Population Pharmacokinetic Study of Amoxicillin-Treated Burn Patients Hospitalized at a Swiss Tertiary-Care Center

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ABSTRACT The objective of this study was to investigate the population pharmacokinetics (PK) of amoxicillin in ICU burn patients and the optimal dosage regimens. This was a prospective study involving 21 consecutive burn patients receiving amoxicillin. PK data were analyzed using nonlinear mixed-effects modeling. Monte-Carlo simulations assessed the influence of various amoxicillin dosage regimens with identified covariates on the probability to achieve a target (PTA) value of time during which free amoxicillin concentrations in plasma exceeded the MIC ($fT > MIC$). A two-compartment model best described the data. Creatinine clearance (CL_{CR}) and body weight (BW) influenced amoxicillin CL and central volume of distribution (V_1), respectively. The median CL_{CR} (Cockcroft-Gault formula) was high (128 ml/min), with 25% of patients having CL_{CR} s of >150 ml/min. The CL, V_1 , and half-life ($t_{1/2}$) values at steady state for a patient with a CL_{CR} of 110 ml/min and BW of 70 kg were 13.6 liters/h, 9.7 liters, and 0.8 h, respectively. Simulations showed that a target $fT > MIC$ of $\geq 50\%$ was achieved ($PTA > 90\%$) with standard amoxicillin dosage regimens (1 to 2 g every 6 to 8 h [q6–8h]) when the MIC was low (<1 mg/liter). However, increased dosages of up to 2 g/4 h were necessary in patients with augmented CL_{CR} s or higher MICs. Prolonging amoxicillin infusion from 30 min to 2 h had a favorable effect on target attainment. In conclusion, this population analysis shows an increased amoxicillin CL and substantial CL PK variability in burn patients compared to literature data with nonburn patients. Situations of augmented CL_{CR} and/or high bacterial MIC target values may require dosage increases and longer infusion durations. (This study has been registered at ClinicalTrials.gov under identifier NCT01965340.)

KEYWORDS amoxicillin, pharmacokinetics, burn patients, population pharmacokinetics

Treating sepsis promptly and adequately in burn patients is crucial, as it is still the predominant cause of morbidity and mortality despite major advances in hemodynamic and respiratory support (1–5). In this context, optimizing antibiotic dosage regimens to improve clinical outcomes and to avoid antibiotic resistance is desirable (6). However, this task is highly complex, as it requires not only a well-trained multidisciplinary team but also extended knowledge of pharmacokinetic (PK) alterations due to burn trauma (7–9). Therapeutic drug monitoring (TDM) appears to be a useful

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TABLE 1 Characteristics of the burn patients who received amoxicillin

Characteristic ^a	Value
No. of patients	21
Male, <i>n</i> (%)	16 (76.2)
Age (yrs), mean (SD)	50.1 (24.3)
Body wt at admission (kg), median (IQR)	72.4 (67.0–83.6)
Initial CL _{CR} (ml/min), median (IQR) ^b	128 (65–150)
TBSA affected (%), median (IQR)	23 (12.5–44)
<20, <i>n</i> (%)	7 (33.3)
20–40, <i>n</i> (%)	9 (42.9)
41–60, <i>n</i> (%)	2 (9.5)
>60, <i>n</i> (%)	3 (14.3)
SAPS II, mean (SD)	35.9 (18.6)
Ryan score, median (IQR)	1 (1–2)
Inhalation lesions, <i>n</i> (%)	16 (76.2)
Length of ICU stay, median (IQR)	23 (13.0–39.5)
Mortality in the burn ICU, <i>n</i> (%)	2 (9.5)

^aIQR, interquartile range; SAPS II, Simplified Acute Physiology Score; TBSA, total body surface area.

^bUsing the Cockcroft and Gault formula.

intervention to ensure attainment of PK/pharmacodynamic (PD) surrogate indicators of antibiotic efficacy to counteract the well-documented interindividual variability in PK observed in this population (10–15). Moreover, TDM might also potentially improve burn patient outcomes (10, 12, 16, 17). Currently, the Bayesian forecasting approach for estimation of individual PK parameters represents the gold standard approach for TDM (18, 19).

Amoxicillin is frequently used as a first-line antibiotic treatment in burn patients during the first weeks of hospitalization (14). It is partially metabolized by the hepatic system and lowly protein bound (18%) (<http://www.swissmedicinfo.ch/>). As this antibiotic is excreted in the urine (60% unchanged), its elimination is slowed in the case of renal impairment. TDM of amoxicillin is available but rarely used in our institution, including in the Burn Centre, as beta-lactam antibiotics are often thought to have few dose-dependent side effects and a wide therapeutic margin (20).

Because significant PK changes of antibacterial agents have been reported for burn patients, such patients are considered a special population in clinical PK studies (9, 21). For beta-lactams, most reports indicate increased values of drug clearance (CL) and volume of distribution in burn patients compared to healthy subjects (9). Because of these alterations, dose requirements of antibiotics may be increased in this population. However, few reports have addressed the PK profile in burn patients, and those that have are based on a small number of individuals. The use of a population approach to characterize amoxicillin PK and its variability and identify sources of variability has rarely been performed for burn patients.

In this context, our study aimed at determining the PK profile of intravenous amoxicillin given to adult patients with severe burns hospitalized in the Burn Centre of our hospital. The population model served to evaluate the PK/PD target attainment using standard and alternative dosage regimens of amoxicillin.

RESULTS

Patient characteristics and microbiological data. A total of 185 amoxicillin concentrations were obtained from 21 burn patients aged from 16 to 93 years. The patients' body weight (BW) on admission ranged from 60 to 132 kg. Table 1 presents the characteristics of the population. There was a large majority of male patients. The median creatinine clearance (CL_{CR}) was high (128 ml/min), with 25% of patients having a CL_{CR} of >150 ml/min. The median BW on admission was 72.4 kg, with a coefficient of variation of 22%. Limited intraindividual variability was observed in BW during the course of amoxicillin (with or without clavulanic acid) therapy, with a median change in BW of −2.3% (minimum, −11.4%; maximum, +10.6%). Of note, some patients had

TABLE 2 Microbiological data

Organisms ^a	No. of isolates	Median MIC (minimum–maximum), mg/liter	Antimicrobial therapy
Gram-negative bacteria	5	2 (1–4)	Amoxicillin + clavulanic acid (<i>n</i> = 5)
Gram-positive bacteria	15	0.5 (0.016–2)	Amoxicillin (<i>n</i> = 3), amoxicillin + clavulanic acid (<i>n</i> = 12)

^aGram-negative species included *Haemophilus influenzae* (*n* = 2), *Klebsiella pneumoniae*, *Citrobacter koseri*, and *Pantoea* spp. Gram-positive species included *Staphylococcus aureus* (*n* = 6), *Streptococcus pneumoniae* (*n* = 5), *Streptococcus bovis*, *Enterococcus faecalis*, *Granulicatella adiacens*, and *Bacillus* spp.

several episodes of infection that were treated by amoxicillin (with or without clavulanic acid). Microorganism identification with susceptibility was obtained at least once for 16 out of 21 patients. Two different bacteria were identified in the same sample for three patients, and one patient presented the same bacterium (*Staphylococcus aureus*) in two distinct tissue samples. As a result, a total of 20 susceptibilities were determined. Table 2 summarizes the microbiological data.

Population PK model. The final model was a two-compartment model with the following parameters: amoxicillin CL, central (V_1) and peripheral (V_2) volumes of distribution, and intercompartmental clearance (Q).

Residual variability was best described by a combined additive and proportional residual error model. The stepwise covariate modeling approach identified CL_{CR} and BW as covariates influencing amoxicillin CL and V_1 , respectively. CL_{CR} was found to influence amoxicillin CL in a linear manner, and V_1 was allometrically scaled to the actual BW. Amoxicillin CL was the only random PK parameter, while the others had fixed, estimated values. Models incorporating between subject variability on V_1 , V_2 , and Q were tested. As they either did not improve the model fit or poorly estimated the corresponding random effects (high standard errors), interindividual variabilities were not estimated for those parameters in the final model. Interoccasion variability could not be tested owing to the sampling design, which prevented from discriminating between interoccasion and inpatient variabilities. Table 3 displays the final estimates of population PK parameters and bootstrap estimates as well as parameter-covariate relationships. All parameters were estimated with acceptable precision.

Figure 1 shows the plots of conditional weighted residuals versus population predictions and time. Most residuals were within the expected range (-2 ; $+2$), and no major trend was observed versus prediction or time. The prediction-corrected visual predictive check obtained with the final model is shown in Fig. 2. As good agreement was observed between measured amoxicillin concentrations and model-based predictions (Fig. 3), the model was deemed to be appropriate for further dosing simulations.

TABLE 3 Population PK parameters of amoxicillin^a

Parameter	Structural model mean estimate (RSE, %)	Covariate model mean estimate (RSE, %)	Bootstrap mean estimate (95% CI)
Fixed effects			
CL (liter/h)	13.1 (12)	13.6 (8)	13.7 (11.5–16.5)
$\theta_{CL_{CR}, CL}$		0.57 (25)	0.53 (0.19–0.79)
V_1 (liters)	10.1 (25)	9.73 (20)	9.6 (4.5–16.4)
Q (liters/h)	20.8 (31)	20.1 (24)	20.2 (12.6–52.5)
V_2 (liters)	16.6 (15)	17.6 (14)	17.4 (13.0–24.2)
Random effect			
ω_{CL} (% CV)	57.3 (16)	37.3 (19)	36.0 (21.7–53.2)
Residual variability			
Proportional error (%)	34.4 (20)	37 (19)	34.6 (22.1–47.5)
Additive error (mg/liter)	0.59 (31)	0.08 (10)	0.08 (0.07–0.93)

^aCL, clearance; V_1 , central volume of distribution; RSE, relative standard error; 95% CI, 95% confidence interval; $\theta_{CL_{CR}, CL}$, proportional increase in CL elimination as a function of CL_{CR} ; ω_{CL} , interpatient variability on CL; CV, coefficient of variation. The final models are as follows: $TV_{CL} = CL \times [1 + \theta_{CL_{CR}, CL} \times (CL_{CR} - 110)]$ and $TV_{V1} = V1 \times (BW/70)$, where CL_{CR} is estimated by the Cockcroft-Gault equation (in milliliters per minute), 110 ml/min is average CL_{CR} in our population, and BW is body weight (in kilograms).

FIG 1 Model-derived conditional weighted residuals versus population predictions (upper graph) and time (lower graph).

Dosage regimen simulations. The Monte-Carlo simulation results are summarized in Fig. 4. As expected, when all other factors were kept constant, the values for free amoxicillin concentrations in plasma exceeding the MIC ($fT > MIC$) and PTA decreased with increasing renal function and MIC values.

Considering $fT > MIC$ of $\geq 50\%$ as the target, in patients with normal renal function ($CL_{CR} = 100$ ml/min), the standard dosage regimens with a 30-min infusion of 1 to 2 g every 6 to 8 h ($q6-8h$) were adequate for MIC values of ≤ 2 mg/liter but failed to achieve 90% PTA for higher MIC values. For a MIC of 8 mg/liter, such a PTA was achieved only with the most intensive dosage of 2 g/4 h and extended infusion (2 h) of 1 g/4 h or 2 g/6 h. The results were quite similar in patients with moderately impaired renal function ($CL_{CR} = 60$ ml/min), with 2 g/8 h administered as a 2-h infusion also being effective for high MIC values. In order to achieve the target $fT > MIC$ of 100%, regimens of 1 to 2 g administered every 4 h were necessary for MIC values of ≤ 2 mg/liter. However, for the high MIC of 8 mg/liter, even an extended infusion of 2 g q4h failed to achieve 90% PTA in simulated patients with CL_{CR} s of 100 ml/min.

In patients with augmented renal clearance (200 ml/min), the minimum dose to achieve the desired 90% PTA for the low target ($fT > MIC \geq 50\%$) with MIC values of ≤ 1 mg/liter was 1g q6h (Fig. 4). For higher MIC values, prolonging the infusion duration was effective for obtaining a higher PTA, and a 2-h infusion of 2 g every 4 h was the only regimen associated with PTA of $>90\%$ for a MIC of 8 mg/liter. None of the tested

FIG 2 Prediction-corrected visual predictive check obtained with the final model. The open circles represent the observed concentrations. The gray solid and dashed lines represent the median and the 5th/95th percentiles of the observed concentrations, respectively. The dark and light gray areas represent the 95% confidence interval (CI) of the simulated median and 5th/95th percentiles, respectively. Of note, three observations greater than 100 mg/liter are not shown for ease of graphical display (105.7, 134.4, and 198 mg/liter).

regimens was associated with acceptable PTA for the high target when the MIC was >2 mg/liter.

In patients with renal impairment (15 and 30 ml/min), reduced dosages (0.5 or 1 g q12h) appeared to be adequate for low MIC values but were not sufficient for MIC values as high as 8 mg/liter. In this case, a standard dosage (1 to 2 g q6–8h) appeared to be necessary to achieve $fT > MIC$ of $\geq 50\%$, while regimens of 1 g q4h and 2 g q4h were necessary to achieve $fT > MIC$ of 100% with acceptable PTA in patients with CL_{CR} s of 15 and 30 ml/min, respectively.

BW had a limited influence on $fT > MIC$ and PTA, as shown in Table 4. The index $fT > MIC$ slightly increased with an increasing BW as a result of a decrease in the distribution (K_{12} [transfer rate constant between compartment 1 {central} and 2 {peripheral}] = Q/V) and elimination rate constants ($k_{el} = CL/V$). However, even a 3-fold increase in BW had only a modest effect on PTA.

FIG 3 Observed versus population/individual predictions (linear scale). White circles represent population predictions, and smaller black circles individual predictions. The line is $y = x$.

FIG 4 Probability of target attainment as a function of the MIC and dosage regimen for six stages of renal function. IT, infusion time. The left and right sides show the results for the low ($fT > MIC \geq 50\%$) and high ($fT > MIC = 100\%$) pharmacodynamic targets, respectively.

TABLE 4 Probability of target attainment stratified by body weight and renal function for a dosage of 1 g q8h and a MIC of 8 mg/liter^a

CL _{CR}	Body wt (kg)	<i>f</i> T>MIC, mean (SD)	PTA <i>f</i> T>MIC ≥ 50%	PTA <i>f</i> T>MIC = 100%
30 ml/min	50	0.65 (0.24)	0.69	0.17
	70	0.68 (0.24)	0.73	0.21
	100	0.72 (0.24)	0.77	0.26
	150	0.76 (0.23)	0.82	0.32
100 ml/min	50	0.34 (0.18)	0.16	0.008
	70	0.36 (0.19)	0.19	0.01
	100	0.38 (0.20)	0.22	0.02
	150	0.42 (0.21)	0.27	0.04
200 ml/min	50	0.18 (0.10)	0.012	0
	70	0.19 (0.10)	0.018	0
	100	0.21 (0.11)	0.03	0
	150	0.23 (0.12)	0.04	0

^aCL_{CR}, creatinine clearance; *f*T>MIC, cumulative percentage of a 24-h period that the unbound fraction of a drug exceeds the MIC under steady-state pharmacokinetic conditions; PTA, probability of target achievement.

DISCUSSION

Although amoxicillin is frequently prescribed for patients with severe burns, to the best of our knowledge, this is the first population analysis carried out in this population. Literature regarding the PK of amoxicillin in nonburn patients exists but is scarce (22–25). Our study provides several insights regarding the PK and dosage requirements of amoxicillin in this population with known distinct PK characteristics. The PK of burn patients is indeed altered due to different phenomena, such as capillary leak syndrome, mechanical ventilation, hypoalbuminemia, extracorporeal circuits, and postchirurgical drains; in addition, significant burn injuries themselves might increase the volume of distribution of hydrophilic drugs (26–31).

Our results are in good agreement with a recent population PK study of amoxicillin-clavulanic acid in 13 ICU adult patients (22), with comparable patient characteristics (except for burns). While the typical values of volumes of distribution were similar (*V*₁ and *V*₂), patients with normal renal function (CL_{CR} of 110 ml/min) in the present study showed a 21% increased CL and a 22% increased Q compared to the ICU patients without burns. Our results confirm the increase in CL reported for other beta-lactam agents in burn patients, irrespective of renal function (10, 15). We did not observe any difference in amoxicillin volume of distribution in comparison to the nonburn population (22, 32, 33). The covariate analysis confirmed that CL_{CR} influences amoxicillin CL, as previously described (22). We also found that BW influences *V*₁, suggesting an approximate doubling of this value from 70 kg to 132 kg. This will be reflected in a slightly longer elimination half-life in overweight patients. Renal function explained 35% of the initial estimated interpatient variability in amoxicillin clearance, which remains still largely unexplained. This large variability could be due to several factors related to patients' characteristics, burn consequences, and medical support that can affect drug concentrations.

Among critically ill patients (including burn patients), evidence suggests that patients may have a higher CL_{CR} even in the presence of normal plasma creatinine concentrations (34, 35). An augmented CL_R (i.e., CL_{CR} of >130 ml/min) has been reported to occur in 15 to 65% of intensive care unit (ICU) patients, including burn patients, therefore increasing the risk of subtherapeutic concentrations in this population (15, 36). Based on our simulations, our data showed that in patients with a CL_{CR} of 150 to 200 ml/min, the standard dosage achieved the desired 90% PTA only for MIC values of <1 mg/liter. In those patients, dosages as high as 2 g or 4 g appear necessary to achieve *f*T>MIC of ≥50% for the highest MIC breakpoint (8 mg/liter). Prolonging the amoxicillin infusion from 30 min to 2 h was also a way to increase the target attainment.

As more aggressive pathogens are commonly found in the ICU, the prescription of antibiotics has to be adapted and carefully monitored among burn patients (10). The standard amoxicillin dose of 1 to 2 g q6–8h results in adequate exposure ($fT > MIC \geq 50\%$) for both low and normal CL_{CR} s in the case of MIC values of ≤ 2 mg/liter. However, a higher dosage should be used to treat burn patients infected by microorganisms with higher MIC values. This may be especially relevant for the treatment of infection caused by *Enterobacteriaceae* with amoxicillin-clavulanic acid, as those bacteria often display high MIC values ranging from 2 to 8 mg/liter.

Achieving $fT > MIC$ of 100% with acceptable PTA for MIC values as high as 8 mg/liter was possible only in patients with severe renal impairment. In burn patients with normal or augmented renal clearance, our results showed that achieving this target was hardly possible even with the highest dosage of amoxicillin (12 g/day) and repeated extended infusions. Yet the clinical benefits of targeting $fT > MIC$ of 100% remain unclear. In the DALI study performed with critically ill patients who received intravenous beta-lactams, there was no difference in the predictive value of positive clinical outcomes following either $fT > MIC$ of 100% or $fT > MIC$ of $\geq 50\%$ (11). In addition, targeting $fT > MIC$ of 100% in all burn patients would require larger doses and concentrations of amoxicillin that could increase its toxicity without a thorough monitoring by TDM. Indeed, adverse reactions such as crystalluria, have been reported with the use of high doses of amoxicillin (37, 38). Unlike renal function, interindividual changes in BW did not appear to have a clinically relevant influence on amoxicillin PD in adult burn patients.

Our simulation results are somewhat different from those described by Carlier et al. (22). They reported that an $fT > MIC$ of 50% or even 100% would be achieved in most ICU patients treated with standard doses of amoxicillin (3 to 4 g in three or four administrations per day), except for the conditions of augmented CL_R combined with the highest bacterial MIC. Our less optimistic results in burn patients are likely due to the increased Q and CL values discussed above. In addition, the proportion of simulated patients associated with successful treatment was not clearly stated in the work by Carlier et al.

As shown for other time-dependent beta-lactam agents (39, 40), increasing the infusion time of amoxicillin may be a simple way to optimize $fT > MIC$ and drug response. Amoxicillin combined with clavulanic acid is less stable than amoxicillin alone due to the degradation of clavulanic acid catalyzed by both acids and bases when dissolved in aqueous solution (41). However, a recent study has demonstrated that amoxicillin alone is stable enough to be administered as a continuous infusion and that the combination of amoxicillin and clavulanic acid is stable for 2 h (41). As our data showed that prolonging the infusion duration was effective for obtaining a higher PTA in burn patients treated with amoxicillin, we recommend that an extended infusion of 2 h could be used in cases of infections caused by microorganisms with high MIC values.

Optimizing antibiotic exposure in the burn population is of high importance, as 60% of these patients fail to reach the PK/PD target $fT > MIC$ of 100% while receiving beta-lactams (12). In order to counteract the PK variability observed in this frail population, TDM is a valuable intervention that should be widely used in order to reach and maintain the antibiotic therapeutic target (10, 12, 14, 15).

Our study had a prospective design and included all burn patients admitted consecutively to a tertiary-care hospital. Nevertheless, this study has several limitations. First, as burn patients constitute a specific and difficult population to study, the sample size is limited compared with population PK standards. However, quite rich amoxicillin concentration data were available, and PK parameters were precisely estimated. Second, as our laboratory could not dose clavulanic acid, only the amoxicillin concentration could be analyzed and studied for this work. Further research is necessary to investigate the potential PK changes of clavulanic acid in burn patients and to define the PK/PD in this population. However, amoxicillin is considered the main therapeutic agent, as clavulanic acid has a very weak antibacterial activity and literature suggests

that clavulanic acid has no influence on amoxicillin PK and vice versa (42). Third, even though research is active on the field, it is still unclear which PK/PD target should be aimed for in critically ill patients (including the burn population) (11, 12, 14), and experimental studies have shown that the values of $fT > MIC$ required to produce a given effect may vary between beta-lactams (43). Fourth, this work focused only on efficacy targets and no upper threshold for toxicity endpoints was evaluated. Owing to the large therapeutic window of amoxicillin, this should not present an important limitation to our results. Finally, simulations performed outside the range of data should be handled cautiously. Nevertheless, this may be an adequate approach when the covariate-parameter relationships have a rational basis. Regarding body weight, the use of allometric scaling to describe the relationship between V_1 and BW is a reasonable approach for simulating the effect of weight on the PK based on the theories of allometry and scaling (44). Regarding renal function, the simulation was based on the linear correlation found between amoxicillin clearance and creatinine clearance, which is in accordance with renal clearance concepts.

In conclusion, to the best of our knowledge, this is the first population analysis of amoxicillin PK data in burn patients showing increased typical values and important variability in amoxicillin CL compared to literature data with nonburn patients. This work highlights the need for a higher dosage and a longer infusion of amoxicillin in burn ICU patients with augmented CL_R infected by microorganisms with high MIC values.

MATERIALS AND METHODS

Ethics. This study was approved by the Institutional Review Board of the Centre Hospitalier Universitaire Vaudois and the Ethics Committee of the State of Vaud, Switzerland (protocol 195/13). Written informed consent was obtained from each patient.

Study design and setting. We prospectively and consecutively enrolled all burn patients admitted to the Burn Centre of our hospital who received a course of intravenous amoxicillin administered either alone or in combination with clavulanic acid from October 2013 to October 2016. The Burn Centre is a five-bed Swiss tertiary-care ICU nested in a 35-bed medical surgical ICU. This study was registered on the ClinicalTrials.gov platform (identifier NCT01965340).

Data collection. Age, sex, weight, CL_{CR} (Cockcroft-Gault formula), and burn characteristics (including total burnt body surface area and Ryan score [45]) were collected from medical records for each burn patient hospitalized during the study period. Data regarding amoxicillin administration (including date and time of administration, dose administered, and duration of infusion) were prospectively collected from our computerized information system (Metavision; IMDsoft, Tel Aviv, Israel). For each episode of infection, the microorganism was systematically identified, if possible. The susceptibility to amoxicillin was determined using Etest for each patient in whom the microorganism was identified (46, 47).

Antimicrobial treatment. Amoxicillin (Clamoxyl [GlaxoSmithKline AG, Münchenbuchsee, Switzerland] or Co-Amoxi-Mepha [Mepha Schweiz AG, Basel, Switzerland]) was dosed in accordance with the manufacturer's recommendations (1 to 2 g every 6 to 8 h in patients with normal renal function) and infused over 2 h (amoxicillin) or 1 h (amoxicillin-clavulanic acid) starting from the second dose (over 30 min for the first dose) according to our local guidelines. Indeed, amoxicillin-clavulanic acid solutions (diluted with 0.9% NaCl) have limited stability, and therefore, the maximal infusion time was set to 1 h. In contrast, as amoxicillin alone is more stable (stability of 3 h or 6 h when diluted in Ringer's lactate solution or 0.9% NaCl, respectively [<http://www.swissmedicinfo.ch/>]), an extended perfusion (2 h) could be used. For patients with renal insufficiency, the dosage was adjusted according to the renal function estimated by the Cockcroft-Gault equation (eGFR [estimated glomerular filtration rate] of <30 ml/min, 500 mg to 2 g q8–12h; eGFR of <15 ml/min, 750 mg to 2 g q24h).

Blood concentration measurements. Blood samplings for the amoxicillin assay were performed at various time points. Trough levels were sampled on days 2, 4, 6, and 8. Additional samples were obtained every 2 days for a few patients who received a course longer than 8 days. Random levels were sampled on day 6 and day 8. A rich amoxicillin kinetic profile was obtained on one occasion for most patients (18 out of 21, including 1 patient with two rich profiles) at the following sampling times: 0, 1, 2, 3, 4, and 5 h after the end of the infusion. The exact times were adjusted according to the infusion duration.

Determination of amoxicillin concentrations. Plasma amoxicillin levels were determined using a multiplex assay by ultraperformance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) requiring 100 μ l of plasma for the simultaneous quantification within 9 min of 12 most recommended and frequently used antibacterial drugs (see the supplemental material) (L. A. Decosterd, B. Ternon, S. Cruchon, N. Guignard, S. Lahrichi, B. Pesse, B. Rochat, T. Calandra, C. Csajka, T. Buclin, A. Mello, N. Widmer, and O. Marchetti, unpublished data). As no assay method was available for clavulanic acid, its concentration was not determined in this study. Blood samples were directly sent to the laboratory after sampling and were stored at -80°C until the analysis. Analyses were performed within 6 h during the week. Samples collected over the weekend were analyzed the following Monday afternoon.

Population PK model building. NONMEM version 7.1.0 (ICON Development Solutions, Ellicott City, MD) was used to analyze amoxicillin plasma concentrations versus time data using a nonlinear mixed-effects modeling approach. The first-order conditional estimation with the interaction algorithm was selected for all runs. We assumed lognormal distributions of PK parameters, i.e., an exponential model for interindividual variability. First, the best structural model and residual error models were identified. One-, two-, and three-compartment open linear models were evaluated. For the residual error, proportional, additive and combined (additive plus proportional) error models were tested.

Next, covariate model building was performed using the stepwise covariate model building tool of Perl Speaks to NONMEM (48). This tool permits forward selection and backward deletion of covariates in a model in a comprehensive manner. The following covariates were investigated: sex, total body surface area, serum albumin, serum creatinine, CL_{CR} (estimated by the Cockcroft-Gault equation), actual body weight (BW), BW on admission (BW_{ADM}), BW gain (defined as $BW - BW_{ADM}$ when $BW > BW_{ADM}$ and zero otherwise), and BW loss (defined as $BW_{ADM} - BW$ when $BW_{ADM} > BW$ and zero otherwise). Linear relationships were tested for categorical covariates, while linear and power functions were tested for continuous covariates. The change in the objective function value (OFV) was used to assess the influence of covariates, assuming a chi-squared distribution of the OFV, with 1 degree of freedom for each addition of a linear power relation. Statistical significance was set at a *P* value of 0.05 for forward selection and 0.01 for backward deletion.

Final model evaluation. Internal model validation was based on standard criteria (49): the OFV as described above, parameter estimates along with their standard errors, plots of observed amoxicillin concentration versus population and individual predictions, and plots of conditional weighted residuals (50). A bootstrap analysis ($n = 1,000$ samples with replacement from the original data set) was carried out with the PsN tool kit to check the uncertainty of parameter estimates and derive the 95% CI. A visual predictive inspection was also performed by comparing the observed amoxicillin concentration with model-based simulations ($n = 1,000$ samples for each patient) (51).

Dosing simulations. PK/PD simulations were performed based on the final model to investigate the influence of amoxicillin dose, dose interval, infusion duration, and covariates (renal function) on the probability to achieve a target exposure for amoxicillin. All simulations and calculations of probabilities of target attainment were done with the Pmetrics R package (52). Mean and variance of parameters of the final model estimated with NONMEM were imported into Pmetrics. We tested three amoxicillin doses (0.5, 1, and 2 g), five dosing intervals (4, 6, 8, 12, and 24 h), and two infusion times (30 min and 2 h). As CL_{CR} was found to influence amoxicillin CL, we considered six levels of renal function: 15, 30, 60, 100, 150, and 200 ml/min. For each condition, 500 virtual patients were created based on parameter estimates and covariates retained in the final NONMEM model. Amoxicillin CL values were randomly sampled based on the final estimates of mean and variance. The Q and V_2 values were fixed to their final NONMEM estimates for all subjects. Since BW influenced V_1 , this value changed as a function of BW. BW was sampled from a lognormal distribution in the form of $89 \times \exp(\eta_{BW})$, with $\eta_{BW} \sim N(0, 0.184^2)$. These values were representative of the average BW (89 kg) during antibiotic therapy and variability in a group of 39 burn patients from our Burn Centre who received a beta-lactam antibacterial agent (data not shown), including the 21 burn patients involved in this study.

Steady-state (i.e., after 10 days of therapy) amoxicillin concentration profiles were simulated for each condition. Then we derived probabilities of target attainment (PTA) using the dedicated function in Pmetrics. The PK/PD target was defined as a percentage of time during which the free amoxicillin concentration in plasma exceeded the MIC ($fT > MIC$). Two targets were considered: a target of $fT > MIC$ of $\geq 50\%$, usually recognized as efficient for beta-lactams (such as penicillins and carbapenems) (43, 53–55), and a more conservative target $fT > MIC$ of 100%, which seems to be associated with better outcomes in critically ill patients (11, 22). We considered MIC values of 0.25, 0.5, 1, 2, 4, 8, and 16 mg/liter as 8 mg/liter is the highest amoxicillin MIC breakpoint value for several Gram-negative organisms, including *Escherichia coli*, according to the European Committee on Antimicrobial Susceptibility Testing (56). We assumed a free fraction of 82% for amoxicillin (57). In addition, we considered 90% as an optimal PTA to be achieved, as suggested by the European Medicines Agency (58).

The influence of BW on PTA was also examined using the same approach. We considered situations of low weight (50 kg), standard weight (70 kg), overweight (100 kg), and obesity (150 kg) as well as three levels of renal function (30, 100, and 200 ml/min) for each weight. A dosage regimen of 1 g q8h (infused over 30 min) was simulated in 500 virtual patients under all 12 conditions, and steady-state concentrations were analyzed.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00505-18>.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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C.C., Y.-A.Q., P.E., P.V., and A.F. designed the study. A.F. and O.P. collected the data.

C.C., A.F., and S.G. analyzed the data. A.F., S.G., C.C., Y.-A.Q., P.E., O.P., and F.S. wrote the manuscript. All authors contributed to and approved the final version of the manuscript.

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