



Population pharmacokinetic modelling of total and unbound flucloxacillin in non-critically ill patients to devise a rational continuous dosing regimen

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ABSTRACT

Objective: This study's objective was to describe the population pharmacokinetics of total and unbound flucloxacillin in non-critically ill patients, and to devise a rational continuous dosing regimen for this population.

Methods: Total and unbound flucloxacillin pharmacokinetics in 30 non-critically ill patients receiving intravenous flucloxacillin were analysed using non-linear mixed-effects modelling. Monte Carlo simulation was used to assess the fraction of the population reaching effective unbound flucloxacillin levels and the fraction reaching potential neurotoxic exposure for various continuous dosing regimens.

Results: The observed protein binding varied between 64.6–97.1%. The unbound fraction was significantly associated with serum albumin and was concentration-dependent. The parameter estimates of the final model were: Cl_{total} 122 L/h, Cl_{renal} 1.41 L/h, V_c 190 L, V_p 33.9 L, Q 16.8 L/h, K_d 9.63 mg/L, θ_{Bmax} 177 mg/L, θ_{alb} 0.054. A continuous dose of 6 g/24 hours was sufficient for 100% of the population to obtain an unbound concentration of > 0.25 mg/L. With 14 g/24 h, 91.2% of the population was predicted to reach concentrations of > 2 mg/L, the clinical breakpoint for *Staphylococcus aureus*. Potential toxic unbound flucloxacillin levels were reached in 2.0% of the population with 6 g/24 h, and 24.1% with 14 g/24 h.

Conclusions: This study showed that a continuous infusion of 6 g/24 h flucloxacillin is sufficient to treat most infections in non-critically ill patients. With this dosing regimen, an unbound serum concentration flucloxacillin > 0.25 mg/L was reached in 100% of the patients, with minimal chance of neurotoxicity.

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1. Introduction

With antibiotic drug pipelines rapidly drying up [1], the available drugs need to be optimally used to retain an antimicrobial armamentarium [2]. In Europe, flucloxacillin is one of the most clinically important anti-staphylococcal penicillins. Flucloxacillin is widely used and recommended for penicillin-resistant methicillin-susceptible *Staphylococcus aureus* (MSSA) infections such as bacteraemia [3], infective endocarditis [4], skin and soft tissue infections [5].

Flucloxacillin has been approved as an intermittent dosing regimen of 1–2 g every 4–6 h [6]. However, continuous infusion of

the same daily dose is common practice. First, because of practical benefits: less frequent preparation and administration saves time, prevents errors, and enables outpatient antimicrobial treatment of infections that require high-dose parental therapy for a prolonged period. Second, continuous infusion is thought to be more effective, as the antibacterial activity of flucloxacillin, like all beta-lactam antibiotics, is associated with the time that the unbound concentration of flucloxacillin remains above the minimum inhibitory concentration (MIC) [$fT_{>MIC}$] at the site of action [7–9].

As flucloxacillin has a short elimination half-life of approximately 1 h, continuous, instead of intermittent, dosing will more likely result in bacterial killing [10]. However, since the approval of flucloxacillin over half a century ago, few studies have investigated flucloxacillin pharmacokinetics and the optimal continuous dose, and several knowledge gaps remain to be bridged.

First, although some pharmacokinetic studies on flucloxacillin have been previously performed, these were performed in healthy

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volunteers or critically ill patients [11–12]. It may be questioned whether these populations are representative enough to develop dosing regimens for non-critically ill patients, since they differ (e.g. volume of distribution). Moreover, flucloxacillin is assumed to be highly protein-bound, with an unbound fraction of approximately 4% [6]. However, previous studies on critically ill and neonates have suggested that flucloxacillin protein binding may be highly variable and dependent on serum albumin concentrations [13,14]. As only the unbound concentration is pharmacologically active, it is pivotal that in vivo flucloxacillin serum protein binding has been fully characterised when developing improved dosing regimens; thus far, this has not been performed.

Second, as flucloxacillin is partly excreted renally, renal function should be taken into account when developing improved dosing regimens [6]. In routine clinical practice, the glomerular filtration rate is usually estimated by measuring serum creatinine concentrations. As this endogenous marker is muscle mass-dependent, using other algorithms to estimate the glomerular filtration rate may improve dose individualisation [15]. For instance, serum cystatin C, a muscle mass-independent endogenous marker, is known to provide better estimations of glomerular filtration rate [16]. Serum cystatin C has not been previously used in pharmacokinetics studies about flucloxacillin.

Third, there is accumulating evidence that high exposure to flucloxacillin is associated with neurotoxicity [17–19], potentially due to its interaction with benzodiazepine receptors in the central nervous system at therapeutically relevant exposure [20]. Therefore, the therapeutic index of flucloxacillin may be smaller than previously thought and this underlines the necessity of dose-optimisation studies.

Therefore, the primary objective of the current study was to describe the unbound and total population pharmacokinetics of flucloxacillin in non-critically ill patients and to propose a rational and continuous dosing regimen, accounting for protein binding and renal function.

2. Materials and methods

2.1. General approach

The study first characterised the population pharmacokinetics of flucloxacillin in non-critically ill patients admitted to the hospital and who were treated with flucloxacillin as part of routine clinical care. Thereafter, a Monte Carlo simulation study was performed to investigate optimal continuous dosing regimens for flucloxacillin in this patient population.

2.2. Patients and ethics

The research was conducted in accordance with the Declaration of Helsinki, and national and institutional standards. This multicentre pharmacokinetic study was approved by the Ethical Committee of Zwolle Isala Clinics (16.06103 dz) and registered in the Dutch trial register (NTR 5934) [21].

Inclusion criteria were: minimum age of 18 years, receiving intravenous therapy with flucloxacillin, and admission to the hospital. Exclusion criteria were admission to the Intensive Care Unit and pregnancy. Written informed consent was obtained from all patients. Patients received intravenous flucloxacillin as indicated by their treating physician. Thirty patients admitted to the Deventer Hospital (Deventer, The Netherlands) or Radboud University Medical Center (Nijmegen, the Netherlands) from January to December 2017 were included.

2.3. Data collection

2.3.1. Pharmacokinetic sample size and sampling design justification

A stochastic simulation and estimation (SSE) study ($n=500$) based on the population pharmacokinetic model by Landersdorfer et al. [11] showed that a study with 30 patients, of which 10 were on a continuous dosing regimen and 20 were on an intermittent dosing regimen, would result in precise ($< 15\%$ relative standard deviation) and unbiased (bias $< 15\%$) estimation of the pharmacokinetic parameters in a sampling scheme. Sampling was performed on two occasions (interval ≥ 24 h) for each individual at $t=0$ h, $t=0.5$ h and $t=3$ h after dosing for patients on an intermittent dosing regimen, and at two random time points in patients on a continuous dosing regimen.

2.3.2. Additional data collection

Beside pharmacokinetic data collection, information about age, sex, weight, height, co-morbidities, indication for flucloxacillin therapy, pathogen, dose and duration of flucloxacillin IV therapy, concomitant medication, serum creatinine, blood urea nitrogen, serum cystatin C, serum albumin, serum triglycerides, C-reactive protein, complications from flucloxacillin therapy, and side effects from flucloxacillin were collected.

2.4. Bioanalysis of total and unbound flucloxacillin concentrations in serum

Blood samples were centrifuged (10 min 1500 g) immediately after collection. To obtain the unbound fraction, 500 μ L serum was immediately centrifuged for 10 min 1500 g at 25°C using a Millipore Centrifree® filter (Merck, Germany). The obtained serum and ultrafiltrate were stored at -80°C until further analysis. Serum proteins were precipitated using 400 μ L acetonitrile. Flucloxacillin concentrations were quantified using a high-pressure liquid chromatography method (Shimadzu, column: Pursuit XRS C18 10cm) with diode array detection. Potassium dihydrogen phosphate (pH 3.0)-acetonitrile (63:37) was used as mobile phase. Flucloxacillin and the internal standard dicloxacillin were detected at 220 nm.

The linearity of flucloxacillin calibrations curves was demonstrated from 6–175 mg/L (total flucloxacillin) and 0.25–4.5 mg/L (unbound flucloxacillin). The between-run accuracy ranged from 98.6–107.7%, variation coefficient (VC) 3.2–7.2% (total flucloxacillin) and 99.4–105.6%, VC 3.9–6.1% (unbound concentration).

The within-run accuracy ranged from 93.4–113.9%, VC 1.28–5.4% (total flucloxacillin) and 87.9–108.6%, VC 1.67–8.22% (unbound concentration). The method was validated in line with the most recent European Medicines Agency guideline on bioanalytical method validation [22].

2.5. Pharmacokinetic analysis

Population pharmacokinetics analysis of flucloxacillin was performed by means of non-linear mixed-effects modelling using the software package NONMEM V7.4.1. Pirana 2.9.4 was used as an interface for Nonmem, Perl Speaks Nonmem, Xpose and R [23]. The first-order conditional estimation (FOCE) method with interaction between random effects and residual variability, when applicable, was used throughout model building. An integrated pharmacokinetic model for total and unbound flucloxacillin pharmacokinetics was developed. The pharmacokinetic parameters were estimated from the unbound pharmacokinetics, and total concentrations were predicted by estimation of protein binding constants. Inter-individual variability was assumed to be log-normally distributed. Additive, proportional, and combined additive-proportional error models were tested to describe the residual error. Parameter uncertainty, presented as 95% confidence intervals (95% CI), was

calculated with the sampling importance resampling procedure, as recently described by Dosne et al. [24].

2.6. Covariate analysis

Covariate analysis was guided by physiological plausibility and statistical significance. Nested models were compared using the likelihood ratio test. A *P*-value of < 0.05, corresponding with a decrease in objective function of > 3.84 points, was considered statistically significant. Non-nested models were compared by computing the Akaike Information Criterion [25]. Because flucloxacillin is partly renally excreted, estimated renal function was tested as covariate on clearance [6]. For this purpose, the current study investigated the estimated glomerular filtration rate (eGFR) as calculated with equations for the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) based on serum creatinine (CKD-EPI creatinine) alone, cystatin C (CKD-EPI cystatin C) alone, and both serum creatinine and cystatin C (CKD-EPI creatinine and cystatin C)

As albumin, triglyceride and urea nitrogen are known to be able to impact protein binding for several drugs [12,26], these were tested as continuous covariates for protein binding, assuming a linear relationship. Spearman's correlation coefficient was used to describe the correlation for relevant continuous covariates.

2.7. Simulation study

With the final model, a Monte Carlo simulation study was performed to predict the unbound flucloxacillin concentrations at steady state in 1000 virtual patients for continuous dosing regimens of 1–14 g/day. Based on this simulation, the probability of target attainment (i.e. the percentage of the population with 100% $f_{T>MIC}$) for each dosing regimen was calculated. For this, the MIC distribution of cloxacillin for MSSA was used [27], since the MIC distribution of flucloxacillin for MSSA is lacking but is known to be similar to that of cloxacillin [28].

Furthermore, the probability of obtaining a toxic flucloxacillin concentration for the simulated dosing regimens was determined. A total through concentration of 125 mg/L is associated with a 50% risk of developing a neurotoxicity event [18]. Based on the simulations from the current study, a total serum flucloxacillin concentration of 125 mg/L corresponded with an unbound concentration of 7.5 mg/L in a typical patient with a serum albumin level of 35 g/L. As only unbound concentrations are pharmacologically active, an unbound flucloxacillin concentration of 7.5 mg/L was therefore used as cut-off for toxic concentrations when evaluating dosing regimens.

3. Results

A total of 30 patients were included. Clinical and demographic data are shown in Table 1. Two patients experienced side effect (phlebitis and temporary aspartate and alanine transaminase elevations) but these did not limit flucloxacillin therapy.

The observed protein binding varied between 64.6–97.1%. The unbound fraction was negatively correlated with serum albumin (Spearman's correlation $r = -0.649$, $P \leq 0.001$) and positively correlated with unbound concentration (Spearman's correlation $r = 0.676$, $P \leq 0.001$). The unbound fraction increased when serum albumin decreased and unbound concentration increased (Figs. 1 and 2). These findings suggest that albumin concentrations affect binding capacity and that protein binding is saturable in the therapeutic range of flucloxacillin concentrations.

3.1. Base model development

Initially, a population pharmacokinetic model was developed for the unbound flucloxacillin concentrations. It was found that

Table 1
Clinical and demographic patient data.

	Study population (n = 30) mean (range)
Gender	76.7 male (%) 23.3 female (%)
Age, years	64 (21–91)
Height, cm	177 (164–190)
Weight, kg	91.1 (63.1–129.0)
Creatinine, $\mu\text{mol/L}$	84 (48–140)
Cystatin C, mg/L	1.19 (0.64–2.53)
CKD CR CYS*, mL/min	91 (32–140)
Albumin, g/L	27.3 (15.7–35.0)
Triglycerides, mmol/L	5.7 (1.3–16.4)
Blood urea nitrogen, mmol/L	1.9 (0.6–3.4)
C-reactive protein day of sampling, mg/L	127 (11–401)
Indication	
- Cellulitis	11
- Prosthetic joint infection	7
- Surgical site infection	3
- Bursitis	2
- Septic arthritis	2
- Osteomyelitis	2
- <i>Staphylococcus aureus</i> bacteraemia	1
- Other	2
Pathogen	
- <i>Staphylococcus aureus</i>	12
- Other	6
- Culture negative	15
Dose (mg/24 h)	
- 4000	10
- 6000	18
- 12 000	2
Duration therapy, days	14.5 (3–56)

* CKD CR CYS: equation from the Chronic Kidney Disease Epidemiology Collaboration based on serum creatinine and cystatin C.

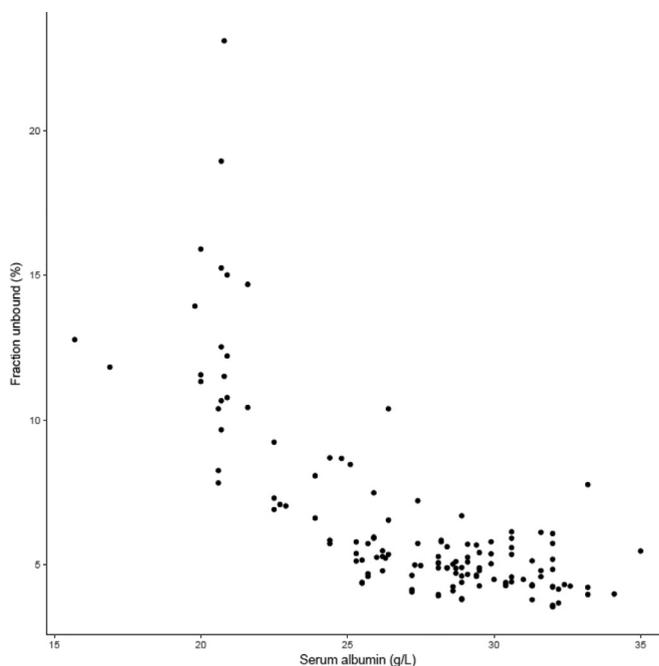
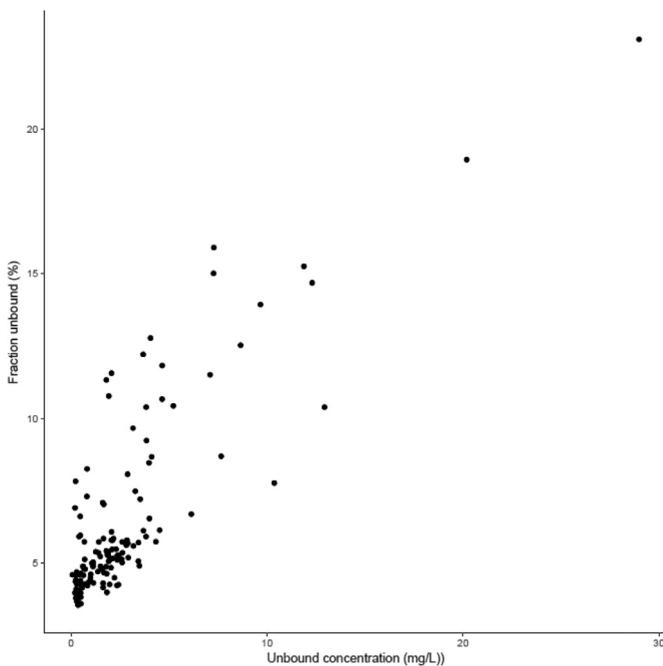


Fig. 1. Unbound fraction flucloxacillin vs. serum albumin.

a two-compartment linear model described the data well. Thereafter, the total concentrations were added to the dataset. Separate proportional error models with residual error correlation for both the unbound and total concentrations were used. Inter-individual variability could be identified for central volume of distribution and clearance (Table 2). Intra-individual variability could not be estimated.

Table 2
Parameter estimates.

	Parameter estimates (95% CI)			
	Base model	CKD-EPI Creatinine	CKD-EPI Cystatin C	CKD-EPI Creatinine and Cystatin C (final model)
Clearance				
$\theta_{\text{clearance}}$ (L/h)	116 (66.2–168)	110 (86.5–134)	130 (102–159)	122 (96.9–149)
θ_{renal}	–	1.42 (0.751–2.09)	1.31 (0.652–1.87)	1.41 (0.788–2.02)
V_c (L)	215 (115–371)	187 (110–273)	192 (109–284)	190 (113–277)
V_p (L)	90.8 (21.0–189)	32.7 (13.2–55.7)	34.2 (13.1–60.6)	33.9 (12.0–59.1)
Q (L/h)	6.07 (2.45–9.63)	16.2 (3.76–32.5)	17.5 (4.07–34.2)	16.8 (4.48–32.3)
K_d (mg/L)	10.2 (6.26–15.3)	9.86 (6.81–13.0)	9.51 (6.87–12.4)	9.63 (6.79–12.6)
$\theta_{B_{\text{max}}}$ (mg/L)	193 (127–277)	181 (136–229)	176 (134–220)	177 (135–224)
θ_{alb}	0.0505 (0.0347–0.0642)	0.0541 (0.0418–0.0650)	0.0541 (0.0422–0.0653)	0.0540 (0.0434–0.0654)
Inter-individual variability V_c (%)	81.4 (42.3–120)	93.5 (56.7–125)	96.3 (56.5–127)	94.7 (57.3–124)
Inter-individual variability CI (%)	74.5 (44.3–101)	55.2 (38.2–68.7)	54.9 (37.6–69.3)	54.3 (37.2–69.1)
Residual error unbound flucloxacillin (%)	46.4 (39.4–53.1)	45.1 (37.3–52.1)	45.4 (38.1–52.6)	45.1 (38.2–52.4)
Residual error total flucloxacillin (%)	42.9 (36.5–48.3)	41.1 (34.8–47.7)	42.0 (35.1–48.9)	41.4 (34.9–48.2)
Correlation	0.919	0.933	0.937	0.932
Difference in objective function	–	–19.75	–21.74	–22.80

**Fig. 2.** Unbound fraction flucloxacillin vs. unbound concentrations.

As protein binding showed to be non-linear, one site-specific binding was implemented to describe the relationship between free and bound flucloxacillin concentrations, assuming the following relationships:

$$C_{\text{tot}} = C_{\text{free}} + C_{\text{bound}} \quad (1)$$

$$C_{\text{bound}} = (C_{\text{free}} * B_{\text{max}}) / (K_d + C_{\text{free}}) \quad (2)$$

In these equations, C_{tot} is the total concentration, C_{free} is the unbound concentration, C_{bound} is the protein bound concentration, B_{max} is the maximum binding capacity, and K_d is the dissociation constant [29]. It was found that this relationship described the protein binding of flucloxacillin well. A second specific or non-specific binding site could not be identified. As flucloxacillin is mainly bound to albumin and since a clear inverse relationship between serum albumin concentrations could be observed, serum albumin was introduced as a linear covariate for B_{max} in the model, as described in Equation 3.

$$B_{\text{max}} = \theta_{B_{\text{max}}} * (1 + \theta_{\text{alb}} * (\text{ALB} - 25.6)) \quad (3)$$

In this equation, $\theta_{B_{\text{max}}}$ is the parameter describing the B_{max} for a typical individual with a serum albumin of 25.6 g/L, θ_{alb} is the parameter describing the gradient in which the B_{max} changes with serum albumin, and ALB is the observed serum albumin concentration. Introduction of serum albumin as a covariate for B_{max} decreased the objective function with > 30 points, corresponding with $P < 0.00001$, and explained approximately half of the observed inter-individual variability in B_{max} (decrease of 28.4% to 14.1%). The parameter estimates for $\theta_{B_{\text{max}}}$ and θ_{alb} are shown in Table 2 together with all model parameter estimates corresponding with the pharmacokinetic parameters for unbound flucloxacillin. A figure depicting the estimated relationship between serum albumin and total and unbound concentrations can be observed in Supplemental material S1. A schematic depiction of the pharmacokinetic model is shown in Fig. 3.

3.2. Covariate analysis

Various glomerular filtration estimation algorithms were tested as covariates for renal clearance as follows:

$$Cl = \theta_{\text{clearance}} + (\theta_{\text{renal}} * (\text{eGFR} - 90)) \quad (4)$$

In this equation, $\theta_{\text{clearance}}$ is the estimated parameter for clearance of unbound flucloxacillin (in L/h) for a typical individual with an estimated glomerular filtration rate of 90 mL/min, θ_{renal} is the for the dependency of clearance on renal function, and eGFR is the estimated glomerular filtration rate with one of the investigated algorithms. In the base model, θ_{renal} was therefore not estimated. It was found that including renal function as a covariate for clearance of unbound flucloxacillin, irrespective of eGFR algorithm, significantly improved model fit with a decrease in objective function of > 18 points, and partly explained inter-individual variability in clearance. Although the relative differences in model fit for the different CKD-EPI equations was marginal, the model using the CKD-EPI creatinine and cystatin C equation was chosen as the final model, as it is known that this equation is most accurate in estimating glomerular filtration rate and since this equation resulted in the greatest reduction in objective function and explained most inter-individual variability in clearance. In the base model, the typical clearance of unbound flucloxacillin was 116 L/h (95% CI 66.2–168 L/h) with an inter-individual variability of 74.5% (95% CI 44.3–101%). In the model with the CKD-EPI creatinine and cystatin C for estimation of the glomerular filtration rate, the typical clearance was estimated to be 122 L/h (95% CI 96.9–149 L/h) with an inter-individual variability of 54.3% (95% CI 37.2–69.1%). This study also tested blood urea nitrogen and serum triglycerides as continuous

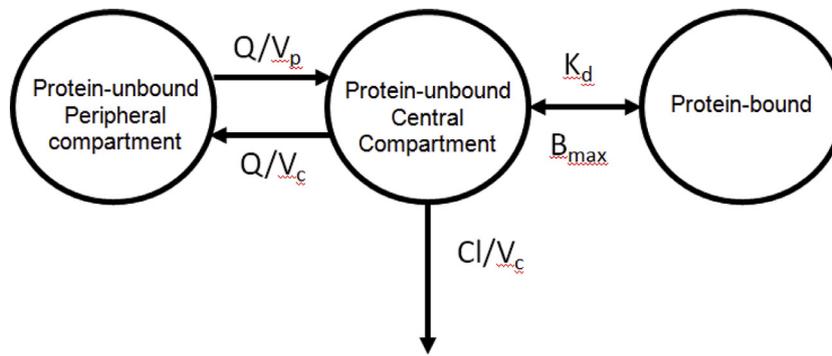


Fig. 3. Schematic depiction of the pharmacokinetic model.

V_c , volume of central compartment; V_p , volume of peripheral compartment; Cl , clearance; Q , intercompartmental clearance; K_d , dissociation constant; B_{max} , maximum binding capacity

covariates for the maximum binding capacity of albumin, assuming a linear relationship, but these did not improve model fit and did not explain inter-individual variability of B_{max} . The model parameter estimates of the base model and the models with different glomerular filtration rate estimation algorithms are shown in Table 2.

The goodness of fit plots are depicted in Fig. 4 A-F. As observed in the plots with observed vs. population and individual predicted concentrations, the data are uniformly scattered around the line of unity. The conditional weighted residual plots show that concentrations can be predicted without bias during a dosing interval and across the population-predicted concentration range, as all residuals are uniformly scattered around 0. Lastly, in the prediction-corrected VPCs, the observed 10th, 50th and 90th percentiles correspond well with the simulated data, showing the internal validity of this model.

3.3. Simulations

The results of the simulation are shown in Fig. 5. The bars demonstrate the MIC distribution of *Staphylococcus aureus* (*S. aureus*); 51.5% of the MSSA isolates had an MIC of 0.25 mg/L. With a continuous dose of 2 g/24 h, 94% of the population with an MIC of 0.25 mg/L had an unbound flucloxacillin concentration > 0.25 mg/L on steady state. With 4 g/24 h, 99.6% reached an unbound flucloxacillin concentration > 0.25 mg/L. To reach 100% target attainment for an MIC of 0.25 mg/L, a dose of 6 g/24 h is needed. A total of 88.9% of the population showed target attainment with a dose of 12 g/24 h for an MIC of 2 mg/L. With a dose of 14 g/24 h, levels > 2 mg/L were reached for 91.2% of the population. The clinical breakpoint of *S. aureus* for (flu)cloxacillin is 2 mg/L and distinguishes between MSSA and methicillin-resistant *S. aureus* (MRSA). Of note, 4.94% of the *S. aureus* isolates had an MIC of 2 mg/L (Fig. 5).

In the current simulation study, no patients had a potential toxic flucloxacillin unbound concentration of > 7.5 mg/L at a dose of 2 g/24 h (Fig. 6). Toxic levels at steady state were reached in 0.2% with a dose of 4 g/24 h, in 2% with 6 g/24 h, and this increased to 18.1% of the population with a dose of 12 g/24 h and 24.1% for 14 g/24 h.

4. Discussion

The present study shows that a continuous flucloxacillin dose of 6 g/24 h in non-critically ill patients is sufficient to reach therapeutic exposure in 100% of infections with pathogens with an MIC of \leq 0.25 mg/L, which is the most common MIC in the pathogen population. At this dose, the risk for toxic exposure is limited (2%).

It is believed that this is the first time that an optimal dose for continuous flucloxacillin administration has been proposed in the non-critically ill population.

This study had several interesting findings. First, it found that protein binding of flucloxacillin was saturable and that the maximum binding capacity depended on serum albumin concentrations. Although previous studies have reported variable and possibly serum albumin concentration-dependent protein binding in neonate and critically ill patients [13,14], in vivo protein binding had thus far not been fully characterised. Drug protein binding was adequately described with Michaelis-Menten kinetics in the current study. Second, it found that of all tested algorithms for calculations of glomerular filtration rate, the CKD-epi equation, incorporating both serum creatinine and cystatin C concentrations, best described variability in clearance. Nonetheless, it only explained a minor part of the inter-individual variability in clearance. The clinical implication of this finding may be that dose adjustments in renal dysfunction are likely not useful. It should be noted, however, that patients with severe renal dysfunction (eGFR < 30 mL/min) were not included in the current study. Extrapolation of the findings to this population should be performed with caution. Third, it showed that in a dose of 12 g/24 h, a dose that is commonly administered in routine practice, a significant part of the population (18.1%) is predicted to develop toxic exposure. Although one may argue that the cut-off for toxic exposure has not yet been fully characterised or validated, it is believed that the current analysis shows that due to high variability in clearance, unnecessary high exposure to flucloxacillin may develop with commonly used dosing regimens [18].

The clinical implication of the current study is that a dose of 6 g/24 h is adequate to treat most common infections with pathogens with an MIC of 0.25 mg/L. In clinical practice, a dosage of 6 g/24 h or 12 g/24 h depending on the indication is often used. In cases of less susceptible micro-organisms with an MIC of 2 mg/L, a dose > 12 g/24 h might be needed to reach 100% target attainment, since 88.9% of the population reached unbound serum levels \geq 2 mg/L with 12 g/24 h. Although it has to be said that 4.94% of the *S. aureus* isolates has an MIC of 2 mg/L. As there appears to be a clear therapeutic index, dictated by pathogen susceptibility and exposure-dependent toxicity, there may be a role for therapeutic drug monitoring to individualise therapy. In cases of insufficient treatment response or presumed toxicity, it is advisable to measure unbound flucloxacillin concentrations to adjust the dose based on the worst-case MIC and below the toxic threshold. Since at a dose of 12 g/24 h a significant proportion of patients is predicted to have a potentially toxic unbound serum level and the MICs of MSSA are well below this concentration, it seems that further tailoring the dose by means of therapeutic

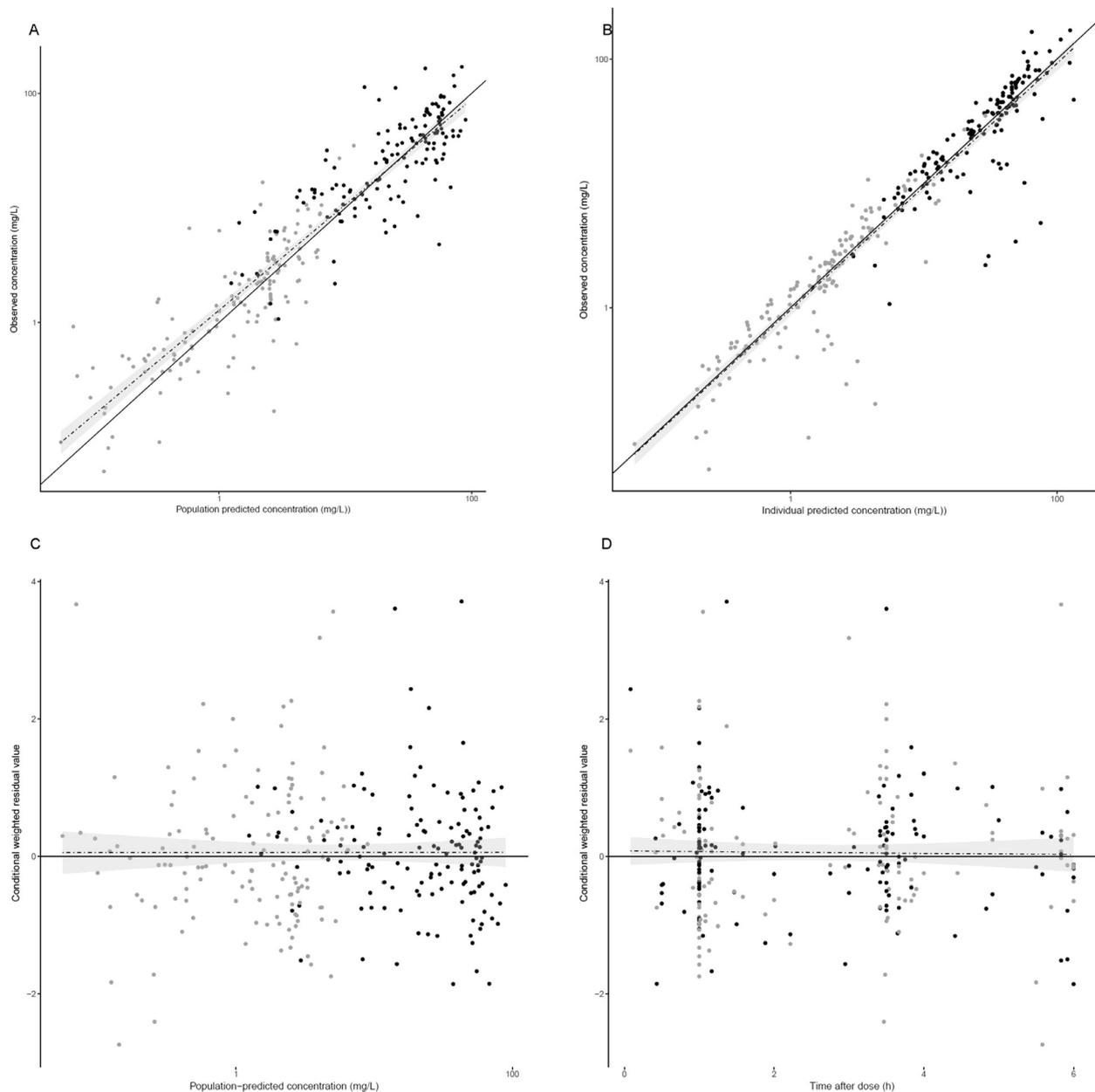


Fig. 4. Goodness of fit plots.

A: Observed vs. population predicted concentrations with x-axis and y-axis in log scale. The grey dots represent the unbound concentrations, the black dots represent the total concentrations. The solid black line is the line of unity. The dashed line with grey area shows the trend line with the 95% confidence interval. Both axes are logarithmically transformed.

B: Observed vs. individual predicted concentrations with x-axis and y-axis in log scale. The grey dots represent the unbound concentrations, the black dots represent the total concentrations. The solid black line is the line of unity. The dashed line with grey area shows the trend line with the 95% confidence interval. Both axes are logarithmically transformed.

C: Conditional weighted residuals vs. population predicted concentration, with x-axis on log scale. The grey dots represent the unbound concentrations, the black dots represent the total concentrations. The dashed line with grey area shows the trend line with the 95% confidence interval. The horizontal axis is logarithmically transformed.

D: Conditional weighted residuals vs. time after dose. The grey dots represent the unbound concentrations, the black dots represent the total concentrations. The dashed line with grey area shows the trend line with the 95% confidence interval.

E: Prediction-corrected visual predictive check of the unbound flucloxacillin concentrations, based on 1000 simulations. The dots represent the observed values, the grey areas correspond with the prediction interval of the 10th, 50th and 90th percentiles of the simulated data, and the dashed black line connects the observed 10th, 50th and 90th percentiles of the observed data.

F: Prediction-corrected visual predictive check of the total flucloxacillin concentrations, based on 1000 simulations. The dots represent the observed values, the grey areas correspond with the prediction interval of the 10th, 50th and 90th percentiles of the simulated data, and the dashed black line connects the observed 10th, 50th and 90th percentiles of the observed data.

drug monitoring may be of use to optimise therapy. If a bioanalytical method for measurement of unbound concentrations is unavailable, the unbound concentration may be calculated from the paired observation of serum albumin and total flucloxacillin concentrations, using the B_{max} and K_d from the current study.

For this purpose, the figure depicting the estimated relationship between serum albumin and total and unbound concentrations (supplemental material S1) can be used.

A limitation of this study is that it did not measure the unbound concentration at the site of infection. However, measuring

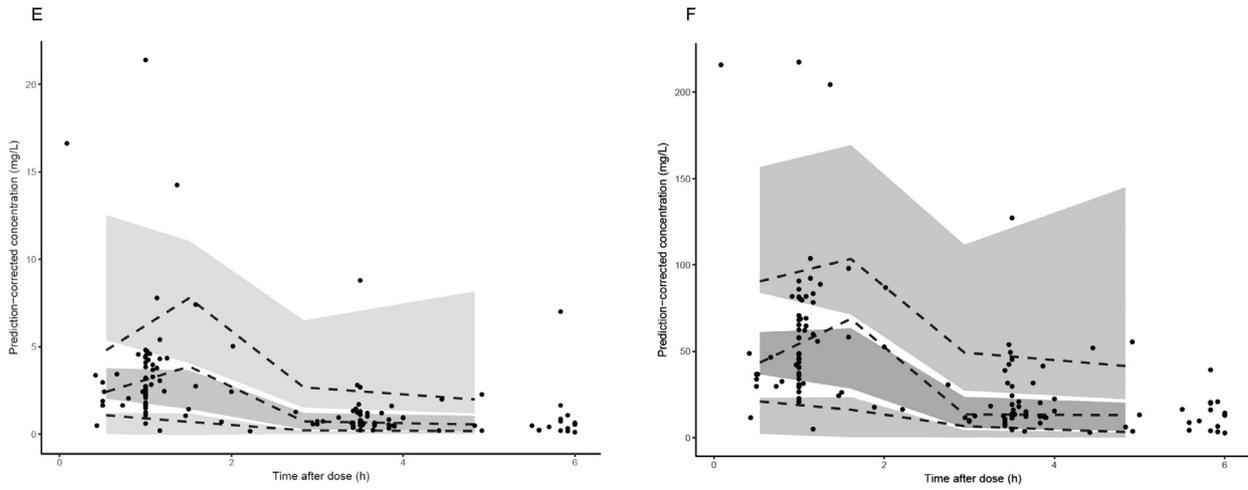


Fig. 4. Continued

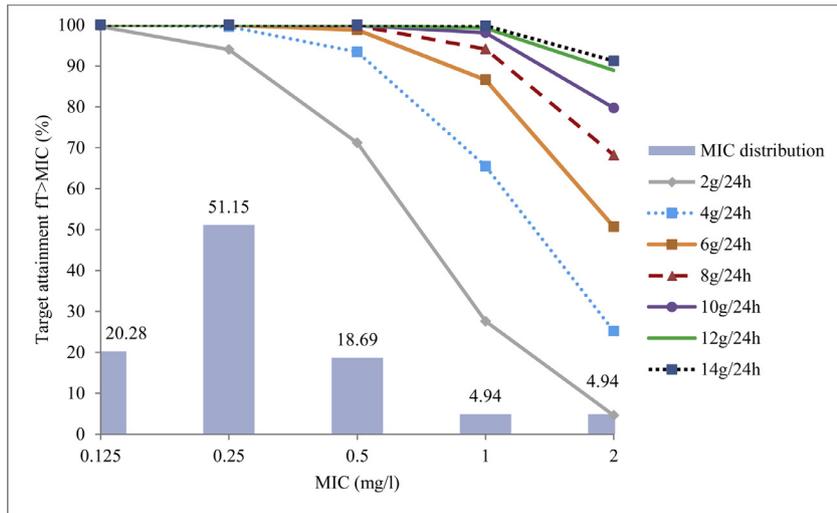


Fig. 5. Percentage target attainment for different doses and MICs at steady state. 100% target attainment means that 100% of the patients reached unbound flucloxacillin serum levels exceeding the MIC. The bars demonstrate the MIC distribution for *Staphylococcus aureus*. For example, 51.15% of MSSA isolates had an MIC of 0.25 mg/L [27].

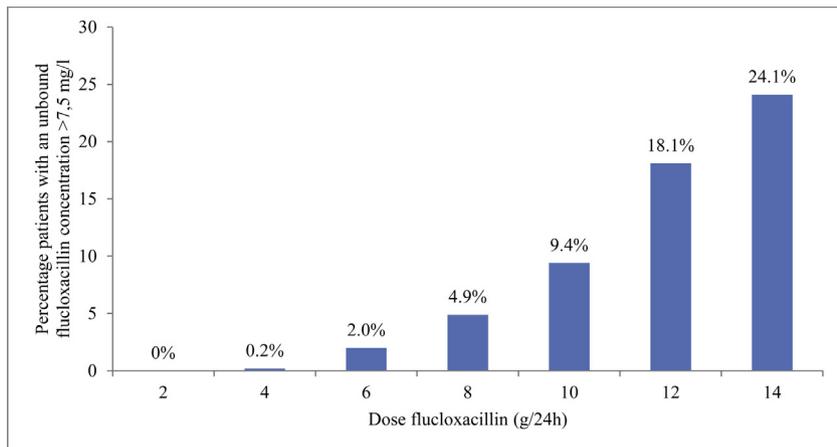


Fig. 6. Percentage unbound toxic flucloxacillin concentrations for different doses at steady state.

drug exposure at the site of infection was simply unfeasible. Since only unbound flucloxacillin can permeate in infected tissue, it is currently considered that the unbound concentration is the best proxy for tissue concentrations. Another limitation may be that the study was based on pharmacokinetic endpoints and that a prospective study based on clinical endpoints (e.g. survival or microbiological cure) was not been performed. Although a prospective study on clinical endpoints should often be considered the gold standard, this is difficult for many antimicrobial drugs, considering the heterogeneous patient populations and infection types. In these cases, pharmacokinetics may be a more sensitive endpoint than clinical endpoints. It is encouraging that the most recent EMA and Food and Drug Administration guidelines for clinical development of antimicrobial drugs underline the use of pharmacokinetic endpoints for dose development, when a pharmacodynamic target for clinical efficacy is known [30,31]. A prospective study to evaluate the dosing strategy in line with these recommendations is therefore warranted.

5. Conclusion

This study found that the optimal continuous dose for flucloxacillin is 6 g/24 h in non-critically ill patients, as the predicted unbound serum concentrations were > 0.25 mg/L for 100% of the population, with a minimal risk for toxic exposure predicted for 2% of the population for this dose.

Declarations

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Competing Interests

None.

Ethical Approval

The study was approved by the Ethical Committee of Zwolle Isala Clinics (16.06103 dz).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2018.11.018.

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