**STATE OF THE ART OF UNBOUND CEFTRIAXONE AS A PHARMACODYNAMIC TOOL: ARE WE READY FOR ITS IMPLEMENTATION IN CLINICAL PRACTICE?**

Johnny MICHELa, Francesco MONTIb, Fabien LAMOUREUXc, Interne 1c, Interne 2c, Manuel ETIENNEd, Muriel QUILLARDe, Fabienne TAMIONf, Sandrine DAHYOTg, Tania PETERSENc\*, Tony PEREIRAc, Martine PESTEL-CARONg, Julien GROSJEANb,h,Thomas DUFLOTi#

aEmergency Department, CHU Rouen, Rouen F-76000 France.

bDepartment of Digital Health, CHU Rouen, Rouen F-76000 France.

cDepartment of Pharmacology, CHU Rouen, Rouen F-76000 France.

dUniv Rouen Normandie, Univ Caen Normandie, INSERM, Normandie Univ, DYNAMICURE UMR 1311, CHU Rouen, Department of infectious diseases, F-76000 Rouen, France.

eDepartment of Biochemistry, CHU Rouen, Rouen F-76000, France.

fDepartment of Medical Intensive Care Unit, CHU Rouen, Rouen F-76000 France.

gUniv Rouen Normandie, Univ Caen Normandie, INSERM, Normandie Univ, DYNAMICURE UMR 1311, CHU Rouen, Department of Bacteriology, F-76000 Rouen, France

hLaboratoire d'Informatique Médicale et d'Ingénierie des Connaissances en e-Santé (LIMICS), U1142, INSERM, Sorbonne Université, Paris, France.

iUniv Rouen Normandie, INSERM, Normandie Univ, EnVI UMR1096, CHU Rouen, Department of Pharmacology, F-76000 Rouen, France.

# Corresponding author: Dr Thomas Duflot

Email: [thomas.duflot@chu-rouen.fr](mailto:thomas.duflot@chu-rouen.fr) Phone: +33(2)32883731 Fax: +33(2)32889094

\*Present address: Tania Petersen, Department of Bacteriology, AP-HP Hôpital Universitaire Pitié Salpêtrière

**Running head:** Unbound ceftriaxone in clinical practice

**Abstract:**

**Background:** Ceftriaxone is pivotal in treating severe infections; however, modeling unbound plasma ceftriaxone (CEFu) from total ceftriaxone (CEFtot) remains challenging.

**Objectives:** This study aimed to (1) predict CEFu from CEFtot, (2) determine optimal thresholds for CEFtot trough concentration in plasma, (3) perform an external validation of published models, and (4) analyze factors influencing CEFtot trough concentration and the probability of target attainment (PTA).

**Methods:** CEFu predictions based on CEFtot were evaluated using previously published models, considering both normal albumin concentrations (35 g/L) and hypoalbuminemia (20 g/L). Optimal CEFtot thresholds for a MIC of 1mg/L were calculated to achieve CEFu concentrations with fT > 1xMIC 100% and fT > 4×MIC 100%. External validation was conducted using prospective data (62 samples). Retrospective data, comprising 408 CEFtot and 222 patients, were analized to identify significant predictors of CEFtot trough concentrations and PTA based on the evaluated models.

**Results:** Optimal CEFtot trough concentration thresholds ranged from 1.8 mg/L to 16.9 mg/L (1xMIC) and from 6.6 mg/L to 56.2 mg/L (4xMIC). External validation suggested that some published model predicted well CEFu. In the retrospective cohort, PTA varied from 94.4% to 98.7% for 1xMIC and from 66.9% to 97.3% for 4xMIC. Age, daily dose, albuminemia and creatininemia were significant predictors of CEFtot concentration. Notably, a dosing regimen of 1 g twice daily improved PTA compared to 2 g once daily.

**Conclusions:** Modeling or quantifying CEFu may enhance patient outcomes but requires standardized analytical approaches and further investigation.

**INTRODUCTION:**

Ceftriaxone (CEF) is a widely-used third-generation beta-lactam antibiotic in the cephalosporin class. It plays a crucial role in preventing and treating severe infections like meningitis, pneumonia, osteoarticular infections, soft tissue infections, and endocarditis. In emergency medical settings, CEF is often the preferred choice for antimicrobial therapy due to its rapid and broad-spectrum activity. 1 However, determining the optimal dosing regimen for individual patients is a challenge, primarily because total ceftriaxone (CEFtot) exhibits nonlinear pharmacokinetics (PK), in contrast to unbound ceftriaxone (CEFu), which follows linear PK. 2,3 In cases of severe sepsis and septic shock, the PK of CEFtot undergoes significant modifications due to altered parameters such as hypoalbuminemia, renal dysfunction, and fluid extravasation. 4,5 Understanding the pharmacodynamic (PD) properties of antibiotics and the potential changes in their PK in such critical conditions is essential for tailoring individualized dosing regimens. 6 CEF has a high, saturable binding affinity to plasma proteins, especially albumin. Consequently, increases in CEFtot concentration and/or hypoalbuminemia, a common condition in critically ill patients, can raise the fraction of CEFu. This increase leads to a higher apparent volume of distribution and enhanced clearance resulting in lower overall drug exposure. Such reductions may compromise time-dependent PD target of CEF. 7

Interestingly, in the pharmacokinetic of CEF, therapeutic drug monitoring (TDM) primarily assesses CEFtot. However, CEF’s activity is mediated by the unbound fraction CEFu, which exhibits high variability both within and between patients. Understanding the dynamics and implications of this variability could provide crucial insights for optimizing dosing regimens and improving therapeutic outcomes.

Several research teams have endeavored to model CEFu using diverse mathematical formulas that incorporate albumin concentrations. Nevertheless, these models have been developed within specific populations, such as adult and pediatric intensive care units, and have suffered from a lack of external validation. Given the potential of CEFu quantitation and modeling to offer new insights into PK, efficacy, and toxicity, this study's objectives are fourfold: (1) to predict CEFu in plasma from CEFtot, based on existing formula in the literature; (2) to establish optimal CEFtot thresholds in plasma to achieve a predefined CEFu target of 1 mg/L; (3) perform an external validation of the previously published models; and (4) to scrutinize predictors of CEFtot and the probability of target attainment (PTA), based on TDM in a comprehensive retrospective cohort.

**RESULTS**

**Literature Search**

A total of 23 publications were identified. Among these, 14 publications were excluded for the following reasons: 3 were outside the scope, 8–10 5 lacked quantitation of CEFu 11–15 and 5 did not involve modeling of CEFu based on CEFtot. 16–20 Notably, one publication met the criteria but could not be used due to discrepancies in the PK parameters. 21 As a result, a total of 9 publications were retained for CEFu modeling (Table 1). CEFtot is defined as the sum of CEFu and bound ceftriaxone (CEFb) (Eq.1). Six of the 9 publications 22–27 used a non-linear protein-binding model (Eq.2) for CEFb resulting in Eq.3. In this equation, Bmax and Kd represented the maximum protein binding capacity and the dissociation constant, expressed in mg/L or mM. Solving for CEFu from Eq.3 yielded Eq.4 which was used by these authors.

(Eq.1)

(Eq.2)

(Eq.3)

(Eq.4)

Two publications employed the calculation of the unbound fraction (fu) using either a polynomial 28 or an exponential approach. 29 Additionally, one publication devised its own transformation to predict CEFu from CEFtot. 30 For the quantitation of CEFu, UF and equilibrium dialysis (ED) were used in 6 and 2 publications respectively whereas the method employed was not explicitly defined in one publication. 40 It is worth noting that 8 out of the 9 formulas used albuminemia as a significant predictor of Bmax.

**Ceftriaxone free fraction modeling & CEFtot optimal thresholds**

The prediction of CEF free fraction according to CEFtot is depicted in Figure 1. The Ulldemolins model is distinguished by its linear relationship between CEF free fraction and CEFtot. The Standing model estimated the higher CEF free fraction values based on CEFtot, both in cases of normal hypoalbuminemia (Figure 1A) and normal albuminemia (Figure 1B). Substantial disparities emerged with increasing CEFtot concentrations. Models developed by Bos, Dreesen, Gijsen, Gregoire, Hartman, Heffernan and Leegwater displayed similar CEF free fraction predictions within the lower range of CEFtot (from 0 to 75 mg/L) in the case of normal albuminemia (35 g/L) (Figure 1B). However, in hypoalbuminemia (20 g/L), differences in the predicted CEF free fraction become more pronounced across the full range of predicted concentrations (Figure 1A). For further analysis, a calculator for determining CEFu concentrations from CEFtot and albumin concentrations is available at: <https://github.com/ThomasDuflot/Ceftriaxone-AAC>.

The objective of CEFu modeling was to determine the CEFtot concentration needed to sustain a CEFu concentration above the MIC of 1 mg/L and 4xMIC (4 mg/L). This threshold reflects the minimum CEFtot trough concentration necessary to achieve the PD target, with distinct values for both normal albumin concentrations (35 g/L) and hypoalbuminemia (20 g/L).

For achieving fT > MIC 100% under normal albumin conditions (35 g/L), substantial variability was observed across models, with the Ulldemolins model requiring the lowest CEFtot concentration (3.3 mg/L) and the Gijsen model the highest (16.9 mg/L). This variability was even more pronounced when targeting fT > 4xMIC 100%: the Ulldemolins model suggested a threshold as low as 13.1 mg/L, while the Heffernan model indicated a much higher concentration of 56.2 mg/L. Across models, CEFtot thresholds showed marked differences depending on the PD target, with the mean CEFtot concentration for fT > MIC 100% at 11.4±5.3 mg/L, and 35.8±14.5 mg/L for fT > 4xMIC 100%. Notably, as shown in Table 2, the coefficient of variation for thresholds under normal albumin conditions was 48.3%.

In cases of hypoalbuminemia (20 g/L), the required CEFtot concentrations decreased overall, yet variability across models remained high. For fT > MIC 100%, the Standing model estimated the lowest CEFtot threshold (1.8 mg/L), while the Gregoire model required the highest concentration (15.1 mg/L). Similarly, for fT > 4xMIC 100%, the lowest CEFtot concentration was 6.6 mg/L (Standing model), and the highest was 50.1 mg/L (Gregoire model), as outlined in Table 2. Hypoalbuminemia notably intensified between-model variability, with the mean CEFtot threshold concentrations averaging 7.2±4.1 mg/L for fT > MIC 100% and 23.4±13.0 mg/L for fT > 4xMIC 100%. The coefficient of variation increased to 62.8% under hypoalbuminemic conditions, highlighting the complex impact of reduced albumin on target attainment and model-dependent threshold disparities.

**External validation and comparison of predictive performance**

A total of 59 patients (26 women and 33 men) receiving ceftriaxone treatment were included in the external validation study, with 62 plasma samples collected in total. The albumin concentrations, CEFtot, and CEFu measurements spanned ranges of 18.9–37.5 g/L, 2.9–259 mg/L, and 0.14–94.70 mg/L, respectively.

Upon analysis, the models developed by Gregoire, Hartmann, and Heffernan provided the most accurate predictions, with favorable metrics across MSE, MPE, RMSE, RMSE%, and R² (Table 3 and Figure 2). This result was further supported by Bland-Altman plots of signed and relative differences, which showed the lowest variability (Supplementary Figure S1). An overview of significant differences between models is provided in Supplementary Table S1.

**Evaluation of CEFu prediction on a retrospective cohort of patient treated by ceftriaxone**

In the retrospective cohort, a total of 408 CEFtot plasma samples and 376 albumin concentrations measurements were collected from 222 patients. Since some patients had multiple CEFtot quantitations (ranging from 1 to 14 samples per patient), patients- and dosing-related variables were categorized accordingly (Table 3). The median albumin concentration was 27.0 g/L, below the normal range. Albumin concentrations ranged from a minimum of 10.4 g/L to a maximum of 42.7 g/L. Among the patients, 27 out of 376 (7.2%) had albumin concentrations below 20 g/L. Most patients were hospitalized in the infectious diseases department, followed by the medical ICU and cardiology. The primary indication for treatment was infectious endocarditis, mainly caused by Enterococcus faecalis (53%), often treated in combination with amoxicillin.

The PTA showed minimal variability across different models for a PD target of fT > 1xMIC 100%, with a mean frequency of 97.3±1.0%. The lowest PTA value was 95.7% (Gregoire model), while the highest was 98.7% (Ulldemolins model) (Figure 3A). For a more stringent PD target of fT > 4xMIC 100%, the mean PTA dropped to 86.4±9.7%, ranging from a minimum of 67.8% (Gregoire model) to a maximum of 97.3% (Ulldemolins model) (Figure 3A).

The concordance matrix for the fT > 1xMIC 100% target indicated strong agreement between models, with concordance rates above 97% (Figure 3B). In contrast, the concordance matrix for the fT > 4xMIC 100% target revealed three distinct groups: the Gregoire and Heffernan models (93% concordance), the Standing and Ulldemolins models (99%), and a cluster of Hartman, Gijsen, Dreesen, Leegwater, and Bos models, each with concordance rates of 96% or higher (Figure 3C).

In addition, clinical, demographic, and biological data from the retrospective cohort were analyzed as predictors of CEFtot concentration at trough. Simple linear mixed-effects regression identified age (p=0.033), intake dose (p<0.001), daily dose (p=0.001), albumin (p=0.002), and creatininemia (p=0.021) as significant predictors. After integrating these predictors into a full model and applying backward variable selection, the final model retained age (p=0.005), daily dose (p<0.001), albumin (p=0.009), and creatininemia (p<0.001) as key predictors of CEFtot concentration at trough (Table 4).

ANOVA revealed that CEFtot trough concentrations increased with higher dosing regimens. Mean CEFtot trough concentrations were 52.6±33.5 mg/L for the 1g x1/day regimen, 62.9±44.6 mg/L for 2g x1/day, 84.2±38.5 mg/L for 1g x2/day, and 126.3±69.1 mg/L for 2g x2/day (Figure 4). The 2g x2/day regimen showed significantly higher CEFtot trough concentrations than the other dosing regimens (p<0.001). Notably, the 1g x2/day regimen produced higher CEFtot trough concentrations than both the 2g x1/day regimen (p=0.029) and the 1g x1/day regimen (p<0.001). However, no statistically significant difference was observed between the 1g x1/day and 2g x1/day regimens (p=0.522) (Figure 4).

PTA curves further supported these findings, showing that increased dosing improved PTA. Notably, the 1g x2/day regimen achieved higher PTA than the 2g x1/day regimen. The model used to predict CEF<sub>u</sub> had a marked impact on PTA for each dosing regimen. The MIC range covered adequately (PTA > 90%) for a 1g once-daily dose varied significantly, from 1 mg/L (Gregoire model) to 8 mg/L (Ulldemolins model) for the same plasma CEFtot concentration. Increasing the dosing regimen generally elevated the CEFtot trough concentration, resulting in broader coverage of higher MIC values. The 2g twice-daily regimen, for example, provided coverage ranging from just below 4 mg/L (Gregoire model) to 16 mg/L (Standing and Ulldemolins models) (Figure 4).

**Discussion:**

The primary aim of the present study was to conduct a comprehensive analysis of literature data concerning CEFu, both in terms of quantification and modeling, in order to perform an external validation and to assess its relevance and potential applicability in clinical practice. The study revealed several noteworthy findings, despite the presence of significant limitations.

The literature search yielded a total of nine models for CEFu modeling based on CEFtot. It should be noted that some data were found to be unsuitable for modeling purposes and were challenging to obtain. Some data were deemed irrelevant 21 while others exhibited inconsistencies, including discrepancies between the original manuscript and supplementary model. 22 However, it is important to clarify that these discrepancies did not appear to impact the overall conclusions drawn in these studies. Furthermore, it is worth highlighting that although efforts were made to accurately translate the models from the literature for use in this study, errors in data interpretation cannot be ruled out. To promote transparency and reproducibility, the code used in this study is available on GitHub at <https://github.com/ThomasDuflot/Ceftriaxone-AAC>.

A notable degree of variability was observed between models when examining CEFu modeling and the determination of optimal thresholds based on minimum inhibitory concentration (MIC) with higher variability of the thresholds when hypoalbuminemia occurs. An interesting key factor is the variation in the studied population among the different research studies. These differences included the age of patients, their critical illness status, the number of samples collected, the timing of sample collection, the presence or absence of hypoalbuminemia, and the use of cardiopulmonary bypass. Additionally, the method employed for sample processing, such as UF or ED, introduced another source of variability. It is noteworthy that a recent paper reported significant differences in the parameters Bmax and Kd between *in vitro* UF and *in vivo* IV microdialysis. 31 The choice between UF and ED is particularly important, as it influences the determination of the free drug fraction. While ED is regarded as the gold standard method, it is also known for its time-consuming nature. Conversely, UF is a more straightforward approach but is sensitive to a range of analytical conditions. Both UF and ED are influenced by temperature, and UF is particularly affected by centrifugation speed and time. It is also important to note that these analytical considerations may vary depending on the physico-chemical properties of the drug being studied. 32

The significance of external validation in ensuring the reliability of the study's findings cannot be overstated. Consequently, the predictive performance of the models under investigation was rigorously assessed, despite the limited sample size in this single-center prospective cohort (N=62). Although this limitation is acknowledged, the use of combined fit metrics provided valuable insights, revealing that certain models demonstrated a higher degree of reliability compared to others. It is important to interpret these results with caution, as their validation requires replication and further extensive investigation.

Transitioning from modeling concepts to clinical implications, the primary objective was to ascertain whether the CEF dosing regimen was sufficient to achieve the therapeutic objectives. Although the retrospective cohort study possessed evident limitations, it has been observed that when employing a CEFu threshold of 1 mg/L, PTA is 95.7% across all models. Consequently, the level of concordance among these models was relatively high. However, as the thresholds increased, inter-model variability may start to impact clinical conclusions regarding the effectiveness of CEF.

Balancing the limitations of retrospective data, it is crucial to emphasize that the significant predictors of CEFtot identified in this cohort, including age, plasma albumin, plasma creatinine, and dose, have been previously highlighted in the literature. 19,27 As demonstrated in the mixed effects regression analysis (Table 4), daily dose was treated as a continuous variable, revealing that CEFtot concentrations increase with higher doses. PARAGRAPHE SUR MODELE PK-PD / PKPOP. It is interesting to observe that CEFtot concentrations were significantly higher with a dosing regimen of 1g administered twice daily compared to 2g administered once daily. This suggests that dividing the daily dose or employing continuous infusion may represent more effective approaches for achieving the therapeutic target but may also elevate the risk of toxicity. 33,34 In addition, the observed disparities in the probability of target attainment (PTA) curves among the various models under evaluation may lead to divergent conclusions regarding the optimal therapeutic management and dosage adjustments for ceftriaxone.

To conclude, determining CEFu offers an intriguing opportunity to enhance our understanding of CEF's PK and PD, as recent publications have emphasized. In line with this, the current study has strived to provide comprehensive results based on several available models, enabling fellow researchers to improve their collective understanding of this topic. From a clinician’s perspective, targeting 4 times the MIC during the interdose period is essential for treating serious infections like infective endocarditis. However, achieving this target depends on the model used, and nutritional status plays a crucial role in dose optimization, with a balance between inefficacy and toxicity. Analysis of dosing regimens in the retrospective cohort revealed that splitting a dose twice daily is more effective than once daily administration. Nevertheless, it is important to stress the need for standardized analytical considerations and rigorous external validation to establish CEFu as a robust PD biomarker in clinical practice. In summary, the application of CEFu in clinical practice may face challenges due to potential analytical biases, which warrant further investigation.

**MATERIALS AND METHODS**

**Literature Search**

A systematic review of population pharmacokinetic (PK) models for both CEFtot and CEFu was conducted using Pubmed, covering the period from January 2000 up to December 2022. The terms “population”, “pharmacokinetics”, “free”, “unbound” and “ceftriaxone” were selected for the literature review and combined to obtain the following search query:

* Population AND pharmacokinetics AND ceftriaxone AND (free OR unbound) AND (("2000/01/01"[Date - Publication] : "2022/12/31"[Date - Publication]))

The query was not limited by age groups or medical conditions, but articles included were required to be in English. Informations from the selected articles were collected, including the number of patients and samples, the studied population, the method used to quantitate CEFu , the formula used to predict the relationship between CEFtot and CEFu, and the values of each parameter of the formula. Formulas were retained for further analysis if all variable and parameter values were provided, allowing for comprehensive CEFu modeling.

**Unbound ceftriaxone (CEFu) modeling**

For each model, the concentration of CEFu was modeled as a function of CEFtot both under normal albumin concentrations (35 g/L) and hypoalbuminemia (20 g/L). To fully appreciate the non-linear relationship between CEFu and CEFtot , figures were generated with CEFtot concentrations ranging from 0 to 300 mg/L, commonly observed in clinical practice.

**Determination of optimal total ceftriaxone (CEFtot** **) thresholds**

A target minimum inhibitory concentration (MIC) of 1 mg/L, which is considered the breakpoint concentration of ceftriaxone against *Enterobacteriaceae* by the European Committee on Antimicrobial Susceptibility Testing, 35 was employed to establish CEFtot thresholds based on MIC. These thresholds were calculated for the criteria of achieving fT > MIC 100% and fT > 4 × MIC 100%.

**External validation and comparison of predictive performance**

For external validation, performance metrics, including signed error, relative error, mean signed error (MSE), mean percentage error (MPE), root mean square error (RMSE), root mean square error of percentage (RMSE%) and determination coefficient (R²) were calculated and employed to compare the predictive performance of each formula. Analysis of variance (ANOVA) followed by Tukey Honest Significant Differences tests were performed for between model comparison. Bland-Altman plots for each model for both signed and relative differences were drawn for a full representation of the data.

**Studied population**

EDSaN solution, 36 a Clinical Data Warehouse (CDW), was used to identify and extract trough plasma CEF concentration requests for trough plasma CEF concentration, spanning from 2016 to 2022. These requests were subsequently obtained, along with the relevant patient data, from the CDW. The extracted data encompassed various blood biology elements, in addition to clinical and demographic data. Moreover, information regarding the CEF dosing regimen was manually retrieved from the medical records of the patients.

**Ethics**

The French Data Protection Authority (CNIL) approved the construction and the usage of the Rouen University Hospital Clinical Data Warehouse (decision DT-2020-007), based on a declaration compatible with the General Data Protection Regulation applicable in France. Following national rules, a global public information was issued and individual information provided for each new patient in the hospital In addition, the prospective study was conducted following approval from our local ethics committee (approval number E2024-19, obtained on February 29, 2024). Due to the non-interventional nature of the study, written informed consent was not mandatory according to the national regulatory framework.

**Statistical analysis**

CEFu free fraction modeling was performed using R software v4.2.237 and the following packages: *ggplot2* v3.4.3, 38 *ggsci* v2.9, 39 *ggpubr* v0.5.0, 40 *reshape2* v1.4.4, 41 and *cowplot* v1.1.1. 42

Concerning patient and sample-related variables, continuous and categorical variables were presented as medians with the interquartile range (IQR) and n (%) respectively, where "n" corresponds to the number of non-missing observations.

Predictors of CEFtot trough concentration were examined through linear mixed effects models using Satterthwaite's degrees of freedom for p-value computation, considering multiple measurements for the same patient. Subsequently, all predictors with a p-value < 0.05 were integrated into a full model. Irrelevant variables were eliminated from the full model using backward variable selection, guided by the Akaike Information Criterion.

Analysis of variance (ANOVA), followed by Tukey's Honestly Significant Differences method for post hoc pairwise comparison, was conducted to assess the impact of the main ceftriaxone dosing regimen on CEFtot trough concentrations. PTA was then calculated for each model, stratified by dosing regimen, across various minimum inhibitory concentration (MIC) values ranging from 0.125 to 32 mg/L.

Statistical analyses and graphs for the retrospective analysis were performed using R v4.2.2, 37 and the following packages: *forcats* v1.0.0, 43 *dplyr* v1.1.2, 44 *gtsummary* v1.7.2, 45 *RcolorBrewer* v1.1.3, 46 *ggcorrplot* v0.1.4, 47 *exact2x2* v1.6.8, 48 *gt* v0.9.0, 49 *multcomp* v1.4.25 50 and *lmerTest* v3.1.3. 51

Raw data and R code are available in the following public repository: <https://github.com/ThomasDuflot/Ceftriaxone-AAC>. In order to maintain patient privacy; age, sex, admission dates and co-morbidities have been removed from the raw data of studied population and considered as “NA”.

**Acknowledgements:**

Authors would like to acknowledge technical staff from the Department of Pharmacology as well as nurses involved in the care of all patients included in this study.

**Funding:**

This research was not supported by any public, commercial or non-profit funding organization.

**Transparency declarations:**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**References :**

1. Kollef MH, Shorr AF, Bassetti M, *et al.* Timing of antibiotic therapy in the ICU. *Crit Care* 2021; **25**: 360.

2. Stoeckel K, McNamara PJ, Brandt R, Plozza-Nottebrock H, Ziegler WH. Effects of concentration-dependent plasma protein binding on ceftriaxone kinetics. *Clin Pharmacol Ther* 1981; **29**: 650–7.

3. Stoeckel K. Pharmacokinetics of Rocephin®, a Highly Active New Cephalosporin with an Exceptionally Long Biological Half-Life. *Chemotherapy* 2009; **27**: 42–6.

4. Lizza BD, Raush N, Micek ST. Antibiotic Optimization in the Intensive Care Unit. *Semin Respir Crit Care Med* 2022; **43**: 125–30.

5. De Paepe P, Belpaire FM, Buylaert WA. Pharmacokinetic and pharmacodynamic considerations when treating patients with sepsis and septic shock. *Clin Pharmacokinet* 2002; **41**: 1135–51.

6. Novy E, Martinière H, Roger C. The Current Status and Future Perspectives of Beta-Lactam Therapeutic Drug Monitoring in Critically Ill Patients. *Antibiotics (Basel)* 2023; **12**: 681.

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

8. Lode H, File TM, Mandell L, *et al.* Oral gemifloxacin versus sequential therapy with intravenous ceftriaxone/oral cefuroxime with or without a macrolide in the treatment of patients hospitalized with community-acquired pneumonia: a randomized, open-label, multicenter study of clinical efficacy and tolerability. *Clin Ther* 2002; **24**: 1915–36.

9. Serafino Wani RL, Filson SA, Chattaway MA, Godbole G. Invasive shigellosis in MSM. *Int J STD AIDS* 2016; **27**: 917–9.

10. Rambaud A, Gaborit BJ, Deschanvres C, *et al.* Development and validation of a dosing nomogram for amoxicillin in infective endocarditis. *J Antimicrob Chemother* 2020; **75**: 2941–50.

11. Nathan BR, Scheld WM. The efficacy of trovafloxacin versus ceftriaxone in the treatment of experimental brain abscess/cerebritis in the rat. *Life Sci* 2003; **73**: 1773–82.

12. Marsot A. Population pharmacokinetic models of first choice beta-lactam antibiotics for severe infections treatment: What antibiotic regimen to prescribe in children? *J Pharm Pharm Sci* 2020; **23**: 470–85.

13. Perry TR, Schentag JJ. Clinical use of ceftriaxone: a pharmacokinetic-pharmacodynamic perspective on the impact of minimum inhibitory concentration and serum protein binding. *Clin Pharmacokinet* 2001; **40**: 685–94.

14. Simon N, Dussol B, Sampol E, *et al.* Population pharmacokinetics of ceftriaxone and pharmacodynamic considerations in haemodialysed patients. *Clin Pharmacokinet* 2006; **45**: 493–501.

15. Wang Y-K, Wu Y-E, Li X, *et al.* Optimal Dosing of Ceftriaxone in Infants Based on a Developmental Population Pharmacokinetic-Pharmacodynamic Analysis. *Antimicrob Agents Chemother* 2020; **64**: e01412-20.

16. Blumer JL, Reed MD, Kaplan EL, Drusano GL. Explaining the poor bacteriologic eradication rate of single-dose ceftriaxone in group a streptococcal tonsillopharyngitis: a reverse engineering solution using pharmacodynamic modeling. *Pediatrics* 2005; **116**: 927–32.

17. Tsai D, Stewart P, Goud R, *et al.* Total and unbound ceftriaxone pharmacokinetics in critically ill Australian Indigenous patients with severe sepsis. *Int J Antimicrob Agents* 2016; **48**: 748–52.

18. Meletiadis J, Turlej-Rogacka A, Lerner A, *et al.* Amplification of Antimicrobial Resistance in Gut Flora of Patients Treated with Ceftriaxone. *Antimicrob Agents Chemother* 2017; **61**: e00473-17.

19. Tang Girdwood S, Dong M, Tang P, *et al.* Population Pharmacokinetic Modeling of Total and Free Ceftriaxone in Critically Ill Children and Young Adults and Monte Carlo Simulations Support Twice Daily Dosing for Target Attainment. *Antimicrob Agents Chemother* 2022; **66**: e0142721.

20. Meenks SD, le Noble JLML, Foudraine NA, de Vries F, Neef K, Janssen PKC. Population pharmacokinetics of unbound ceftriaxone in a critically ill population. *Int J Clin Pharmacol Ther* 2022; **60**: 373–83.

21. Cheng V, Abdul-Aziz MH, Burrows F, *et al.* Population Pharmacokinetics and Dosing Simulations of Ceftriaxone in Critically Ill Patients Receiving Extracorporeal Membrane Oxygenation (An ASAP ECMO Study). *Clin Pharmacokinet* 2022; **61**: 847–56.

22. Standing JF, Ongas MO, Ogwang C, *et al.* Dosing of Ceftriaxone and Metronidazole for Children With Severe Acute Malnutrition. *Clin Pharmacol Ther* 2018; **104**: 1165–74.

23. Bos JC, Prins JM, Mistício MC, *et al.* Pharmacokinetics and pharmacodynamic target attainment of ceftriaxone in adult severely ill sub-Saharan African patients: a population pharmacokinetic modelling study. *J Antimicrob Chemother* 2018; **73**: 1620–9.

24. Leegwater E, Kraaijenbrink BVC, Moes DJ a. R, Purmer IM, Wilms EB. Population pharmacokinetics of ceftriaxone administered as continuous or intermittent infusion in critically ill patients. *J Antimicrob Chemother* 2020; **75**: 1554–8.

25. Heffernan AJ, Curran RA, Denny KJ, *et al.* Ceftriaxone dosing in patients admitted from the emergency department with sepsis. *Eur J Clin Pharmacol* 2021; **77**: 207–14.

26. Hartman SJF, Upadhyay PJ, Hagedoorn NN, *et al.* Current Ceftriaxone Dose Recommendations are Adequate for Most Critically Ill Children: Results of a Population Pharmacokinetic Modeling and Simulation Study. *Clin Pharmacokinet* 2021; **60**: 1361–72.

27. Dreesen E, Gijsen M, Elkayal O, *et al.* Ceftriaxone dosing based on the predicted probability of augmented renal clearance in critically ill patients with pneumonia. *J Antimicrob Chemother* 2022; **77**: 2479–88.

28. Grégoire M, Dailly E, Le Turnier P, *et al.* High-Dose Ceftriaxone for Bacterial Meningitis and Optimization of Administration Scheme Based on Nomogram. *Antimicrob Agents Chemother* 2019; **63**: e00634-19.

29. Ulldemolins M, Bastida C, Llauradó-Serra M, *et al.* Once-daily 1 g ceftriaxone optimizes exposure in patients with septic shock and hypoalbuminemia receiving continuous veno-venous hemodiafiltration. *Eur J Clin Pharmacol* 2021; **77**: 1169–80.

30. Gijsen M, Dreesen E, Van Daele R, *et al.* Pharmacokinetic/Pharmacodynamic Target Attainment Based on Measured versus Predicted Unbound Ceftriaxone Concentrations in Critically Ill Patients with Pneumonia: An Observational Cohort Study. *Antibiotics (Basel)* 2021; **10**: 557.

31. Sanz-Codina M, Wicha SG, Wulkersdorfer B, *et al.* Comparison of ultrafiltration and microdialysis for ceftriaxone protein-binding determination. *J Antimicrob Chemother* 2023; **78**: 380–8.

32. Metsu D, Lanot T, Fraissinet F, *et al.* Comparing ultrafiltration and equilibrium dialysis to measure unbound plasma dolutegravir concentrations based on a design of experiment approach. *Sci Rep* 2020; **10**: 12265.

33. Heffernan AJ, Sime FB, Kumta N, *et al.* Multicenter Population Pharmacokinetic Study of Unbound Ceftriaxone in Critically Ill Patients. *Antimicrob Agents Chemother* 2022; **66**: e0218921.

34. Alasmari F, Alasmari MS, Muwainea HM, *et al.* Physiologically-based pharmacokinetic modeling for single and multiple dosing regimens of ceftriaxone in healthy and chronic kidney disease populations: a tool for model-informed precision dosing. *Front Pharmacol* 2023; **14**: 1200828.

35. Anon. Comité de l’Antibiograme de la Société Française de Microbiologie. *Société Française de Microbiologie*. Available at: https://www.sfm-microbiologie.org/boutique/comite-de-lantibiograme-de-la-sfm-casfm/. Accessed February 24, 2023.

36. Pressat-Laffouilhère T, Balayé P, Dahamna B, *et al.* Evaluation of Doc’EDS: a French semantic search tool to query health documents from a clinical data warehouse. *BMC Med Inform Decis Mak* 2022; **22**: 34.

37. Anon. R Core Team (2022) R A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. - References - Scientific Research Publishing. Available at: https://www.scirp.org/(S(lz5mqp453ed%20snp55rrgjct55))/reference/referencespapers.aspx?referenceid=3456808. Accessed September 13, 2023.

38. Wickham H. *ggplot2*. Cham: Springer International Publishing; 2016. Available at: http://link.springer.com/10.1007/978-3-319-24277-4. Accessed September 3, 2023.

39. Xiao [aut N, cre, Cook J, Jégousse C, Li M. ggsci: Scientific Journal and Sci-Fi Themed Color Palettes for ‘ggplot2’. 2023. Available at: https://cran.r-project.org/web/packages/ggsci/. Accessed September 13, 2023.

40. Kassambara A. ggpubr: ‘ggplot2’ Based Publication Ready Plots. 2023. Available at: https://cran.r-project.org/web/packages/ggpubr/index.html. Accessed September 4, 2023.

41. Wickham H. Reshaping Data with the reshape Package. *Journal of Statistical Software* 2007; **21**: 1–20.

42. Wilke CO. cowplot: Streamlined Plot Theme and Plot Annotations for ‘ggplot2’. 2020. Available at: https://cran.r-project.org/web/packages/cowplot/index.html. Accessed September 4, 2023.

43. Wickham H, RStudio. forcats: Tools for Working with Categorical Variables (Factors). 2023. Available at: https://cran.r-project.org/web/packages/forcats/index.html. Accessed September 4, 2023.

44. Wickham H, François R, Henry L, *et al.* dplyr: A Grammar of Data Manipulation. 2023. Available at: https://cran.r-project.org/web/packages/dplyr/index.html. Accessed September 4, 2023.

45. Sjoberg DD, Whiting K, Curry M, Lavery JA, Larmarange J. Reproducible Summary Tables with the gtsummary Package. *The R Journal* 2021; **13**: 570–80.

46. Neuwirth E. RColorBrewer: ColorBrewer Palettes. 2022. Available at: https://cran.r-project.org/web/packages/RColorBrewer/index.html. Accessed September 4, 2023.

47. Kassambara A, Patil (@patilindrajeets) I. ggcorrplot: Visualization of a Correlation Matrix using ‘ggplot2’. 2022. Available at: https://cran.r-project.org/web/packages/ggcorrplot/index.html. Accessed September 4, 2023.

48. Fay MP. Two-sided Exact Tests and Matching Confidence Intervals for Discrete Data. *The R Journal* 2010; **2**: 53–8.

49. Iannone R, Cheng J, Schloerke B, *et al.* gt: Easily Create Presentation-Ready Display Tables. 2023. Available at: https://cran.r-project.org/web/packages/gt/index.html. Accessed September 4, 2023.

50. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biom J* 2008; **50**: 346–63.

51. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* 2017; **82**: 1–26.

**TABLE 1:** Description of the different formulas used to predict unbound ceftriaxone (CEFu) from total ceftriaxone (CEFt) concentrations.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Model** | **Population** | **CEFu** | **Formula** | **Parameter values** | **Reference** |
| **A** | Severely ill sub-Saharan African adults (N=88 patients, 277 samples for CEFt and 276 samples for CEFu). | UF |  | mM  mM | Bos et al23 |
| **B** | Children with severe acute malnutrition (N=81 children, 244 samples for CEFt and CEFu) | UF |  | mg/L  mg/L | Standing et al22 |
| **C** | Adults with suspected or proven bacterial meningitis (N=153 patients, 301 samples for CEFt and 214 for CEFu) | UF |  |  | Gregoire et al28 |
| **D** | Critically ill adults (N=55 patients, 110 samples for CEFt and CEFu) | - |  | mg/L  mg/L | Leegwater et al24 |
| **E** | Critically ill adults with pneumonia (N=31 patients, 72 samples for CEFt and CEFu) | ED |  | mM  mM | Gijsen et al30 |
| **F** | Critically ill children (N=45 patients, 205 samples for CEFt and 45 samples for CEFu) | UF |  | mg/L  mg/L | Hartman et al26 |
| **G** | Adults with septic shock, hypoalbuminemia and hemodiafiltration (N=50 patients, 50 samples for CEFt and CEFu) | UF |  |  | Ulldemolins et al29 |
| **H** | Critically ill adults with augmented clearence (N=33 patients, 259 samples for CEFt and 76 for CEFu) | ED |  | mM | Dreesen et al27 |
| **I** | Critically ill adults (N=36 patients, 267 samples for CEFt and 207 samples for CEFu) | UF |  | mg/L  mg/L | Heffernan et al25 |

ALB: Albuminemia, Bmax: Maximum binding capacity , CEFt: Total ceftriaxone, CEFu: Unbound Ceftriaxone, ED: Equilibrium dialysis, fu: fraction unbound, Kd: Dissociation constant, Koff: Dissociation rate constant, Kon: Association rate constant, UF: Ultrafiltration.

**TABLE 2:** Total ceftriaxone thresholds for a MIC of 1 mg/L in case of normal albumin concentration (35 g/L) and hypoalbuminemia (20g/L).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Model** |  | **fT > MIC 100%** | |  | **fT > 4 x MIC 100%** | |
|  | **Normal albumin concentration** | **Hypoalbuminemia (% decrease)** |  | **Normal albumin concentration** | **Hypoalbuminemia (% decrease)** |
| **Bos** |  | 15.7 | 8.1 (-48%) |  | 43.3 | 23.0 (-46%) |
| **Dreesen** |  | 8.5 | 5.3 (-38%) |  | 31.0 | 19.5 (-37%) |
| **Gijsen** |  | 16.9 | 9.1 (-46%) |  | 36.0 | 23.3 (-35%) |
| **Gregoire** |  | 15.1 | 15.1 (0%) |  | 50.1 | 50.1 (0%) |
| **Hartman** |  | 10.3 | 6.3 (-39%) |  | 37.8 | 23.3 (-38%) |
| **Heffernan** |  | 16.6 | 10.0 (-40%) |  | 56.2 | 33.8 (-40%) |
| **Leegwater** |  | 12.3 | 6.8 (-45%) |  | 40.2 | 22.7 (-44%) |
| **Standing** |  | 4.0 | 1.8 (-55%) |  | 14.6 | 6.6 (-55%) |
| **Ulldemolins** |  | 3.3 | 2.0 (-39%) |  | 13.1 | 7.9 (-40%) |

fT: Fraction of time, MIC: Minimal inhibitory concentration.

**TABLE 3:** MSE, MPE, RMSE, RMSE of percentage error and R² of the external validation (N=62)

| **Model** | **Mean Signed Error (MSE)** | **Mean Percentage Error (MPE)** | **Root Mean Square Error (RMSE)** | **RMSE of Percentage error** | **R-squared (R²)** |
| --- | --- | --- | --- | --- | --- |
| Bos | -18.32 | -110.81 | 30.34 | 168.67 | 0.76 |
| Dreesen | -4.63 | -56.39 | 9.47 | 83.45 | 0.82 |
| Gijsen | -2.52 | -38.11 | 9.72 | 77.19 | 0.75 |
| Gregoire | 6.63 | 31.29 | 13.90 | 43.74 | 0.66 |
| Hartmann | 0.13 | -21.92 | 7.94 | 52.42 | 0.84 |
| Heffernan | 3.21 | 14.55 | 8.17 | 37.00 | 0.86 |
| Leegwater | -8.76 | -59.20 | 16.71 | 95.97 | 0.81 |
| Standing | -28.79 | -255.08 | 39.10 | 298.03 | 0.71 |
| Ulldemolins | -15.93 | -205.95 | 19.07 | 246.80 | 0.73 |

**TABLE 4:** Patient and dosing characteristics.

| **Patient related variables** | **n** | **Overall**  **(N=222)** |  | **Dosing related**  **variables** | **n** | **Overall**  **(N=408)** |
| --- | --- | --- | --- | --- | --- | --- |
| **Age**, years | 222 | 74 [63 - 82] |  | **Total Ceftriaxone** (mg/L) | 408 | 69 [43 - 105] |
| **Sex** | 222 |  |  | **Albumin** (g/dL) | 376 | 27.0 [23.4 - 30.1] |
| Man |  | 139 (62.6%) |  | **Bilirubin** (µmol /L) | 403 | 6 [5 - 11] |
| **Department** | 221 |  |  | **Creatinine** (µmol/L) | 407 | 121 [81 - 202] |
| ID |  | 53 (24.0%) |  | **C-reactive protein** (mg/L) | 404 | 49 [22 - 97] |
| Medical ICU |  | 45 (20.4%) |  | **Neutrophils** (x109/L) | 408 | 6.3 [4.7 - 9.2] |
| Cardiology |  | 41 (18.6%) |  | **GGT** (U/L) | 404 | 92 [42 - 171] |
| Medicine |  | 22 (10.0%) |  | **Hemoglobin** (g/dL) | 408 | 9.5 [8.6 - 10.9] |
| Other |  | 18 (8.1%) |  | **Protein** (g/L) | 407 | 65 [60 - 69] |
| Geriatrics |  | 17 (7.7%) |  | **ASAT** (U/L) | 404 | 30 [22 - 43] |
| Nephrology |  | 11 (5.0%) |  | **ALAT** (U/L) | 404 | 24 [16 - 41] |
| HGE |  | 8 (3.6%) |  | **Urea** (mmol/L) | 407 | 11 [6 - 17] |
| Neurology |  | 6 (2.7%) |  | **Concomitant Antibiotics** | 404 |  |
| **BMI**, (kg/m²) | 166 | 28 [24 - 32] |  | Betalactams |  | 225 (55.7%) |
| **Diabetes** | 219 | 79 (36.1%) |  | Both |  | 71 (17.6%) |
| **Hypertension** | 220 | 134 (60.9%) |  | Other |  | 52 (12.9%) |
| **Kidney failure** | 220 | 54 (24.5%) |  | None |  | 50 (12.4%) |
| **Hepatic failure** | 220 | 15 (6.8%) |  | Aminoglycosides |  | 6 (1.5%) |
| **Heart failure** | 222 | 58 (26.1%) |  | **Dosing regimen** | 355 |  |
| **Infection** | 218 |  |  | 1g once a day |  | 87 (24.5%) |
| Endocarditis |  | 86 (39.4%) |  | 1g twice daily |  | 83 (23.4%) |
| Bacteremia |  | 44 (20.2%) |  | 2g twice daily |  | 79 (22.3%) |
| Other |  | 37 (17.0%) |  | 2g once daily |  | 75 (21.1%) |
| UTI |  | 24 (11.0%) |  | Other |  | 31 (8.7%) |
| Suspected |  | 14 (6.4%) |  |  |  |  |
| Pneumopathy |  | 13 (6.0%) |  |  |  |  |
| **Length of stay (days)** | 222 | 27 [17 - 46] |  |  |  |  |
| **Bacteria** | 183 |  |  |  |  |  |
| *E. faecalis* |  | 97 (53.0%) |  |  |  |  |
| Other |  | 37 (20.2%) |  |  |  |  |
| *E. coli* |  | 33 (18.0%) |  |  |  |  |
| *K. pneumoniae* |  | 9 (4.9%) |  |  |  |  |
| *S. pneumoniae* |  | 7 (3.8%) |  |  |  |  |

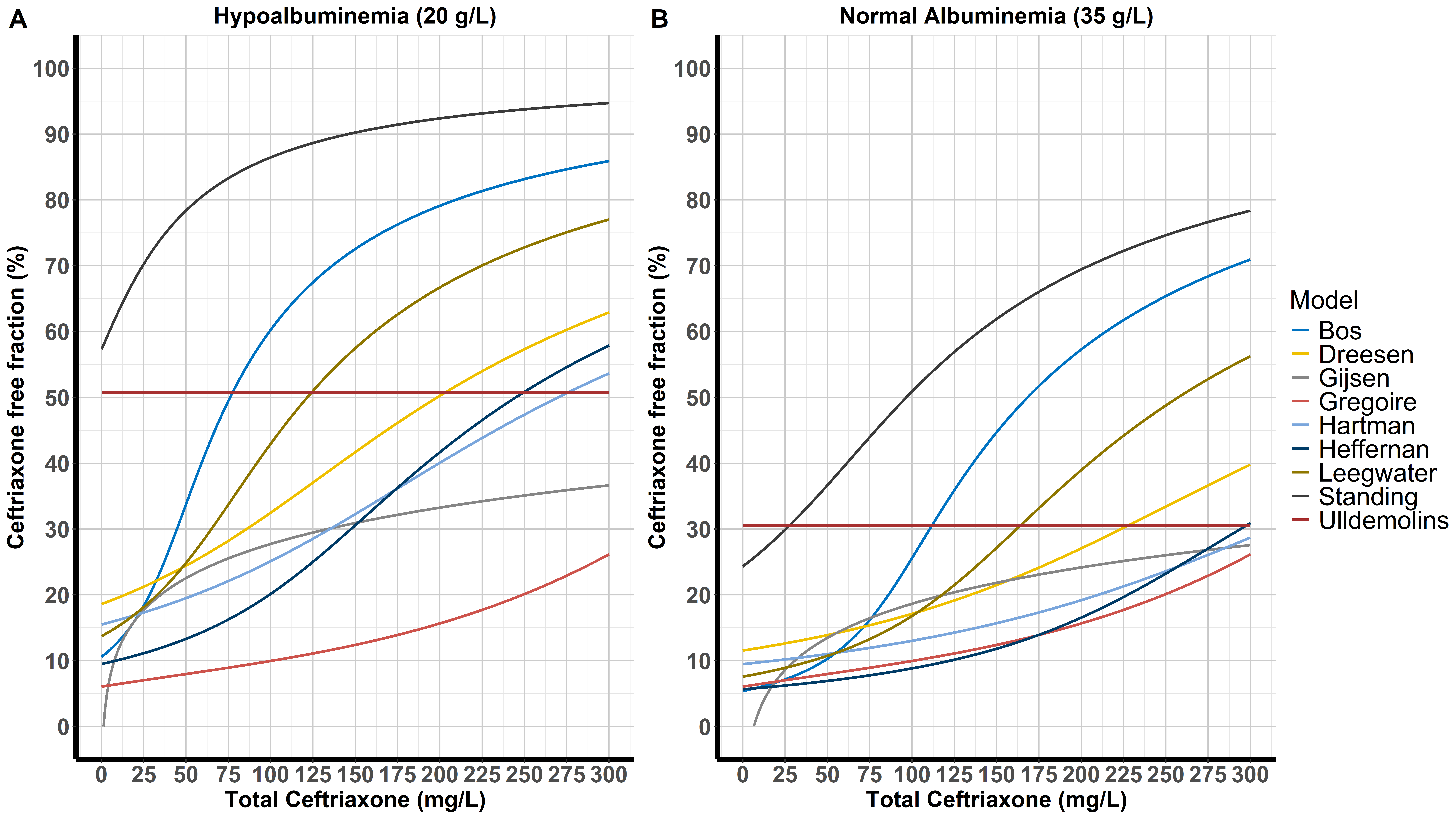
Data are expressed as median [IQR] for continuous variables and as n (%) for categorical variables. ALAT: L-alanine aminotransferase, ASAT: L-aspartate aminotransferase, BMI: Body mass index, *E. coli: Escherichia coli*, *E. faecalis: Enterococcus faecalis*, GGT: Gamma-Glutamyl Transferase, HGE: Hepato-gastro-enterology, ICU: Intensive care unit, ID: Infectious diseases, *K.pneumoniae: Klebsiella pneumoniae*, N: Total number of observations, n: Number of non-missing observations, *S. pneumoniae: Streptococcus pneumoniae*, UTI: urinary tract infection.

**TABLE 4:** Predictors of total ceftriaxone concentration.

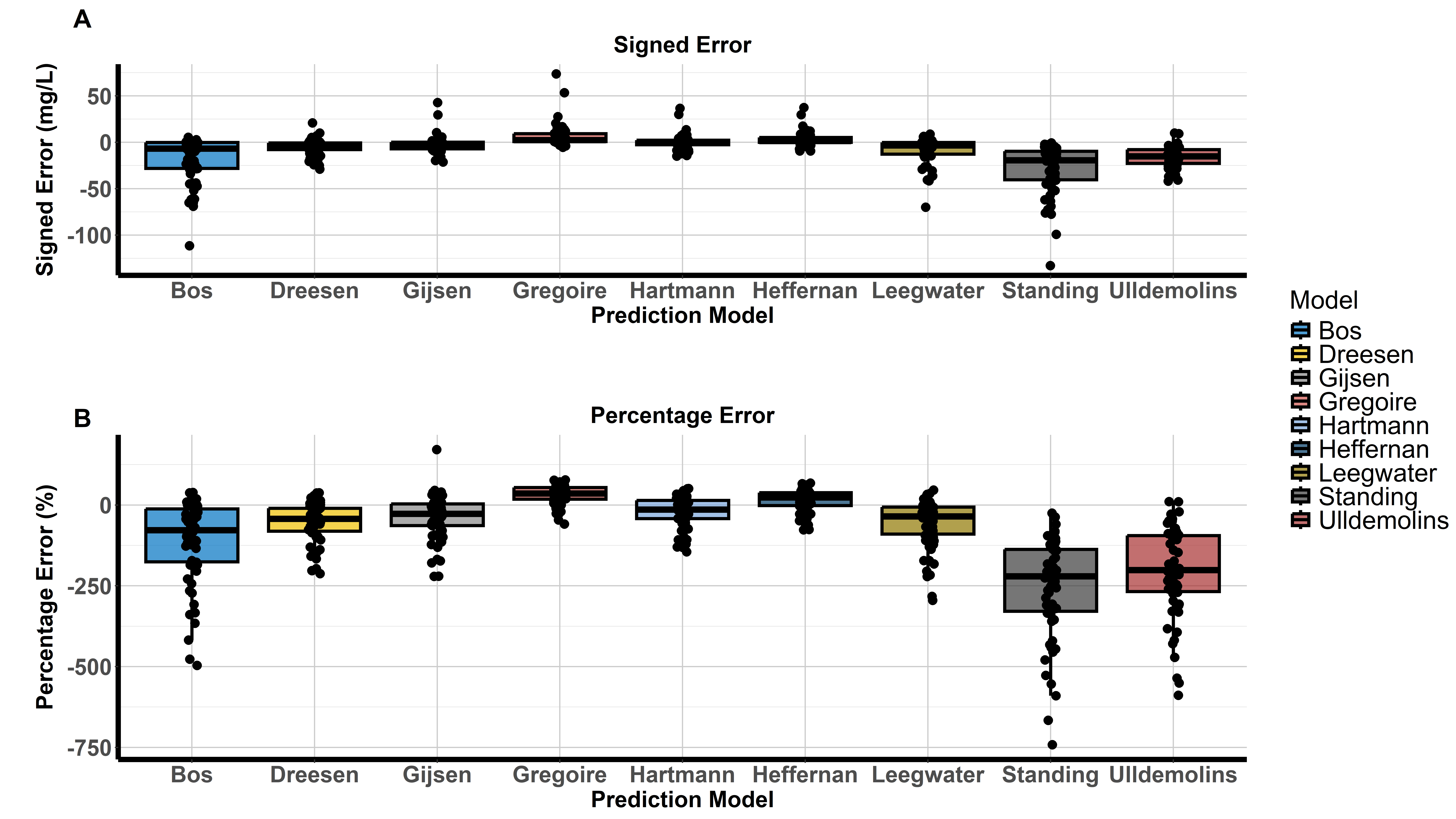
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Predictors | Unadjusteda | | Full modelb (N=325) | | Final modelc(N=325) | |
| **β ± s.e** | **P-valued** | **β ± s.e** | **P-valued** | **β ± s.e** | **P-valued** |
| Age (per year increase) | 0.445 ± 0.208 | **0.033** | 0.523 ± 0.229 | **0.024** | 0.618 ± 0.216 | **0.005** |
| Weight (per kilogram increase) | 0.336 ± 0.159 | 0.036 | 0.296 ± 0.162 | 0.068 |  |  |
| Sex (ref=woman) | 6.17 ± 6.49 | 0.342 |  |  |  |  |
| Diabetes (ref=no) | 10.65 ± 6.24 | 0.089 |  |  |  |  |
| Hypertension (ref=no) | 3.02 ± 6.25 | 0.630 |  |  |  |  |
| Intake dose (per gram increase) | 24.92 ± 4.88 | **<0.001** | -10.00 ± 7.39 | 0.177 |  |  |
| Daily dose (per gram increase) | 16.60 ± 1.95 | **0.001** | 20.38 ± 3.04 | **<0.001** | 17.57 ± 1.90 | **<0.001** |
| Albumin (per gram/L increase) | 1.86 ± 0.58 | **0.002** | 1.36 ± 0.60 | **0.023** | 1.53±0.57 | **0.009** |
| Bilirubin (per µmol/L increase) | -0.017 ± 0.07 | 0.818 |  |  |  |  |
| Creatininemia (per µmol/L increase) | 0.045 ± 0.020 | **0.021** | 0.100 ± 0.020 | **<0.001** | 0.108±0.020 | **<0.001** |
| Urea (per mmol/L increase) | 0.28 ± 0.30 | 0.346 |  |  |  |  |

a Simple linear mixed effects regression, b Multiple linear mixed effects regression for variables with P-value below 0.05, c Backward variable selection from the full model, d Satterthwaite's degrees of freedom for p-value computation. β: coefficient estimate, ref: reference, s.e: standard error.

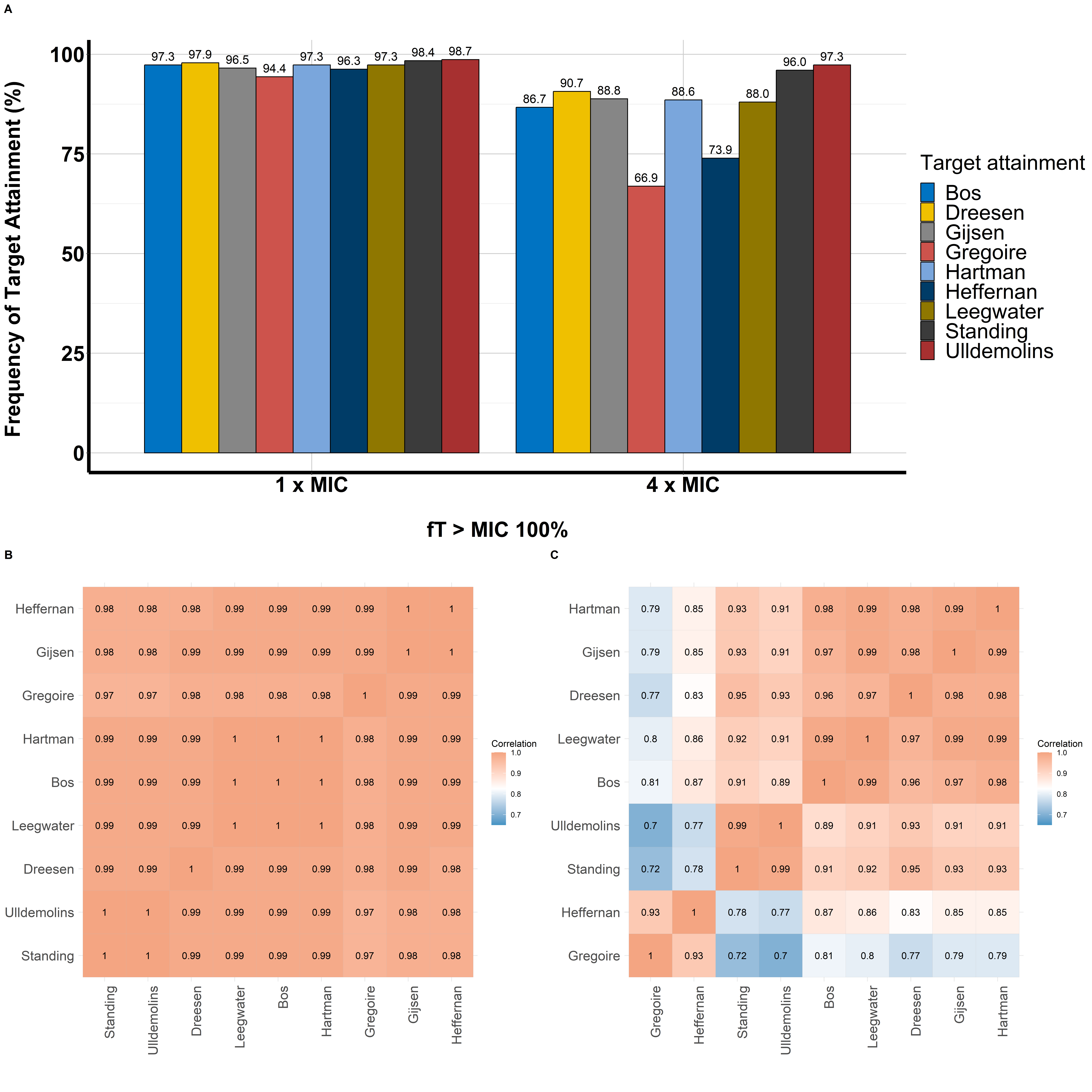




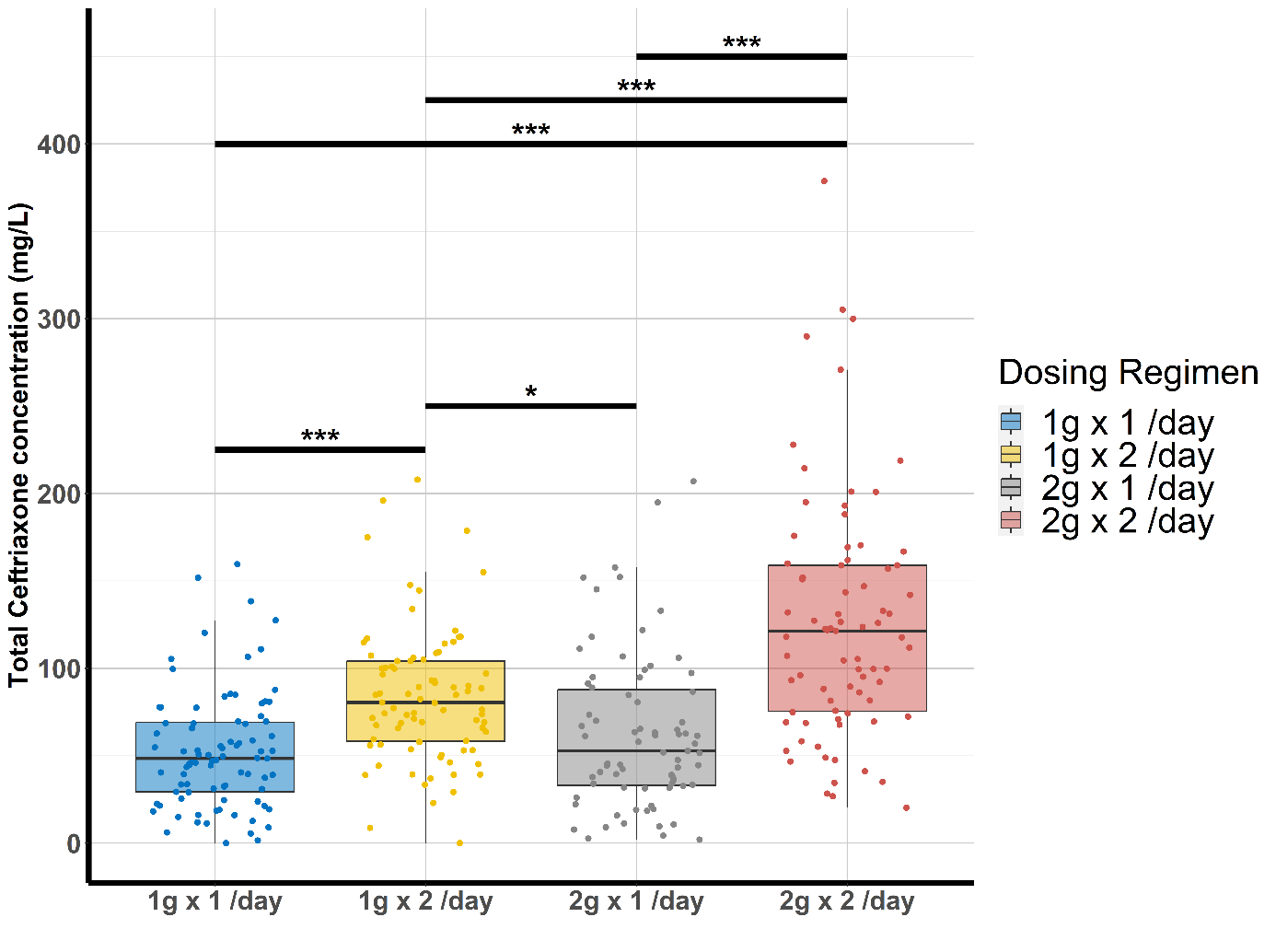
**Figure 1:** Modeling of ceftriaxone free fraction based on total ceftriaxone in the case of hypoalbuminemia (20 g/L - 1A) and normal albumin concentration (35 g/L – 1B).



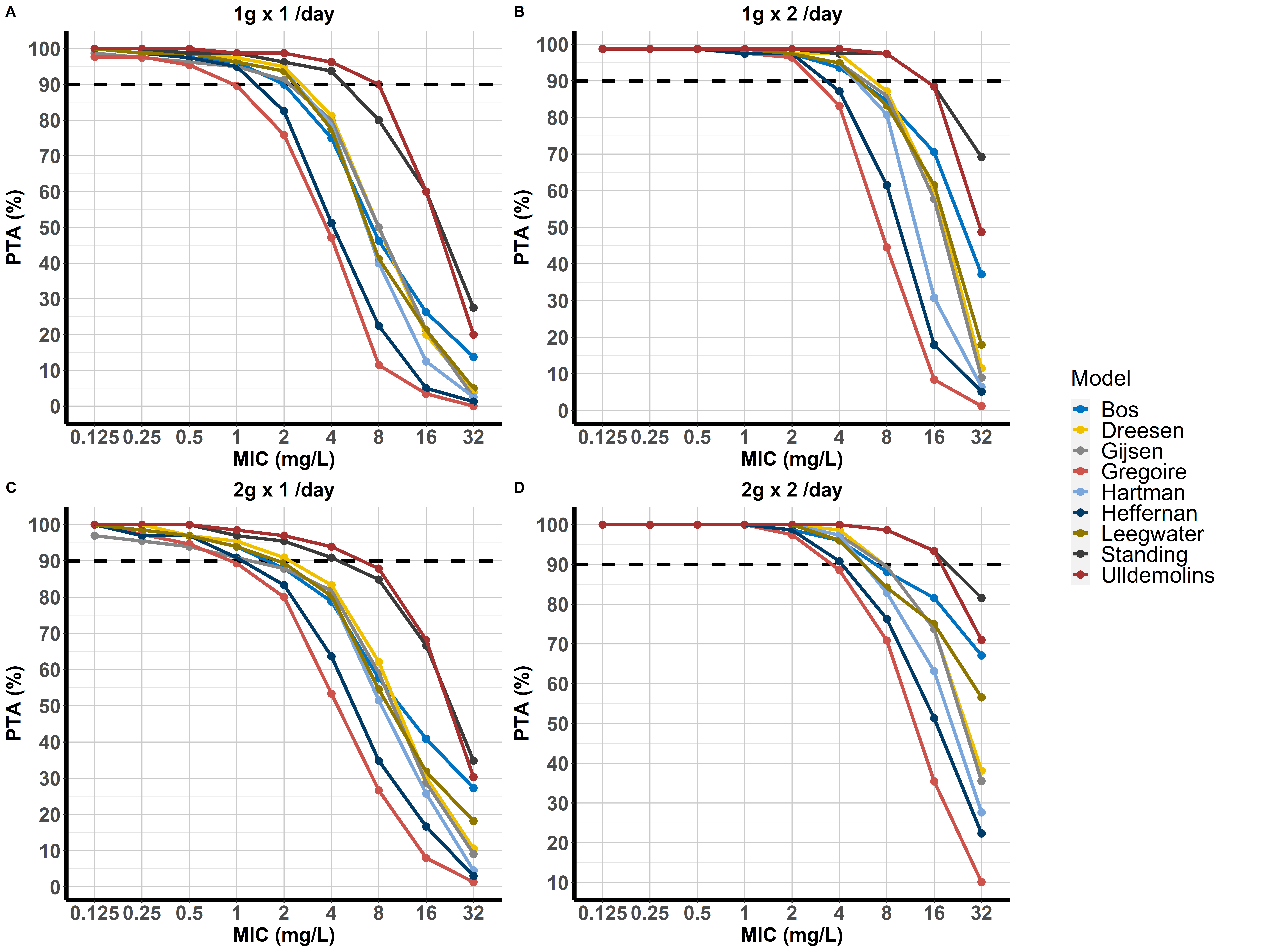
**Figure 2:** Signed **(A)** and percentage error **(B)** for the 9 studied model during external validation (N=62)

****

**Figure 3:** Frequency of target attainment for 1 x MIC and 4 x MIC using fT > MIC 100% according to the different models **(A)** and concordance matrix for 1 x MIC **(B)** and 4 x MIC **(C)**.



**Figure 4:** Boxplots of total ceftriaxone trough concentration (mg/L) according to dosing regimen. \*p<0.05, \*\*\*p<0.001. N=324 observations (87, 83, 75 and 79 observations respectively).



**Figure 5:** Probability of target attainment curves according to the different models and stratified by dosing regimen. N=87 for **(A)**, N=83 for **(B)**, N=75 for **(C)** and N=79 for **(D)**. Horizontal dotted lines indicate 90% PTA values. MIC: Minimum inhibitory concentration, PTA: Probability of target attainment (fT > MIC 100%).