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Probability of pharmacological target attainment with flucloxacillin in *Staphylococcus aureus* bloodstream infection: a prospective cohort study of unbound plasma and individual MICs

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Objectives: MSSA bloodstream infections (BSIs) are associated with considerable mortality. Data regarding therapeutic drug monitoring (TDM) and pharmacological target attainment of the β-lactam flucloxacillin are scarce.

Patients and methods: We determined the achievement of pharmacokinetic/pharmacodynamic targets and its association with clinical outcome and potential toxicity in a prospective cohort of 50 patients with MSSA-BSI. Strain-specific MICs and unbound plasma flucloxacillin concentrations (at five different timepoints) were determined by broth microdilution and HPLC–MS, respectively.

Results: In our study population, 48% were critically ill and the 30 day mortality rate was 16%. The median flucloxacillin MIC was 0.125 mg/L. The median unbound trough concentration was 1.7 (IQR 0.4–9.3), 1.9 (IQR 0.4–6.2) and 1.0 (IQR 0.6–3.4) mg/L on study day 1, 3 and 7, respectively. Optimal (100% $fT_{>\mathrm{MIC}}$) and maximum (100% $fT_{>4\times\mathrm{MIC}}$) target attainment was achieved in 45 (90%) and 34 (68%) patients, respectively, throughout the study period. Conversely, when using the EUCAST epidemiological cut-off value instead of strain-specific MICs, target attainment was achieved in only 13 (26%) patients. The mean unbound flucloxacillin trough concentration per patient was associated with neurotoxicity (OR 1.12 per 1 mg/L increase, $P=0.02$) and significantly higher in deceased patients (median 14.8 versus 1.7 mg/L, $P=0.01$).

Conclusions: Flucloxacillin pharmacological target attainment in MSSA-BSI patients is frequently achieved when unbound flucloxacillin concentrations and strain-specific MICs are considered. However, currently recommended dosing regimens may expose patients to excessive flucloxacillin concentrations, potentially resulting in drug-related organ damage.

Introduction

MSSA bloodstream infections (BSIs) are associated with a high mortality rate, ranging between 18% and 30%.[1] Appropriate antibiotic therapy, identification of infectious foci and consultation of an infectious diseases specialist are the cornerstones in the management of MSSA-BSI.[2] The β-lactam flucloxacillin is a recommended antimicrobial agent for its treatment.[3,4] Approximately 95% of the drug is bound to serum proteins. Hence, the total flucloxacillin plasma concentrations poorly reflect the unbound concentrations.[5] The extent of plasma protein binding is highly relevant, because the unbound fraction of the drug is responsible for its pharmacological effect.[6] The time that the unbound drug concentration remains above the MIC for the microorganisms ($fT_{>\mathrm{MIC}}$) is recognized as the pharmacological parameter that best

correlates with antimicrobial activity and the outcome of β-lactam antibiotic treatment. Clinical outcome may be improved if concentrations of flucloxacillin are maintained above the MIC for the entire dosing interval ($\geq$100% $fT_{>MIC}$ or even $\geq$100% $fT_{>4\times MIC}$).[7,8] However, data on target attainment of unbound flucloxacillin in plasma are scarce. Simulation studies based on a small number of patients with MSSA-BSI have demonstrated flucloxacillin underdosing[9,10] and high variability of the unbound flucloxacillin fraction, particularly in critically ill patients.[5,11] In these studies, the MSSA oxacillin standard epidemiological cut-off value (ECOFF; 2 mg/L) and not the strain-specific flucloxacillin MIC value was used to calculate target attainment. Considering these reports, current data on flucloxacillin dose recommendations may derive from imprecise flucloxacillin plasma concentrations and exaggerated MSSA MICs. The aim of this study was to determine the probability of optimal pharmacological target attainment ($\geq$100% $fT_{>MIC}$) by prospectively measuring (i) total and unbound flucloxacillin plasma concentrations and (ii) MSSA strain-specific flucloxacillin MICs in patients with MSSA-BSI.

Patients and methods

This prospective observational cohort study was conducted at a 750 bed tertiary care hospital between January 2018 and December 2019. It was approved by the Ethics Committee of Northwest and Central Switzerland (EKNZ Project-ID: 2017-02072) and in accordance with the Declaration of Helsinki. All patients provided written informed consent for participation in the study.

Patient characteristics and management

All adult patients $\geq$18 years with at least one MSSA-positive blood culture result and who received flucloxacillin treatment were screened for eligibility. Exclusion criteria were age <18 years, previous inclusion in the study within the last 30 days, haemodialysis treatment, pregnancy, outpatient treatment, polymicrobial BSI, and termination of flucloxacillin treatment within 24 h or discharge within the next 48 h after eligibility screening. Demographic, clinical and laboratory data were prospectively collected. Plasma albumin and creatinine concentrations [including estimated glomerular filtration rate (eGFR) calculated by the Chronic Kidney Disease Epidemiology Collaboration study equation] and flucloxacillin concentrations were determined from the same blood sample. Flucloxacillin (2 g) was administered as a standard intermittent bolus infusion over 30 min, every 6 h (standard dose for MSSA-BSI) or every 4 h (in case of suspected or confirmed infective endocarditis or CNS infection). The dosage was 1 g every 6 to 8 h in the presence of an eGFR of less than 10 mL/min/1.73 m^2.

MSSA identification and MIC determination

MALDI-TOF MS-based analysis of colonies grown on subcultures of positive blood cultures (bioMérieux BacT/ALERT® FA/FN Plus system) was used for the identification of *Staphylococcus aureus*. Susceptibility testing was performed with the VITEK2 system (bioMérieux). Oxacillin MICs for MSSA were determined by MIC test strips (Liofilchem Diagnostici). Flucloxacillin MICs were determined by microdilution with CAMHB. *S. aureus* strains were considered susceptible to oxacillin and flucloxacillin if the MIC was $\leq$2 mg/L (according to EUCAST). In case of missing flucloxacillin MIC values (*n* = 13), an MIC of 0.25 mg/L was defined as the reference, because this was the highest of all measured flucloxacillin MICs in our strain population.

Plasma sampling and drug assay

Plasma samples were drawn on study day 1 (flucloxacillin mid-dose and trough concentration), 3 (mid-dose and trough) and 7 (trough) (Figure S1, available as Supplementary data at *JAC* Online). Samples were centrifuged, aliquotted and stored at $-80°$C immediately after blood collection and subsequently analysed in batches. Total and unbound plasma concentrations of flucloxacillin were measured with a validated HPLC–MS method using isotope dilution.[12] Free plasma concentrations were determined using ultracentrifugation prior to analysis. Sample preparation consisted of manual protein precipitation and an online turbo flow extraction (TLX) step. After chromatography, the analyte and the deuterated internal standard were separated and fragmented by positive electrospray ionization and three major fragments were detected. The lower limit of quantification was 1.0 mg/L for the measurement of total flucloxacillin and 0.05 mg/L for unbound flucloxacillin. The assay was linear in the range of 1–60.0 mg/L for total flucloxacillin and 0.05–20 mg/L for unbound flucloxacillin. The intra- and inter-assay precision experiments over the entire concentration range resulted in a coefficient of variation of <8.9% for total flucloxacillin and <5.6% for unbound flucloxacillin.

Outcomes

The primary outcome measure was defined as the proportion of optimal target attainment (100% $fT_{>MIC}$) in blood plasma at all measured timepoints. Target attainment was defined as a measured trough plasma concentration of flucloxacillin above the MIC measured by microdilution. In addition, target attainment was determined by the EUCAST ECOFF for oxacillin of 2 mg/L.

Secondary outcome measures included the proportion of pharmacological target attainment for the minimum ($\geq$50% $fT_{>MIC}$) and maximum (100% $fT_{>4\times MIC}$) targets in plasma. Secondary outcome target attainment was defined as a measured mid-dose and trough plasma concentration of flucloxacillin above the MIC and above at least 4$\times$ the MSSA MIC at all measured timepoints, respectively.

Additional outcome measures included: (i) intra-individual variability of flucloxacillin plasma concentrations and the unbound fraction; (ii) association of the following parameters with target attainment (100% $fT_{>MIC}$): age, Charlson comorbidity score, renal function, total daily dosage, requirement of dialysis/vasopressors/ventilation, serum albumin and inflammatory protein levels, and Pitt bacteraemia and SOFA scores; and (iii) association of 100% $fT_{>MIC}$ or the patient-individual mean unbound flucloxacillin trough concentrations with clinical outcome (30 day mortality rate).

Association of flucloxacillin plasma concentrations and toxicity

The toxicity of flucloxacillin was evaluated in a post-hoc analysis.[13] An unbound trough flucloxacillin concentration of more than 10 times the ECOFF value (i.e. >20 mg/L) was defined as the threshold for a potentially toxic drug concentration.[14] The renal toxicity of flucloxacillin was evaluated using the acute kidney injury (AKI) index[15] and hepatic toxicity was evaluated using drug-induced liver injury criteria.[16] To determine neurotoxicity, we assessed multiple variables.[17] These included the need for intubation, alterations in the Glasgow Coma Scale or nSOFA, electroencephalograms and signs or symptoms associated with drug-associated encephalopathy (e.g. seizures, delirium, hallucinations, confusion, focal deficits and myoclonia). Deterioration in scores or impairment in neurological function for any of these parameters was defined as potential drug-associated neurotoxicity. The association of flucloxacillin concentrations and toxicity was evaluated starting on the first day of flucloxacillin treatment and ending 5 days beyond the last dose.

Statistical analysis

We estimated that 50 cases would be necessary to obtain reasonably robust predictions to achieve the primary outcome and include up to three covariates in the analyses.

The Mann–Whitney *U*-test, Student's *t*-test, the χ^2 test, Fisher's exact test and the Spearman correlation coefficient test were used where appropriate. A mixed-effect model with the restricted maximum likelihood method was used to analyse the intra-individual variability of unbound flucloxacillin trough concentrations and the unbound fraction.

Multivariable stepwise logistic regression models that included potentially confounding variables with a univariate *P* value of less than 0.1 were performed to analyse associations between patient variables and target attainment (using EUCAST ECOFF), toxicity or outcome.

We considered statistical significance to occur if the two-sided *P* value was less than 0.05. All analyses were performed with the use of SPSS Version 26 (IBM SPSS Statistics for Windows. Armonk, NY, USA) and Prism Version 8 (GraphPad Software, San Diego, CA, USA).

Results

Patients and clinical characteristics

Between January 2018 and December 2019, 118 patients were screened for eligibility and 50 were included in the study (Figure S2). The median time from the first positive blood culture result to administration of flucloxacillin was 2 (IQR 1–3) days and from the first positive result to inclusion of the patient (study visit 1) was 4 (IQR 3–6) days.

Table 1 summarizes the baseline patient characteristics. Bone and joint infections were the most common sources of BSI (*n* = 16;

Table 1. Characteristics and outcome of 50 patients with a BSI caused by MSSA

Variable	*n* (%) or median (IQR)	
Female	13 (26)	
Age (years)	64.7 (49.9–76.8)	
BMI (kg/m^2)	25.7 (22–29.5)	
Length of stay (days)	26 (16–50.5)	
Combination antibiotic treatment for >3 days[a]	4 (8)	
Comorbidities		
diabetes mellitus	12 (24)	
liver cirrhosis	4 (8)	
chronic renal disease	13 (26)	
cardiovascular disease	20 (40)	
chronic lung disease	5 (10)	
malignancies	6 (12)	
IV drug use	9 (18)	
Charlson comorbidity score	3 (1.5-6)	
Disease severity	at BSI onset	at study inclusion
Pitt bacteraemia score	1 (0–2)	1 (0–1.5)
SOFA score	3 (2–5.5)	3 (1.5–6)
Laboratory results	at BSI onset	at study inclusion
C-reactive protein (mg/L)	179 (45–281)	112 (69–171)
leucocytes (10^6/L)	11 (8–15)	10 (7–14)
creatinine (µmol/L)	89 (63–153)	82 (58–122)
eGFR (mL/min/1.73 m^2)	79 (37–104)	81 (47–111)
albumin (g/L)	28 (23–34)	21 (18–26)
Focus of BSI		
osteomyelitis or arthritis	16 (32)	
endocarditis	11 (22)	
skin and soft tissue	10 (20)	
catheter or foreign material	4 (8)	
respiratory tract	1 (2)	
primary origin	8 (16)	
Severity and outcome		
vasoactive treatment	6 (12)	
ICU admission	24 (48)	
30 day mortality rate	8 (16)	

[a]Rifampicin and gentamicin in three patients for confirmed prosthetic valve endocarditis and daptomycin in one patient.
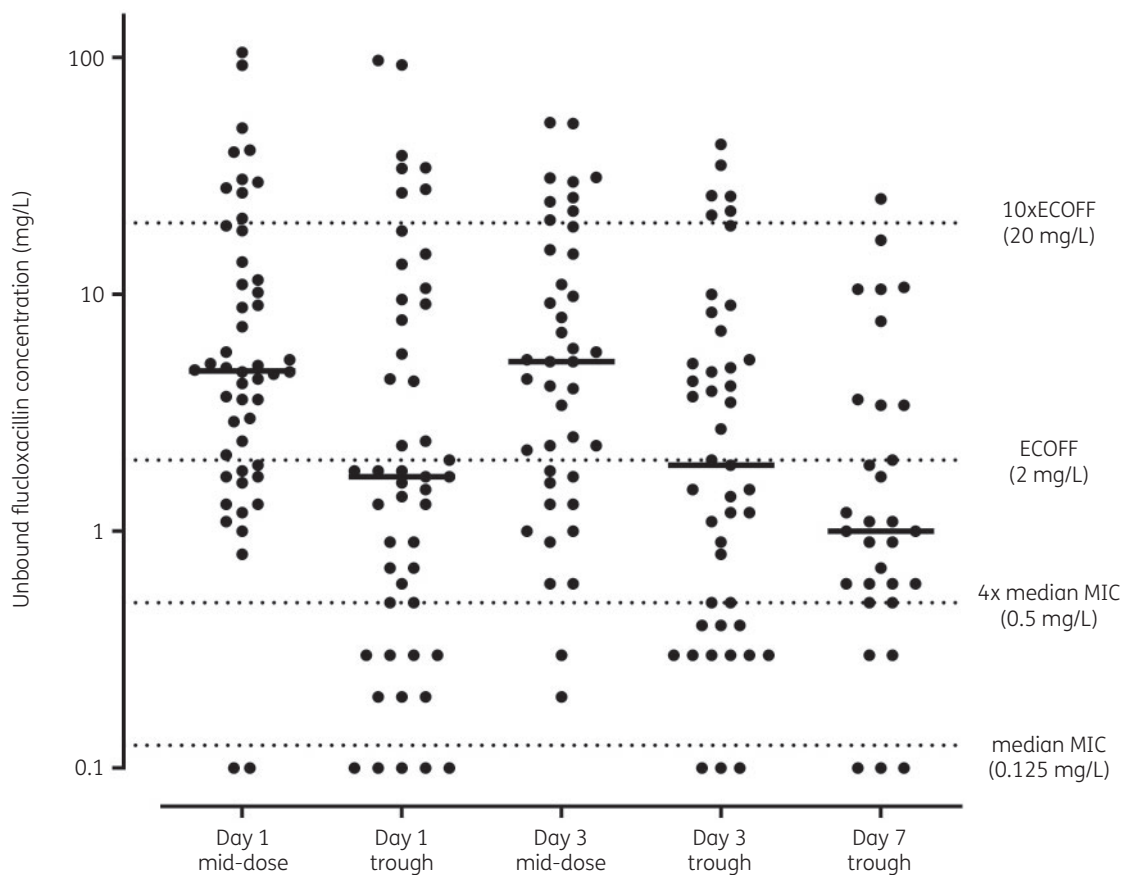
Figure 1. Unbound flucloxacillin concentration during the 7 day study period. The black solid lines depict the median flucloxacillin concentration at the respective timepoints. The dotted lines depict the median measured flucloxacillin MIC or ECOFF.

32%), followed by infective endocarditis ($n = 11$, 22%) and skin and soft tissue infections ($n = 10$; 20%). The median length of hospital stay was 26 (IQR 16.0–50.5) days. Eight (16%) patients died within 30 days after the onset of BSI.

Forty-six (92%) patients received a β-lactam antibiotic as empirical treatment (most frequently amoxicillin/clavulanic acid). In 34 (68%) patients, flucloxacillin treatment was initiated at 12 g IV per day, and 16 (32%) patients received lower dosages.

Pharmacological data

The median mid-dose unbound flucloxacillin concentration was 4.8 (IQR 1.9–14.9) mg/L on study day 1 and 5.2 (IQR 1.7–16.4) mg/L on study day 3. The median unbound trough concentration was 1.7 (IQR 0.4–9.3), 1.9 (IQR 0.4–6.2) and 1.0 (IQR 0.6–3.4) mg/L on study day 1, 3 and 7, respectively (Figure 1). The median unbound flucloxacillin fraction ranged from 7.3% (trough on study day 7) to 13.5% (mid-dose on study day 1), with a minimum of 1.1% and a maximum of 64.7% [median of all values 11.0% (IQR 7.1–20.3)]. A significant correlation was observed between the unbound fraction and serum albumin levels ($r = -0.67$), serum creatinine levels ($r = 0.47$), eGFR ($r = -0.4$), total flucloxacillin concentration ($r = 0.49$), C-reactive protein ($r = 0.41$), platelet count ($r = -0.45$) and disease severity (SOFA score, $r = 0.49$; and Pitt bacteraemia score, $r = 0.49$)

(all *P* values <0.005) (Figure 2). Results of the multivariable regression model are presented in Table S1.

Variations of intra-individual trough unbound flucloxacillin concentrations were small, despite the fact that flucloxacillin total daily dosages were changed in 11/50 (22%) patients ($P > 0.05$) during the 7 day treatment period. Although significant, intra-individual variation in the unbound flucloxacillin fraction over time was modest, including all mid-dose and trough levels ($P = 0.02$). The median intra-individual coefficient of variation was 27%, with a range from 0% to 69% (Figure 3). There was no significant correlation between the intra-individual coefficient of variation and age, disease severity, mean eGFR and mean serum albumin levels (data not shown).

Unbound flucloxacillin plasma concentrations and the unbound fraction were higher in critically ill patients than in those who were non-critically ill [median unbound flucloxacillin trough concentration 4.2 (IQR 0.9–23.6) versus 1.1 (IQR 0.3–10.9) mg/L, respectively, $P = 0.008$; and median unbound flucloxacillin fraction (mid-dose and trough) 16.0 (IQR 9.7–30.4) versus 8.0 (IQR 6.7–12.8) mg/L, respectively, $P = 0.001$]. In contrast, there was no difference in intra-individual variability (data not shown).

Microbiological data

MICs of flucloxacillin and oxacillin were determined for 37 (74%) and 44 (88%) bacterial isolates, respectively. The median MIC of
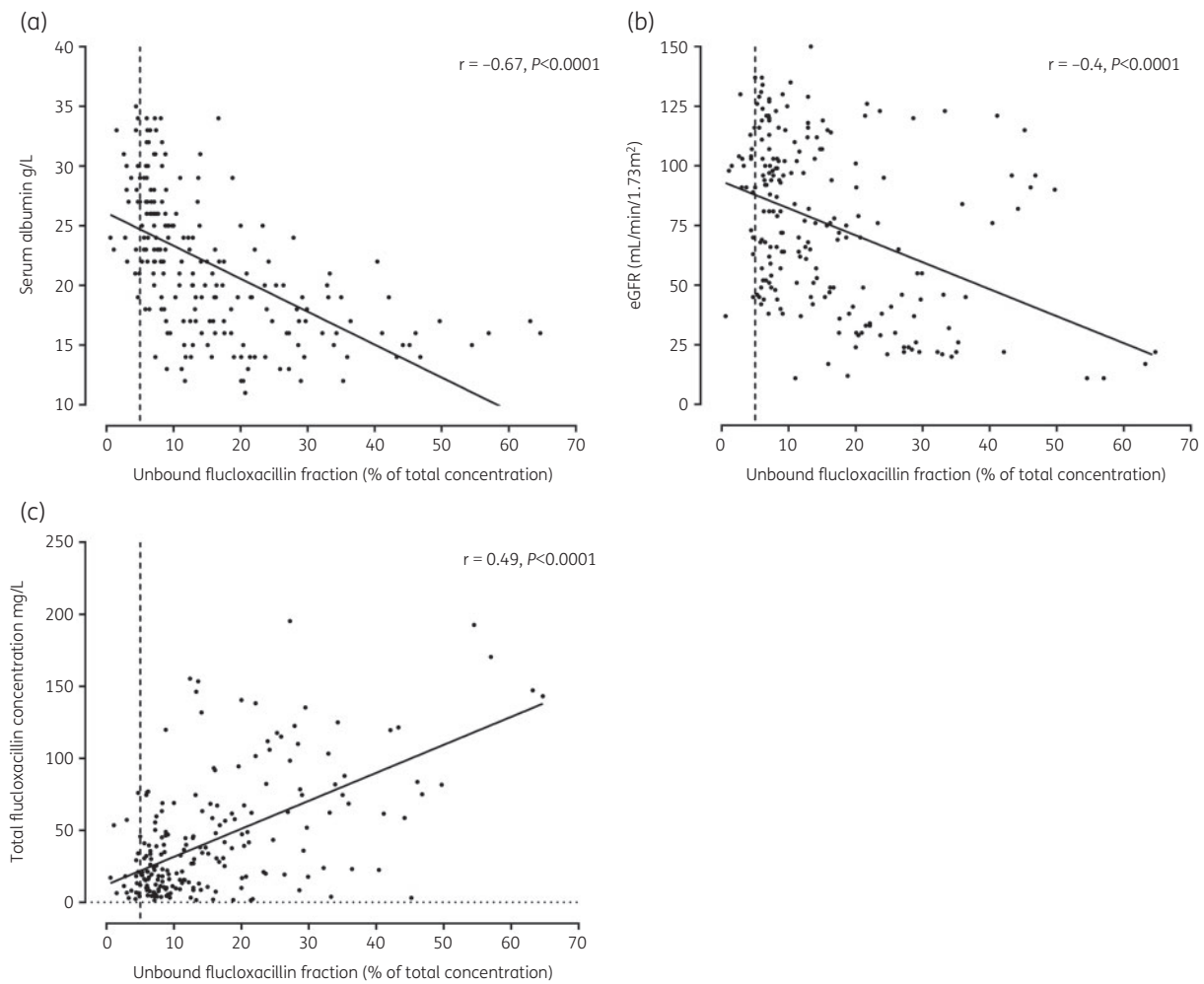
Figure 2. Correlation of (a) serum albumin, (b) eGFR and (c) total flucloxacillin concentration with unbound flucloxacillin fraction. The dashed line represents the published unbound flucloxacillin fraction (5%) in healthy volunteers.
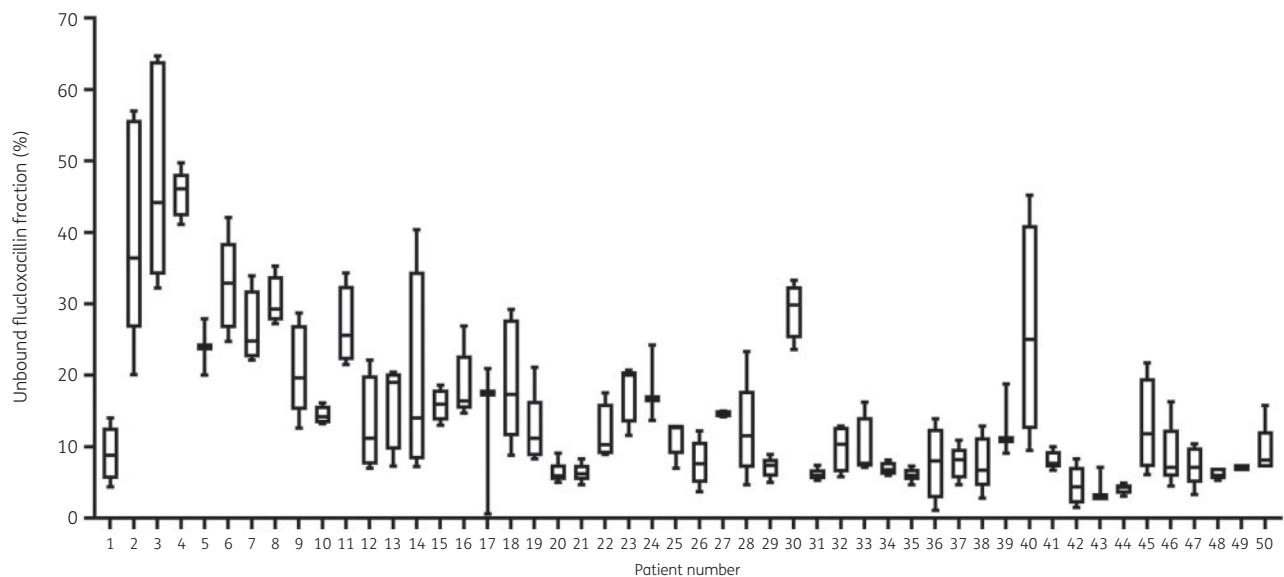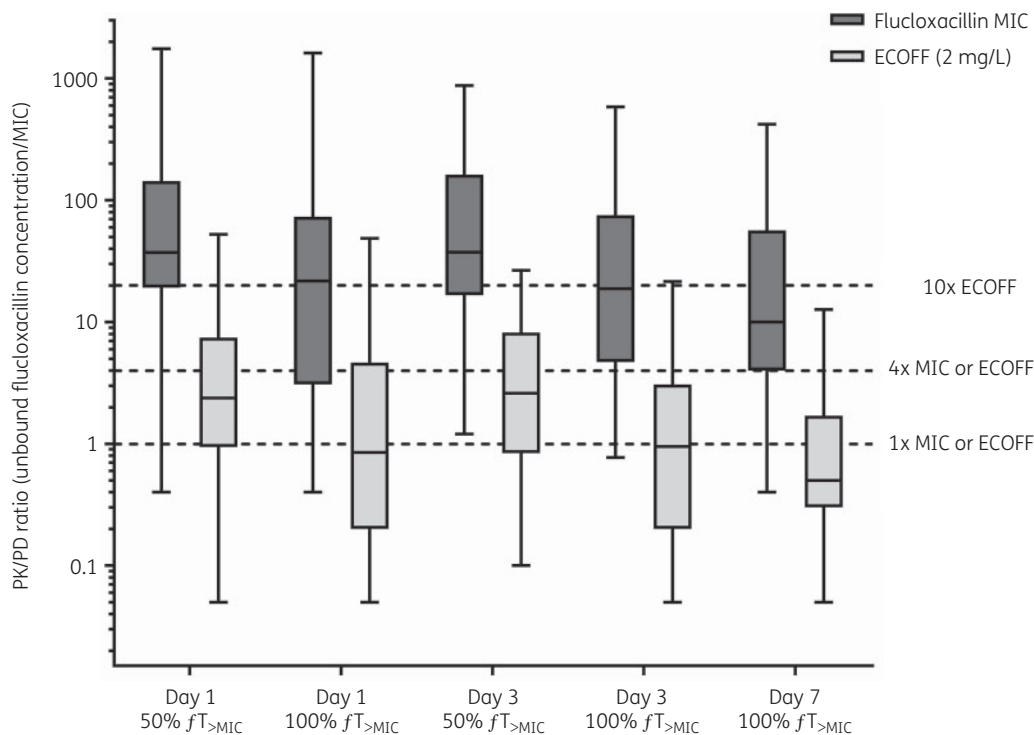

Figure 3. Individual patient unbound flucloxacillin fraction (%), including all mid-dose and trough measurements during the 7 day study period.

Table 2. Target attainment at different timepoints and during the entire period (cumulative)

	Study day 1		Study day 3		Study day 7		Cumulative	
	flucloxacillin MIC	ECOFF	flucloxacillin MIC	ECOFF	flucloxacillin MIC	ECOFF	flucloxacillin MIC	ECOFF
≥50% $fT_{>MIC}$	96%	74%	100%	71.4%			96%	64%
≥50% $fT_{>4\times MIC}$	94%	36%	90.5%	38.1%			90%	30%
≥100% $fT_{>MIC}$	91.8%	40.8%	97.8%	48.9%	93.5%	32.3%	90%	26%
≥100% $fT_{>4\times MIC}$	71.4%	26.5%	82.2%	22.2%	83.9%	16.1%	68%	14%

% $fT_{>n\times MIC}$, percentage of dosing period in which the unbound concentration of flucloxacillin is *n* times above the MIC.

Figure 4. Pharmacokinetic/pharmacodynamic (PK/PD) ratios [unbound flucloxacillin concentrations divided by individual flucloxacillin MIC or EUCAST ECOFF (2 mg/L)] at 50% and 100% of the dosing interval during the 7 day study period.

flucloxacillin was 0.06 (IQR 0.06–0.1) mg/L and it was 0.125 (IQR 0.06–0.25) mg/L when a value of 0.25 mg/L was assigned for missing MICs for worst-case scenario calculations. The median MIC of oxacillin was 0.38 (IQR 0.3–0.5) mg/L.

Pharmacological target attainment

The primary target (100% $fT_{>MIC}$) was attained in 45 patients (90%) at all timepoints (Table 2). Thirty-four patients (68%) achieved the maximum target (100% $fT_{>4\times MIC}$). Overall, unbound trough flucloxacillin plasma concentrations were on average 15 times the strain-specific MIC. Flucloxacillin trough concentrations were above the EUCAST ECOFF (100% $fT_{>ECOFF}$) in 13 (26%) patients at all timepoints and when using 50% $fT_{>ECOFF}$ only in 32 (64%) patients (Table 2 and Figure 4). Critically ill patients were more likely to achieve pharmacological targets than were non-critically ill patients (100% versus 81%, respectively, for 100% $fT_{>MIC}$, P = 0.05;

83% versus 54%, respectively, for 100% $fT_{>4\times MIC}$, P = 0.04; and 38% versus 15%, respectively, for 100% $fT_{>ECOFF}$, P = 0.1).

Logistic regression analyses with a worst-case scenario (MIC = ECOFF) identified disease severity (as measured by the Pitt bacteraemia score on the day of the first study visit) and renal function as independent predictors of optimal target achievement (100% $fT_{>ECOFF}$) at all timepoints (Table 3).

Clinical endpoints

Target attainment (100% $fT_{>MIC}$) was not associated with length of stay or 30 day mortality rate. However, the 30 day mortality rate was higher when using 100% $fT_{>ECOFF}$ as the target value [5/13 (39%) versus 3/37 (8%), P = 0.02] in univariate analysis. Along the same lines, the mean unbound flucloxacillin trough concentration over the study period was significantly higher in deceased than in surviving patients [median 14.8 (IQR 1.2–31.8) versus 1.7

Table 3. Predictors (measured at BSI onset) of cumulative flucloxacillin target attainment of 100% $fT_{>ECOFF}$ (worst-case scenario) during the study period; univariate and multivariate analysis

| Predictor	Univariate OR (95% CI)	P	Multivariate OR (95% CI)	P
Age (years)	1.03 (0.99–1.07)	0.1		
Female	1.38 (0.34–5.59)	0.7		
BMI (kg/m^2)	0.99 (0.86–1.15)	0.9		
eGFR (mL/min/1.73 m^2)	0.96 (0.94–0.98)	0.002	0.95 (0.92–0.99)	0.006
SOFA score	1.63 (1.20–2.22)	0.002		
Pitt bacteraemia score	2.38 (1.22–4.65)	0.01	3.6 (1.2–11.0)	0.03
Charlson comorbidity score	1.03 (0.82–1.30)	0.8		
Daily flucloxacillin dose 12 g versus <12 g IV	8.2 (0.96–69.75)	0.06		
ICU admission	3.3 (0.86–12.71)	0.08		

(IQR 0.6–5.1) mg/L, respectively, $P = 0.01$]. After adjusting for age and the Pitt bacteraemia score on admission, these associations were not significant. The OR was 1.6 (95% CI 0.2–12.8) for 100% $fT_{>ECOFF}$ ($P = 0.6$) and 1.4 (95% CI 0.96–1.13) for the mean unbound flucloxacillin trough concentration ($P = 0.3$).

Flucloxacillin toxicity

The unbound trough concentration of flucloxacillin exceeded the predefined threshold for potential drug toxicity (20 mg/L) in 9 patients [18%; the majority (89%) were critically ill patients] at least once during their hospital stay (median 34.2 mg/L, IQR 27–68). When using a threshold of more than 10 times the strain-specific MIC, the number of patients fulfilling the toxicity threshold increased to 33 patients (60% of them critically ill). Excessive unbound flucloxacillin concentrations (100% $fT_{>10\times ECOFF}$) were more frequently observed in critically ill than in non-critically ill patients [8/24 (33%) versus 1/26 (4%), respectively, $P = 0.01$] and associated with 30 day mortality [4/9 (44%) versus 4/41 (10%), respectively, $P = 0.03$].

AKI and neurotoxicity, but not acute liver injury, occurred significantly more frequently in patients with 100% $fT_{>10\times ECOFF}$ than in patients who had lower flucloxacillin plasma concentrations [AKI 8/9 (89%) versus 14/41 (34%), respectively, $P = 0.007$; neurotoxicity 7/9 (78%) versus 9/41 (22%), respectively, $P = 0.003$; and acute liver injury 2/9 (22%) versus 2/41 (5%), respectively, $P = 0.1$]. The median creatinine peak concentration was 304 (IQR 210–514) μmol/L in these patients versus 89 (IQR 70–139) μmol/L in those without trough concentrations above this threshold ($P < 0.001$). However, when we considered the plasma creatinine level at the start of flucloxacillin treatment as the baseline for the calculation of AKI, the association of excessive unbound flucloxacillin concentration and development of AKI was not significant [3/9 (33%) versus 9/41 (22%), $P = 0.7$].

Increased mean unbound flucloxacillin trough concentrations of individual patients during the study period were associated with potential neurotoxicity ($P < 0.0001$), but not acute liver injury and AKI (Figure 5), in univariate analysis. They remained an independent predictor of neurotoxicity (OR 1.12 per 1 mg/L increase, 95% CI 1.02–1.23, $P = 0.02$) after adjusting for age, baseline renal
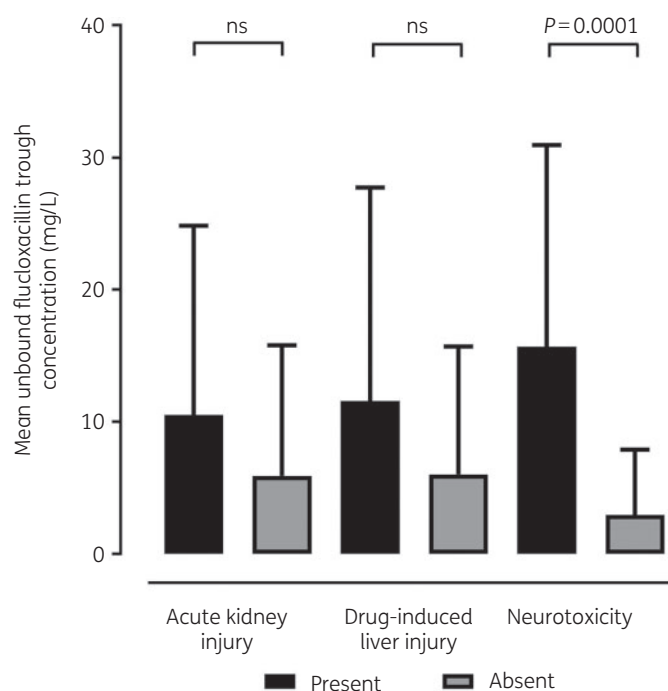
Figure 5. Mean unbound flucloxacillin trough concentration (three study visits) in patients with AKI, acute liver injury and potential neurotoxicity occurring after the start of flucloxacillin treatment. ns, not significant.

function, the Charlson comorbidity score and the Pitt bacteraemia score.

Discussion

Flucloxacillin is recommended as the treatment of choice for MSSA-BSI in various national guidelines in Europe, Asia and Australia.[4,18,19] Two grams four to six times per day is usually administered for at least 14 days (up to 6 weeks in infective endocarditis).[4] Critically ill patients with MSSA-BSI may be at risk for flucloxacillin overdosing as a consequence of impaired renal function and low serum albumin levels. In this prospective study,

we systematically assessed the proportion of pharmacological target attainment in patients with MSSA-BSI by measuring both the unbound fraction of flucloxacillin at several timepoints and the MSSA strain-specific flucloxacillin MICs.

Using this approach, we observed that 90% of all patients and 100% of critically ill patients attained the optimal target (100% $fT_{>MIC}$), whereas, with 100% $fT_{>ECOFF}$, only 38% of critically ill patients attained the target. In pharmacokinetic/pharmacodynamic studies, the ECOFF[20] or the EUCAST clinical species-specific breakpoint value[14] for the targeted bacterium is applied to define the target concentration of the antibacterial drug. In our study, measured flucloxacillin MICs ranged from <0.06 to 0.25 mg/L with a median value of 0.125 mg/L. These values are 16 times, or four dilutions, lower than the MSSA ECOFF. Variations in MIC determinations have to be considered for the definition of pharmacological targets and dosing adjustments guided by therapeutic drug monitoring.[21] However, the fact that MSSA MICs in the present and previous studies[22] were considerably lower than 2 mg/L implies that the use of ECOFF to define optimal target flucloxacillin concentration is imprecise, as there is a risk of exposing patients to higher and potentially toxic drug concentrations. In our strain population, the flucloxacillin MIC distribution closely resembles that of cloxacillin rather than oxacillin. Of note, EUCAST defines a 4-fold lower ECOFF for cloxacillin (0.5 mg/L) than for oxacillin (2 mg/L), although MSSA-BSI dosing recommendations are similar for all isoxazolyl penicillins. Consequently, optimal target attainment may vary depending on the MIC value used. A previous study reported optimal target attainment (100% $fT_{>MIC}$) in only 52% of patients who were receiving a median of 12 g of flucloxacillin per day when the oxacillin EUCAST clinical breakpoint was used (2 mg/L).[14] In our view, strain-specific flucloxacillin MICs—or, alternatively, oxacillin MICs or the (lower) cloxacillin ECOFF—should be preferred whenever possible to define the optimal flucloxacillin concentration in MSSA-BSI.

Using calculated (derived from total concentrations and fixed protein binding) instead of measured unbound flucloxacillin plasma concentrations for modelling studies may be inappropriate. The probability of optimal target attainment (100% $fT_{>0.5\,mg/L}$) with a cloxacillin dosage of 2 g every 4 and 6 h was only 38% and 9%, respectively, when protein binding of 95% was assumed.[22] Conversely, our results demonstrate 90% target attainment when we used the measured unbound flucloxacillin plasma concentration. Similarly, pharmacological target attainment (50% $fT_{>2\,mg/L}$) with a flucloxacillin dosage of 2 g every 4 h was only 10% in the study of Landersdorfer *et al.*,[23] which again did not account for the variation in protein binding, whereas our results demonstrate 70%–80% target attainment for the same dosing regimen. Calculation of the unbound concentrations, assuming 95% protein binding, may therefore result in considerable overdosing, in particular in critically ill patients with hypoalbuminaemia and renal impairment.[5,11] In the present study, the inter-individual unbound plasma fraction of flucloxacillin varied widely from 1.1% to 64.7%, showing a substantially higher median value (11%) than reported for healthy individuals (5%).[24]

Previously, more aggressive dosing recommendations have been made based on total flucloxacillin concentrations or the oxacillin ECOFF.[10,20,22] The present data showing high target achievement with the use of the strain-specific MIC, as well as the lack of association between outcome and target achievement

with the use of MSSA ECOFF, challenge current flucloxacillin dosing recommendations. In addition, dosages should only be adjusted according to the measured unbound flucloxacillin concentration in patients with MSSA-BSI,[20] as models that are able to accurately predict the unbound concentration from the total measured flucloxacillin concentration are lacking.[5,25]

We identified disease severity and renal function as independent predictors of 100% $fT_{>ECOFF}$, which is consistent with findings of previous studies.[26] Disease severity is likely associated with a catabolic condition and hypoalbuminaemia, and renal function is associated with the renal elimination proportion of flucloxacillin ($Q_0 = 0.3$).[10] Similarly, unbound flucloxacillin concentrations and unbound fractions were substantially higher in critically ill patients than in those who were non-critically ill. In our study population, critically ill patients often received high doses of flucloxacillin (12 g/day), in accordance with international guidelines.[27] These results call for improved flucloxacillin dosing strategies, particularly in critically ill patients with MSSA-BSI. This may include the use of continuous infusions and therapeutic drug monitoring coupled with software based on Bayesian forecasting.[28] Given the modest variability in intra-individual unbound flucloxacillin concentrations, our data emphasize that measurement of a single value might be sufficient to evaluate target attainment provided that albumin and creatinine levels remain stable.

Although we observed a higher 30 day mortality rate in patients with optimal target attainment when we used the oxacillin ECOFF or considered the mean trough unbound flucloxacillin concentration of each patient over time, this was not significant after adjustment for disease severity and age. Hence, our data are not sufficient to identify increased flucloxacillin concentrations as an independent determinant of a worse outcome. Nonetheless, the lack of flucloxacillin dose adaptions in critically ill patients with hypoalbuminaemia and renal impairment is likely responsible for the increased unbound concentrations observed in our population. In our cohort, 68% of patients initially received 12 g of flucloxacillin per day. In addition, our findings demonstrate the risk of overdosing and organ toxicity, in particular in frail or critically ill patients. The infective endocarditis guidelines of the BSAC recommend the use of lower flucloxacillin dosages than what was administered in our cohort, even in patients with confirmed infective endocarditis.[4] Others have recommended evaluating a dosage reduction in patients with moderate AKI after 48–72 h[20] and argue against maintaining the dosage as long as the creatinine clearance is above 10 mL/min/1.73 m^2.[29]

In critically ill patients, the proportion of those with excessive unbound flucloxacillin concentrations (100% $fT_{>10\times ECOFF}$) was significant (33%). When 10× the strain-specific MIC was used as the threshold for toxicity, more than 60% of patients exceeded this level. This illustrates that dose reductions may be considered in the majority of patients, as there is little benefit to be expected when the exposure is above 10× the MIC. We observed a substantially higher incidence of neurotoxicity in patients with excessive unbound flucloxacillin concentrations (100% $fT_{>10\times ECOFF}$). However, we acknowledge the limitations of our analysis given the post-hoc assessment and the presence of many potential confounders for this endpoint. Considering the polypharmacy in our cohort and broad definition for the presence of potential drug-associated neurotoxicity, we may have overestimated the association of unbound plasma flucloxacillin levels and neurotoxicity. Hence,

our data should be viewed as hypothesis-generating and require confirmation in a larger and prospective study. Our results are in line with the findings of Imani et al.,[15] who observed an increased incidence of neurotoxicity in patients with a total flucloxacillin concentration of >125 mg/L.

The strengths of our study include the prospective design with complete follow-up and assessment of disease severity at multiple timepoints, the inclusion of patients with different levels of disease severity, the measurements of unbound and total flucloxacillin concentrations and concomitant albumin and creatinine levels at several timepoints and the determination of strain-specific flucloxacillin MIC.

The limitations of our study include its single-centre design and the small sample size of only 50 patients. Another limitation concerns the exclusion of patients undergoing haemodialysis and the post-hoc analysis of toxicity. The pharmacological cut-off chosen to assess toxicity has not been validated for flucloxacillin. However, validated unbound flucloxacillin concentration thresholds are lacking. The chosen threshold (20 mg/L) should be viewed as indicative of an exposure that may warrant a dose reduction, as the benefit of concentrations beyond this threshold is probably absent. Lastly, the study period for flucloxacillin measurement comprised only 7 days.

In conclusion, pharmacological target attainment in patients with MSSA-BSI is remarkably higher with consideration of measured unbound flucloxacillin concentrations and strain-specific MICs than with calculated unbound flucloxacillin concentrations and the oxacillin ECOFF. Current dosing regimens may expose patients to excessive flucloxacillin concentrations and potentially result in drug-related organ damage.

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Transparency declarations

None to declare.

Supplementary data

Figures S1 and S2 and Table S1 are available as Supplementary data at JAC Online.

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