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ORIGINAL ARTICLE

Variable temocillin protein binding and pharmacokinetics in different clinical conditions: Implications for target attainment

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Abstract

Aims: The beta-lactam antibiotic temocillin is increasingly used to treat extended-spectrum beta-lactamase (ESBL-producing) strains; however, its protein binding is complex. This study aims to predict unbound temocillin concentrations in various participant groups to determine its impact on the probability of target attainment (PTA) and to improve dosing recommendations.

Methods: The plasma pharmacokinetics were analysed using non-linear mixed-effects modelling. Data from individuals in four groups: healthy volunteers (HV), urinary tract infection patients (UTI), ventriculitis patients and sepsis-ICU patients were included. Simulations were performed to compare the PTA for different dosing regimens and participant-groups.

Results: A two-compartment protein-binding model best fitted the 1085 concentrations (543 unbound, 542 total). Temocillin clearance was influenced by creatinine clearance, serum albumin (ALB) and C-reactive protein (CRP). For 2 g q8h intermittent infusion, the PTAs at an MIC of 16 mg/L were 2.3%, 39.5%, 10.0% and 72.5%, for HV, UTI, ventriculitis and sepsis-ICU patients, respectively. The effects of the covariates on the PTA were simulated for two example patients with intermittent infusion: the PTAs at an MIC of 8 mg/L for a sepsis-ICU patient (CRP 300 mg/L, albumin 15 g/L) and a mild-UTI patient (CRP 30 mg/L, albumin 35 g/L) were 94.3% and 62.4%, respectively. Continuous infusion consistently outperformed intermittent infusion in achieving the desired pharmacodynamic target (time above MIC).

Conclusions: Our study underscores the significant variation in temocillin clearance and unbound fractions among different participant groups, challenging the efficacy of traditional 2 g q12h dosing. For patients with enhanced renal function and lower inflammation, continuous infusion emerges as a more effective strategy to achieve optimal target attainment.

The authors confirm that the Principal Investigator for this paper is Francoise van Bambeke for the overall study. Per institution there was a responsible physician (Xavier Wittebole, Pierre-François Laterre, Steven Vervaeke).

KEYWORDS
dose individualization, pharmacokinetics, protein binding, PTA, unbound fraction

1 | INTRODUCTION

Temocillin is a parenteral β-lactam antibiotic recognized for its effectiveness in treating many Gram-negative bacteria, including extended-spectrum beta-lactamase (ESBL)-producing strains. It is often used for urinary tract infections (UTI), lower respiratory tract infection and sepsis in several European countries.[1–4] Unlike carbapenem antibiotics, it demonstrates less disturbances of the intestinal microbiota, relatively low risk on *Clostridioides difficile* infection, and the potential for selecting multi-drug resistant bacteria is relatively low.[5–7] With the increasing prevalence of ESBL-positive isolates, the interest in temocillin has also increased.

Over the last decades two dosing regimens have been used. The common regimens are 2 g IV q12h and 2 g IV q8h administered either by intermittent or continuous infusion.[8] The 2 g q12h dose has been used mainly in the treatment of uncomplicated urinary tract infections caused by bacteria with beta-lactam resistance mechanisms. Current European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints only apply to infections originating from the urinary tract and only the high dosing regimen of 2 g q8h is recommended.[9]

As the efficacy of antibiotics is correlated with the exposure to the unbound concentrations, protein binding is very important. Currently recommended regimens for temocillin were based on a fixed percentage of protein binding (70–85%).[5,10–12] Although it is known for many beta-lactam antibiotics that the protein binding might differ substantially in different clinical situations (e.g., different renal failure, age, albumin levels and comedications), recently it has been shown that protein binding of temocillin in plasma in various patient groups is also complex with various non-linear relationships.[13]

The efficacy of temocillin is best correlated to the percentage of time of the dosing interval that the unbound drug concentration remains above the MIC (%*f*T>MIC). Recent knowledge on the complexity of temocillin protein binding raises the question whether the recommended dosing regimens are optimal in different patient groups, including patients with UTI on the general ward and two groups of intensive care unit (ICU) patients (cerebral ventriculitis and sepsis),[13] as protein binding in these populations differs from that reported in the summary of product characteristics (SmPC) for healthy volunteers. The aim of the current study was to determine the effect of the protein binding in different patient groups *vs.* healthy volunteers on the probability of target attainment (PTA) and thereby to optimize dosing regimens using a population pharmacokinetic analysis based on total and unbound concentrations. The influence on the PTA of relevant covariates was also assessed.

What is already known about this subject

- Target attainment using a fixed percentage of protein binding in ICU patients is often low.
- Protein binding of temocillin is complex.
- This study aims to determine the impact of differences in protein binding between various disease states on the target attainment.

What this study adds

- Differences in protein binding have a major influence on the target attainment.
- Target attainment in patients at a general ward is relatively low.
- Target attainment in septic ICU-patients is relatively high.

2 | METHODS

2.1 | Data collection

2.1.1 | Study population

This prospective, multicentre, open-label and non-randomized pharmacokinetic study was conducted within the context of a previously reported non-randomized controlled trial in Belgium.[13] This study was conducted in accordance with the principles of the Declaration of Helsinki (version: October 2008) and approved by the Institutional Review Board. The ad hoc authority of each clinical institution approved and registered the study protocol (AZ Delta: Commissie medische ethiek [no. B403201938914]); CU St-Luc: Comité d'Ethique hospitalo-facultaire (no. 1737/2015); Medical University of Vienna: Ethic Committee (no. 1737/2015)], which was then globally approved and given a unique Belgian registration no. (B403201629439) by the Comité d'Ethique hospitalo-facultaire of the Health Sciences Sector of the Université catholique de Louvain. The study protocols were registered at ClinicalTrials.gov (NCT03440216 and NCT03557840) and EudraCT (number 2015–003457-18). Informed consent was obtained from each participant and/or each legally authorized representative. This study recruited a diverse group of participants, consisting of four groups, namely healthy male volunteers, patients with UTI on the general ward (UTI patients), individuals with suspected ventriculitis on the

ICU (ventriculitis patients), and patients with sepsis/septic shock on the ICU (sepsis ICU patients).

The included individuals had the following characteristics. All individuals were ≥18-years-old. UTI patients were hospitalized for cUTI (including cystitis, pyelonephritis, prostatitis, bacteraemia and urosepsis, but excluding septic shock requiring admission to the ICU). Ventriculitis patients were admitted to the intensive care and required an external ventricular drain. They were included when diagnosed with, or showing clinical signs of, cerebral ventriculitis with cerebrospinal fluid (CSF) cultures positive for Enterobacterales with a temocillin MIC ≤ 8 mg/L. Sepsis ICU patients were diagnosed with septic shock originating from an intra-abdominal infection (spontaneous bacterial peritonitis, secondary peritonitis, pancreatic infected necrosis, liver abscess). The pathogens were expected to be susceptible to temocillin.

2.2 | Study design

The following intravenous dosing schemes were applied: for healthy volunteers, a single dose of 2 g was administered over 0.5 h; for UTI patients, 2 g q12h were administered over 0.5 h; for ventriculitis patients and sepsis ICU patients, a loading dose (2 g) was administered over 0.5 h, followed by a continuous infusion of 6 g/24 h. All blood samples (5 mL) were drawn from an arterial catheter, collected in ethylenediaminetetraacetic acid (EDTA) tubes, and centrifuged at 2000*g* for 15 min at a temperature of 4 °C, following a precise schedule of timed intervals. The sampling times differ per group and are summarized in Data S1 in the Supporting Information.

Total and unbound temocillin concentrations were measured after protein precipitation with methanol and on centrifuge-generated ultrafiltrates (exclusion: 30 kDa), respectively, using a previously validated high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method for analysis in serum[14] or plasma[15] samples. All temocillin-spiked samples were incubated at 37 °C for 30 min (a time sufficient to reach equilibrium of drug PPB11 and used in most studies).[16] Lack of binding of temocillin to surfaces of the ultrafiltration devices and negligible influence of temperature during the centrifugation step (at 25 °C, as in all published studies with temocillin PPB, *vs.* 37 °C, often selected in PPB studies for other drugs) were demonstrated using matrix analysis approaches.[13] The assay had a lower limit of quantification (LLOQ) of 1.00 mg/L for total temocillin and 0.50 mg/L for unbound temocillin. The lower limit of detection (LLOD) was 0.10 mg/L and 0.05 mg/L for total and unbound temocillin, respectively.[14] The plasma samples were stored at −80 °C until analysis.

2.3 | Data analysis

2.3.1 | Model building

A pharmacokinetic analysis of temocillin was conducted using the non-linear effects modelling approach in NONMEM® with first-order conditional estimates (FOCE) with interaction (version 7.5, ICON Development Solutions, MD, USA). Pirana version 3.0.0 (Certara, NJ, USA) was utilized to support the NONMEM® analysis, and the resulting data were further analysed in R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

Several structural models were fitted to the data and parameters such as the volume of distribution (V), clearance (CL), intercompartmental clearance (Q) were estimated. Different models were evaluated by considering changes in the objective function values (dOFV), Akaike information criterion (AIC), visual evaluations, such as goodness-of fit plots and VPCs, and the precision of estimated pharmacokinetic parameters.

The unbound fraction (Fu) can be derived from equation 1 (Eq. 1). Since temocillin is predominantly bound to albumin and exhibits a non-linear protein binding, the unbound and total concentration are linked by the modified Hill–Langmuir equation (Eq. 2).[13] The inter-individual variability (IIV) was tested on CL, V and temocillin binding affinity (Kd) and total number of binding spots (B_{max}). Proportional, additive and combined (proportional and additive) residual error models were tested to describe the residual error in the model predicted concentrations.

$$Fu = C_{unbound}/C_{total}. \quad (1)$$

$$C_{total} = C_{unbound} + albumin^{*}B_{max}{}^{*}C_{unbound}/(Kd + C_{unbound}). \quad (2)$$

where Fu is the unbound fraction, $C_{unbound}$ is the unbound temocillin concentration, C_{total} is the total temocillin concentration, B_{max} is the total number of binding spots and Kd is the temocillin binding affinity.

2.3.2 | Covariate analysis

A stepwise covariate model-building strategy was employed including forward inclusion and backward deletion. Covariate selection and analysis were guided by both physiological plausibility and change in OFV. In the forward univariate inclusion, a reduction exceeding 3.84 OFV was considered statistically significant ($P < 0.05$) for one degree of freedom. For the backward elimination process, a reduction greater than 6.63 OFV served as the criterion for significance ($P < 0.01$).

Physiological factors, including age, gender, weight, height, body mass index (BMI) and body surface area (BSA), along with biochemical indicators such as creatinine clearance (CLCR) calculated based on 24-h urine creatinine, CRP, albumin (ALB), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels were examined as covariates on PK parameters using power models. Continuous covariates that were screened were normalized to the median value and incorporated into the power function equation for parameter estimation. To evaluate categorical covariates such as presence of sex or group label, indicator functions were used to establish the relationship between these covariates and the pharmacokinetic parameters.

2.4 | Model evaluation

We evaluated different structural models using diagnostic goodness-of-fit plots and visual predictive checks (VPCs) on 1000 simulated datasets via R packages. The relative standard error (RSE) of population parameters was kept below 60%, and a parameter shrinkage threshold of less than 30% was set for accuracy.[17,18] To assess parameter estimation robustness, we employed the bootstrap method within Pirana/PsN, creating 1000 bootstrap datasets, comparing original dataset parameters with median values and 90% confidence intervals from bootstrap replicates. To evaluate the model's predictive capacity for unbound concentrations based on total plasma concentrations, we initially omitted the unbound concentrations, fixed all parameters, and compared model predictions with traditional method estimates, aiming to ascertain better prediction performance.[19,20]

2.5 | Pharmacodynamic targets and Monte Carlo simulations

According to the EUCAST breakpoint table, the current breakpoints only apply to *Escherichia coli*, *Klebsiella species* (excluding *Klebsiella aerogenes*) and *Proteus mirabilis*. The epidemiological cutoff (ECOFF) values for these pathogens are 16 mg/L for *E. coli* and 8 mg/L for *Klebsiella pneumoniae* and *P. mirabilis*.[9,21] The ECOFF for a specific pathogen-antibiotic is similar in all countries and will in principle not change over time.[22] Several pharmacodynamic (PD) targets have been described in a murine infection model.[23] To determine or evaluate dosing regimens, preferably 1-$\log_{10}$ kill PD targets are used. In the previous study, the PD target for the murine thigh infection model was higher compared to the lung infection model. We used the most conservative median values from the murine thigh infection model of 85%*f*T>MIC for *E. coli* and 75%*f*T>MIC for *K. pneumoniae*. This also covers a target of 50%*f*T>MIC as used previously.[24–26] As in clinical practice 100%*f*T>MIC is frequently used for ICU patients, the PTA was also determined for this target in the different groups.

To determine the impact of significant covariates on temocillin plasma concentrations in the final model and to evaluate the recommended dosing regimen in the four participant groups, Monte Carlo simulations (MCS) were performed in NONMEM. The following dosing regimens were used: a 2 g loading dose infused over 0.5 h, followed by a continuous infusion (CI) of 6 g every 24 h; 2 g intermittent infusion (II) over 0.5 h three times daily; or 2 g II over 0.5 h twice daily. The %*f*T>MIC was calculated and the PTA of reaching the PD targets was determined using 1000 simulated individuals for a range of MICs (0.5–64 mg/L). To compare the PTA between the four participant groups, the model that reflects the clinical situation as much as possible was used (simulation based on the subgroup covariates features and distributions to include the amount of variability in the population as in the original dataset). Additionally, to illustrate the impact of important covariates, the PTA for the most conservative murine target of 85%*f*T>MIC for a range of MICs between an example sepsis ICU patient and an example UT -patient using the three dosing regimens was compared.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/24.

3 | RESULTS

3.1 | Study population

In total, 74 participants were included in the analysis. Their characteristics are summarized in Table 1. In total, 1085 temocillin concentrations (543 unbound, 542 total) were included and no samples were below the LLOQ. On average, seven blood samples were collected from each participant, ranging from six to ten.

Only for the participants in the sepsis ICU group was information on co-morbidities available. The main co-morbidities of the patients in the sepsis ICU group were: hypertension or cardiomyopathy ($n = 14$), liver cirrhosis ($n = 12$), kidney disease ($n = 7$), alcohol/tobacco abuse ($n = 6$), diabetes mellitus ($n = 5$), chronic obstructive pulmonary disease (COPD) ($n = 3$) and malignancy ($n = 3$). Overall, none of the patients included in the study was on dialysis and/or mechanical ventilation.

3.2 | Pharmacokinetic model

3.2.1 | Pharmacokinetic basic model

The pooled data were best described by a two-compartment model with first-order elimination and the modified Hill–Langmuir equation (Eq. 2) for the protein binding. A combined proportional and additive error was used for the residual error and exponential form of IIV was included on CL, V1, V2, Kd and B_{max}. The results of the basic model are presented in Table 2.

3.2.2 | Influence of covariates

The forward univariate analysis resulted in the following significant covariates: age, gender, ALB, CRP, CLCR and ASAT. After the backward deletion procedure, only ALB, CRP and CLCR remained as significant covariates in our final model. CLCR and ALB showed positive correlation with CL while CRP showed a small but significant negative correlation. Albumin also showed a positive correlation with

TABLE 1 Summary of patient characteristics and number of concentrations.

Characteristic (unit)	Group 1 Healthy volunteers	Group 2 General ward: urinary tract infection	Group 3 ICU: ventriculitis	Group 4 ICU: sepsis
Participants (*n*)	14	23	10	27
Gender (M/F)	14/0	12/11	4/6	12/15
Age (years)	27 (23–55)	72 (35–91)	55 (20–60)	56 (21–80)
Weight (kg)	77 (66–105)	78 (52.7–127)	77.5 (59–120)	66 (45–104)
Height (m)	1.76 (1.73–1.93)	1.68 (1.48–1.9)	1.7 (1.5–1.86)	1.7 (1.5–1.85)
BMI (kg/m^2)	24.2 (20.7–29)	26.8 (21.1–45)	26 (21.9–45.2)	23.9 (15–35.2)
Albumin (g/L)	44 (41–48.5)	30 (22.9–50.6)	35 (24.8–40.5)	22 (13.7–32)
Total protein (g/L)	71 (64.4–77)	64 (52.7–78.3)	67 (30–70.8)	52 (29.6–75)
CRP (mg/L)	1.8 (0.38–3.5)	80 (0.9–385)	49 (15–131)	124 (20–365)
ASAT (U/L)	24.5 (13–30)	25 (13–63)	46 (17–235)	41.5 (14–198)
ALAT (U/L)	26.5 (11–45)	21.5 (12–116)	30 (9–221)	28 (5–120)
CLCR (ml/min)	116 (83–153)	69 (34–100)	118 (66–221)	41 (20–149)
Conc (*n*)	291	305	175	314
Conc range (mg/L) (unbound)[a]	1.1–60.8	0.5–113.5	1.6–80	15–125.5
Conc range (mg/L) (total)[a]	20.8–308.6	8.3–286.5	7.6–166.3	38.5–232.5

Note: Values are median (range) unless stated otherwise; conc: concentrations.
[a]Minimum and maximum values are measured in all participants in the respective groups.

V1, but on the other hand, CRP showed a negative correlation with V2. And CRP also hade a negative correlation with B_{max}. Compared to the basic model, the final model reduced the IIV in CL, V1, V2 and B_{max} by approximately 30%, 7.2%, 26% and 4.4%, respectively (Table 2).

3.2.3 | Model evaluation

The final model showed only minor changes in the parameter estimates compared to the basic model. However, it demonstrated reduced residual error and IIV estimates. In addition, the bootstrap analysis of the final model indicated that the parameter estimates in the final model were consistent with the 90% lower and upper percentile range of the bootstrap results. The final estimates of both models and the bootstrap are shown in Table 2.

The model evaluation showed that the final model adequately described the data, as is confirmed by the VPCs (Figure 1). Both the fit on the bound and unbound concentrations stratified per patient group showed that the majority of observed median and percentile values were contained within the 95% confidence intervals derived from the VPC simulation. Although final decisions were based on the VPCs, for completeness the goodness-of-fit plot is shown in Data S2 in the Supporting Information. Figure 2 illustrates the difference in protein binding in the four groups and the adequate model fit. Healthy volunteers exhibited the lowest free fraction, followed by UTI patients and ventriculitis patients, while sepsis ICU patients showed the largest free fraction with the highest variation.

3.2.4 | Probability of target attainment

The PTA for the four groups for the three dosing regimens and three PD targets are shown in Figure 3. Considering a PD target of 75% *f*T>MIC and a 16 mg/L MIC, the PTA varies significantly among different groups and dosing regimens. For sepsis ICU patients, UTI patients, ventriculitis patients and healthy volunteers, the PTAs at a dose of 2 g q12h II were 45.7%, 10.7%, 0.7% and 0.1%, respectively. For 2 g q8h II, these values increased to 80.8%, 49.2%, 17.2% and 4%, respectively. A 6 g q24h CI resulted in the highest PTAs, with 98.4%, 90.7%, 73.5% and 41.7% for the respective groups. Considering PD targets of 85% and 100%, we observed similar differences in the PTA across the groups. The PTA in the sepsis ICU patients is the highest among the groups for all three PD targets. For a PD target of 75% $fT{>}MIC_{ECOFF\ 8\ mg/L}$ against *K. pneumoniae*, more than 90% of sepsis ICU patients achieved this target using a dosing regimen of 2 g q8h II. This target was met by over 90% of individuals in all groups under the CI regimen. In the case of *E. coli*, with an 85% $fT{>}MIC_{ECOFF\ 16\ mg/L}$ target, the same high success rate of over 90% was observed for sepsis ICU patients with the 2 g q8h II, and across all groups with CI. In ICU patients CI resulted in a PTA of 98.4% in sepsis ICU patients and 73.3% in ventriculitis patients taking into account the ECOFF for *E. coli* of 16 mg/L.

The PTA shown in Figure 4 illustrated the impact of the covariates ALB and CRP, the two main covariates, apart from the renal function. To illustrate this, we compared the PTA in an example sepsis ICU patient with a CRP at 300 mg/L (high value of the range in this group) and albumin at 15 g/L (low value of the range in this group) and an example UTI patient with CRP at 30 mg/L (slightly elevated CRP in

TABLE 2 Parameter estimates of the basic, final model and the bootstrap.

Basic model				Final model			Bootstrap of the final model		
Parameter		RSE%	Shrinkage%		RSE%	Shrinkage%	Median	95% percentile (lower)	95% percentile (upper)
CL (L/h)	9.00	8.00		9.20	5.00		9.10	8.3	10.3
V1 (L)	29.00	6.00		28.90	5.00		29.00	26.4	32.6
V2 (L)	15.50	15.00		13.80	13.00		13.50	9.5	17.6
Q (L/h)	19.00	11.00		19.80	12.00		19.70	15.7	24.4
Kd	27.80	13.00		21.50	16.00		22.00	16.1	33.2
B_{max}	4.90	8.00		4.20	8.00		4.30	3.7	5.2
CLCR on CL	—	—		0.40	20.00		0.39	0.22	0.55
ALB on CL	—	—		0.57	38.00		0.56	0.14	0.93
CRP on CL	—	—		−0.06	39.00		−0.06	−0.11	−0.01
ALB on V1	—	—		0.72	25.00		0.73	0.39	1.03
CRP on V2	—	—		−0.23	22.00		−0.24	−0.34	−0.14
CRP on B_{max}	—	—		−0.12	21.00		−0.12	−0.17	−0.05
IIV-CL (%)	70.10	7.00	1.00	43.10	12.00	1.00	44.40	34.3	57.9
IIV-V1 (%)	51.40	9.00	4.00	44.20	11.00	5.00	45.70	35.5	57.7
IIV-V2 (%)	88.40	12.00	10.00	62.40	33.00	13.00	72.60	42.6	127.2
IIV-Kd (%)	66.00	9.00	10.00	54.30	13.00	17.00	59.90	42.5	82.2
IIV-B_{max} (%)	35.40	11.00	11.00	31.00	13.00	12.00	30.20	21.4	39.7
G1 UB prop	0.14	19.00		0.14	18.00		0.13	0.08	0.18
G1 TOT prop	0.10	10.00		0.10	10.00		0.10	0.08	0.12
G2 UB prop	0.10	18.00		0.10	18.00		0.10	0.06	0.14
G2 UB add	0.77	30.00		0.80	31.00		0.81	0.37	1.34
G2 TOT prop	0.04	33.00		0.05	29.00		0.05	0.02	0.08
G2 TOT add	5.00	22.00		4.50	25.00		4.55	1.96	6.96
G3G4 UB prop	0.06	48.00		0.05	49.00		0.06	0.01	0.11
G3G4 UB add	4.30	37.00		4.50	36.00		4.24	1.37	6.85
G3G4 TOT add	9.60	8.00		9.70	10.00		9.63	8.29	10.9

Note: Model equations:
Eq. 1: $CL_{i,j} = CL_{pop} * \exp(\eta_i)$; $V_{i,j} = V_{pop} * \exp(\eta_i)$; $Kd_{i,j} = Kd_{pop} * \exp(\eta_i)$; $B_{maxij} = B_{maxpop} * \exp(\eta_i)$.
Eq. 2: $CL_{i,j} = [CL_{pop}*(COV/median\ COV)^{\Theta cov}]* \exp^{(\eta i)}$; $V_{i,j} = [V_{pop}*(COV/median\ COV)^{\Theta cov}]* \exp^{(\eta i)}$; $Kd_{i,j} = [Kd_{pop}*(COV/median\ COV)^{\Theta cov}]* \exp^{(\eta i)}$; $B_{maxij} = [B_{maxpop}*(COV/median\ COV)^{\Theta cov}]* \exp(\eta_i)$.
Eq. 3: $CL_{i,j} = CL_{pop}*(\Theta_{cat})^{FLAG} * \exp^{(\eta i)}$; $V_{i,j} = V_{pop}*(\Theta_{cat})^{FLAG} * \exp^{(\eta i)}$; $Kd_{i,j} = Kdpop*(\Theta_{cat})^{FLAG} * \exp^{(\eta i)}$; $B_{maxij} = B_{maxpop}*(\Theta_{cat})^{FLAG} * \exp^{(\eta i)}$.
Abbreviations: Θcov: continuous effect on PK parameters; Θ_{cat}: categorical covariate effect on PK parameters; Add: additive error; B_{max}: total number available of binding spots; CL: clearance; $CL_{i,j}$, $V_{i,j}$,$Kd_{i,j}$,B_{maxij}: individual value of the parameters; CL_{pop}, V_{pop}, Kd_{pop}, B_{max}: population typical value of the parameters; COV: continuous covariate values; FLAG: indicators of presence of the categorical covariates; G1-G4: group 1 to group 4; IIV: inter-individual variability (η); Kd: temocillin binding affinity; Prop: proportional error; Q: inter-compartment clearance; RSE: Relative standard error; TOT: total concentrations; UB: unbound concentrations; V1: central volume of distribution; V2: peripheral volume of distribution.

this group) and albumin at 35 g/L (lowest value of the normal albumin range) for a CLCR of 90 mL/min. With a PD target of 85% *f*T>MIC and a 16 mg/L MIC, the PTA varied across different dosing regimens and patient profiles. For a dosing regimen of 2 g q12h II, the PTA was only 0.8% in UTI patients (CRP 30 mg/L, ALB 35 g/L), compared to 12.3% in sepsis ICU patients (CRP 300 mg/L, ALB 15 g/L) at a CLCR of 90 mL/min. Increasing the dose to 2 g q8h II improved the PTA to 16.3% in UTI patients and 61.2% in sepsis ICU patients. A 6 g q24h CI regimen resulted in a PTA of 79.3% in UTI patients and 98.8% in sepsis ICU patients, both at the same CLCR. The need for CI is more pronounced in UTI patients as compared to sepsis ICU patients. In sepsis ICU patients, the influence of CrCL on temocillin CL is evident. Patients with a CrCL of 20 mL/min achieved a PTA of 91%, whereas those with a CrCL of 149 mL/min had a PTA of only 19% for a PD target of 85% *f*T>$MIC_{16mg/L}$, with a dosing regimen of 2 g every 8 h and median values for other covariates (ALB = 22 g/L, CRP = 124 mg/L).

4 | DISCUSSION

The pharmacokinetics of temocillin, both bound and unbound concentrations, in four different groups were well described by a population

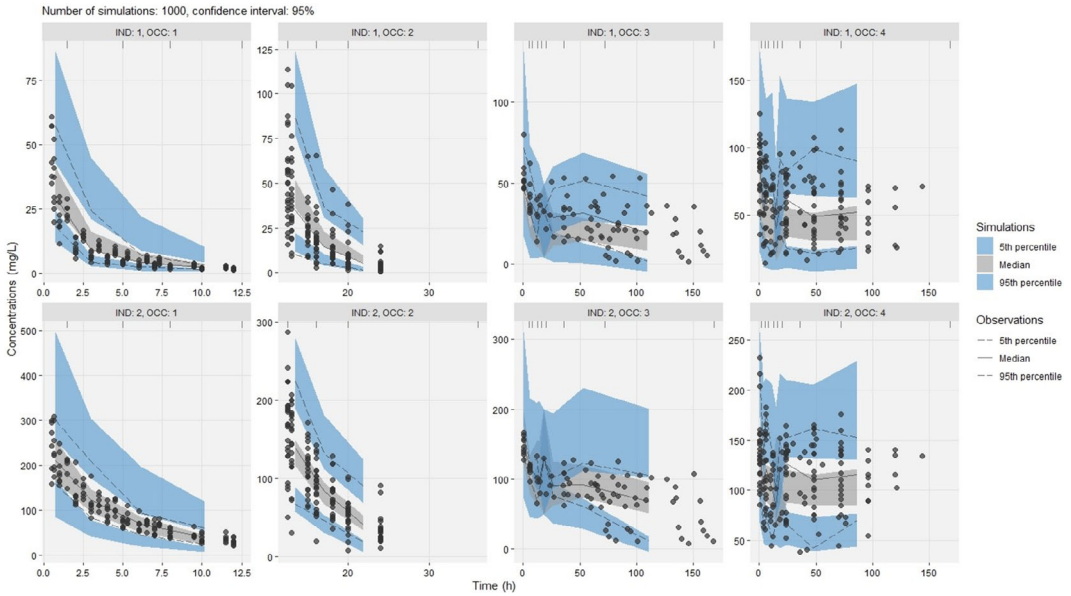

FIGURE 1 Observed unbound and total temocillin concentration–time data and the visual predictive check (VPC) in four different groups. OCC: group labels (1: healthy volunteers, 2: UTI patients, 3: ventriculitis patients, 4: sepsis ICU patients), IND: 1: unbound concentration, 2: total concentration.

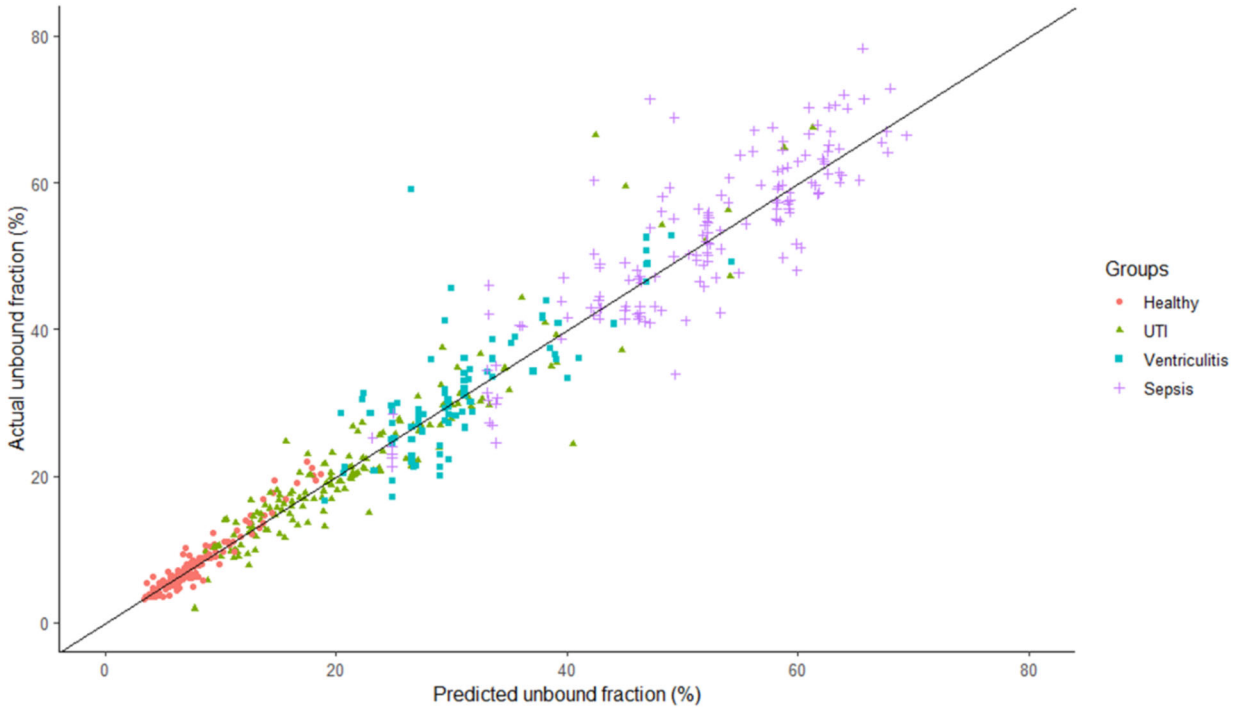

FIGURE 2 Observed unbound fraction *vs.* predicted for different groups. UTI: urinary tract infection. Solid line = line of identity.

pharmacokinetic model. MCS showed clinically relevant differences in the PTA between the types of participants. The highest PTA was achieved in sepsis ICU patients and the lowest in healthy volunteers due to considerable differences in protein binding. Furthermore, the PTA was significantly influenced by CrCL, CRP and albumin. The use of 2 g q12h should not be encouraged in infections requiring adequate systemic temocillin exposure. In contrast to current practice, especially in patients on the general ward with mild infections, the PTA could be enhanced by using continuous infusion.

The estimation of temocillin pharmacokinetic parameters based on total concentrations in this study was in line with previous studies (Table 3). Inclusion of unbound concentrations in the analysis showed the importance of the protein binding in the analysis of the PTA. In current clinical practice on the ICU, doses are increased and

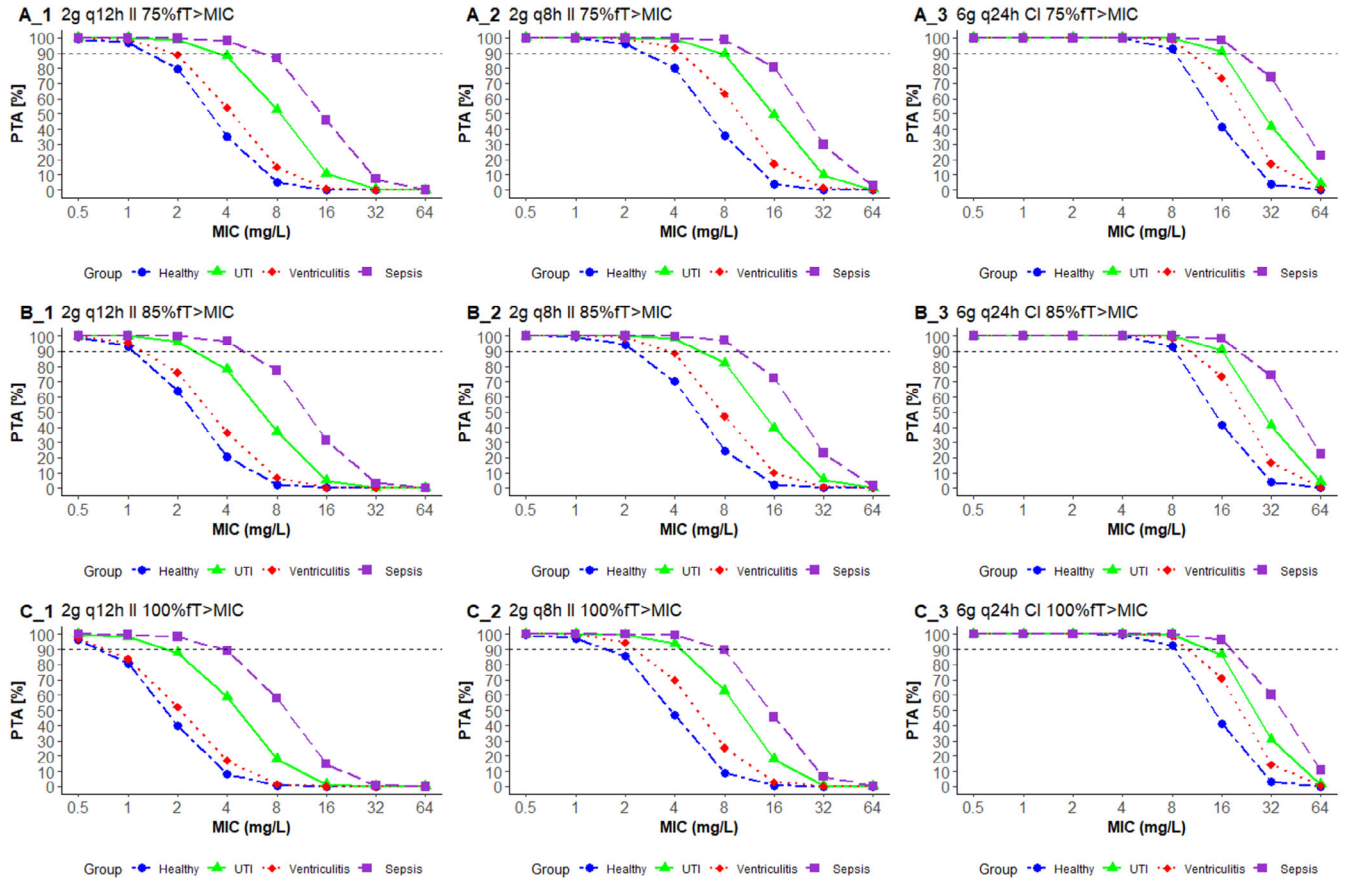

FIGURE 3 PTA of three dosing regimen (1: 2 g 30 min infusion per 12 h, 2: 2 g 30 min infusion per 8 h, 3: 2 g 30 min loading dose followed by 6 g 24 h continuous infusion) for three PD targets (A: 75%*f*T>MIC, B: 85%*f*T>MIC, C: 100%*f*T>MIC) with four patient group (blue: healthy volunteers; green: UTI patients; red: ventriculitis patients; purple: sepsis ICU patients) with their typical covariate (ALB, CLCR, CRP) distributions. The horizontal dashed line indicates the PTA of 90%.

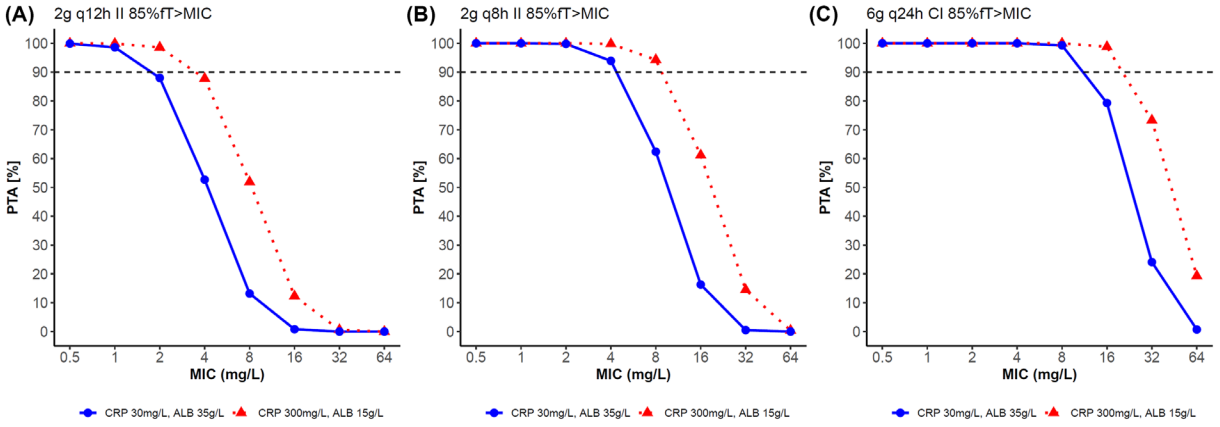

FIGURE 4 PTA for a UTI patient (blue solid line with circle points) and sepsis ICU patient (red dashed line with triangle points) with different dosing regimen (2 g 30 min infusion per 12 h, 2 g 30 min infusion per 8 h, 2 g 30 min loading dose followed by 6 g 24 h continuous infusion) for a CLCR of 90 mL/min. The horizontal dashed line indicates the PTA of 90%.

continuous infusion is more frequently used, as compared to patients with mild infections on general wards.[32,33] This is also in line with the dosing regimens included in the current study with intermittent dosing on the general ward and continuous infusion on the ICU. The study showed the counterintuitive phenomenon that the PTA in sepsis ICU patients was higher compared to patients with a mild infection on the wards, due to the differences in protein binding. The added value of continuous infusion instead of intermittent infusion is

TABLE 3 Comparison of our study and previous temocillin pharmacokinetic studies.

	Layios[27]	Ngougni Pokem[28]	Miranda Bastos[29]	Vandecasteele[30]	Laterre[8]	De Jongh[31]	Ours
Type	Critically-ill patients	Critically-ill patients	HD patients	Patients with ESRD	Critically ill patients	ICU patients	Pooled dataset
No. of patients	32	19	16	16	29	12	74
Samples	142TOT + 142UB + 32ELF	114 blood + 114 ascitic fluid	429	448	329	126	1084
CL (L/h)	15	5.36 (2.45 + 2.91)	CL: 1.4 L/h/70 kg CL_{dial}:7.67	CL: 1.56CL_{HD}: 8.1	3.69	2.43	9.2
V (L)	V1: 31; V2: 26.6 Q: 13.3	V1: 14.4; V2: 13.2; Q: 71	V1: 22.7; V2: 18.6; Q: 3.99Kd: 34.3; B_{max}: 117	V: 43.9	V1: 14; V2: 21.7; Q: 8.45	14.3	V1: 29; V2: 14 Q: 20Kd: 21.5; B_{max}: 4.23
Half-life (hour)	3.42	3.60	10.00	23.6 hHD: 3.6 h	7.89	4.03	3.4
Covariate	CRCL on CL	CRCL on CL	Dry BW scaled CL and V				CRCL, ALB, CRP

Abbreviations: ALB: albumin; B_{max}: total number of binding spots; BW: bodyweight; CL: clearance; CLdial: temocillin dialysis clearance; CLHD: haemodialysis clearance; CRCL: creatinine clearance; CRP: C-reactive protein; ELF: epithelial lining fluid; ESRD: end-stage renal disease; HD: haemodialysis; Kd: binding affinity; Q: distribution clearances between central and peripheral compartments; TOT: total concentration; UB: unbound concentration; V: volume of distribution; V1: volumes of central compartment; V2: volumes of peripheral compartment.

therefore more pronounced in patients with mild infections on the regular ward.

In the PTA analysis, the choice of the PD target is crucial. In previous studies a target of 50%*f*T>MIC was used. However, more recently a study on the pharmacodynamics in a murine thigh and lung model showed higher targets of 85% *f*T>MIC for *E. coli* and 75% *f*T>MIC for *K. pneumoniae*, associated with 1-log kill in the thigh model. In the thigh model, 2-log kill was not reached, not even with 100% *f*T>MIC.[23] Target values in the lung model were lower as compared to the thigh model. For our study, we used the most conservative targets from the thigh model. The target of 100% *f*T>MIC was presented as well since it is frequently used in the ICU, though based on the murine data its added value is uncertain. The difference in PTA between *E. coli* and *K. pneumoniae* is increased since the ECOFF values are different as well (16 mg/L and 8 mg/L, respectively). Due to these differences in target and ECOFF, higher PTAs were reached for *K. pneumoniae* than for *E. coli*.

The choice of the model to be used for the MCS is also of importance. Several covariates were included in the final model and the variability in the covariates was found to be different per group. Using the final model including these covariates would therefore result in unrealistically low variability and too high PTA values. To ensure that the variability in our simulated patients accurately mirrors that found in actual patient groups, we performed MCS using the model including the covariate distributions (CRP, ALB, CLCR) observed in each of the patient groups.

Several covariates were included in the final model. As temocillin is predominantly eliminated renally,[24] CLCR was found to be a covariate on CL. Albumin and CRP were also important covariates. CRP and albumin are commonly used biomarkers for assessing inflammatory conditions.[34] B_{max} was negatively correlated with CRP. And CRP exhibited a modest but statistically significant negative relationship with CL. This finding aligns with previous research on meropenem, which has also shown a negative correlation between CL and CRP.[35] What is particularly intriguing is that, in contrast to the concept that low plasma albumin levels would result in increased total drug clearance,[36] our study revealed a positive correlation between albumin and unbound temocillin clearance. There could be several explanations for this phenomenon. Firstly, in our study, patients with low protein levels are often severely ill, which might lead to pharmacokinetic changes, potentially impacting drug clearance. Another contributing factor could be with higher albumin levels resulting in a larger Vc, an increase in CL can coincide with a higher Vc.

This study, while comprehensive, does encounter certain limitations. Primarily, we were unable to identify covariates that could account for the considerable unexplained variability observed in protein binding, especially regarding the Kd component. This limitation may be explained by the restricted number of covariates examined. Furthermore, while unbound concentrations can be well predicted using total concentrations in this study, in real clinical settings, patients may present with complexities that do not align perfectly with the four defined groups in our study. While there is a difference in PTA between the groups, this does not directly translate into a difference in clinical outcome. The small numbers of patients in the groups in this study did not allow us to compare clinical outcome between the groups. Despite these limitations, our analysis effectively highlights the significant impact of variations in protein binding on the pharmacotherapeutic target achievement.

Human unbound plasma PK profiles were compared with preclinical murine PD targets, based on murine unbound PK corrected

with PD effects in tissue. For beta-lactams these PD targets are believed to correlate quite well with clinical outcomes in humans.[25] Nevertheless, there is increased interest in concentrations at the site of infection. Such information on site-specific concentrations might increase our knowledge on local exposure, for example if the exposure in the urine is increased as compared to plasma. However, these data cannot be interpreted using the known PD targets. Site-specific PD targets might differ from classical PD targets[37,38] and for temocillin they are unavailable. PD targets used were determined in immunoincompetent mice. Patients with mild infections might have a better immune system, as compared to the critically ill, which potentially might in part compensate for the lower PTAs reached in patients with mild infections.

In summary, our study indicates that a temocillin dosage 2 g every 12 h may result in low target attainment. Based on the plasma PK, the use of continuous infusion could improve target attainment in all patients For the regimen using continuous infusion, the 85% $f\text{TMIC}_{8\ mg/L}$ was reached in all patient groups. PTA values of >90% for the 85% $f\text{TMIC}_{16\ mg/L}$ target were only reached in the sepsis ICU patients and UTI patients, but not in the ventriculitis patients and healthy volunteers. These counterintuitive findings, that target attainment is higher in sepsis ICU patients as compared to UTI patients, can be explained by the differences in protein binding between the patient groups.

AUTHOR CONTRIBUTIONS

F.V.B., P.N.P. and M.Z. designed the study; X.W., S.V. and P.F.L. acquired the data. L.L., S.D.T.S. and A.E.M. analysed and interpreted the data. All authors have made substantial contributions to drafting or revising the article and all have approved the final version of the article.

CONFLICT OF INTEREST STATEMENT

P.N.P. was an employee of the Université catholique de Louvain. F.V.B. is Research Director of the Fonds de la Recherche Scientifique (F.R.S.-FNRS). None of the authors has a conflict of interest for the content of this article.

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplement 1

Patient dosing and sampling times:

For Group 1, a 2-gram dose of temocillin is administered intravenously over 40 minutes. Following the infusion, sampling occurs immediately and continues at timed intervals: 40 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours.

In Group 2, a 2-gram infusion administered over 30 minutes is administered every 12 hours. Sampling took place at 0.5, 1, 3, 4, 6, 8, and 12 hours following the start of last infusion.

In Group 3, patients initially receive a 2-gram loading dose administered over 30 minutes. This is followed by a continuous infusion of 6 grams per day. When the infusion is discontinued, sampling continues to assess the residual drug concentration in the bloodstream. Post-infusion sampling occurs at 1, 3, 6, and 12 hour after stopping the infusion.

In Group 4, patients are treated with a 2-gram loading dose administered over 30 minutes, followed by a continuous daily infusion of 6 grams. Samples were taken at specific intervals: 0.5 hours after the infusion starts, and then at 6, 24, 48, 72, 96, 120, and 144 hours.

Supplement 2: Goodness-of-fit plots of the final model.