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

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**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 analyzed 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 models 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) 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. (5) 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. (6) Such reductions may compromise time-dependent PD target of CEF.

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, (7–9) 5 lacked quantitation of CEFu (10–14) and 5 did not involve modeling of CEFu based on CEFtot. (15–19) Notably, one publication met the criteria but could not be used due to discrepancies in the PK parameters. (20) 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 (21–26) 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 (27) or an exponential approach. (28) Additionally, one publication devised its own transformation to predict CEFu from CEFtot.(29)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. (23) 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, 2.9–259.0 mg/L, and 0.14–94.70 mg/L, respectively.

Upon analysis, the models developed by Gregoire, Hartman, 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 quantitation (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 (20) while others exhibited inconsistencies, including discrepancies between the original manuscript and supplementary model. (21) 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. (30) 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. (31) It is also important to note that these analytical considerations may vary depending on the physico-chemical properties of the drug being studied.

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.

Interestingly, although the Gregoire, Heffernan, and Hartman models demonstrated satisfactory metrics during external validation, we observed differences in concordance between Hartman and the other two models. Gregoire and Heffernan formed a concordance group with high similarity (93%), while Hartman showed lower concordance—85% with Heffernan and 79% with Gregoire. This intriguing result may be attributed to Hartman’s higher MSE and MPE. Given the greater variability in MPE for the Hartman model, we hypothesize that the Gregoire and Heffernan models offer better predictive performance, with Heffernan being the strongest overall due to its lowest MPE, RMSE, RMSE%, and highest R².

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. (18, 26) 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. Of note, a population pharmacokinetic (popPK) analysis would allow a more thorough evaluation of covariates and CEFu estimation using nonlinear mixed-effects modeling. However, due to the retrospective nature of our data, the low number of patients with repeated ceftriaxone concentration measurements, and the heterogeneity of our patient population (encompassing both ICU and general ward patients), developing a popPK model for our cohort would likely introduce bias and yield inconclusive results. Despite this limitation, 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. (32, 33) 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.

**Total (CEFtot) and unbound (CEFu) quantitation**

*Chemicals and reagents*

Ceftriaxone and the internal standard ceftriaxone-d4 were purchased from Alsachim® (Illkirch-Graffenstaden - France). HPLC-grade methanol and water were supplied by Carlo Erba Reagents® (Val de Reuil, France). Centrifugal filter units (Amicon® Ultra 0.5 mL 30K) for CEFu determination were provided by Merck Millipore (Cork, Ireland).

*Sampling and analysis*

Blood samples for therapeutic drug monitoring (TDM) purposes were collected using dry collection tubes and were promptly subjected to centrifugation at 1,700 x *g* for 10 min. For the determination of CEFu, 500 µL of serum was processed through ultrafiltration (UF) utilizing centrifugal filter units at room temperature, following the manufacturer’s guidelines (centrifuged at 14,000 *x g* for 10 minutes). The resulting filtrates underwent the same sample preparation procedure as CEFtot. Detailed analytical procedures for quantifying both CEFtot and CEFu can be found in Supplementary Material S1.

**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, (34) 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, (35) 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**

Statistical analysis was performed using R software v4.4.1, (36) RStudio v2024.4.2.764, (37) and the following packages: *ggplot2* v3.5.1, (38) *ggsci* v3.2, (39) *ggpubr* v0.6.0, (40) *reshape2* v1.4.4, (41) *cowplot* v1.1.3, (42) *forcats* v1.0.0, (43) *dplyr* v1.1.4, (44) *flextable* v0.9.6, (45) *gridExtra* v2.3, (46) *gt* v0.11.1, (47) *gtsummary* v2.0.3, (48) *officer* v0.6.6, (49) *ggcorrplot* v0.1.4.1, (50) *exact2x2* v1.6.9, (51) *lmerTest* v3.1.3, (52) *RColorBrewer* v1.1.3 (53) and *multcomp* v1.4.26. (54)

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.

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”.

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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.

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Model** | **Population** | **CEFu** | **Formula** | **Parameter values** | **Reference** |
| **A** | Severely ill sub-Saharan African adults (N=88 patients, 277 samples for CEFtot and 276 samples for CEFu). | UF |  | mM  mM | Bos et al(22) |
| **B** | Children with severe acute malnutrition (N=81 children, 244 samples for CEFtot and CEFu) | UF |  | mg/L  mg/L | Standing et al(21) |
| **C** | Adults with suspected or proven bacterial meningitis (N=153 patients, 301 samples for CEFtot and 214 for CEFu) | UF |  |  | Gregoire et al(27) |
| **D** | Critically ill adults (N=55 patients, 110 samples for CEFtot and CEFu) | - |  | mg/L  mg/L | Leegwater et al(23) |
| **E** | Critically ill adults with pneumonia (N=31 patients, 72 samples for CEFtot and CEFu) | ED |  | mM  mM | Gijsen et al(29) |
| **F** | Critically ill children (N=45 patients, 205 samples for CEFtot and 45 samples for CEFu) | UF |  | mg/L  mg/L | Hartman et al(25) |
| **G** | Adults with septic shock, hypoalbuminemia and hemodiafiltration (N=50 patients, 50 samples for CEFtot and CEFu) | UF |  |  | Ulldemolins et al(28) |
| **H** | Critically ill adults with augmented clearence (N=33 patients, 259 samples for CEFtot and 76 for CEFu) | ED |  | mM | Dreesen et al(26) |
| **I** | Critically ill adults (N=36 patients, 267 samples for CEFtot and 207 samples for CEFu) | UF |  | mg/L  mg/L | Heffernan et al(24) |

ALB: Albuminemia, Bmax: Maximum binding capacity , CEFtot : 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% and R² of the external validation (N=62)

| **Model** | **Mean Signed Error (MSE)** | **Mean Percentage Error (MPE)** | **Root Mean Square Error (RMSE)** | **RMSE%** | **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 |
| Hartman | 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%) |  |  |  |  |

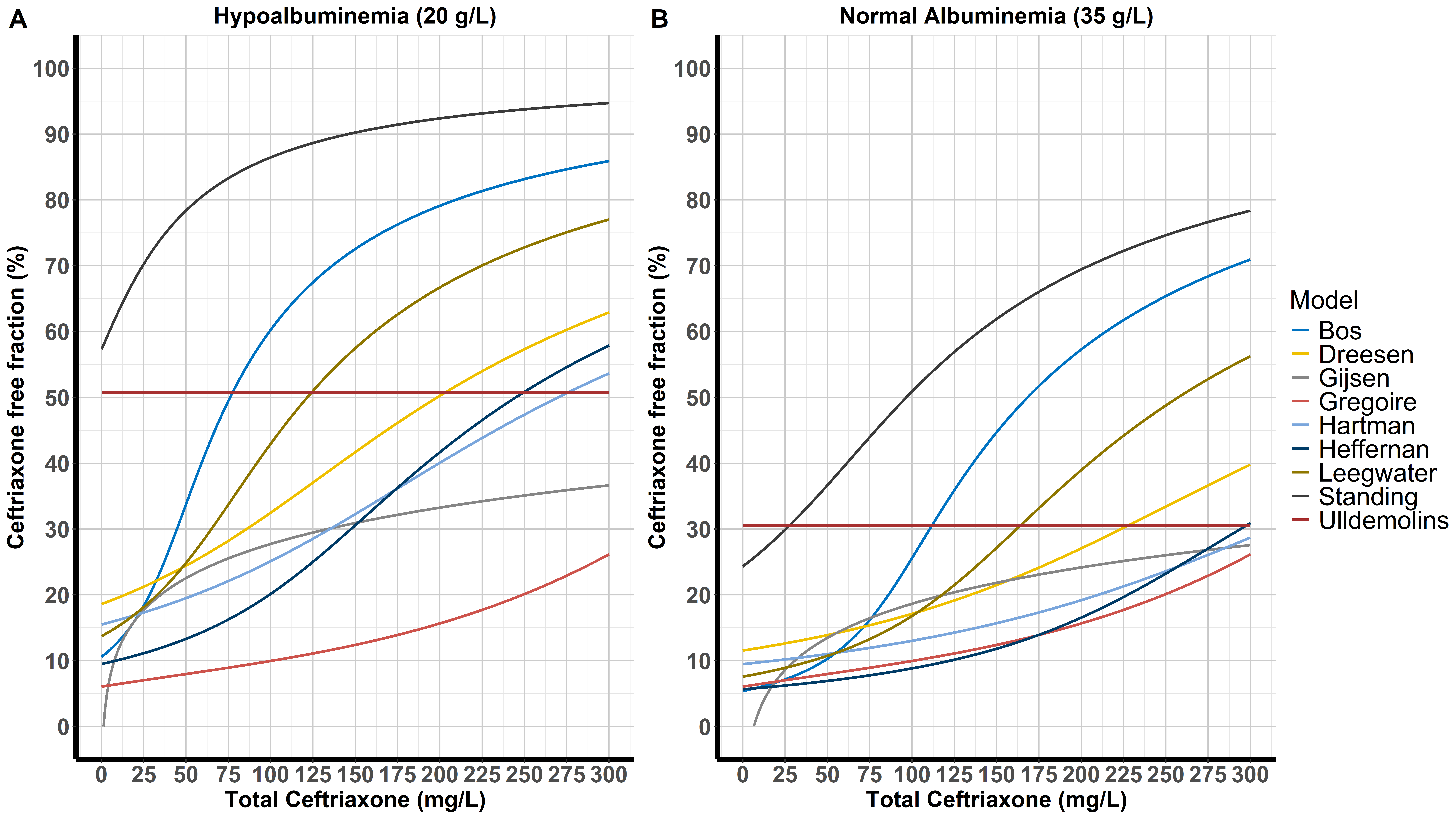
Data are expressed as median [IQR] for continuous variables and as n (%) for categorical variables. ALAT: L-alanine aminotransferase, ASAT: L-aspartate aminotransferase, BMI: Body mass index, *E. coli: Escherichia coli*, *E. faecalis: Enterococcus faecalis*, GGT: Gamma-Glutamyl Transferase, HGE: Hepato-gastro-enterology, 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.

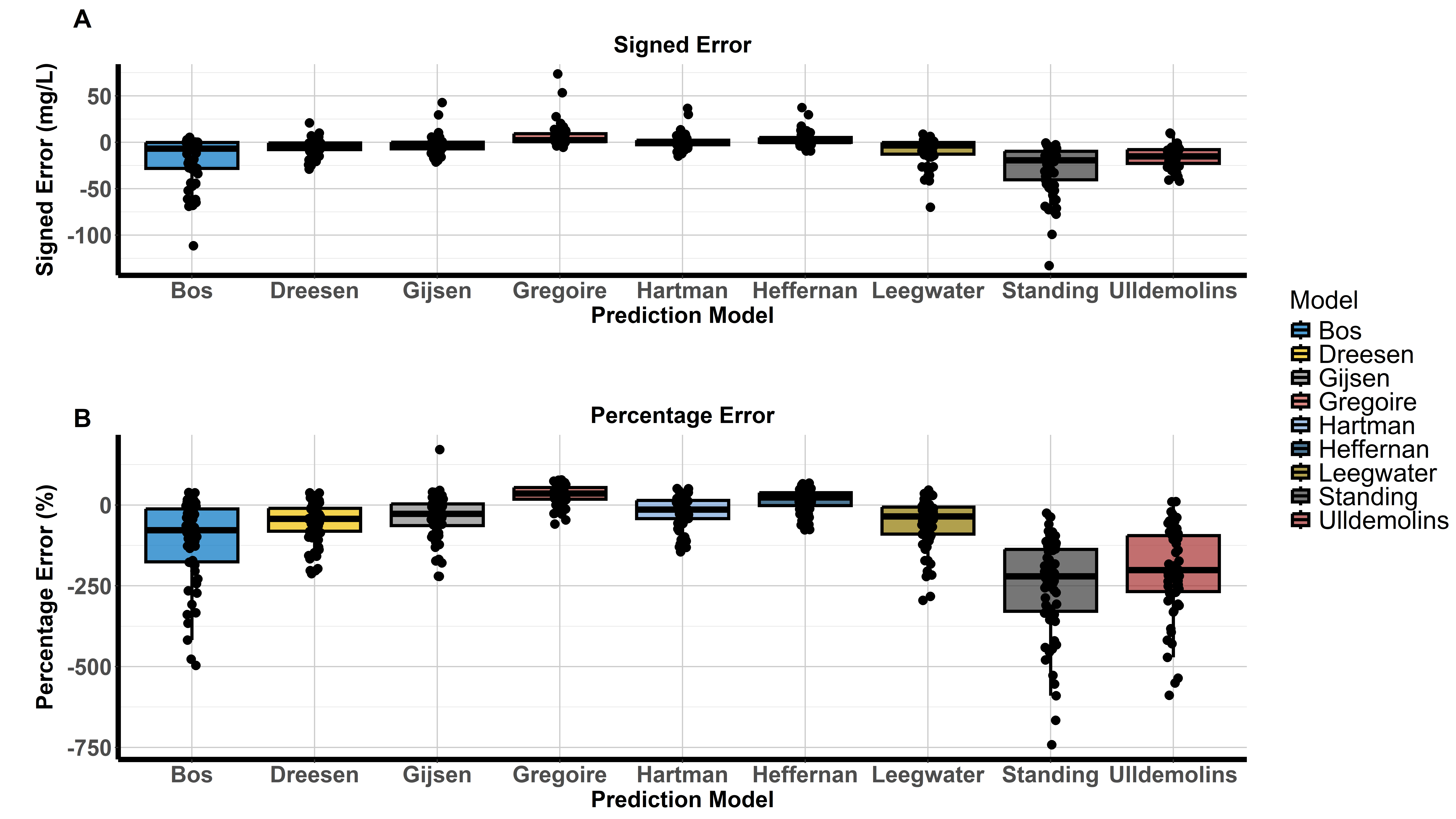
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Predictors | Unadjusteda | | Full modelb (N=325) | | Final modelc(N=325) | |
| **β ± s.e** | **P-valued** | **β ± s.e** | **P-valued** | **β ± s.e** | **P-valued** |
| Age (per year increase) | 0.445 ± 0.208 | **0.033** | 0.523 ± 0.229 | **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.

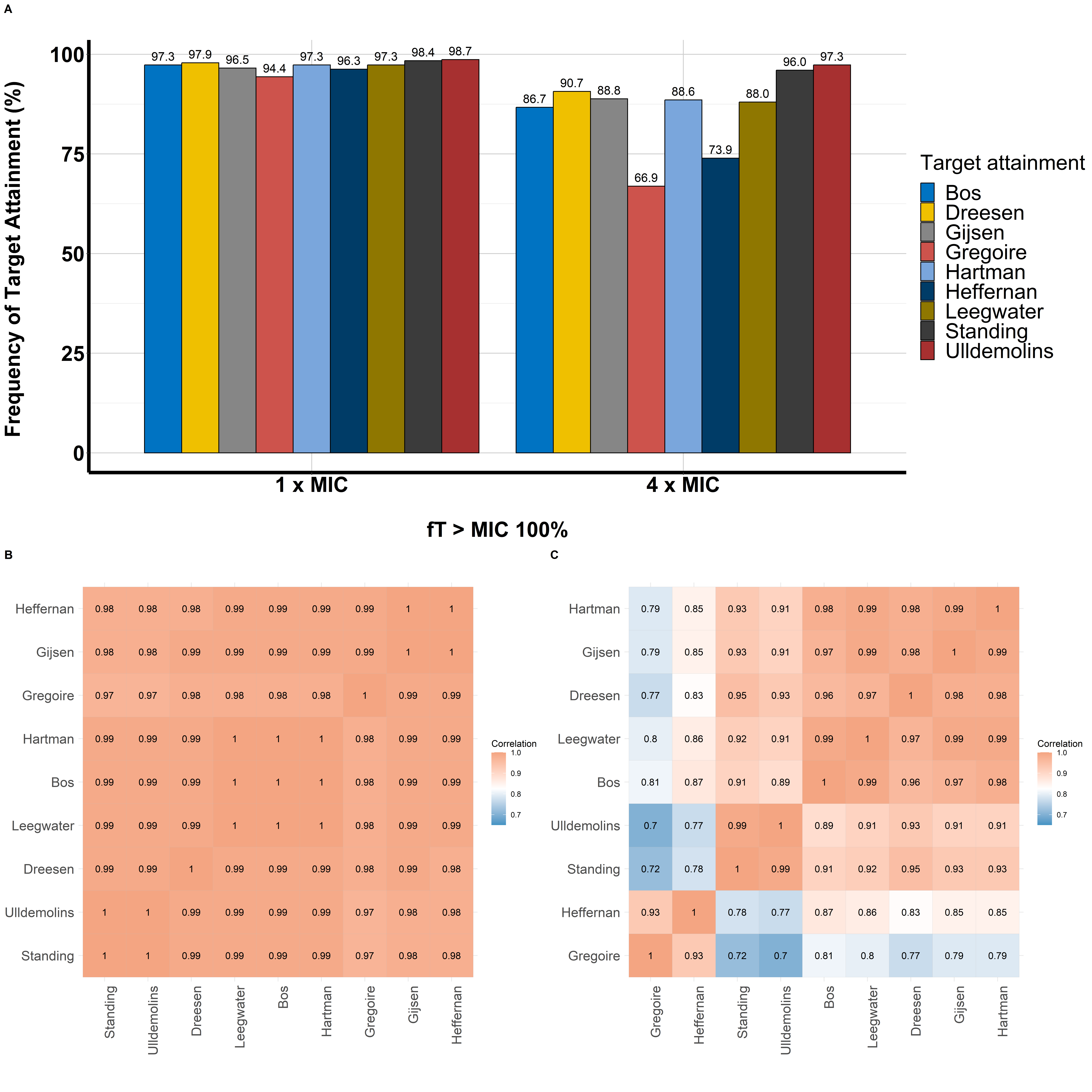




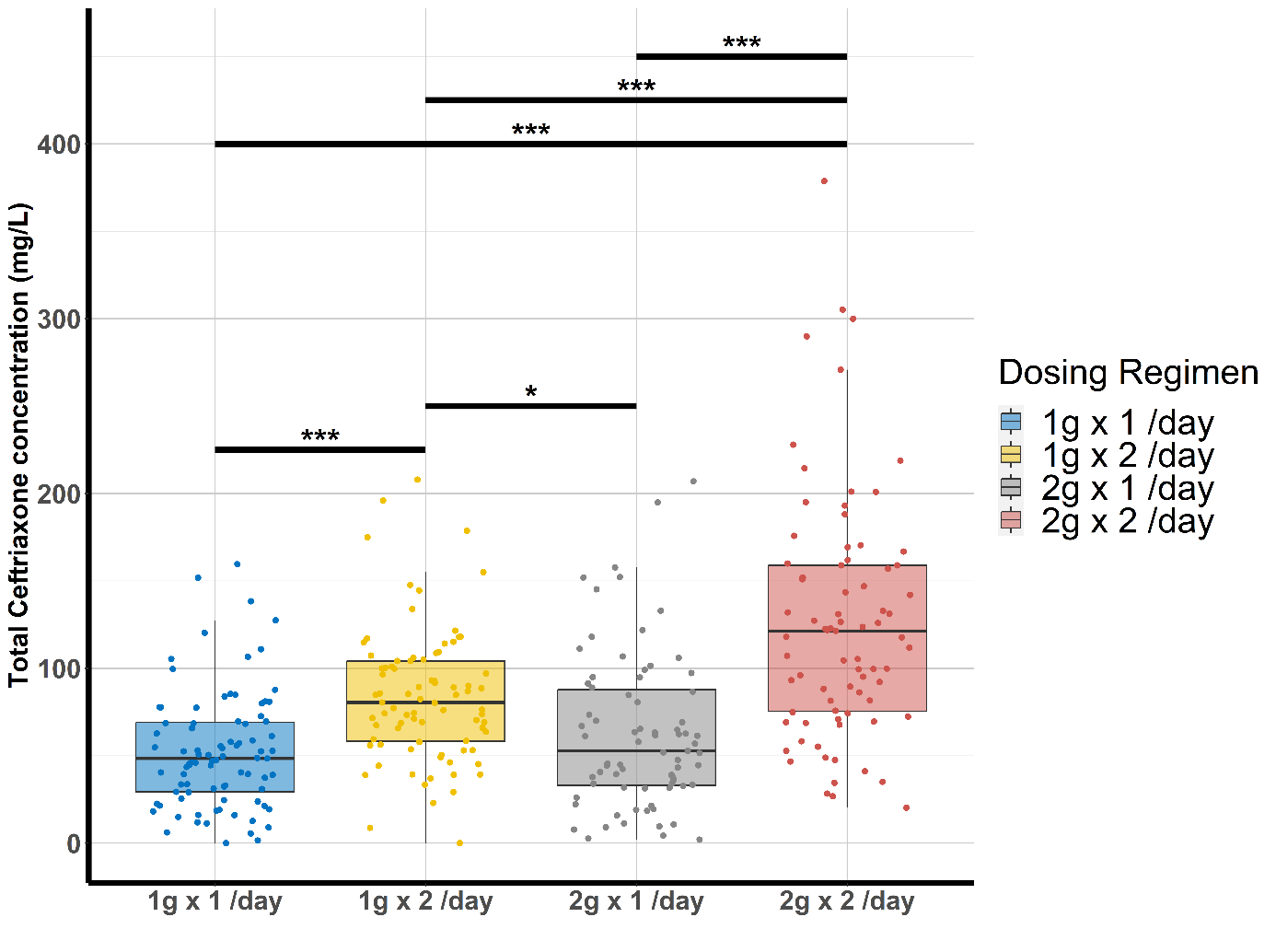
**Figure 1:** Modeling of ceftriaxone free fraction based on total ceftriaxone in the case of hypoalbuminemia (20 g/L - 1A) and normal albumin concentration (35 g/L – 1B).



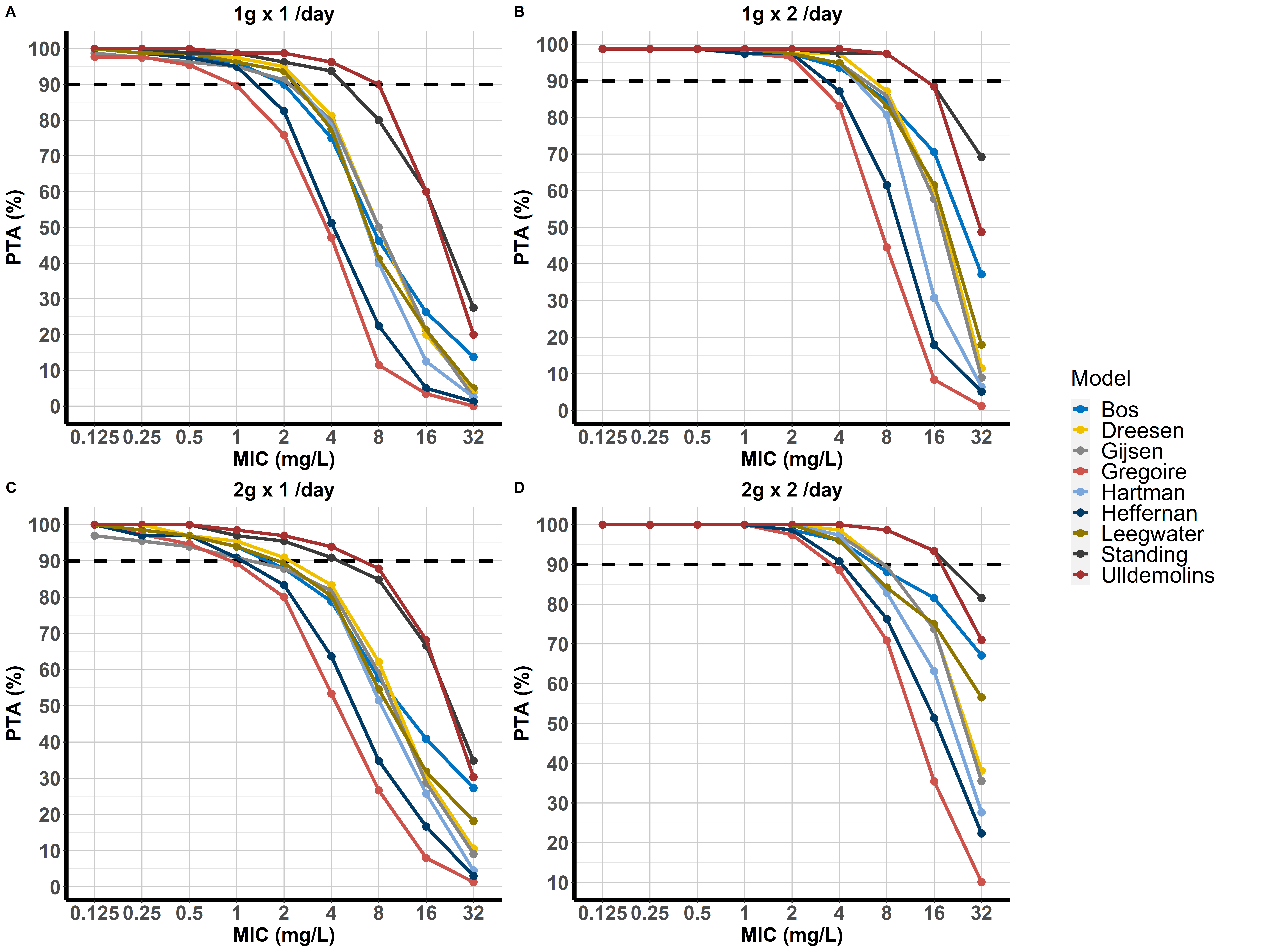
**Figure 2:** Signed **(A)** and percentage error **(B)** for the 9 studied model during external validation (N=62)

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**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%).