Diagnosis and treatment of common infectious diseases in severely ill sub-Saharan African patients
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Chapter 5

Pharmacokinetics and pharmacodynamic target attainment of ceftriaxone in an adult severely ill sub-Saharan African patient population

Bos JC, Prins JM, Mistício MC, Nunguiane G, Lang CN, Beirão JC, Mathôt RA, van Hest RM

Submitted
Abstract

Background In sub-Saharan Africa (SSA), the β-lactam ceftriaxone (CEF) is frequently used for the empirical treatment of severe bacterial infection. Systemic drug exposure of CEF can be altered in critically ill intensive care (ICU) patients, but pharmacokinetic variability and pharmacodynamic target attainment data for non-ICU SSA populations are lacking.

Methods We performed a prospective observational population pharmacokinetic study in an adult Mozambican hospital population, treated with CEF from October 2014 until November 2015. One trough, one peak and two random blood samples were collected per patient for the measurement of total CEF (CEFt) and unbound CEF (CEFu) plasma concentrations. We developed a PPK model through non-linear mixed effect analysis and performed Monte Carlo simulations for different patient variable and dosing regimen scenarios, with the primary endpoint being a CEFu concentration above MIC throughout the dosing interval (100% \( f_T \geq \text{MIC} \)).

Results Eighty-eight participants yielded 277 CEFt and 276 CEFu concentrations. The median age was 35 years, body mass index 18.9, albumin 29 (range 11-44) g/L and creatinine clearance (CLcr) 91 (range 4-261) mL/min. A one-compartment model with non-linear protein binding best described CEFt/CEFu pharmacokinetics. CLcr was positively correlated with CEFu CL, and albumin concentration with maximum CEF binding. For microorganisms with an MIC of 1 mg/L, simulations showed that with a 1 g twice-daily and 2 g once-daily regimen, 58.2% and 16.5% of patients respectively, would have a 100% \( f_T \geq \text{MIC} \).

Conclusions Severely ill adult non-ICU SSA patients are at substantial risk for underexposure to CEFu during routine intermittent bolus dosing with a 1 g twice-daily regimen, especially when their renal function is intact. The use of a 2 g once daily regimen should probably be discouraged.
Background

In sub-Saharan African (SSA), a region of the world with high HIV infection rates, 10-34% of patients admitted with fever suffer from bacterial bloodstream infections or sepsis.1-3 A high proportion of these infections is caused by *Streptococcus pneumonia* and non-typhoidal *Salmonella* serovars and mortality rates can be as high as 46-72%.4,5 The 3rd generation cephalosporin ceftriaxone (CEF) is among the most frequently used antibiotics for the empirical antibiotic treatment of adults in this situation.

Evidence from resource-rich intensive care unit (ICU) settings suggests that appropriateness of antibiotic treatment should not only be reviewed in terms of antibiotic drug choice, but also in terms of optimal systemic drug exposure, especially when treating critically ill patients.6,7 Pharmacokinetics (PK) of antibiotics in this group of patients can be substantially altered and β-lactam antibiotics are particularly vulnerable in this respect, as they are hydrophilic drugs with predominant renal clearance (CL). CEF is also highly bound to albumin at therapeutic concentrations, and during sepsis and hypoalbuminaemia, a lower protein bound drug fraction, an increased volume of distribution, and increased total drug clearance (CL) may change the total as well as the unbound, active drug concentration.4,7 Such alterations may give rise to sub-optimal drug exposure, an inability to attain pharmacodynamic (PD) targets, and ultimately, to adverse clinical outcome.8

In SSA, highly prevalent chronic diseases such as tuberculosis (TB), hepatitis B and hypertension may also have the potential to influence PK of antibiotics by means of cachexia and liver and kidney dysfunction, respectively.9-11 How coinciding acute and chronic conditions in severely ill (non-ICU) SSA patients influence the PK of β-lactam antibiotic drugs, including CEF, has been poorly investigated. What is clear is that underexposure to the active, unbound antibiotic drug may not only pose a threat to a patient’s health, but also to public health, as it contributes to the emergence of antimicrobial resistance, a phenomenon that is already highly prevalent in South Africa as well as in other SSA countries.12,13

In view of the above, we performed a population pharmacokinetic (PPK) study of CEF in a Mozambican, severely ill, adult hospital medicine ward population. The specific aims of the study were to describe the population PK of unbound CEF (CEFu) and total CEF (CEFt) in order to identify sources of PK parameter variability. Additionally, we aimed to assess the probability of PK/PD target attainment (PTA) of CEFu for the treatment of bacterial pathogens commonly causing sepsis in SSA.
Methods

Setting
Mozambique is a SSA country with an estimated adult HIV prevalence of 10.6%.14 The Beira Central Hospital (HCB) is a 733-bed governmental referral health facility with 260 internal medicine beds, admitting up to 1500 patients monthly. The proportion of patients infected with HIV on its medicine ward may be as high as 74%, and up to 32% of patients may die during hospital stay.15

Study design
The current study was a prospective, observational, population pharmacokinetic (PPK) study of CEF, as part of a PPK study of antibiotics in adult patients admitted to the HCB medicine ward. In this study, PK data were collected from October 2014 until November 2015 from patients who were treated with intravenously administered benzyl-penicillin, ampicillin, gentamicin or CEF. The PPK study was reviewed and approved by the Mozambican National Committee for Bio-ethics in Health (CNBS: study registration nr. 118/CNBS/2013). Additionally, a letter of approval was obtained from the general director of the HCB. Participants gave written informed consent. Those unable to read, write and/or understand Portuguese gave a thumbprint and an impartial, literate witness observed the entire informed consent process and subsequently co-signed the informed consent form.

Recruitment and data collection
Patients were eligible for study entry if they were hospitalized on the medicine ward of the HCB and were being treated with one or more of the study antibiotics, as documented in a patient’s medication record. Inclusion criteria were age ≥ 18 years and being willing and able to give informed consent. Exclusion criteria were the use of drugs known to significantly affect PK of the different study antibiotics (probenecid, phenylbutazon, acetylsalicylic acid, indomethacin), a haemoglobin level ≤ 6 g/dL as measured by the HCB’s laboratory, any condition necessitating a blood transfusion irrespective of haemoglobin level, and an altered level of consciousness. PPK study participants with an intravenous CEF prescription (Nirlife, Nirma Ltd., Gujarat, India) were selected for the present study.

Two trained research nurses captured baseline characteristics and CEF dosing information and measured body weight and length of all study participants. Doses of one gram of ceftriaxone powder for injection were dissolved in 10 ml of sterile water for injection, and subsequently injected intravenously via a venous catheter in half a minute, according to the responsible physician’s prescription. During a minimum of two days, a maximum of four blood samples were collected for the
measurement of CEF concentrations. Sample times were pre-dose (through level), 30-120 minutes after intravenous administration (peak level) and at 2 random time points during the dosing interval (random levels). CEF administration procedures were observed where possible, and a maximum of 19 ml of blood was collected over a time period of two or more days. One blood sample was also used for the measurement of albumin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyl transpeptidase (GGT) and creatinine concentrations. Bilirubin levels were not measured, for local practical reasons in relation to bilirubin’s high photosensitivity. Creatinine clearance ($C_{\text{CR}}$) was estimated using the Cockroft and Gault (CRGT) formula.¹⁶

**Sample handling and drug assay**

EDTA anti-coagulated blood samples were refrigerated at 4-8°C immediately after collection until laboratory processing, which took place within two hours of collection. Samples were centrifuged and plasma was stored at -80°C in the local research laboratory until shipment on dry ice to the Netherlands for biochemical marker and drug concentration analysis. Plasma was ultrafiltrated (centrifugal filters: Millipore Amicon Ultra 0.5 ml/30K, Merck Millipore, Darmstadt, Germany) and the ultrafiltrated plasma was subsequently processed as a typical plasma sample to obtain the unbound concentration. $C_{\text{EF}_u}$ and $C_{\text{EF}_t}$ concentrations were measured using a validated high-performance liquid chromatography mass spectrometry (LC-MS; LC: LC30 UPLC, Shimadzu, Kyoto, Japan & MS: Qtrap 5500 system, Sciex, Framingham, MA, USA). The lower limit of quantification (LLQ) was 1 mg/L and the higher limit of quantification was 200 mg/L. Within and between assay variability was smaller than 5.6% and 6.8%, respectively. The accuracy of the assay was between 90 and 105%.

**Population pharmacokinetic analysis**

**Model development**

The PPK analysis was performed using the non-linear mixed-effect modelling software package NONMEM (7.1.2; Icon Development Solutions, Ellicott City, Maryland, USA). Model building was performed using a stepwise approach. For detailed methodological model building information see the online appendix. In brief, during a first step, a structural, compartmental PPK model was developed in which the PK of $C_{\text{EF}_u}$ and $C_{\text{EF}_t}$, as well as the protein binding of CEF were described, including their between-patient variability (BPV). One and two compartment models were tested for $C_{\text{EF}_u}$ and CEF protein binding was tested in a linear and non-linear protein-binding model. In the second step, an attempt was made to explain BPV by building a covariate model in which patient demographics and pathophysiological factors were tested for their correlation with the estimated
PK parameters volume of distribution, CL and CEF protein binding. Tested covariates included age, sex, weight, length, BMI, haemoglobin, albumin, creatinine, creatinine clearance, and gamma-GT, ALAT and ASAT concentrations. In the third and last step the robustness of the final model resulting from the 2nd step was tested using a so-called bootstrap analysis in which the dataset was resampled 1000 times. To validate the final model visual predictive checks (VPCs) were performed that investigated whether the final model could adequately predict the observed concentration-time course of CEFu and CEFt, including the observed variability. Bootstrap and VPC analyses were performed using Perl-speaks-NONMEM version 3.5.3 software (PsN4, Uppsala, Sweden).

Monte Carlo dosing simulations
Using the final validated PPK model, CEFu concentration-time profiles were predicted based on Monte Carlo simulations following three dosing regimens (1 g every 12 hours (BID), 2 g every 24 hours (QD) and 2 g BID). Each simulation generated concentration time profiles for 1000 virtual patients per drug regimen. Based on these data, the PTA, being the percentage of patients with a CEFu remaining above a specified MIC during a specified percentage of time of the dosing interval (fT>MIC), was calculated for different fT>MIC targets. The primary target to be tested was fT>MIC=100% and secondary targets were fT>4xMIC=100%, fT>MIC=50% and fT>4xMIC=50%. The choice of PD targets was based on the conclusions of a recent review and critical appraisal of data concerning β-lactam administration and β-lactam PD targets in critically ill patients.6,17 The choice of the target MIC was based on the EUCAST MIC clinical breakpoints for susceptibility to CEF of Enterobacteriaceae (1mg/L) and S. pneumoniae (0.5 mg/L) being.18

Results
Patients and ceftriaxone concentrations
We screened a total of 762 patients for the larger PPK study and excluded 366 patients (Figure 1). The most common reasons for exclusion were a haemoglobin level ≤6 g/dL, having received or being scheduled to receive a blood transfusion, or having an altered level of consciousness.
We included 98 patients in the current study on CEF and remained with 88 participants for analysis, after removing six patients with insufficient data and four patients from whom no blood samples were collected. A large majority of patients (93.1%) had a 1 g BID CEF dosing schedule. Patient characteristics are presented in Table 1. A total of 277 plasma samples yielded 277 CEFt and 276 CEFu concentrations. 43/88 (48.9%) participants had less than four plasma samples available, with the most common explanations being a participant’s unforeseen discharge, CEF use being stopped, and receiving a blood transfusion after inclusion (Figure 1).

We screened a total of 762 patients for the larger PPK study and excluded 366 patients (Figure 1). The most common reasons for exclusion were a haemoglobin level ≤ 6 g/dL, having received or being scheduled to receive a blood transfusion, or having an altered level of consciousness.

Patients screened n = 762

Patients included n = 396

Patients on ceftriaxone n = 98

Patients on ceftriaxone analyzed n = 88

277 samples

Patients excluded: n = 366

• 150: Hb ≤ 6 g/dL
• 122: blood transfusion
• 103: altered level of consciousness
• 15: refused participation
• 28: other
  • 10: age < 18 years
  • 6: study drug never administered
  • 4: handcuffed (prisoner)
  • 2: deaf
  • 2: extensive skin lesions
  • 1: no Hb
  • 1: no witness
  • 2: unknown

Patients with < 4 samples: n = 43

• 21: unforeseen discharge
• 8: ceftriaxone stopped
• 4: blood transfusion
• 3: no venous access
• 3: patient left without notice
• 1: transferred
• 1 died
• 2: withdrawal

Figure 1. Study profile.
albumin concentration, explaining 17% and 31% of the BPV respectively. The final model had an adequate fit and VPCs are shown in Figure 2. The residual variability, an estimate of unexplained variability relating to measurement error, errors in data collection, intra-patient variability and model misspecification, was 9.9 mg/L (relative standard error (RSE) 12%) for the CEFu, and 24 mg/L (RSE: 6%) for the CEFt concentration.

Figure 2. Observed ceftriaxone concentration-time data and visual predictive checks (VPC) of the final model.

The black dots are the observed concentrations. The black line is the observed median and the grey lines the 5th and 95th percentile of the observed data. The dark and light blue lines are the corresponding simulated percentiles with their 95% confidence intervals. Panel A: total ceftriaxone; Panel B: unbound ceftriaxone.

Table 1. Baseline characteristics of study population (n=88).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex (n/%)</td>
<td>48 (54.5)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35 (18-76)</td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td>50 (29-70)</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>18.9 (11.3-26.4)</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>9.4 (6.3-16.4)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>29 (11-44)</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>49 (6-1276)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>25 (0-676)</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>50 (11-594)</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>63 (19-971)</td>
</tr>
<tr>
<td>Creatinine clearance* (mL/min)</td>
<td>91 (4-261)</td>
</tr>
<tr>
<td>Ceftriaxone dose prescribed (n/%)</td>
<td></td>
</tr>
<tr>
<td>1 g BID</td>
<td>82 (93.1)</td>
</tr>
<tr>
<td>2 g QD</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>2 g BID</td>
<td>4 (4.6)</td>
</tr>
</tbody>
</table>

Results expressed as median (range) unless specified otherwise. *Creatinine clearance estimated using Cockroft-Gault equation. ASAT: aspartate aminotransferase; ASAT: alanine aminotransferase; GGT: gamma-glutamyl transpeptidase, BID: twice-daily; QD: once-daily.

Sixty-seven of 88 (76.1%) participants had three or four plasma samples available for analysis. There were 33/276 (12%) CEFu samples with a concentration below the LLQ. The median unbound fraction was 19% (interquartile range (IQR): 13-29). The observed CEFt and CEFu concentrations are shown in Figure 2. The relationship between the CEFt concentration and the CEFu concentration is shown in figure S1 (Supplementary file).

Population pharmacokinetic analysis

Model development

For detailed modelling information, including the PK parameter values belonging to the different modelling steps, see the appendix. In brief, during step 1 of the analysis, the CEFu data were fitted to several compartmental models and a one-compartmental model best fitted the data, while a non-linear protein-binding model best described CEFt concentration and protein binding (Figure 3). The covariate analysis, based on 100% availability of covariate results, yielded a model with significant associations between CEFu CL and CLCR and between maximum CEF binding (maximum estimated CEF concentration bound to albumin: Bmax) and
albumin concentration, explaining 17% and 31% of the BPV respectively. The final model had an adequate fit and VPCs are shown in Figure 2. The residual variability, an estimate of unexplained variability relating to measurement error, errors in data collection, intra-patient variability and model misspecification, was 9.9 mg/L (relative standard error (RSE) 12%) for the CEFu, and 24 mg/L (RSE: 6%) for the CEFt concentration.

**Figure 2.** Observed ceftriaxone concentration-time data and visual predictive checks (VPC) of the final model.

The black dots are the observed concentrations. The black line is the observed median and the grey lines the 5th and 95th percentile of the observed data. The dark and light blue lines are the corresponding simulated percentiles with their 95% confidence intervals. Panel A: total ceftriaxone; Panel B: unbound ceftriaxone.
Figure 3. Covariate relationship between albumin and the unbound ceftriaxone fraction.

Monte Carlo dosing simulations
For the dosing regimens investigated, patients with a higher CLCR and lower albumin concentrations had lower CEFt concentrations. As for CEFu, simulations indicated that only patients with higher CLCR were likely to have lower CEFu concentrations (Figure 4A-D). For microorganisms with an MIC of 1 mg/L, the PTA of the most frequently used CEF regimen of 1 g BID for patients with the median CLCR of 91 mL/min was 58.2% for the primary PD target of 100% fT>MIC. The PTA of the secondary PD target of 50% fT>MIC was 95.1%. For a 2 g QD CEF regimen, the PTA was 16.5% and 74.7% for the PD targets of 100% fT>MIC and 50% fT>MIC, respectively (Table 2, Figure 5). When treating a pathogen with an MIC of 0.125 mg/L, the PTA of the target 100% fT>MIC was approximately 95% for a 1 g BID regimen and around 50% for a 2 g QD CEF regimen, in patients with the median albumin concentration of 29 g/L and the median CLCR (Figure 4).
Simulations of steady state ceftriaxone concentration-time profiles of patients with all median characteristics of the population, but with three different plasma albumin concentrations and two creatinine clearances. For albumin, the observed median, 25th and 75th percentile were used, and for creatinine clearance, the median and 25th percentile. Panel A: total CEF following a 1 g BID injection; Panel B: unbound CEF following a 1 g BID injection; Panel C: total CEF following a 2 g QD injection; Panel D: unbound CEF following a 2g QD injection.
The present study describes the development of a PPK model of CEF in a SSA hospital population with presumptive severe infection, based on observed CEFu and CEFt plasma concentrations. The model suggests that substantial numbers of severely ill patients, especially the ones with a preserved kidney function, are at risk of underexposure to CEFu.

The study population was generally young and severely ill, as illustrated by its low median BMI and low median haemoglobin and plasma albumin concentrations. A one-compartment model with non-linear, saturable protein binding best described CEFt/CEFu PK. Similar to other studies with critically ill patients, the BPV was high. As can be expected with an antibiotic with predominant renal CL, CEFu CL was significantly associated with CLCR and explained a substantial part of this BPV.19,20 Additionally, a significant relationship was found between maximum CEF binding, Bmax, and albumin concentration. Results from other studies have also suggested that CEF is subject to non-linear protein binding.21 The relationship between Bmax and albumin concentration implies that the maximum CEF concentration bound to albumin is lower at lower albumin concentrations. With a higher CEFu fraction present at such lower albumin concentrations, more CEFu will be available for clearance, thereby leading to a lower CEFt concentration and not to a lower CEFu concentration, at steady state. The result is that CEFt concentrations become less representative of CEFu concentrations with a decreasing albumin concentration, as can be seen in our study’s Monte Carlo dosing simulations, which clearly visualize how albumin concentration and CLCR interact to produce an effect on the CEFu concentration time profiles that is different from the one on the CEFt concentration time profiles. Our findings match results from earlier PK studies with CEF in non-SSA critically ill ICU patients that also implied a predominant dependency of CEFu exposure on CLCR.19,20,22-24 However, all but one of those studies found a 1 g BID or 2 g QD regimen to reach appropriate CEFu concentrations when applying a PD target of 100% fT>MIC 1 mg/L. Only Joynt et al. suggested an inability to reach the same target with a 2 g BID prescription.22 With the median CLCRs (ranges) in these studies appearing more or less similar to the current study’s estimated values, the difference in results between studies may be explained by the fact that not all studies based their conclusions on directly measured CEFu concentrations and that some may have been too small to draw meaningful conclusions.

Although none of our measured liver enzymes proved to have a significant relationship with CEFu PK, other investigators have found that hepatic dysfunction as measured by bilirubin levels negatively correlated with albumin binding and non-renal clearance of CEFu.24 In addition, two more recent studies found an elevated time of the unbound ceftriaxone concentration above the MIC. BID: twice-daily; QD: once-daily.

Table 2. Probability of target attainment with three different ceftriaxone dosing regimens.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>fT&gt;MIC=100%</th>
<th>fT&gt;MIC=50%</th>
<th>fT&gt;4xMIC=100%</th>
<th>fT&gt;4xMIC=50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 g BID</td>
<td>58.2</td>
<td>95.1</td>
<td>19.2</td>
<td>63.6</td>
</tr>
<tr>
<td>2 g QD</td>
<td>16.5</td>
<td>74.7</td>
<td>3.6</td>
<td>35.1</td>
</tr>
<tr>
<td>2 g BID</td>
<td>75.8</td>
<td>98.4</td>
<td>37.9</td>
<td>84.9</td>
</tr>
</tbody>
</table>

Probability of target attainment, i.e., the percentage of 1000 simulated patients predicted to achieve four different PD targets with three different ceftriaxone dosing regimens, for patients with a median creatinine clearance of 91 mL/min and an albumin concentration of 29 g/L, and assuming a pathogen’s ceftriaxone minimal inhibitory concentration (MIC) of 1 mg/L (EUCAST clinical breakpoint for Enterobacteriaceae).18 fT>MIC: time of the unbound ceftriaxone concentration above the MIC. BID: twice-daily; QD: once-daily.

Figure 5. Probability of target attainment with three different ceftriaxone dosing regimens.

Percentages of 1000 simulated patients with a median albumin concentration (29 g/L) and a median estimated creatinine clearance (91 mL/min) achieving an unbound CEF concentration above the minimal inhibitory concentration throughout the dosing interval (fT>MIC=100%), for three different ceftriaxone dosing regimens and a range of MICs. Clinical breakpoint for Enterobacteriaceae according to EUCAST is 1 mg/L.18
Discussion

The present study describes the development of a PPK model of CEF in a SSA hospital population with presumptive severe infection, based on observed CEF\textsubscript{u} and CEF\textsubscript{t} plasma concentrations. The model suggests that substantial numbers of severely ill patients, especially the ones with a preserved kidney function, are at risk of underexposure to CEF\textsubscript{u}.

The study population was generally young and severely ill, as illustrated by its low median BMI and low median haemoglobin and plasma albumin concentrations. A one-compartment model with non-linear, saturable protein binding best described CEF\textsubscript{t}/CEF\textsubscript{u} PK. Similar to other studies with critically ill patients, the BPV was high. As can be expected with an antibiotic with predominant renal CL, CEF\textsubscript{u} CL was significantly associated with CL\textsubscript{CR} and explained a substantial part of this BPV.\textsuperscript{19,20} Additionally, a significant relationship was found between maximum CEF binding, B\textsubscript{max}, and albumin concentration. Results from other studies have also suggested that CEF is subject to non-linear protein binding.\textsuperscript{21} The relationship between B\textsubscript{max} and albumin concentration implies that the maximum CEF concentration bound to albumin is lower at lower albumin concentrations. With a higher CEF\textsubscript{u} fraction present at such lower albumin concentrations, more CEF\textsubscript{u} will be available for clearance, thereby leading to a lower CEF\textsubscript{t} concentration and not to a lower CEF\textsubscript{u} concentration, at steady state. The result is that CEF\textsubscript{t} concentrations become less representative of CEF\textsubscript{u} concentrations with a decreasing albumin concentration, as can be seen in our study’s Monte Carlo dosing simulations, which clearly visualize how albumin concentration and CL\textsubscript{CR} interact to produce an effect on the CEF\textsubscript{u} concentration time profiles that is different from the one on the CEF\textsubscript{t} concentration time profiles. Our findings match results from earlier PK studies with CEF in non-SSA critically ill ICU patients that also implied a predominant dependency of CEF\textsubscript{u} exposure on CL\textsubscript{CR}.\textsuperscript{19,20,22-24} However, all but one of those studies found a 1 g BID or 2 g QD regimen to reach appropriate CEF\textsubscript{u} concentrations when applying a PD target of 100\% fT$>$MIC 1 mg/L. Only Joyn et al. suggested an inability to reach the same target with a 2 g BID prescription.\textsuperscript{22} With the median CL\textsubscript{CR}s (ranges) in these studies appearing more or less similar to the current study’s estimated values, the difference in results between studies may be explained by the fact that not all studies based their conclusions on directly measured CEF\textsubscript{u} concentrations and that some may have been too small to draw meaningful conclusions.

Although none of our measured liver enzymes proved to have a significant relationship with CEF\textsubscript{u} PK, other investigators have found that hepatic dysfunction as measured by bilirubin levels negatively correlated with albumin binding and non-renal clearance of CEF\textsubscript{u}.\textsuperscript{24} In addition, two more recent studies found an elevated
CEF_u fraction in patients with (severe) hyperbilirubinaemia.\textsuperscript{20,23} Since we did not include bilirubin in our analysis, it cannot be excluded that liver dysfunction is a source of CEF_u PK variability, and that part of the BPV may have resulted from a relationship between liver function and CEF_u PK that was left unaccounted for.

For once daily dosing regimens, the PTA for the PD target of 100% fT>MIC was just above 50% for pathogens with an MIC of 0.125 mg/L in patients with median values of albumin concentration and ClCR. Importantly, simulations with the use of the commonly prescribed 1 g BID CEF regimen demonstrated that the use of this regimen may also lead to CEF_u underexposure, especially for patients with a preserved kidney function, whereas the risk for underexposure in patients with an impaired renal function appears to occur mostly when more stringent PD targets are applied. For pathogens with an MIC of 1 mg/L, both QD and BID dosing in this population are very likely to lead to CEF_u underexposure, for conservative and more stringent PD targets alike. Although clinical and microbiological evidence is still limited, available data do seem to support the idea that in patients with severe disease, who may be at higher risk for impaired drug distribution and tissue penetration, the use of the more stringent PD target of 100% fT>MIC improves clinical outcome and reduces selective pressure and the emergence of antimicrobial resistance, as compared to the most conservative target of 50% fT>MIC.\textsuperscript{8,17,25,26}

The CEF_u concentration time simulations in our study could not be tested against the background of locally derived bacterial pathogens’ MICs, which means that the actual PTA of the use of certain CEF dosing regimens could differ from what is suggested on the basis of the MICs we used for S. pneumoniae and Enterobacteriaceae (1 mg/L). Results from a surveillance trial of regional and global antimicrobial susceptibility showed that in Africa, 32% of S. pneumoniae isolates collected from 2009-2012 were resistant against penicillin (MIC >2 mg/L), and that in this group, roughly 25% of isolates were non-susceptible to ceftriaxone (MIC >1 mg/L).\textsuperscript{27} Multi-drug resistance of non-thyphoid Salmonella serovars, including resistance against 3rd generation cephalosporins, is emerging.\textsuperscript{28} Third generation cephalosporin resistance still appears to be restricted to certain geographical African areas, but very little susceptibility surveillance data are currently available.

There are limitations to our study. Firstly, although the study population was generally severely ill, it may however not have been entirely made up of patients with sepsis, as patient selection was based on the use of CEF as prescribed by the responsible local doctor and a microbiological diagnosis is not routinely made. Secondly, we did not collect information about a patient’s fluid balance and the presence of excess fluid in the interstitial and trans-cellular space, and we are
therefore not informed about how hydration status, oedema and so called ‘third spacing’ may have affected PK BPV. It is our experience that administration of sufficient amounts of fluids in adult hospitalized patients in a resource poor setting as ours is rare, and our observations are supported by repeated reports from Uganda showing that fluid therapy was being severely underprovided for in-patients with presumptive sepsis. This may have led to an inadequate hydration status in a substantial amount of cases and may imply that CEFu concentrations as well as PTAs could turn out to be lower than what was found in the current study once proper volume therapy is applied. Thirdly, there was substantial residual PK variability as expressed by the CEFu and CEFt additive error. An explanation may lie in drug dispensing and administration procedures, even though these procedures were observed where possible.

Although it is not unlikely that our study population’s high risk of CEFu underexposure is indicative of what happens to PK/PD in critically ill patients in general, extrapolations to non-SSA severely ill (ICU) patient populations should be made with caution, as our study population’s underlying disease, as well as other treatment aspects, including antibiotic infusion time and volume therapy, are likely to be different.

To summarize, the current study provides the first population pharmacokinetic modeling data on the use of the highly albumin bound CEF in an adult severely ill, non-ICU, SSA hospital patient population with a presumptive severe bacterial infection or sepsis. The study’s Monte Carlo simulations, which were based on a model that was built using observed total and unbound CEF concentrations, demonstrate that the probability of pharmacodynamic target attainment is very low when using a 2 g QD dosing regimen, but is also low when using the commonly applied 1 g BID regimen, especially in patients with a preserved kidney function and when dealing with microorganisms with reduced susceptibility. Intermittent bolus dosing of the β-lactam antibiotic ceftriaxone in severely ill adult SSA hospitalized patients may therefore lead to underexposure, which is likely to pose a threat to an individual patient’s health as well as to public health, as it is known to contribute to the emergence of antimicrobial resistance. The use of a 2 g QD dosing regimen should probably be discouraged in this patient setting, although further prospective SSA antibiotic PK data against the background of locally derived MICs are needed, so that well-known and relatively cheap antibiotic drugs can be preserved for use in this region of the world.
Contributors
JCB, RM and JP designed the study. JCB performed the literature search. JCB obtained ethical approval. JCB, MM, GN and MD implemented the study and JCB supervised data collection and study progress on a daily basis. JCB and RVH analyzed the data. JCB and RVH drafted the manuscript. JP and RM critically examined the analysis and findings and all authors critically read and commented on draft versions of the report. All authors approved the final version.

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References


Supplementary files
Population pharmacokinetic model development

Structural model

For model building purposes, observed concentrations of unbound ceftriaxone (CEF_u) and total ceftriaxone (CEF_t) were converted from mg/L to mmol/L by dividing observed concentrations by the molar mass of ceftriaxone of 661.6 g/mol. Concentrations of albumin, CEF's binding protein, were converted from g/L into mmol/L in a comparable fashion, dividing the albumin concentration by the molar mass of albumin of 69000 g/mol and multiplying it by 1000.

Estimated PK parameters for CEF_u were clearance (CL), central volume of distribution (V_1) and, in case of a two-compartment model, peripheral volume of distribution (V_2) and intercompartmental clearance (Q). BPV in CL and V_1 was modelled using exponential models. CEF_t and CEF protein binding were subsequently modelled according to equation (Eq.1),

$$[\text{CEF}_t] \text{ (mmol/L)} = [\text{CEF}_u] + [\text{CEF}_u] * b$$

Eq.1

where \(b\) stands for CEF's binding properties, and \([\text{CEF}_u] * b\) for the bound CEF concentration. The following models for protein binding were tested: 1: a linear protein binding model, in which \(b\) is an estimated parameter, \(\theta_{\text{bind}}\), that correlates to the number of unoccupied binding places, and 2: a nonlinear protein-binding model, in which \(b\) becomes \(B_{\text{max}}/(K_m + [\text{CEF}_u])\). In this model, \(B_{\text{max}}\) represents protein binding defined as the maximum estimated concentration of CEF bound to albumin, and \(K_m\) represents the CEF_u concentration at which albumin binding is half maximal. Both \(b\) parameters versions were tried for the estimation of protein binding, including BPV.

The residual variability, i.e. the difference between measured CEF_u and CEF_t concentrations and the corresponding CEF_u and CEF_t concentrations predicted by the model, was modelled with additive or proportional models or a combination of both. The so-called M3 method was used for the handling of CEF_t and CEF_u concentrations below the lower level of quantification (LLQ).

Covariate model

Patient demographics and pathophysiological factors were tested for their correlation with the estimated PK parameters V_1 (V_2) and CL (Q), as well as with CEF protein binding (Table S1). Tested covariates included age, sex, weight, height, BMI, hemoglobin concentration, albumin concentration, creatinine, creatinine clearance, and gamma-GT, ALAT and ASAT concentrations. Continuous variables were
modelled in an exponential way, and the effect of a categorical variable was estimated by quantification of the fractional difference, relative to the reference category. All covariates were first screened for significance of the correlation between the covariate and the PK parameter by univariate analysis, using a p-value cut-off of 0.05. Furthermore, a reduction in BPV or residual variability, as well as biological plausibility of a covariate-PK parameter relationship was used as a criterion for covariate selection. All covariates selected during the univariate analysis subsequently entered an intermediate model for a backward elimination procedure (multivariate analysis) with a cut-off for statistical significance of 0.001, which yielded the final model.

Potential improvement of the model by adding a compartment or by introducing a correlation between a covariate and a PK parameter was evaluated using the likelihood ratio test, in which the difference between the minimum objective function value (OFV) generated by NONMEM® for two hierarchical models is determined. Model performance was also evaluated by visual inspection of diagnostic ‘goodness-of-fit’ plots. These were generated using Pirana (version 2.9.0) and Xpose (version 4.3.2) software (Nicolas Jonsson and Mats Karlsson, Uppsala, Sweden).6,7

Model robustness and predictive performance
The robustness of the parameter estimates from the final model resulting from the 2nd step was tested using a bootstrap analysis. The predictive performance was evaluated using visual predictive checks (VPC: Figure 3 main manuscript). In the bootstrap analysis, the dataset was resampled 1000 times. VPCs investigated whether the final model could adequately predict the observed time course of CEFu and CEFt, including the observed variability. Bootstrap and VPC analyses were performed using Perl-speaks-NONMEM version 3.5.3 software (PsN4, Uppsala, Sweden).8

Results Population Pharmacokinetic Model Development
Structural model
During step 1 of the analysis, the CEFu data were fitted to several compartmental models and a one-compartmental model best fitted the data. BPV estimates of CEFu CL and CEFu V1 were explored separately, but they were highly correlated. Therefore, BPV of CEFu V1 was modelled as a function of the BPV of CEFu CL, as described by Bonate.7 Instead of directly estimating the BPV of CEFu V1, the ratio of the standard deviations in CEFu CL and CEFu V1 was used, and was estimated to be 0.41. Using this parameter’s value and the estimated BPV of CEFu CL of 93%, BPV of V1 was calculated to be 33%. CEFt concentration and protein binding were best
described by the nonlinear protein-binding model. $K_m$ was estimated to be 0.01 mmol/L while $B_{max}$ was 0.11 mmol/L. The estimated BPV for $B_{max}$ was 31%.

**Covariate model**

The covariate analysis, based on 100% availability of covariate results, yielded a model with the following significant associations after univariate analysis: CEFu CL with $CL_{CR}$, CEFu $V_1$ and albumin concentration, bodyweight, BMI and $CL_{CR}$, $B_{max}$ with albumin concentration and age, and $K_m$ with albumin concentration and age. After multivariate analysis, the associations between CEFu CL and $CL_{CR}$, and between $B_{max}$ and albumin concentration remained statistically significant. All correlations were positive ones, as showed by equations 2 and 3:

$$CEF_u\ CL\ (L/h) = 11 * (CL_{CR}/91)^{0.37}$$ \hspace{1cm} \text{Eq. 2} \\
$$B_{max} = 0.12 * (\text{albumin concentration}/0.42)^{1.3}$$ \hspace{1cm} \text{Eq. 3}

in which 11 (L/h) and 0.12 mmol/L are the typical estimates for CEFu CL and $B_{max}$, respectively, for a patient with the population median values of 91 ml/min for $CL_{CR}$ and 0.42 mmol/L for albumin concentration. The exponents are the estimated parameters determining the shape of the covariate associations. Consequently, a patient with all median population characteristics, but with a low albumin concentration of 0.35 mmol/L will have a low $B_{max}$ of 0.09 mmol/L, while a patient with the same median characteristics but with a higher albumin concentration of 0.48 mmol/L, has a higher $B_{max}$ of 0.14 mmol/L. In a similar fashion, a patient with all median population characteristics, but a with a $CL_{CR}$ of 120 mL/min, has a CEFu CL of 13 L/h, while a patient with a $CL_{CR}$ of 58 mL/min has a CEFu CL of 9.7 L/h.

Using the $B_{max}$ and albumin concentration equation (Eq. 3), equation 1 for the calculation of CEFt is transformed into equation 5:

$$[CEF_t] \ (mmol/L) = [CEF_u] + [CEF_u] * (0.12 * (\text{albumin}/0.42)^{1.3}) / (0.0092 + [CEF_u])$$ \hspace{1cm} \text{Eq. 5}

The relationship between observed as well as predicted CEFu and CEFt concentrations is shown graphically in Figure S1. Upon inclusion of the covariate associations, the estimates for BPV of CEFu CL changed from 93% to 77% and in CEFu $V_1$ from 33% to 37%, which means that a fair part of BPV in CEFu CL is explained. BPV of $B_{max}$ could however no longer be reliably estimated after inclusion of the association between $B_{max}$ and albumin level. This does not mean that BPV of $B_{max}$ is fully explained, but rather that there is not enough information in the data to support a reliable estimate after inclusion of the covariates.
Table S1. Parameter estimates of the different model building steps.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model without covariates</th>
<th>Model with covariates</th>
<th>Bootstrap of model with covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>RSE (%)</td>
<td>Estimate</td>
</tr>
<tr>
<td>CEFu CL (L/h)</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>CEFu V1 (L)</td>
<td>48</td>
<td>10</td>
<td>48</td>
</tr>
<tr>
<td>Km (mmol/L)</td>
<td>0.010</td>
<td>28</td>
<td>0.0092</td>
</tr>
<tr>
<td>Bmax (mmol/L)</td>
<td>0.11</td>
<td>12</td>
<td>0.12</td>
</tr>
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Between-patient variability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE (%)</th>
<th>Estimate</th>
<th>RSE (%)</th>
<th>Estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEFu CL (%CV)</td>
<td>93</td>
<td>15</td>
<td>77</td>
<td>14</td>
<td>73</td>
<td>37-108</td>
</tr>
<tr>
<td>CEFu V1* (%CV)</td>
<td>33</td>
<td>*</td>
<td>37</td>
<td>*</td>
<td>38</td>
<td>7.9-112</td>
</tr>
<tr>
<td>Bmax (%CV)</td>
<td>31</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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Residual variability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE (%)</th>
<th>Estimate</th>
<th>RSE (%)</th>
<th>Estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additive error CEFu (mmol/L)</td>
<td>0.015</td>
<td>12</td>
<td>0.015</td>
<td>12</td>
<td>0.015</td>
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<tr>
<td>Additive error CEFt (mmol/L)</td>
<td>0.036</td>
<td>7</td>
<td>0.036</td>
<td>6</td>
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<td>0.031-0.040</td>
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Covariate effects

<table>
<thead>
<tr>
<th>Covariate effects</th>
<th>Estimate</th>
<th>RSE (%)</th>
<th>Estimate</th>
<th>RSE (%)</th>
<th>Estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRGT on CEFu CL</td>
<td>-</td>
<td>-</td>
<td>0.37</td>
<td>41</td>
<td>0.38</td>
<td>0.083-0.79</td>
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<tr>
<td>Albumin on Bmax</td>
<td>-</td>
<td>-</td>
<td>1.30</td>
<td>41</td>
<td>1.3</td>
<td>0.93-1.70</td>
</tr>
</tbody>
</table>

CL: clearance; V: volume of distribution; Km: CEFu concentration at which albumin binding is half maximal; Bmax: maximum estimated concentration of CEF bound to albumin; CV: coefficient of variation; CLcr: creatinine clearance; RSE: relative standard error; CI: confidence interval. *: The BPV of CEFu V was modelled as a function of the BPV of CEFu.², leaving the BPV without an RSE; ¶: see Eq. 2 and 3.
Figure S1. Relationship between total and unbound ceftriaxone concentrations in the final model.

Total versus unbound observed (black dots) and individual predicted (blue dots) ceftriaxone concentrations with its model predicted relationship (black line) for a patient with the median albumin concentration of 29 g/L.
References supplementary files


