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Empirical models for dosage optimization of four β-lactams in critically ill septic patients based on therapeutic drug monitoring of amikacin

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ABSTRACT

Objectives: The study aims to develop empirical models able to predict the pharmacokinetics (PK) of four β-lactams using the amikacin (AMK) therapeutic drug monitoring (TDM), in order to optimize their dosage regimens.

Design and methods: 69 critically ill septic patients were included. All received a first dose of AMK combined with piperacillin/tazobactam, ceftazidime, cefepime or meropenem. A multivariate analysis was performed to predict the β-lactam PK using AMK PK parameters estimated from TDM and using pathophysiological variables.

Results: An optimal prediction model was identified for each PK parameter of each β-lactam. The best predictor of each model was one of the AMK PK parameters estimated from TDM. Other variables included colloid solution, renal and hepatic biomarkers, age and body weight.

Conclusion: PK of the four β-lactams could be easily and rapidly predicted in critically ill septic patients using the AMK TDM. These predictions could improve the β-lactam dosages in clinical practice.

Introduction

Mortality rates in intensive care unit (ICU) patients with severe sepsis or septic shock remain unacceptably high, reaching 30% to 50% [1–4]. Prompt initiation of the right antibiotic therapy is the cornerstone to maximize the successful outcome of treatment in critically ill patients [5–10], and there is evidence over the last decade that the optimal antibiotic dosage regimen is at least as important [11–14]. The individual optimization of the antibiotic dosages should ideally involve both pharmacokinetic (PK) and pharmacodynamic (PD) parameters, the so-called efficacy indices [15,16].

During severe sepsis or septic shock, there are two important PK modifications, i.e. an increase in volume of distribution (Vd), due to leaky capillaries and decreased protein binding, and a reduction in total body clearance (CL), associated with renal and/or hepatic dysfunction [17–22]. Regrettably, in severely septic patients, the antibiotic dosage regimens are often based on PK data obtained in healthy volunteers or non-critically ill patients, without consideration for the sepsis-induced PK changes, particularly critical at the onset of the treatment (first 24 h). This may result in inadequate drug concentrations and consequently affect the outcome of patients. Moreover, large interindividual PK variations have been reported in critically ill patients, making empirical fixed dose strategy difficult because of the unpredictability of drug concentrations [23]. Individual optimization of the antibiotic dosage regimens needs therefore to be considered for patients with severe sepsis, reinforcing the role of therapeutic drug monitoring (TDM).

Antibiotic therapy in critically ill septic patients usually consists of an aminoglycoside combined with a broad-spectrum β-lactam [24]. In clinical practice, the aminoglycoside dosage regimens are routinely adjusted by serum concentration monitoring. Among PK-based dosing methods dedicated to aminoglycoside TDM, the non-Bayesian least-squares method has the advantage to not require knowledge of the PK parameter distribution in the population [25,26]. Initially described by Sawchuk and Zaske [27,28], this approach is applicable when the PK can be adequately described by a one-compartment model with first-

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order elimination and when it is required to maintain drug concentrations within a desired range. Individual PK parameters can be obtained from at least two blood samples drawn during the elimination phase. Using the appropriate efficacy index, they are then used to estimate the optimal patient dosage regimen.

Unlike aminoglycosides, β-lactam dosage regimens are frequently inferred and roughly adapted only from the patient's pathophysiological status. The lack of TDM for these antibiotics may limit their clinical use optimization. However, due to chemicophysical and PK similarities between aminoglycosides and β-lactams (i.e. hydrophilicity, low molecular weight, low Vd, renal elimination, low plasma protein binding) [18], knowledge of aminoglycoside PK obtained from TDM could be used to predict β-lactam PK parameters, allowing therefore individualized dosage adjustment in a cost-effective strategy, without additional blood sampling.

Since amikacin (AMK) is largely used in Europe, the objectives of the study are: (i) to describe the first dosing PK of AMK and four β-lactams (piperacillin/tazobactam (PIP/TAZ), ceftazidime (CAZ), cefepime (FEP) and meropenem (MEM)) in critically ill septic ICU patients, from serum concentrations obtained after full validation of their analytical methods, and (ii) to develop empirical models able to predict the β-lactam PK from AMK TDM and from variables such as demographic data (age, weight, ...), routine biochemistry markers (creatinine, albumin, …) and blood cells counting. The choice of β-lactam empirical treatment is based on the clinical recommendations for nosocomial infections in Belgium (e.g. MEM is preferred to imipenem due to its lower toxicity) [29].

Methods

Study design, patients, antibiotic treatment and data collection

The open-labeled, observational, clinical study was conducted between January 2005 and April 2007 in the ICU of four Belgian university hospitals (Cliniques Universitaires St-Luc, Erasme Hospital and Universitair Ziekenhuis Brussel in Brussels, and St-Pierre Hospital in Ottignies) after approval by the respective Ethics Committees. Before enrollment, written consent was obtained from patients or their legal representatives.

Eligible patients were diagnosed with severe sepsis or septic shock at their ICU admission or during their ICU stay. Severe sepsis and septic shock were defined according to standard criteria [30]. Patients meeting one of the following criteria were excluded: (i) age <18 years or >90 years, (ii) pregnancy or lactation, (iii) previous administration of any of the investigated antibiotics during the week before inclusion, (iv) chronic renal failure requiring dialysis and (v) allergy to any of the investigated drugs. The study period was limited to the first 24 h of antibiotic therapy, considered as the most critical period.

All patients were treated with a first dose of AMK (25 mg/kg) combined with one of the four following broad-spectrum β-lactams: PIP/TAZ (4 g/500 mg), CAZ (2 g), FEP (2 g) or MEM (1 g) selected according to the local clinical practice. Further β-lactam administration was based on standard dosage regimens and adapted to renal function: 4 g/500 mg of PIP/TAZ every 6 h, 2 g of CAZ, 2 g of FEP and 1 g of MEM every 8 h. Next doses of AMK were determined according to TDM. The antibiotic combination was given by two separate intravenous lines as a 30-min infusion. 3-ml blood samples were collected without anticoagulant immediately before and at 1, 1.5, 4.5, 6 or 8, and 24 h after onset of the first infusion; these blood collection time points are supposed to belong to the elimination phase of all five antibiotics. The exact sampling time was recorded by the nursing or medical staff. Blood samples were centrifuged at 4000×*g* for 10 min at 4 °C after blood clotting. Taking into account the possible drug instability at room temperature, serum samples were stored at −70 °C until shipment in dry ice to a reference laboratory for analysis.

For each patient, demographic and biometric data, complete blood count and routine biochemistry markers were recorded at the baseline (i.e. before start of antibiotic therapy). Similarly, the Acute Physiology And Chronic Health Evaluation (APACHE) II and Sepsis-related Organ Failure Assessment (SOFA) scores were determined [31,32]. Infused volumes of resuscitation fluids (crystalloid and colloid solutions) were also recorded in the database.

Reagents, sample preparation and analytical methods

All antibiotic quantitative analyses were centralized in a reference laboratory.

Reagents

Calibrations were performed using each drug generously provided by Wyeth Pharmaceuticals, Madison, NJ, USA, for piperacillin; GlaxoSmithKline Pharmaceuticals, Genval, Belgium, for ceftazidime; Bristol-Myers Squibb, NY, USA, for cefepime; and AstraZeneca, London, UK, for meropenem. Visnadine and cefazolin, used as internal standard, were obtained from Indena (Milan, Italy) and Sandoz (Holzkirchen, Germany), respectively. Acetonitrile HPLC-grade and triethylammonium phosphate buffer solution (1 M, pH 3) were purchased from Sigma-Aldrich (Munich, Germany), and sodium 1-octanesulfonate monohydrate from Acros Organics (Geel, Belgium). Sulfuric acid and all the chemicals used for protein precipitation and extraction were supplied by Merck (Darmstadt, Germany). Ultrapure water was obtained from a MilliQ® UF-Plus apparatus (Millipore Corp, Bedford, MA, USA).

Analytical method for amikacin

The AMK serum concentrations were quantified by Fluorescence Polarization ImmunoAssay (FPIA) using the TDx analyzer (Abbott Laboratories, Abbott Park, IL, USA) [33,34], according to the manufacturer recommendation, with a limit of quantification (LOQ) of 0.8 μg/mL.

Sample preparation and analytical methods for β-lactams

The β-lactam concentrations were determined by High Performance Liquid Chromatography (HPLC) with Diode Array Detection (DAD). The antibiotics were diluted in water in order to reach stock solution aliquots of 1 mg/mL, stored at −20 °C. Before each assay, a fresh calibration curve was prepared from the stock solution and blank serum, at the following concentrations: 0.75, 1, 2, 5, 10, 25 and 50 μg/mL for PIP; 5, 10, 25, 50 and 100 μg/mL for CAZ; 0.1, 0.25, 0.5, 1, 5, 10, 25 and 50 μg/mL for FEP or MEM. The liquid-liquid extraction procedures and chromatographic conditions are described below.

PIP. 400 μL of serum sample and 200 μL of IS solution (visnadine 50 μg/mL in acetonitrile) were added in a 5-mL glass tube and vortex-mixed for 15 s before a 10-min incubation at 4 °C. 50 μL of a an aqueous solution of perchloric acid 15% (w/v) and 50 μL of an aqueous solution of sodium tungstate 25% (w/v) were then sequentially added, followed by vortex-mixing. The tube was then incubated at 4 °C for 10 min and centrifuged at 4000×*g* for 7 min. 100 μL of the supernatant were transferred in an injection vial. Analyses were performed using a HPLC HP1090 Series II, coupled to a DAD (Hewlett Packard, Palo Alto, CA, USA). 20 μL were injected on a LiChroCART Superspher® 100 RP-18 endcapped column (125×4 mm ID, 5 μm particle size) (Merck, Darmstadt, Germany), equipped with a pre-column (4×4 mm ID, C-18) and maintained at 40 °C. The mobile phase, consisting of solvent A (25 mM triethylammonium phosphate buffer solution, pH 3) and solvent B (acetonitrile), was delivered at a flow rate of 1 mL/min according to the following gradient: 0 min, 85/15%; 10 min, 60/40%;

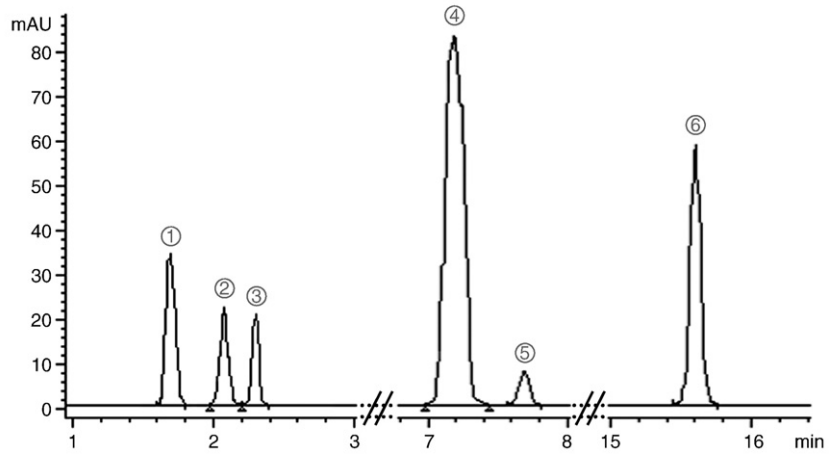

Fig. 1. Adapted HPLC chromatogram from 4 chromatograms obtained from plasma spiked with 10 μg/mL of each analyte. ① cefepime (time of retention: 1.72 min, wavelength: 254 nm); ② ceftazidime (time of retention: 2.10 min, wavelength: 254 nm); ③ meropenem (time of retention: 2.29 min, wavelength: 320 nm); ④ cefazolin (internal standard at 1 mg/mL, time of retention: 7.20 min, wavelength: 254 nm); ⑤ piperacillin (time of retention: 7.69 min, wavelength: 230 nm); ⑥ visnadine (internal standard at 50 μg/mL, time of retention: 15.63 min, wavelength: 320 nm).

13.5 min, 30/70%; 17.5 min, 0/100%, for solvents A/B respectively. The 230- and 320-nm bands were used to detect PIP and its IS (visnadine), respectively, during a 18 min run-time.

CAZ. The extraction procedure was similar to the one used for PIP. 50 μL of the IS solution (cefazolin 1 mg/mL in water) were added to 400 μL of serum sample together with 200 μL of acetonitrile in a 5-mL

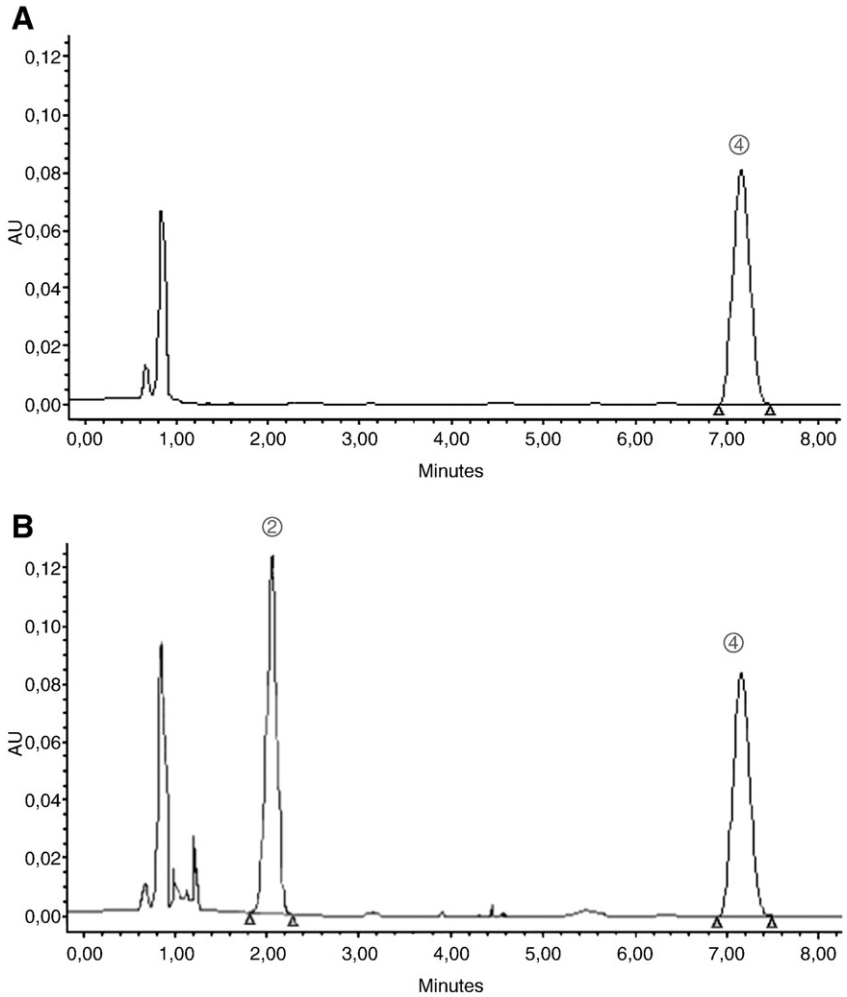

Fig. 2. HPLC chromatograms of (A) a plasma blank, and (B) a plasma sample from a patient treated with ceftazidime (52 μg/mL). The internal standard (cefazolin 1 mg/mL) was added to both samples.

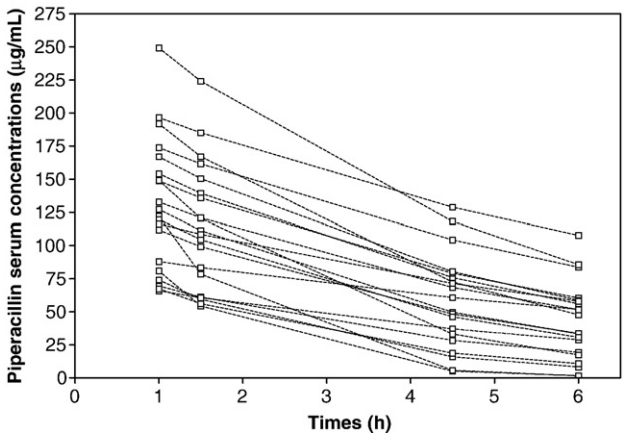

Fig. 3. Individual 320-nm concentration-time profiles of piperacillin in critically ill septic patients ($n=20$).

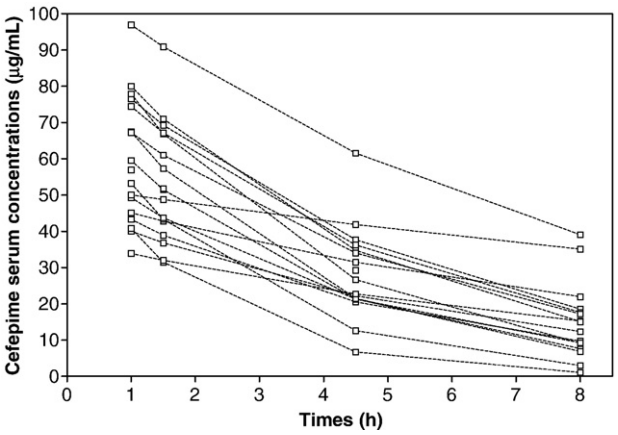

Fig. 5. Individual 320-nm concentration-time profiles of cefepime in critically ill septic patients ($n=17$).

glass tube. Analyses were performed on a HPLC Waters Alliance 2795 Separation Module, coupled to a PhotoDiode Array detector (Waters, Milford, MA, USA). 20 µL were injected on an Ultrasphere® ODS column (250×4.6 mm ID, 5 µm particle size) (Beckman Coulter, Fullerton, CA, USA), maintained at 40 °C. The mobile phase was isocratically delivered at a flow rate of 1 mL/min and consisted of 88% 25 mM triethylammonium phosphate buffer solution (pH 3) and 12% acetonitrile. The 254-nm band was used to detect both CAZ and its IS (cefazolin), in a 9 min run-time.

FEP. To a 500 µL of serum sample, 10 µL of IS solution (MEM 1 mg/mL in water) and 750 µL of acetonitrile were added in a 5-mL glass tube and vortex-mixed for 1 min before a 45-min incubation at 4 °C. After appropriate centrifugation (4000×*g*, 7 min), 950 µL of supernatant were transferred to a second 5-mL glass tube, centrifuged at 4000×*g* for 7 min. 800 µL of the resulting supernatant were further transferred together with 3 mL of dichloromethane in a third 5-mL glass tube, vortex-mixed for 1 min and centrifuged at 4000×*g* for 7 min. Finally, 100 µL of the supernatant were transferred in a HPLC injection vial. Analyses were performed using a HPLC HP 1090 Series II, coupled to a DAD. 5 µL were injected on the same pre-column and analytical column as described for PIP. The mobile phase was isocratically delivered at a flow rate of 1 mL/min and consisted of 79% 5 mM sodium 1-octanesulfonate monohydrate (adjusted to pH 3 with 2.5 M sulphuric acid) and 21% acetonitrile. The 254- and 320-nm bands were used to detect FEP and its IS (MEM), respectively. The run-time was 4 min.

MEM. The extraction procedure, the apparatus and the chromatographic conditions were identical to those used for FEP, except for the IS solution (50 µL of FEP 1 mg/mL in water) and the wavelength bands (320-nm to detect both MEM and FEP).

All methods were validated according to the published acceptance criteria for specificity, linearity, accuracy, precision (intra-day (repeatability), inter-day (intermediate precision)) and sensitivity (LOD (limit of detection) and LOQ) [35,36]. The specificity was investigated for possible interference with a number of drugs commonly administered in the ICU (sedatives (midazolam, propofol, ketamine), analgesics (morphine, remifentanil, fentanil), insulin, antibiotics (vancomycin), antipyretics, antacids), by analysing serum samples collected before the antibiotic infusion. In addition, the carry-over effect was tested.

Pharmacokinetic analysis

PK of the five investigated antibiotics was individually assessed using WinNonlin® Professional version 5.0.1. software (Pharsight Corporation, Mountain View, CA, USA). Due to the absence of sampling in the distribution phase, a one-compartment model with first-order elimination was selected to fit data. Investigated PK parameters included maximal serum concentration (C_{max}), Vd, CL, elimination half-life ($t_{1/2}$) and area under the serum concentration-time curve (AUC). Vd and CL were normalized to the body weight.

AMK PK parameters were further obtained using PharMonitor [37], a TDM software based on the Sawchuk–Zaske method, from serum concentrations determined 1 h and 6 or 8 h after the start of the

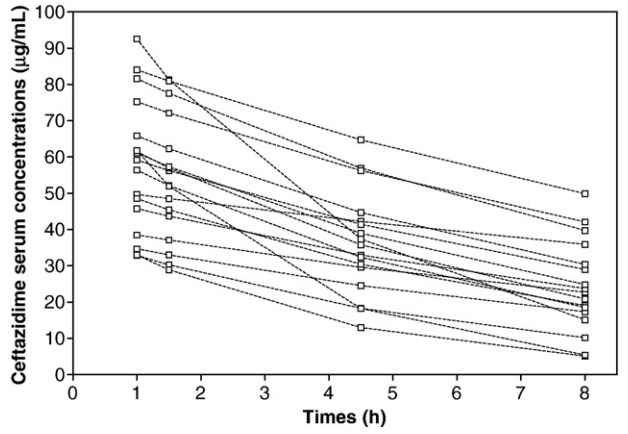

Fig. 4. Individual (–□–)concentration-time profiles of ceftazidime in critically ill septic patients ($n=17$).

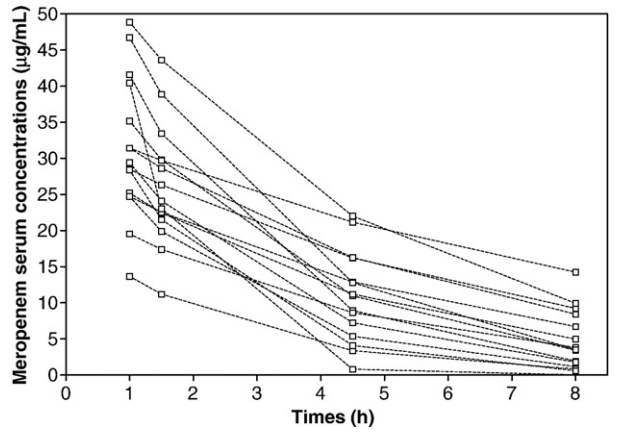

Fig. 6. Individual (–□–)concentration-time profiles of meropenem in critically ill septic patients ($n=15$).

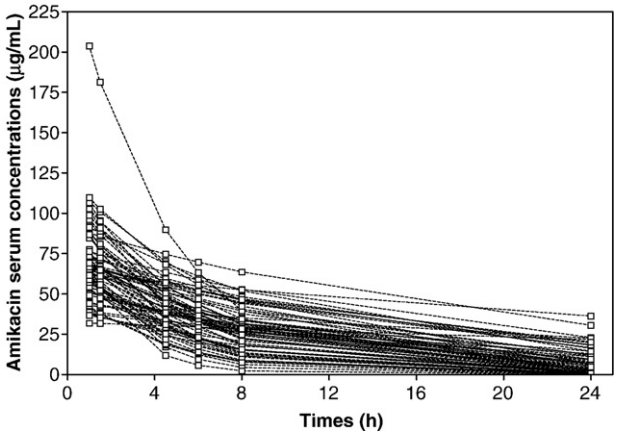

Fig. 7. Individual µg.h concentration-time profiles of amikacin in critically ill septic patients ($n=69$).

infusion. These time-points have been selected after testing different combinations.

Statistical analysis

The performance of PharMonitor to adequately generate the AMK PK parameters of each patient was first assessed using the relative mean prediction error (MPE [%]) (Eq. 1) and the relative root mean square prediction error (RMSE [%]) (Eq. 2) [38]. MPE measures the magnitude of the systematic component of the prediction error (PE); the smaller the absolute MPE value, the smaller will be the bias of the prediction. RMSE measures the standard deviation of the prediction error; the smaller the RMSE value, the greater will be the precision of the prediction.

$$\begin{aligned}\text{MPE } [\%] &= \frac{1}{N}\bullet\sum_{i=1}^{N}\left(\left(\frac{\text{Value}_{\text{pred}}-\text{Value}_{\text{ref}}}{\text{Value}_{\text{ref}}}\right)_{\text{i}}\bullet 100\right)\\ &= \frac{1}{N}\bullet\sum_{i=1}^{N}\text{PE}_{\text{i}}\ [\%]\end{aligned} \tag{1}$$

$$\text{RMSE } [\%] = \sqrt{\frac{1}{N}\bullet\sum_{i=1}^{N}\text{PE}_i^2\ [\%]} \tag{2}$$

The AMK PK parameters obtained from PharMonitor were considered in the calculation of MPE and RMSE as predicted values ($\text{Value}_{\text{pred}}$), and those obtained from WinNonlin as reference values ($\text{Value}_{\text{ref}}$).

In a second step, a multiple linear regression analysis was performed in order to predict Vd, CL, and AUC of each β-lactam. The following variables were tested: PharMonitor AMK PK parameters, demographic and biometric data (sex, age, body weight), renal function markers (creatinine, urea, potassium, glomerular filtration rate estimated either from the Cockcroft and Gault formula [39], or from the simplified Modification of Diet in Renal Disease formula (MDRD) [40]), hepatic function markers (albumin, total protein, total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, prothrombin time, activated partial thromboplastin time), disease severity scores (APACHE II, SOFA), inflammatory marker (CRP), and infused volumes of resuscitation fluids (crystalloid and colloid solutions). The multivariate analysis was carried out by testing different data transformations, such as original versus log-transformed data, original versus categorized data, and PK parameters (Vd, CL and AUC) with or without normalization to body weight. The prediction models were built using a stepwise selection of significant variables using both the forward inclusion and the backward exclusion [41]. To limit the propagation error, the maximal number of variables included in the final model was three. The sensitivity of developed models to outliers was assessed by computing the distribution of residuals and the Cook's influence, and by performing a jack-knife analysis [42]. For each β-lactam PK parameter, the best model was selected using the two predictive performance indices: MPE [%] and RMSE [%] [38]. The β-lactam PK parameters obtained from WinNonlin were investigated in the calculation of these indices as reference values. A third performance criterion was considered, i.e. the percentage of patients with clinically unsatisfactory RPE's, fixed at ≥/≤20%/−20%, 30%/−30% and 40%/−40%. For all prediction models, determination coefficients (r^2) were computed.

Table 2
Prediction models of the β-lactam Vd, CL and AUC.

Multiple linear regression equations	r^2
PIP Vd = 0.5000–0.0030 AMK C_{max} + 0.1000 Cd	0.58
CAZ Vd = 1.6303 × (AMK Vd)$^{1.1788}$	0.84
FEP Vd = 0.4983–0.0022 AMK C_{max} + 0.0525 Cd	0.76
MEM Vd = 0.4112–0.0019 AMK C_{max} + 0.1532 Cd	0.56
PIP CL = 23.4420 × (AMK CL)$^{0.7724}$ × (Ur)$^{-0.1845}$ × (aPTT)$^{-0.4345}$	0.90
CAZ CL = 24.7237 × (AMK CL)$^{0.9146}$ × (Ur)$^{-0.3466}$ × (K)$^{-0.9109}$	0.89
FEP CL = 3.5000 × (AMK CL)$^{0.6735}$ × (Ur)$^{-0.1789}$	0.71
MEM CL = 0.8466 × (AMK CL)$^{0.5404}$ × (Prot_{tot})$^{0.8321}$ × 0.6196*	0.85
PIP AUC = 2.0285 × (AMK AUC)$^{0.8002}$ × (aPTT)$^{0.2523}$ × 0.4967**	0.94
CAZ AUC = 0.3758 × (AMK AUC)$^{1.1290}$ × 0.8086**	0.90
FEP AUC = 3.1356 × (AMK AUC)$^{0.7813}$ × 0.5828**	0.81
MEM AUC = 2.3357 × (AMK AUC)$^{0.6009}$ × 1.6607* × 0.8238**	0.92

PIP, piperacillin; CAZ, ceftazidime; FEP, cefepime; MEM, meropenem; AMK, amikacin; Vd, volume of distribution (L/kg); CL, total clearance (mL/min/kg); AUC, area under the serum concentration-time curve (µg.h/mL); C_{max}, maximal serum concentration (µg/mL); Cd, colloid solution (L); Ur, plasma urea (mg/dL); aPTT, activated partial thromboplastin time (s); K, plasma potassium (mmol/L); Prot_{tot}, plasma total protein (g/dL).
* if ≥70 years.
** if ≥70 kg.

Table 1
Median (range) antibiotic PK parameters in a population of critically ill septic patients, according to antibiotic therapy.

	C_{max} [µg/mL]	Vd [L/kg]	CL [mL/min/kg]	$t_{1/2}$ [h]	AUC [µg.h/mL]
PIP ($n=20$)	145.87 (71.51–273.37)	0.42 (0.23–0.86)	1.61 (0.63–4.91)	3.06 (0.79–6.53)	693.18 (185.84–1766.19)
CAZ ($n=17$)	61.65 (35.78–115.40)	0.51 (0.28–1.03)	0.91 (0.43–3.23)	6.28 (1.99–14.98)	523.49 (147.23–1190.30)
FEP ($n=17$)	66.56 (35.87–103.44)	0.37 (0.25–0.68)	1.26 (0.41–2.87)	3.37 (1.34–13.65)	324.02 (116.06–1024.45)
MEM ($n=15$)	34.54 (15.60–71.07)	0.37 (0.11–0.68)	1.73 (0.69–4.53)	2.08 (0.62–6.14)	128.96 (44.35–302.86)
AMK ($n=69$)	71.69 (32.05–229.16)	0.34 (0.10–0.73)	0.67 (0.15–2.20)	5.09 (1.45–41.89)	599.39 (189.65–2706.92)

PIP, piperacillin; CAZ, ceftazidime; FEP, cefepime; MEM, meropenem; AMK, amikacin; C_{max}, maximal serum concentration; Vd, volume of distribution; CL, total clearance; $t_{1/2}$, elimination half-life; AUC, area under the serum concentration-time curve.

The statistical analyses were performed using JMP Statistical Discovery™ 6.0.0. software (SAS Institute Inc., Cary, NC, USA). All PK values were expressed as median and range. *P*-values<0.05 were considered to be statistically significant.

Results

Under the chromatographic conditions described above, PIP, CAZ, CEF, MER and their respective IS were identified by sharp and well resolved peaks (Figs. 1 and 2). No interference was identified in the analytical methods, either by endogenous compounds or by co-administered drugs as described above. The linearity was statistically confirmed over the concentration range tested for each β-lactam and was associated with r^2 >0.999. The four analytical methods were accurate and precise. LOD and LOQ respectively were 0.50 and 0.75 μg/mL for PIP, 2.00 and 5.00 μg/mL for CAZ, 0.07 and 0.10 μg/mL for FEP and MEM. The methods did not display any carry-over. Appropriate dilution was performed for clinical samples with concentrations above the upper analytical range.

The study included 69 adult patients (44 men) with severe sepsis ($n=17$) or septic shock ($n=52$), with a median age of 65 years (range 22–89 years) and a median weight of 70 kg (range 38–120 kg). At the onset of the antibiotic therapy, median APACHE II and SOFA scores were 20 (range 6–45) and 8 (range 1–19), respectively. Blood cultures were positive in 68% of patients; Gram-negative bacteria were the most frequent isolated micro-organisms (41%).

A total of 20 PK profiles were completed for PIP, 17 for CAZ, 17 for FEP, 15 for MEM and 69 for AMK; they are illustrated in Figs. 3–7, respectively. The corresponding median PK parameters are presented in Table 1. Antibiotic serum concentrations and PK parameters were characterized by a large variability.

When assessing the PharMonitor performance to adequately estimate AMK PK from serum concentrations determined 1 h and 6 or 8 h after onset of the infusion, the following MPE [%] and RMSE [%] were respectively reported: −5.25% and 5.57% for C_{max}; 4.71% and 5.43% for Vd; −4.18% and 19.88% for CL; 5.31% and 18.07% for $t_{1/2}$; 1.08% and 15.98% for AUC.

The best models predicting Vd, CL and AUC of the four β-lactams are listed in Table 2 with their respective r^2, and are illustrated in Figs. 8–10, respectively. For each model, no outlier in the population dataset and patient influencing covariate selection was identified: residuals were normally distributed, and the Cook's influence and jack-knife analysis gave satisfactory results (Cook's distances <1 and CV <5%, respectively). β-lactam Vd and CL were better predicted when normalized to body weight. AMK C_{max} and colloid solution for fluid resuscitation were retained as significant predictive variables of β-lactam Vd, except for CAZ. Elimination of PIP, CAZ and FEP was influenced by both AMK CL and renal biomarker(s) (i.e. urea, potassium). Moreover, hepatic biomarkers (i.e. activated partial thromboplastin time, total protein) were found to significantly affect PIP and MEM CL. Age was retained as a significant predictive variable of MEM CL and AUC. Based on the inclusion and exclusion probabilities, AMK PK parameters appeared the best predictors in all models. Table 3 displays the predictive performance of each developed model.

Discussion

In the present study, PK of AMK and four β-lactams (PTZ, CAZ, FEP and MEM) have been characterized in 69 patients with severe sepsis or septic shock, following the first dose of antibiotics, considering the

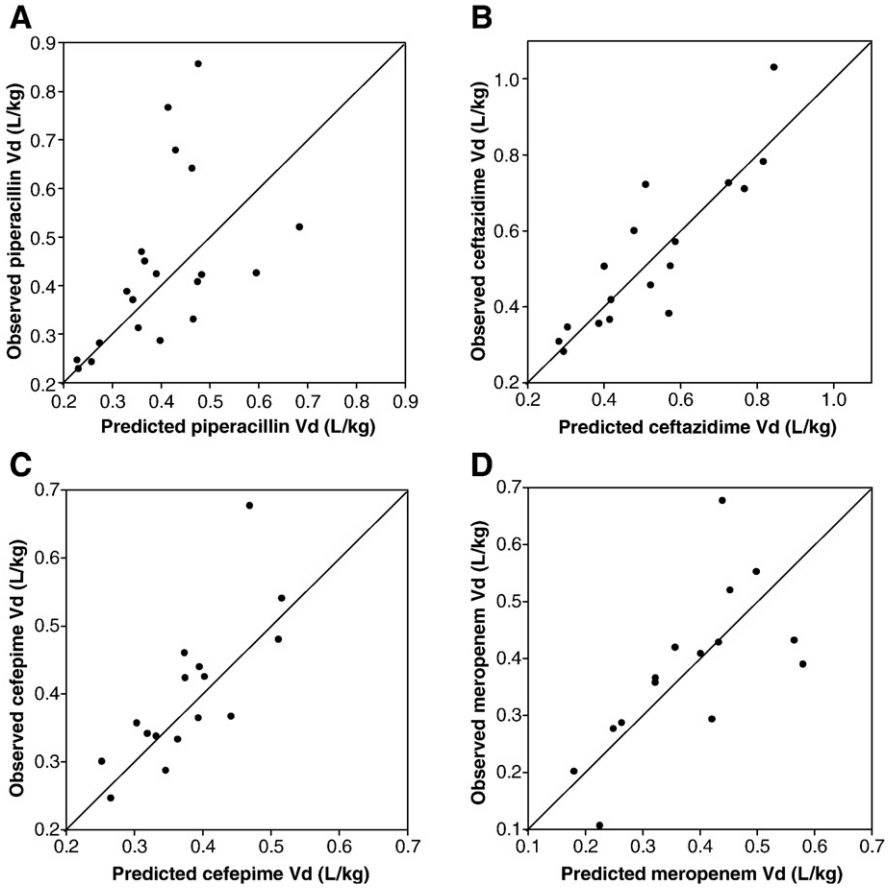

Fig. 8. Observed versus predicted volume of distribution (Vd) of (A) piperacillin, (B) ceftazidime, (C) cefepime and (D) meropenem in critically ill septic patients.

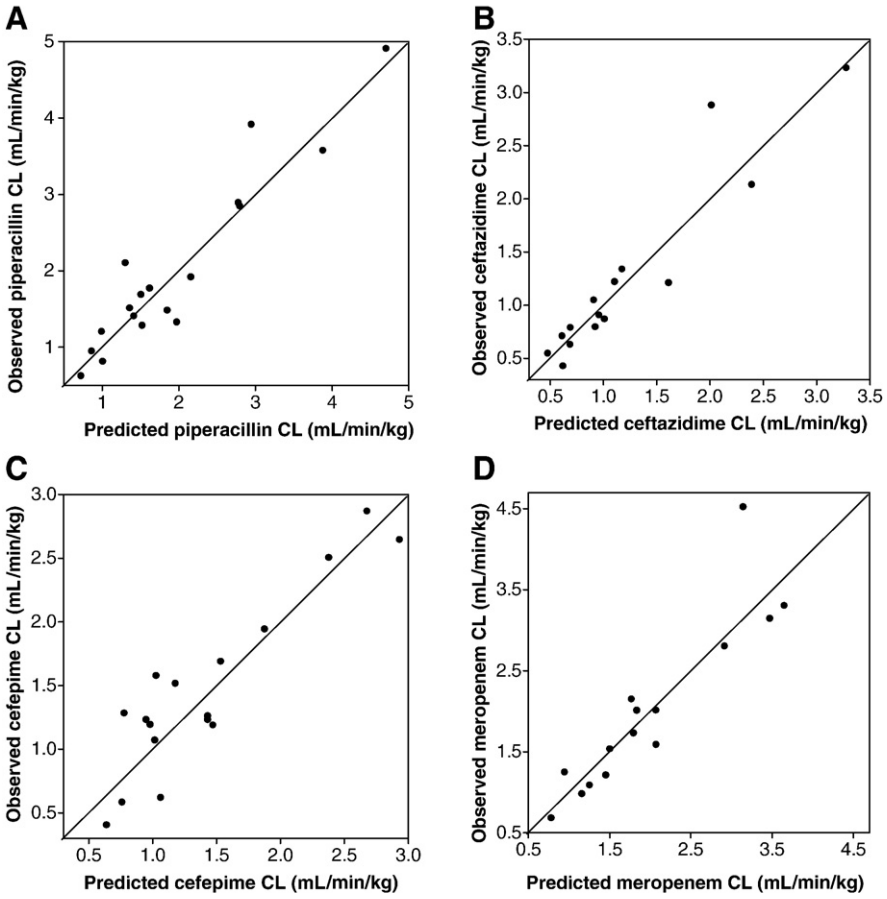

Fig. 9. Observed versus predicted total clearance (CL) of (A) piperacillin, (B) ceftazidime, (C) cefepime and (D) meropenem in critically ill septic patients.

first 24 h as a particularly important period in the management of these patients. This is the first report proposing empirical models for dosage optimization of four β-lactams based on AMK concentration monitoring. These models have been validated using reliable statistical tools: distribution of residuals, Cook's influence, jack-knife analysis [42], and predictive performance parameters as suggested by Sheiner and Beal [38]. The main advantage of these models predicting the β-lactam PK is the ease of their use in clinical practice; while predictive biometric variables are easily available, predictive AMK PK and biochemistry parameters are rapidly measured and can be obtained from the same blood sample. In a recent paper, Panomvana et al. [43] have explored the association between the PK of AMK and CAZ. They have proposed bivariate equations allowing prediction of the PK parameters. Although their models give clinicians helpful guidance in adjusting the dosage regimens of AMK and CAZ, a careful statistical validation should be required to assess sensitivity and predictive performance of their equations.

Severe sepsis is known to affect drug disposition, as a consequence of alterations in capillary permeability, protein binding and organ function [17–22]. Although it is established that free drug fraction is related to the pharmacological effect [44–46], only total concentrations have been measured in this study. Indeed, protein binding of the five investigated antibiotics is low (0–11% for AMK, 21% for PIP, ±10% for CAZ, <16.4% for FEP, ±2% for MEM) [47], and any change in free fraction is not expected to cause significant impact in therapeutic efficacy [48]. The present study has confirmed the sepsis-induced PK modifications; when compared to healthy subject values, Vd and $t_{1/2}$ were increased, and CL was reduced [49,50]. In addition, an important interindividual PK variability has been observed, highlighting therefore the need for TDM to individually adjust the antibiotic dosage regimens (Fig. 3–7, Table 1).

The Sawchuk–Zaske method is a practical TDM approach; it only requires two blood sampling during the elimination phase to adequately estimate the individual patient PK parameters [27,28]. Moreover, it has been widely used and validated in various populations, including patients with extreme PK parameter values [27,51-53]. Its robustness and independency from population PK knowledge make this approach very attractive for ICU patients with severe sepsis or septic shock. Satisfactory accuracy and precision (i.e. MPE and RMSE, respectively) have been obtained when investigating the performance of this method to adequately assess the AMK PK forecasting. Unlike aminoglycosides, β-lactam concentration monitoring is difficult to implement in clinical practice for analytical and economical reasons [54]. The development of models able to predict β-lactam PK from AMK TDM, by performing a multivariate analysis, could therefore be of clinical interest.

From the multivariate analysis, PIP, FEP and MEM Vd were found to be negatively associated with AMK C_{max} and positively associated with fluid resuscitation volume (infused colloid solution). Fluid resuscitation aims to restore circulating intravascular volume (lost due to vasodilatation, venous pooling and capillary leakage), haemodynamic stability and organ perfusion. The infused colloid volume contributes to increase the intravascular volume but may affect drug concentrations by dilution. Antibiotic PK is therefore not only affected by pathophysiological status but also by associated treatments. Unlike other β-lactams, CAZ Vd has been directly associated with AMK Vd. This could be explained by the similar physicochemical properties of both drugs; they are soluble in water and have equivalent molecular weights (585.6 g/mol for AMK, 546.6 g/mol for CAZ). The observed association between hepatic (activated partial thromboplastin time and total protein) and renal

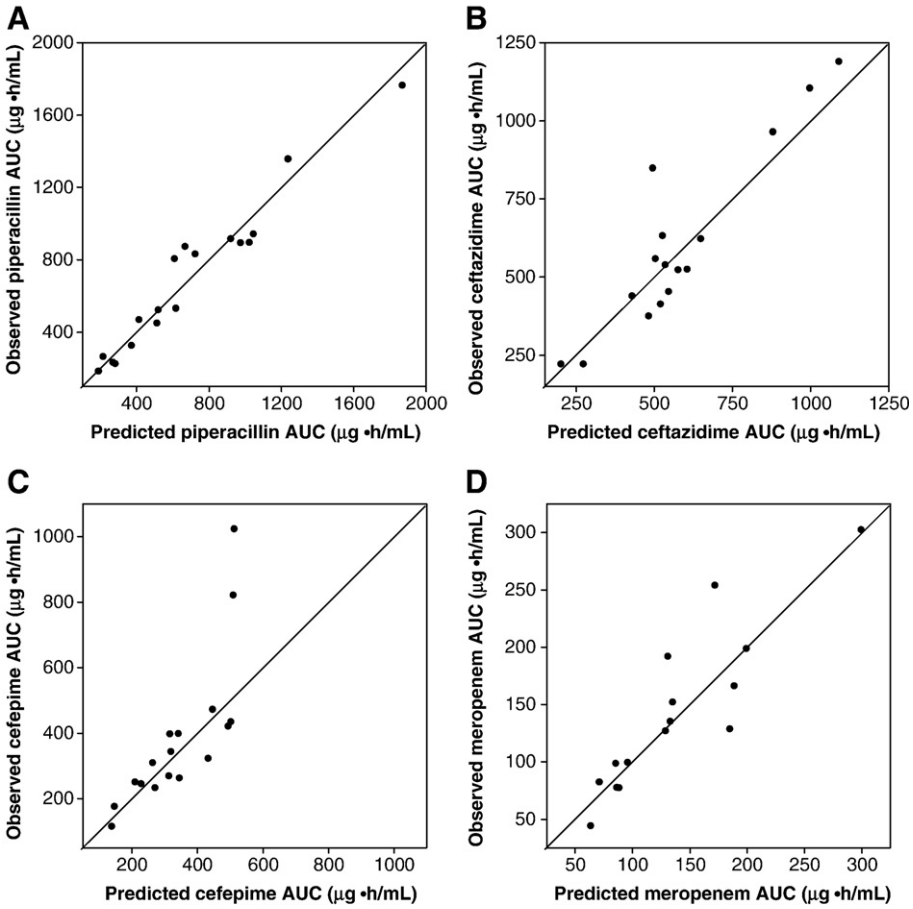

Fig. 10. Observed versus predicted area under the serum concentration-time curve (AUC) of (A) piperacillin, (B) ceftazidime, (C) cefepime and (D) meropenem in critically ill septic patients.

(urea and potassium) biomarkers, retained as variables, and antibiotic CL could be explained by the drug elimination route(s). Indeed, while PIP and MEM are known to be eliminated both via renal and hepatic routes, CAZ and FEP are mainly excreted by the kidneys [47]. For MEM, older age (>70 years) has been found to be a predictor of lower CL and higher AUC, reflecting the relationship between aging and drug disposition [55]. Our results have also stressed the strong predictive value of body weight in the prediction of β-lactam Vd, CL and AUC.

Table 3
Predictive performance of models predicting the β-lactam Vd, CL and AUC.

Predicted PK parameters	*n*	MPE [%]	RMSE [%]	Patients [%] with PE [%] ≥*X* or ≤ −*X*		
				X = 20%	*X* = 30%	*X* = 40%
PIP Vd	20	2.09	26.92	45	35	15
PIP CL	18	−0.85	20.29	28	11	6
PIP AUC	18	−1.07	14.86	17	0	0
CAZ Vd	17	−0.06	18.27	24	6	6
CAZ CL	15	−1.74	19.89	20	20	7
CAZ AUC	17	−0.52	18.03	24	6	6
FEP Vd	16	3.33	14.20	6	6	0
FEP CL	17	−2.84	29.90	47	24	12
FEP AUC	17	2.78	23.56	29	24	6
MEM Vd	15	0.41	23.90	33	33	13
MEM CL	15	−2.67	17.01	20	7	0
MEM AUC	15	−0.94	22.03	27	27	13

PIP, piperacillin; CAZ, ceftazidime; FEP, cefepime; MEM, meropenem; Vd, volume of distribution; CL, total clearance; AUC, area under the serum concentration-time curve; n, number of patients; MPE, mean prediction error; RMSE, root mean square error; PE, prediction error.

The present models provide the PK information necessary for achieving the so-called dual dosage individualization. Such a practice actually takes into account both PK and PD parameters, named efficacy indices, when designing the dosage regimen [15,16]. Three efficacy indices have received increased emphasis by clinicians: the ratio of maximal concentration to minimal inhibitory concentration (MIC) of the infecting pathogen (C_{max}/MIC), the percentage of the dosage interval with concentrations exceeding the MIC (T [%] >MIC) and the ratio of AUC during a 24-h interval to MIC (AUC_{24}/MIC). To perform dual dosage individualization in clinical practice, it is necessary to select the appropriate efficacy index that correlates best with the therapeutic efficacy. While C_{max}/MIC and AUC_{24}/MIC are believed to better predict the therapeutic outcome for aminoglycosides, T [%] >MIC is considered to be the most relevant index indicating the β-lactam efficacy [11–14,56–58]. Furthermore, Schentag et al. [59] support the validity of AUC_{24}/MIC as a universal efficacy index. When the efficacy index target and the MIC breakpoint of the infecting pathogen are known, rational drug dosage can be designed using Eqs. (3) [27], (4) and (5) [23]:

$$\frac{C_{max}}{\text{MIC}} = \frac{\text{Dose}}{t_{inf} \bullet k_e \bullet \text{Vd} \bullet \text{MIC}} \bullet \left(\frac{1 - e^{-k_e \bullet t_{inf}}}{1 - e^{-k_e \bullet \tau}} \right) \tag{3}$$

$$T[\%] > \text{MIC} = ln\left(\frac{\text{Dose}}{\text{Vd} \bullet \text{MIC}} \right) \bullet \frac{t_{1/2}}{0.693} \bullet \frac{100}{\tau} \tag{4}$$

$$\frac{\text{AUC}_{24}}{\text{MIC}} = \frac{\text{Dose}}{\text{Vd} \bullet \text{MIC}} \bullet \frac{t_{1/2}}{0.693} \bullet \frac{24}{\tau} \tag{5}$$

where t_{inf} is the infusion duration (h), k_e is the elimination rate constant (h^{-1}), τ is the dosage interval (h), dose is expressed in mg/kg, Vd in L/kg, $t_{1/2}$ in h, and MIC in mg/L.

In our institution, the Sawchuk–Zaske method is daily applied in a TDM software–PharMonitor–to optimally adjust the dosage regimens of four aminoglycosides (AMK, gentamicin, tobramycin and netilmicin) [37]. By integrating the present empirical models in the software, easy, rapid and cost-effective optimization of the β-lactam dosage regimens could be performed using the appropriate efficacy index. It should be emphasized that the dosage regimen obtained from such PK modeling equations remain to be validated by appropriate medical expertise taking into account the clinical status of the patient.

AMK, frequently prescribed in Europe, has been selected in our study. However, due to close chemicophysical and PK properties among aminoglycosides, it is likely that similar conclusions could be reached from the use of other aminoglycosides such as gentamicin, whose the consumption is larger in USA and Japan.

In an attempt to promote optimal use of antibiotics in critically ill septic patients, the authors have developed empirical models to predict four β-lactams PK from amikacin routine clinical PK monitoring. In these models, the best predictor was one of the amikacin PK parameters obtained from PharMonitor. Other variables included fluid resuscitation, renal and hepatic biomarkers, age and body weight. The application of such models could contribute to a better therapeutic management of these patients.

Acknowledgments

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References

[1] Vincent JL, Sakr Y, Sprung CL, et al. Sepsis occurrence in acutely ill patients investigators. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006;34:344–53.
[2] Linde-Zwirble WT, Angus DC. Severe sepsis epidemiology: sampling, selection, and society. Crit Care 2004;8:222–6.
[3] Alberti C, Brun-Buisson C, Burchardi H, et al. Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. Intensive Care Med 2002;28:108–21.
[4] Dombrovskiy VY, Martin AA, Sunderram J, Paz HL. Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003. Crit Care Med 2007;35:1244–50.
[5] Kollef MH, Sherman G, Ward S, Fraser VJ. Inadequate antimicrobial treatment of infections: a risk factor for hospital mortality among critically ill patients. Chest 1999;115:462–74.
[6] Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest 2000;118:146–55.
[7] Leibovici L, Shraga I, Drucker M, Konigsberger H, Samra Z, Pitlik SD. The benefit of appropriate empirical antibiotic treatment in patients with bloodstream infection. J Intern Med 1998;244:379–86.
[8] Harbarth S, Garbino J, Pugin J, Romand JA, Lew D, Pittet D. Inappropriate initial antimicrobial therapy and its effect on survival in a clinical trial of immunomodulating therapy for severe sepsis. Am J Med 2003;115:529–35.
[9] MacArthur RD, Miller M, Albertson T, et al. Adequacy of early empiric antibiotic treatment and survival in severe sepsis: experience from the MONARCS trial. Clin Infect Dis 2004;38:284–8.
[10] Deresinski S. Principles of antibiotic therapy in severe infections: optimizing the therapeutic approach by use of laboratory and clinical data. Clin Infect Dis 2007;45:S177–83.
[11] Scaglione F. Can PK/PD be used in everyday clinical practice. Int J Antimicrob Agents 2002;19:349–53.
[12] Hyatt JM, McKinnon PS, Zimmer GS, Schentag JJ. The importance of pharmacokinetic/pharmacodynamic surrogate markers to outcome. Focus on antibacterial agents. Clin Pharmacokinet 1995;28:143–60.
[13] Pea F, Viale P. The antimicrobial therapy puzzle: could pharmacokinetic-pharmacodynamic relationships be helpful in addressing the issue of appropriate pneumonia treatment in critically ill patients. Clin Infect Dis 2006;42:1764–71.
[14] Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis 1998;26:1–10.
[15] Schentag JJ, Swanson DJ, Smith IL. Dual individualization: antibiotic dosage calculation from the integration of *in-vitro* pharmacodynamics and *in-vivo* pharmacokinetics. J Antimicrob Chemother 1985;15:S47–57.
[16] Schentag JJ, Ballow CH, Paladino JA, Nix DE. Dual individualization with antibiotics: integrated antibiotic management strategies for use in hospitals. In: Evans WE, Schentag JJ, Jusko WJ, editors. Applied pharmacokinetics. Principles of therapeutic drug monitoring. 3rd ed. Vancouver (WA): Applied Therapeutics, Inc.; 1992. p.17.1-20.
[17] Roberts JA, Lipman J. Antibacterial dosing in intensive care: pharmacokinetics, degree of disease and pharmacodynamics of sepsis. Clin Pharmacokinet 2006;45:755–73.
[18] Pea F, Viale P, Furlanut M. Antimicrobial therapy in critically ill patients: a review of pathophysiological conditions responsible for altered disposition and pharmacokinetic variability. Clin Pharmacokinet 2005;44:1009–34.
[19] 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.
[20] Power BM, Forbes AM, van Heerden PV, Ilett KF. Pharmacokinetics of drugs used in critically ill adults. Clin Pharmacokinet 1998;34:25–56.
[21] Mehrotra R, De Gaudio R, Palazzo M. Antibiotic pharmacokinetic and pharmacodynamic considerations in critical illness. Intensive Care Med 2004;30:2145–56.
[22] Bodenham A, Shelly MP, Park GR. The altered pharmacokinetics and pharmacodynamics of drugs commonly used in critically ill patients. Clin Pharmacokinet 1988;14:347–73.
[23] Mohr JF, Wanger A, Rex JH. Pharmacokinetic/pharmacodynamic modeling can help guide targeted antimicrobial therapy for nosocomial gram-negative infections in critically ill patients. Diagn Microbiol Infect Dis 2004;48:125–30.
[24] Bochud PY, Glauser MP, Carlet J, Calandra T. Empirical antibiotic therapy for patients with severe sepsis and septic shock. In: Vincent JL, Carlet J, Opal SM, editors. The Sepsis Text. Boston: Kluwer Academic Publishers; 2002. p. 539–58.
[25] Tod MM, Padoin C, Petitjean O. Individualising aminoglycoside dosage regimens after therapeutic drug monitoring: simple or complex pharmacokinetic methods. Clin Pharmacokinet 2001;40:803–14.
[26] Erdman SM, Rodvold KA, Pryka RD. An updated comparison of drug dosing methods. Part III: aminoglycoside antibiotics. Clin Pharmacokinet 1991;20:374–88.
[27] Sawchuk RJ, Zaske DE. Pharmacokinetics of dosing regimens which utilize multiple intravenous infusions: gentamicin in burn patients. J Pharmacokinet Biopharm 1976;4:183–95.
[28] Sawchuk RJ, Zaske DE, Cipolle RJ, Wargin WA, Strate RG. Kinetic model for gentamicin dosing with the use of individual patient parameters. Clin Pharmacol Ther 1977;21:362–9.
[29] Sanford JP, Gilbert DN, Moellering RC, et al, editors. The Sanford guide to antimicrobial therapy. 19th ed. Belgian: Luxembourg edition; 2005-2006.
[30] Levy MM, Fink MP, Marshall JC, et al. SCCM/ESICM/ACCP/ATS/SIS. 2001 SCCM/ESICM/ACCP/ATS/SIS. International Sepsis Definitions Conference. Crit Care Med 2003;31:1250–6.
[31] Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 1985;13:818–29.
[32] Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 1996;22:707–10.
[33] Jolley ME. Fluorescence polarization immunoassay for the determination of therapeutic drug levels in human plasma. J Anal Toxicol 1981;5:236–40.
[34] Abbott Laboratories Diagnostics Division. TDxFLx/TDx assays manual. 1990.
[35] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline. Q2(R1); Validation of analytical procedures: text and methodology. Nov 2005. Available at: http://www.ich.org/LOB/media/MEDIA417.pdf. Accessed November 2, 2005.
[36] US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). Guidance for industry: bioanalytical method validation. May 2001. Available at: http://www.fda.gov/cder/Guidance/4252fnl.pdf. Accessed November 2, 2005.
[37] Leal T, Parez JJ, Vanbinst R, Wallemacq PE. Computerized approach to monitoring aminoglycosides. Clin Chem 1991;37:1415–9.
[38] Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm 1981;9:503–12.
[39] Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41.
[40] Levey AS, Greene T, Kusek JW, Beck GJ. A simplified equation to predict glomerular filtration rate from serum creatinine [abstract]. J Am Soc Nephrol 2000;11:A0828.
[41] Glantz SA, Slinker BK. Selecting the best regression model. In: Glantz SA, Slinker BK, editors. Primer of Applied Regression and Analysis of Variance. 2nd ed. New York (NY): McGraw-Hill, Inc.; 2000. p. 241–73.
[42] Brendel K, Dartois C, Comets E. Are population pharmacokinetic and/or pharmacodynamic models adequately evaluated? A survey of the literature from 2002 to 2004. Clin Pharmacokinet 2007;46:221–34.
[43] Panomvana D, Kiatjaroensin SA, Phiboonbanakit D. Correlation of the pharmacokinetic parameters of amikacin and ceftazidime. Clin Pharmacokinet 2007;46:859–66.
[44] Craig WA, Kunin CM. Significance of serum protein and tissue binding of antimicrobial agents. Annu Rev Med 1976;27:287–300.

[45] Rolinson GN. The significance of protein binding of antibiotics in antibacterial chemotherapy. J Antimicrob Chemother 1980;6:311–7.
[46] Shyu WC, Quintiliani R, Nightingale CH, Dudley MN. Effect of protein binding on drug penetration into blister fluid. Antimicrob Agents Chemother 1988;32: 128–30.
[47] Association Générale de l'Industrie du Médicament asbl. Compendium21st ed. ; 2003.
[48] Lovich MA, Creel C, Hong K, Hwang CW, Edelman ER. Carrier proteins determine local pharmacokinetics and arterial distribution of paclitaxel. J Pharm Sci 2001;90:1324–35.
[49] Taeschner W, Vozeh S. Pharmacokinetic drug data. In: Holford NHG, editor. Clinical Pharmacokinetics. Drug data handbook. 3rd ed. Auckland (NZ): Adis International; 1998.
[50] Wise R, Logan M, Cooper M, Ashby JP, Andrews JM. Meropenem pharmacokinetics and penetration into an inflammatory exudate. Antimicrob Agents Chemother 1990;34:1515–7.
[51] Zaske DE, Cipolle RJ, Strate RJ. Gentamicin dosage requirements: wide interpatient variations in 242 surgery patients with normal renal function. Surgery 1980;87: 164–9.
[52] Zaske DE, Cipolle RJ, Rotschafer JC, Solem LD, Mosier NR, Strate RG. Gentamicin pharmacokinetics in 1,640 patients: method for control of serum concentrations. Antimicrob Agents Chemother 1982;21:407–11.
[53] Zaske DE, Irvine P, Strand LM, Strate RG, Cipolle RJ, Rotschafer J. Wide interpatient variations in gentamicin dosage requirements for geriatric patients. JAMA 1982;248:3122–6.
[54] Touw DJ, Neef C, Thomson AH, Vinks AA. Cost-Cost-Effectiveness of Therapeutic Drug Monitoring Committee of the International Association for Therapeutic Drug Monitoring and Clinical Toxicology. Cost-effectiveness of therapeutic drug monitoring: a systematic review. Ther Drug Monit 2005;27:10–7.
[55] Mayersohn MB. Special pharmacokinetic considerations in the elderly. In: Evans WE, Schentag JJ, Jusko WJ, editors. Applied pharmacokinetics. Principles of therapeutic drug monitoring. 3rd ed. Vancouver (WA): Applied Therapeutics, Inc.; 1992. p.9.1-43.
[56] Turnidge JD. The pharmacodynamics of beta-lactams. Clin Infect Dis 1998;27: 10–22.
[57] Moore RD, Lietman PS, Smith CR. Clinical response to aminoglycoside therapy: importance of the ratio of peak concentration to minimal inhibitory concentration. J Infect Dis 1987;155:93–9.
[58] Lacy MK, Nicolau DP, Nightingale CH, Quintiliani R. The pharmacodynamics of aminoglycosides. Clin Infect Dis 1998;27:23–7.
[59] Schentag JJ, Nix DE, Forrest A, Adelman MH. AUIC—the universal parameter within the constraint of a reasonable dosing interval. Ann Pharmacother 1996;30: 1029–31.