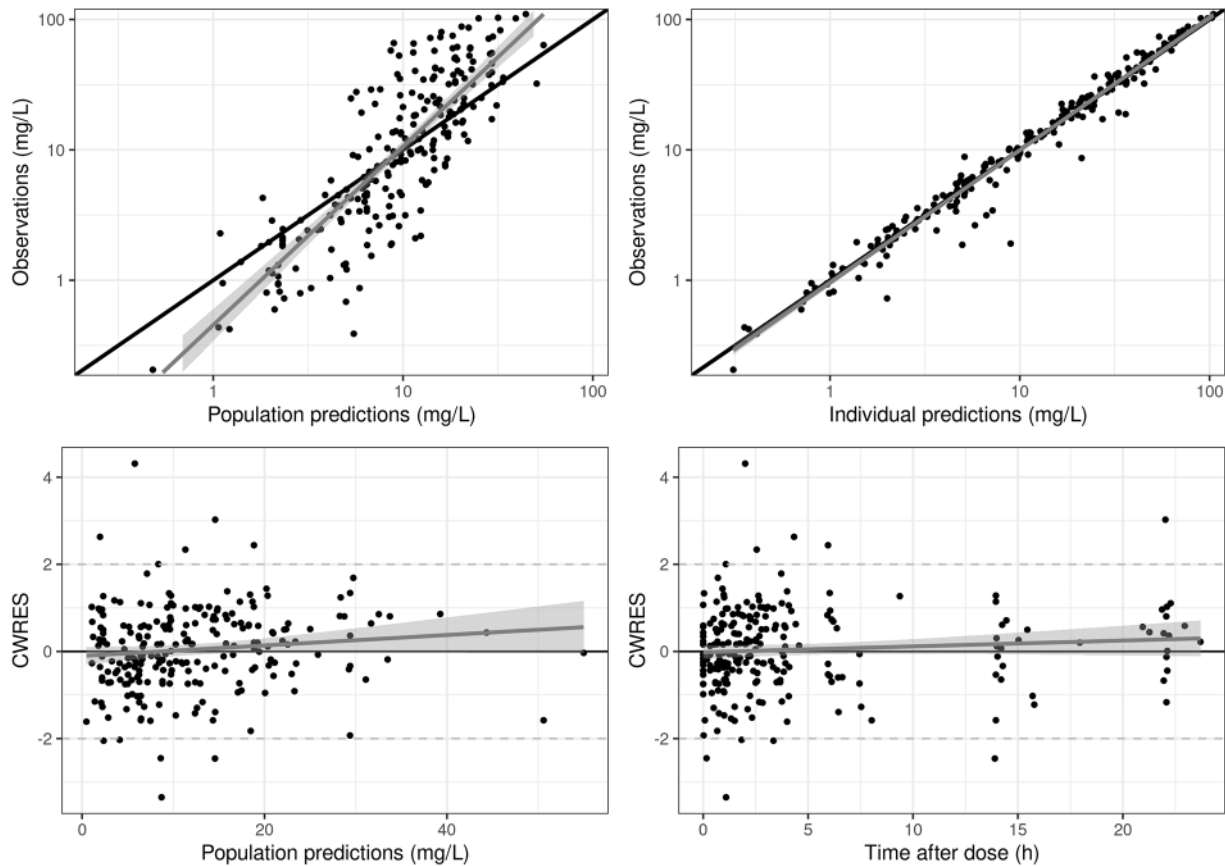
Figure 3. Goodness-of-fit diagnostics for the final pharmacokinetic model of unbound flucloxacillin in critically ill patients. The population and individual predicted concentrations are in concordance with the observed concentrations; the discrepancy between predictions and observations is small. Furthermore, the conditional weighted residuals indicate no model misspecification, the distribution is homogeneous and the majority of the data lie within the (-2 to $+2$) interval.

similar to those described before in critically ill patients. Yet, the estimate for flucloxacillin clearance was lower than previously reported.[3,4] We found a total clearance of 39.5 L/h (for a patient with a median CL_{CR} of 65 mL/min), versus a total clearance of 55.2 L/h and 97.4 L/h. This might be partially explained by the fact that more subjects in the current study had severe renal impairment. The estimate for B_{max} in the current study was lower than previously reported in non-critically ill and in critically ill patients,[4,11] which is probably caused by the lower albumin levels in our patients.

Renal function was a significant covariate for clearance of unbound flucloxacillin. CL_{CR} based on 24 h urine collection described the variability in clearance best. Our data do not support that cystatin C may be used to better predict flucloxacillin clearance, in contrast to what was described previously.[11] No statistically significant relationship between the presence of CRRT and flucloxacillin clearance could be identified. This observation, although based on a small sample size ($n = 7$), matches previous reports by Jager et al.[4] Theoretically, the high protein binding rate of flucloxacillin makes it more difficult to eliminate flucloxacillin by CRRT. Yet, adsorption of flucloxacillin to the polyamide membrane might occur, causing decreased flucloxacillin levels.[26] More research is necessary to evaluate the impact of CRRT on flucloxacillin clearance.

We found that protein binding of flucloxacillin is saturable in the therapeutic concentration range, which is in line with previous findings.[4,11,13] Furthermore, the unbound fraction increased with lower serum albumin. This is especially relevant for critically ill patients, with reported incidences of hypoalbuminaemia as high as 40%–50%.[27]

The absence of safety data is a limitation of this study. A total flucloxacillin concentration of 125 mg/L was previously associated with an increased risk of developing neurotoxicity.[7] In our study, a total of 18% had a total trough or steady-state concentration of more than 125 mg/L. Moreover, 59% of the simulated population is predicted to have an unbound concentration of more than 20 mg/L. Unfortunately, we did not collect data to confirm a relationship between concentration and toxicity. More research is necessary to establish unbound concentration targets for toxicity.

Another limitation of this study is that all patients had a low serum albumin level (≤ 30 g/L). Although hypoalbuminaemia is very common in critically ill patients, extrapolation of our results to patients with higher serum albumin concentrations should be done with caution.

Often, the use of β-lactam antibiotics in critically ill patients has been associated with subtherapeutic exposure, especially in case of Gram-negative infections.[28] We found that the majority of the
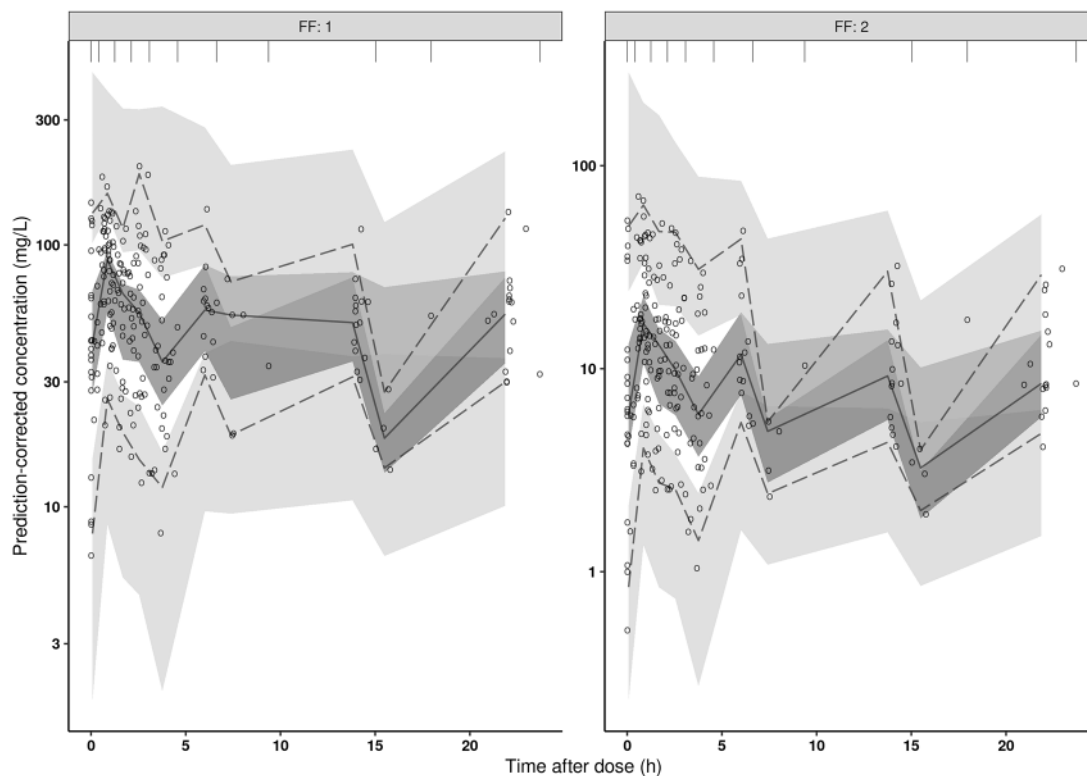
Figure 4. Visual predictive check (VPC) for the final pharmacokinetic model stratified for total (FF = 1) and unbound (FF = 2) concentrations. Prediction-corrected simulated (shaded) areas and observed (open circles) concentrations versus time after dose. The upper and lower lines connect the 5th and 95th percentiles of the observations. Light grey shaded areas are the 95% CIs of the 5th and 95th percentiles. Dark grey shaded areas indicate the CI of the median. The distribution of observed concentrations was consistent with the distribution of the predicted concentrations, suggesting a good internal validity of the model to the data.

population (99.9%) treated with flucloxacillin 12 g/day, reached a target of 100% $fT_{>5\times MIC}$ for infections caused by pathogens with an MIC of 0.5 mg/L. The risk of underexposure might be relevant for patients with augmented renal clearance (CL$_{CR}$ >130 mL/min), with reported incident rates of 20%–65% in ICU patients.[29] Therefore, it is important to identify patients with augmented renal clearance.

Therapeutic drug monitoring (TDM) of flucloxacillin in critically ill patients might be of potential value. We found a high inter-individual variability and a moderate intra-individual variability on clearance of 29%, which leaves sufficient room for TDM to be of value. The range of unbound concentrations could be largely reduced and could prevent out-of-bound exposures. TDM should be based on the unbound flucloxacillin concentration because the unbound concentration cannot be adequately estimated from the total concentration.[30] It must be noted that more information on an upper threshold associated between free drug concentration and neurotoxicity is urgently needed. Furthermore, TDM could assure optimal exposure for patients with augmented renal clearance and for those treated for infections caused by less susceptible pathogens.

We are of the opinion that the time is imminent to start an investigation into the benefits of TDM in the ICU populations.

If more knowledge is gained about the safety and tolerability of high flucloxacillin exposure, infections caused by pathogens with higher MICs might be treatable as well. Occasionally oxacillin MIC

values are high in *S. aureus* in absence of *mec*-gene-mediated resistance.[24,31] These strains have been called BORSA (borderline oxacillin-resistant *S. aureus*). Currently, BORSA strains with an MIC of >2 mg/L have been reported as resistant, but our data suggest that it might be possible to treat infections caused by strains with MICs up to 4 mg/L.
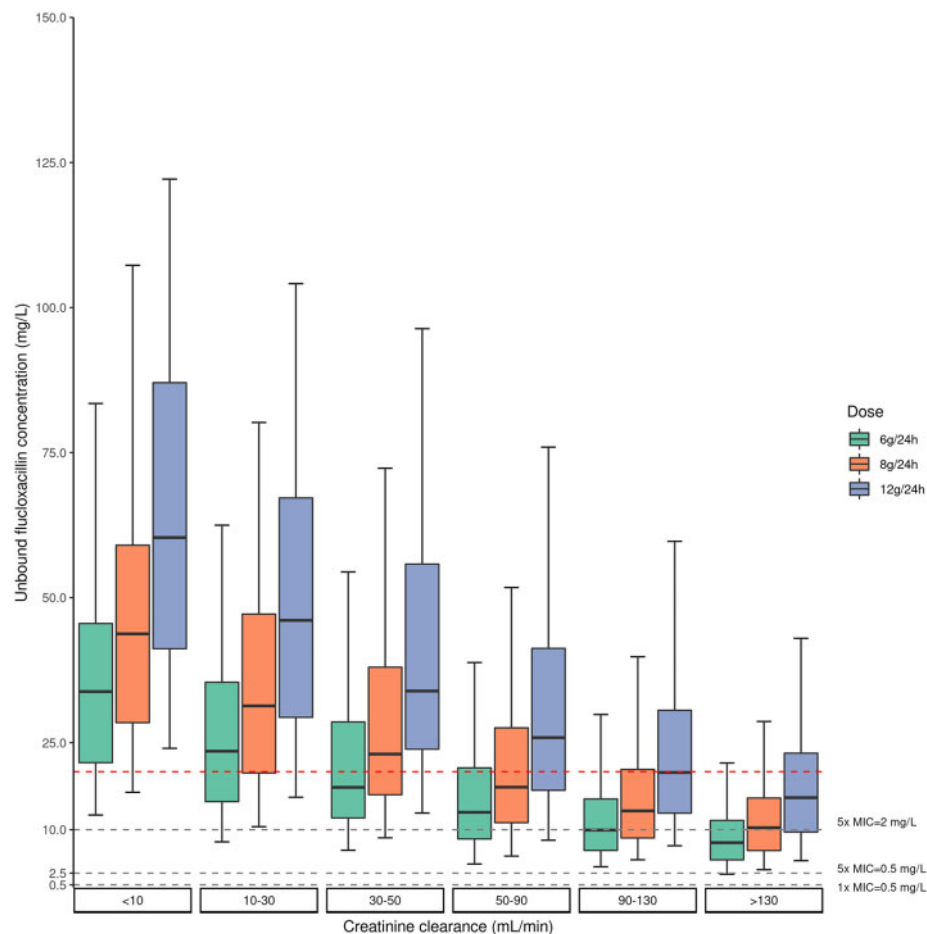
In conclusion, critically ill patients show higher unbound flucloxacillin fractions and concentrations than previously thought. Consequently, the risk of subtherapeutic exposure is low. If more information on the concentration–toxicity relationship is gained, TDM of the unbound flucloxacillin concentration could be of value.

Figure 5. Boxplots showing the simulated flucloxacillin unbound concentrations for ICU patients on dosing regimens of 6, 8 and 12 g/24 h at steady-state for different CL_{CR} categories. The boxplots present the 5th, 25th, median, 75th and 95th percentiles of data. The grey dashed horizontal lines at 0.5, 2.5 and 10 mg/L correspond to a target of 100% fT>MIC, assuming an MIC of 0.5 mg/L, and 100% fT>5×MIC, assuming an MIC of 0.5 and 2 mg/L, respectively. The red dashed horizontal line represents the upper limit of 20 mg/L. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Transparency declarations

None to declare.

Author contributions

E.W., R.t.H. and R.J.B. designed the study. T.F., D.W.d.L., H.v.L., J.A.S. and P.P. recruited patients for this study. E.W., R.t.H. and R.J.B. analysed the data. E.K. supervised the microbiology results. D.M.B., M.M.d.M., J.t.O. and E.M.G. participated in the writing of the article. All authors have read and approved the manuscript for publication.

References

1 Summary of product characteristics Floxapen®, poeder voor oplossing voor injectie 250 mg, 500 mg en 1 g. 2021. https://www.geneesmiddeleninforma tiebank.nl/smpc/h05990_smpc.pdf.

2 Rijnders MI, Deurenberg RH, Boumans ML *et al.* Flucloxacillin, still the empirical choice for putative *Staphylococcus aureus* infections in intensive care units in the Netherlands. *J Antimicrob Chemother* 2009; **64**: 1029–34.

3 Ulldemolins M, Roberts JA, Wallis SC *et al.* Flucloxacillin dosing in critically ill patients with hypoalbuminaemia: special emphasis on unbound pharmacokinetics. *J Antimicrob Chemother* 2010; **65**: 1771–8.

4 Jager NGL, van Hest RM, Xie J *et al.* Optimization of flucloxacillin dosing regimens in critically ill patients using population pharmacokinetic modelling of total and unbound concentrations. *J Antimicrob Chemother* 2020; **75**: 2641–9.

5 Roberts JA, Abdul-Aziz MH, Lipman J *et al.* Individualised antibiotic dosing for patients who are critically ill: challenges and potential solutions. *Lancet Infect Dis* 2014; **14**: 498–509.

6 Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *P T* 2015; **40**: 277–83.

7 Imani S, Buscher H, Marriott D *et al.* Too much of a good thing: a retrospective study of β-lactam concentration-toxicity relationships. *J Antimicrob Chemother* 2017; **72**: 2891–7.

8 Smith JM, Isoxazolyl penicillins: oxacillin, cloxacillin, dicloxacillin, and flucloxacillin. In: Grayson ML, ed. *Kucers' the Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic, and Antiviral Drugs*. CRC Press, 2017; 143–61.

9 Levey AS, Inker LA, Coresh J. GFR estimation: from physiology to public health. *Am J Kidney Dis* 2014; **63**: 820–34.

10 Inker LA, Schmid CH, Tighiouart H *et al*. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012; **367**: 20–9.

11 Wilkes S, van Berlo I, Ten Oever J *et al*. Population pharmacokinetic modelling of total and unbound flucloxacillin in non-critically ill patients to devise a rational continuous dosing regimen. *Int J Antimicrob Agents* 2019; **53**: 310–7.

12 Roder BL, Frimodt-Moller N, Espersen F *et al*. Dicloxacillin and flucloxacillin: pharmacokinetics, protein binding and serum bactericidal titers in healthy subjects after oral administration. *Infection* 1995; **23**: 107–12.

13 Wong G, Briscoe S, Adnan S *et al*. Protein binding of β-lactam antibiotics in critically ill patients: can we successfully predict unbound concentrations? *Antimicrob Agents Chemother* 2013; **57**: 6165–70.

14 Drusano GL. Antimicrobial pharmacodynamics: critical interactions of 'bug and drug'. *Nat Rev Microbiol* 2004; **2**: 289–300.

15 Delattre IK, Taccone FS, Jacobs F *et al*. Optimizing β-lactams treatment in critically-ill patients using pharmacokinetics/pharmacodynamics targets: are first conventional doses effective? *Expert Rev Anti Infect Ther* 2017; **15**: 677–88.

16 Abdul-Aziz MH, Alffenaar JC, Bassetti M *et al*. Antimicrobial therapeutic drug monitoring in critically ill adult patients: a Position Paper. *Intensive Care Med* 2020; **46**: 1127–53.

17 Wong G, Briscoe S, McWhinney B *et al*. Therapeutic drug monitoring of β-lactam antibiotics in the critically ill: direct measurement of unbound drug concentrations to achieve appropriate drug exposures. *J Antimicrob Chemother* 2018; **73**: 3087–94.

18 Moser S, Rehm S, Guertler N *et al*. Probability of pharmacological target attainment with flucloxacillin in *Staphylococcus aureus* bloodstream infection: a prospective cohort study of unbound plasma and individual MICs. *J Antimicrob Chemother* 2021; **76**: 1845–54.

19 Holford NHG, Anderson BJ. Allometric size: the scientific theory and extension to normal fat mass. *Eur J Pharm Sci* 2017; **109S**: S59–S64.

20 Dosne AG, Bergstrand M, Harling K *et al*. Improving the estimation of parameter uncertainty distributions in nonlinear mixed effects models using sampling importance resampling. *J Pharmacokinet Pharmacodyn* 2016; **43**: 583–96.

21 Levey AS, Bosch JP, Lewis JB *et al*. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461–70.

22 Levey AS, Stevens LA, Schmid CH *et al*. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604–12.

23 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16**: 31–41.

24 EUCAST. Data from the EUCAST MIC distribution website: European Committee on Antimicrobial Susceptibility Testing. http://www.eucast.org.

25 Sutherland R, Croydon EA, Rolinson GN. Flucloxacillin, a new isoxazolyl penicillin, compared with oxacillin, cloxacillin, and dicloxacillin. *Br Med J* 1970; **4**: 455–60.

26 Meyer B, Ahmed el Gendy S, Delle Karth G *et al*. How to calculate clearance of highly protein-bound drugs during continuous venovenous hemofiltration demonstrated with flucloxacillin. *Kidney Blood Press Res* 2003; **26**: 135–40.

27 Ulldemolins M, Roberts JA, Rello J *et al*. The effects of hypoalbuminaemia on optimizing antibacterial dosing in critically ill patients. *Clin Pharmacokinet* 2011; **50**: 99–110.

28 Roberts JA, Paul SK, Akova M *et al*. DALI: defining antibiotic levels in intensive care unit patients: are current β-lactam antibiotic doses sufficient for critically ill patients? *Clin Infect Dis* 2014; **58**: 1072–83.

29 Bilbao-Meseguer I, Rodriguez-Gascon A, Barrasa H *et al*. Augmented renal clearance in critically ill patients: a systematic review. *Clin Pharmacokinet* 2018; **57**: 1107–21.

30 Wallenburg E, Bruggemann RJM, Roberts JA *et al*. A meta-analysis of protein binding of flucloxacillin in healthy volunteers and hospitalized patients. *Clin Microbiol Infect* 2021; https://doi.org/10.1016/j.cmi.2021.06.039.

31 Hryniewicz MM, Garbacz K. Borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) - a more common problem than expected? *J Med Microbiol* 2017; **66**: 1367–73.