

# Development of a Physiologically Based Pharmacokinetic Modelling Approach to Predict the Pharmacokinetics of Vancomycin in Critically Ill Septic Patients

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## Abstract

**Background and Objectives** Sepsis is characterised by an excessive release of inflammatory mediators substantially affecting body composition and physiology, which can be further affected by intensive care management. Consequently, drug pharmacokinetics can be substantially altered. This study aimed to extend a whole-body physiologically based pharmacokinetic (PBPK) model for healthy adults based on disease-related physiological changes of critically ill septic patients and to evaluate the accuracy of this PBPK model using vancomycin as a clinically relevant drug.

**Methods** The literature was searched for relevant information on physiological changes in critically ill patients with sepsis, severe sepsis and septic shock. Consolidated information was incorporated into a validated PBPK vancomycin model for healthy adults. In addition, the model was further individualised based on patient data from a study including ten septic patients treated with intravenous vancomycin. Models were evaluated comparing predicted concentrations with observed patient concentration–time data.

**Results** The literature-based PBPK model correctly predicted pharmacokinetic changes and observed plasma concentrations especially for the distribution phase as a result of a consideration of interstitial water accumulation. Incorporation of disease-related changes improved the model prediction from 55 to 88% within a threshold of 30% variability of predicted vs. observed concentrations. In particular, the consideration of individualised creatinine clearance data, which were highly variable in this patient population, had an influence on model performance.

**Conclusion** PBPK modelling incorporating literature data and individual patient data is able to correctly predict vancomycin pharmacokinetics in septic patients. This study therefore provides essential key parameters for further development of PBPK models and dose optimisation strategies in critically ill patients with sepsis.

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### Key Points

A physiologically based pharmacokinetic model was successfully developed to predict the pharmacokinetics in a cohort of critically ill septic patients treated with vancomycin considering relevant disease-related changes in physiology.

Incorporation of literature-based physiological changes obtained from a structured literature survey led to a significant improvement in model prediction for vancomycin, whereas individual creatinine clearance data were necessary to better predict the clearance of this primarily renally cleared drug.

The gathered key parameters for physiological alterations during sepsis will allow for a further physiologically based pharmacokinetic modelling extension in this subpopulation of patients with the aim to optimise drug dosing in critically ill septic patients.

## 1 Introduction

Despite improvements in critical care medicine, sepsis remains a leading cause of death with about seven million fatalities per year [1]. The mortality rate for septic shock can be as high 50% [2] and an increase of incidence by approximately 8.5% per year is expected for the future [3, 4]. According to the definition of the American College of Chest Physicians/Society of Critical Care Medicine consensus conference, sepsis is a microbiologically proven or clinically suspected infection, which is accompanied by a systemic inflammatory response syndrome [5]. This inflammatory state is associated with a multitude of changes in physiological and biochemical processes. Among others, an increase of endothelial permeability ('capillary leakage syndrome') [6, 7], vasodilatation through release of nitric oxide (NO) and changes in macro- and microcirculation owing to the procoagulant effects of cytokines [8], as well as changes in protein biosynthesis [9, 10] are typically observed. The resultant multi-organ dysfunction can peak in life-threatening complications.

The main principles in sepsis therapy are prompt hemodynamic stabilisation, surgical therapy of the infectious focus where applicable, support of organ failure as clinically needed in the intensive care unit (ICU) and the initiation of antimicrobial drug therapy [11]. However, physiological changes during sepsis may influence the pharmacokinetics (PK) of the administered drugs, resulting

in an uncertainty of pharmacotherapeutic success. In the past, several studies were able to demonstrate major changes in the PK of drugs administered to septic patients and a number of sepsis-related alterations in physiology with probable influence on PK were described [12–14]. However, the clinical relevance of these changes remains so far unknown. Hence, a more profound understanding of the impact of physiological changes on PK in sepsis is necessary to optimise pharmacotherapy.

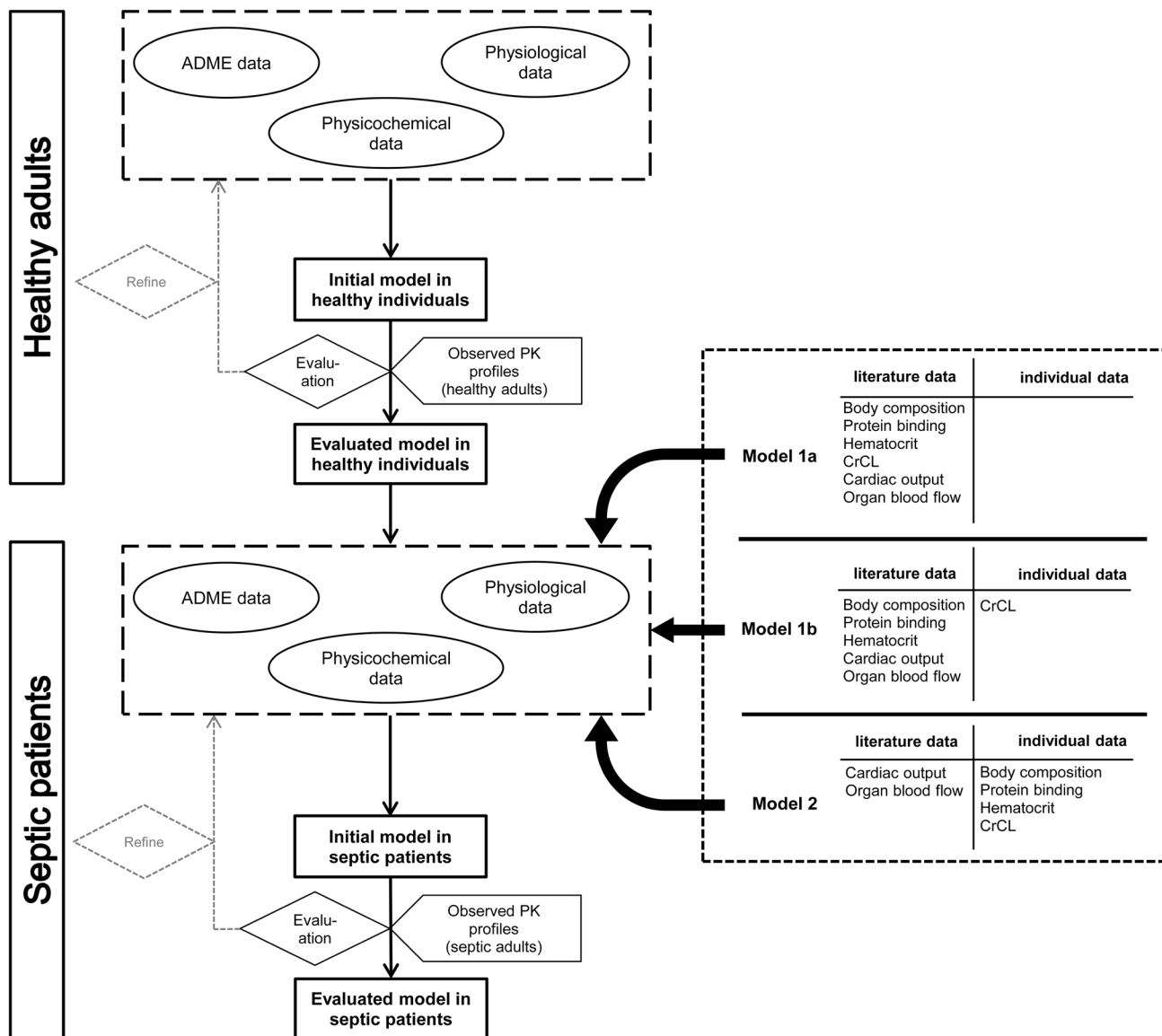
Whole-body physiologically based pharmacokinetic (PBPK) modelling is an ideal *in silico* technique to predict the impact of physiological processes on drug PK owing to (non-)pathological states. In comparison to classical pharmacokinetic modelling, PBPK incorporates drug-specific parameters and patient characteristics to predict whole-body PK [15]. While physicochemical parameters can be determined experimentally, the large number of physiological input parameters, though not always easy to obtain, allows for physiologically based predictions and translation of results. Databases such as the International Commission on Radiological Protection [16] offer a vast source of information on physiological and anatomical data to allow for PBPK modelling in healthy individuals. Detailed knowledge on disease-related physiological, anatomical and biochemical alterations can be used for the prediction of PK in special populations by implementing these changes into the model. So far, PBPK models have been successfully developed for patients with liver cirrhosis [17], patients in perioperative settings [18], as well as individuals in non-pathological conditions, such as pregnant women [19] and children [20], showing that PBPK modelling is capable of simulating special populations.

The aim of the current investigation was to develop a whole-body PBPK model to reflect the disease-related physiological changes of critically ill septic patients and to evaluate the accuracy of the model using *in vivo* data of intravenously administered vancomycin in septic patients. We implemented physiological data extracted from literature as well as individual patient data to improve model prediction during sepsis and compared it with a model considering no disease-related physiological changes. To our knowledge, this is the first study examining the effects of sepsis-related physiologic alterations on PK using a PBPK approach.

## 2 Materials and Methods

### 2.1 Workflow

The workflow for scaling of a vancomycin PBPK model in critically ill septic patients is described in Fig. 1.



**Fig. 1** Schematic workflow for the development of a PBPK model for septic patients based on a model for healthy adults (model 0). Model 1a represents anatomical and physiological data adaptation for septic subjects based on literature values only. For model 1b, the literature-based adaptations were modified by patient CrCL values

from the study. In model 2, additional adjustments for readily accessible patient data from the study were implemented. *ADME* administration, distribution, metabolism, excretion, *CrCL* creatinine clearance, *PBPK* physiologically based pharmacokinetic, *PK* pharmacokinetics

### 2.2 Physiologically Based Pharmacokinetic Modelling

PK-Sim® (Bayer Technology Services, Leverkusen, Germany) is a commercially available PBPK modelling software to describe the fate of xenobiotics in various laboratory animals and humans. The generic structure of human PBPK modelling in PK-Sim® consists of 18 compartments, representing relevant organs and tissues of the body, defined by their physiologic volumes and blood flow rates. Organs are physiologically linked via arteries and veins to enable inter-compartmental mass transport. Each compartment is further

subdivided into four sub-compartmental structures, namely plasma and red blood cells (which form the intravascular space), the interstitial space and the intracellular space. To describe the rate and extent of drug disposition in a compartment, PK-Sim® estimates drug-related model parameters (permeability, organ/plasma partition coefficients) based on readily accessible physicochemical information (e.g. molecular weight, protein binding, lipophilicity) as well as known organ composition data (e.g. lipid, water, protein). Additionally, parameters for compound-specific renal and hepatic elimination processes are required to finally solve differential equations and to obtain concentration–time data of the

**Table 1** Input parameters for the basic PBPK model of vancomycin

Parameters	Value	References
Model settings	Standard model for small molecules	
Model parameters: partition coefficient	PK-Sim <sup>®</sup> standard	
Model parameters: cellular permeabilities	Charge-dependent Schmitt	
Molecular weight (g/mol)	1449	[86]
logP	2.45	Modified according to predicted value from [78]
$f_{u_p}$	0.67	[87–89]
$pK_a$	2.18 (acid), 7.75 (base), 8.89 (base)	[90]
$CL_{renal}$ (mL/min/kg)	0.95	[24, 25]
$CL_{hepatic}$ (mL/min/kg)	0.11	[24, 25]

$CL_{hepatic}$  hepatic plasma clearance of vancomycin,  $CL_{renal}$  renal plasma clearance of vancomycin,  $f_{u_p}$  fraction unbound of vancomycin in plasma,  $logP$  logarithm of octanol/water partition coefficient, *PBPK* physiologically based pharmacokinetic,  $pK_a$  negative decadic logarithm of acid dissociation constant

modelled substance. A more detailed insight into the generic model structure of PK-Sim<sup>®</sup> is given by Willmann et al. [21, 22] or the software manual [23].

### 2.3 Development and Evaluation of a PBPK Model for Vancomycin in Healthy Individuals

PK-Sim<sup>®</sup> (version 6.0) was used for the development and evaluation of a vancomycin PBPK model in healthy volunteers. Model-building parameters are listed in Table 1. The elimination process of vancomycin was divided into a renal (90%) and a hepatic clearance process (10%). For the evaluation of the initial model in healthy adults, two datasets from the literature were used. The first was a study conducted by Cutler et al. who investigated the age dependency of vancomycin PK [24]. Stated pharmacokinetic parameters from the younger adult group were used to calculate the plasma concentration profiles of vancomycin for each individual. One subject (subject 4) was excluded from the evaluation dataset because micro rate constants needed to calculate the plasma concentration profile could not be determined by means of the given pharmacokinetic parameters. The second dataset by Blouin et al. studied the difference between normal and morbidly obese healthy subjects [25]. Measured data from both subgroups were used as observed values.

### 2.4 Study Design and Patient Recruitment

Plasma concentration data for model evaluation and individual physiological parameters were collected in critically ill septic patients from June 2014 to May 2016, treated in the ICUs of the University Hospital of Muenster. The study was approved by the local ethics committee and written informed consent was obtained from the patient or the

patient's representatives before admission to the study. Inclusion criteria were as follows: (1) septic adult patients (>18 years of age) as defined by the American College of Chest Physicians/Society of Critical Care Medicine criteria [5]; (2) necessity for treatment in an ICU; (3) indication for intravenous treatment with vancomycin for a suspected or confirmed infection. Patients were excluded if they experienced dialysis-dependent renal failure. Moreover, patients with preexisting liver cirrhosis or any form of preexisting hepatic impairment were also excluded.

The indication for vancomycin therapy and the dosing regimen was as determined by the physician in attendance. The vancomycin dose ranged between 1 and 2 g and was administered two to three times a day via an intravenous infusion over a period of approximately 1 h. Blood samples for vancomycin quantification were collected during the first (after completion of infusion, 2, 4 and 6 h after start of infusion, pre-dose), second (after completion of infusion, 4 h after start of infusion, pre-dose) and third (randomly after completion of infusion, pre-dose) administration. All blood samples were collected via an indwelling arterial catheter into heparinised tubes. The tubes were centrifuged to obtain plasma, which was stored at  $-20\text{ }^{\circ}\text{C}$  until analysis of vancomycin. The exact vancomycin dose, infusion times and sample collection times were recorded and patient demographic, clinical, as well as laboratory data were extracted from the electronic medical records. Creatinine clearance (CrCL) was estimated according to the Cockcroft–Gault formula [26].

### 2.5 Drug Assay Method

Vancomycin plasma concentrations were determined using a modified high-performance liquid chromatography method [27, 28]. In brief, 100  $\mu\text{L}$  of plasma was mixed

with 50  $\mu\text{L}$  of 2.5% phosphoric acid, 400  $\mu\text{L}$  acetonitrile and 10  $\mu\text{L}$  of 70% perchloric acid. After incubation, the mixture was centrifuged and the supernatant was transferred and extracted with 900  $\mu\text{L}$  dichloromethane. After centrifugation, 40  $\mu\text{L}$  of the upper aqueous layer was injected onto the column. High-performance liquid chromatography instrumentation and the mobile phase were identical to a previously described method [27]. The limit of quantification of the method was 1 mg/L and the intra- and inter-day assay coefficients of variation were <11% over the entire calibration range of 1–80 mg/L. Samples were stable for at least 7 days at 4 °C and 5 weeks at –20 °C.

For determination of unbound vancomycin, a previously described ultrafiltration method was used [29]. Recovery of vancomycin in the ultrafiltrate ranged between 96.8 and 98.4% and freezing at –20 °C for 6 weeks showed no influence on protein binding.

## 2.6 Collection of Physiological Parameters in Sepsis

A literature search was conducted to qualify and quantify physiological, anatomical and biochemical parameter alterations in adults during sepsis, capable of affecting the PK of drugs. Because sepsis can be further subdivided into different degrees of severity (namely sepsis, severe sepsis and septic shock), only studies presenting an explicit classification of the respective septic state, according to the official definition of the American College of Chest Physicians/Society of Critical Care Medicine consensus conference or a comparable definition, were taken into account. Patients aged <18 years, as well as patients experiencing dialysis-dependent renal failure were excluded.

To merge parameter values of different studies, the weighted mean value  $\bar{X}$  was calculated using Eq. (1):

$$\bar{X} = \frac{\sum_{j=1}^J n_j \cdot x_j}{\sum_{j=1}^J n_j} \quad (1)$$

where  $n_j$  is the number of study subjects in the  $j$ th study and  $x_j$  is the reported mean value of the  $j$ th study. The pooled standard deviation (SD) was calculated using Eq. (2):

$$\text{Pooled SD} = \sqrt{\frac{\sum_{j=1}^J ((n_j - 1) \cdot \text{SD}_j^2) + \sum_{j=1}^J (n_j \cdot (x_j - \bar{X})^2)}{\sum_{j=1}^J n_j}} \quad (2)$$

where  $\text{SD}_j$  is the standard deviation of the  $j$ th study. Data presented as median values and the interquartile range or minimum and maximum values were considered by prior conversion into the mean and SD as described by Hozo

et al. and Wan et al. [30, 31], assuming a normal distribution of respective parameters.

Based on the quantitative physiological data identified from the literature search, a scaling factor for each parameter was calculated to allow PBPK model adaptations in septic patients. Mean values of the respective physiological parameters from healthy adults were used as control values. The scaling factor was calculated according to Eq. (3):

$$\text{Scaling factor (parameter)} = \frac{\text{mean value}_{\text{sepsis}}(\text{parameter})}{\text{mean value}_{\text{healthy}}(\text{parameter})} \quad (3)$$

## 2.7 Sepsis-Related Scaling of PBPK Model Parameters

### 2.7.1 Model 0

For model 0, no sepsis-related physiological alterations were implemented into the initial vancomycin model for healthy adults. To allow for comparison among the different sepsis-related models described in the subsequent sections, the elimination process was adapted according to the approximation formula by Pea et al., showing a linear relation between total body vancomycin clearance ( $\text{CL}_{\text{van}}$ ) and CrCL [32]. For model 0,  $\text{CL}_{\text{van}}$  calculation was performed by setting CrCL to a standard value of 120 mL/min for healthy adults.

### 2.7.2 Model 1

In the first PBPK approach for septic patients, two different sub-models, model 1a and model 1b, were developed. Both refer to literature-based changes in physiology. Differing from model 1a, individual study patient data on CrCL were additionally taken into account for model 1b, as shown in Fig. 1. Calculated mean physiological scaling factors for hematocrit as well as organ blood flows were implemented into the healthy adult model for vancomycin. Organs with no information on changes of blood flow during sepsis were scaled by sepsis-related alterations in cardiac index (CI), assuming that blood flow changes linearly with cardiac activity. Model adaptation to protein binding, body composition and drug elimination are described in the following. All disease-related physiological changes were implemented manually in the model for each study patient included.

**2.7.2.1 Protein Binding** Determination of the unbound fraction of vancomycin under septic conditions was applied using the modified Eq. (4) of McNamara and Alcorn [33]:



$$f_{u,sepsis} = \frac{1}{1 + \frac{P_{sepsis}}{P_{healthy}} \times \frac{(1-f_{u,healthy})}{f_{u,healthy}}} \quad (4)$$

in which scaling of the unbound fraction in septic patients ( $f_{u,sepsis}$ ) has been conducted on the basis of the unbound fraction in healthy adults ( $f_{u,healthy}$ ) and of the mean molar binding protein concentration in septic and healthy patients ( $P_{sepsis}$  and  $P_{healthy}$ ). Because albumin is the dominant binding protein of vancomycin [34], literature data on serum albumin concentrations of the respective septic state were used to scale the fraction unbound of vancomycin.

**2.7.2.2 Body Composition** To account for changes in body composition in septic patients, seemingly ‘healthy’ subjects were initially modelled in PK-Sim<sup>®</sup> considering age, sex, body height and body weight at ICU admission. For each of the 18 organs presented in PK-Sim<sup>®</sup>, the volume of sub-compartmental structures (vascular [ $V(\text{organ})_{\text{vascular}}$ ], interstitial [ $V(\text{organ})_{\text{interstitial}}$ ], intracellular [ $V(\text{organ})_{\text{intracellular}}$ ]), as well as the volume of protein ( $V(\text{organ})_{\text{protein}}$ ), fat ( $V(\text{organ})_{\text{fat}}$ ) and interstitial, plasmatic and intracellular water ( $V(\text{organ})_{\text{water,interstitial}}$ ,  $V(\text{organ})_{\text{water,plasma}}$ ,  $V(\text{organ})_{\text{water,intracellular}}$ ) were calculated. The volumes for water, protein and fat were scaled by mean literature information on extracellular water (ECW), intracellular water (ICW), total body protein (TBP) and total body fat (TBF) in septic patients, consequently leading to new organ weights ( $OW_{\text{septic}}$ ) and the new body weight of the septic patients. Because capillary leakage in sepsis profoundly contributes to redistribution of administered fluids and plasma proteins, the majority of ECW was assumed to be located in the interstitial space of the modelled patient with a minor part remaining in the intravascular space. This results in an adaptation of the initially calculated sub-compartmental volume structures, which was implemented by maintaining the intracellular volume, as well as the vascular fraction [ $f(\text{organ})_{\text{vascular}}$ ] to the values from initially modelled healthy subjects (Eqs. 5–7):

$$V(\text{organ})_{\text{intracellular},\text{'septic'}} = V(\text{organ})_{\text{intracellular},\text{'healthy'}} \quad (5)$$

$$V(\text{organ})_{\text{vascular},\text{'septic'}} = OW_{\text{septic}} \times f(\text{organ})_{\text{vascular},\text{'healthy'}} \quad (6)$$

$$V(\text{organ})_{\text{interstitial},\text{'septic'}} = OW_{\text{septic}} - V(\text{organ})_{\text{intracellular},\text{'septic'}} - V(\text{organ})_{\text{vascular},\text{'septic'}} \quad (7)$$

Moreover, the protein ratio between the interstitial space and plasma was adjusted from a default value of 0.37 to a value of 1, assuming an equalisation of proteins as a result of capillary leakage.

**2.7.2.3 Elimination** Total  $CL_{\text{van}}$  was calculated as described for model 0, whereas CrCL was scaled by a calculated mean physiological scaling factor for model 1a and individual patient CrCLs were considered for model 1b.

### 2.7.3 Model 2

Model 2 included additional patient variables that were obtained during routine clinical care (Fig. 1; Table 2). As organ perfusion and CI were not determined individually during the study, scaling of blood flows was considered as described for model 1 using the mean scaling factor for CI. Model adaptation of protein binding, body composition and elimination are described in the following. As described for model 1, all disease-related physiological changes were implemented manually in the model for each study patient included.

**2.7.3.1 Protein Binding** Protein binding of vancomycin was determined individually for each patient, applying the validated high-performance liquid chromatography method.

**2.7.3.2 Body Composition** As described for model 1, seemingly ‘healthy’ subjects were initially modelled in PK-Sim<sup>®</sup> considering sex, age, body height and body weight at ICU admission. This was followed by scaling each of the 18 organs by means of an organ weight scaling factor ( $OW_{SF}$ ) calculated according to Eq. (8):

$$OW_{SF} = \frac{\text{Body weight at study inclusion}}{\text{Body weight at ICU inclusion}} \quad (8)$$

If body weight was not measured on study inclusion day, it was calculated supposing a linear relationship between body weight at ICU admission and the body weight measured at the earliest possible time after study inclusion. The sub-compartmental structures for septic patients were calculated as described in Eqs. 5–7. Finally, sub-compartmental water distribution of each organ was adjusted according to Eqs. (9–11), with a minor ICW shift of 10% to the interstitial space as a result of the interdependency of proteolysis and intracellular dehydration in critical illness assumed by Häussinger et al. [35]:

$$V(\text{organ})_{\text{water, intracellular, 'septic'}} = V(\text{organ})_{\text{water, intracellular, 'healthy'}} \times 0.9 \quad (9)$$

$$V(\text{organ})_{\text{water, plasma, 'septic'}} = V(\text{organ})_{\text{water, plasma, 'healthy'}} + (V(\text{organ})_{\text{vascular, 'septic'}} - V(\text{organ})_{\text{vascular, 'healthy'}}) \quad (10)$$

**Table 2** Characteristics of septic patients attended in the study

Patient	Age (years)	Sex	Weight at ICU admission (kg)	Weight at study inclusion (kg)	Height (cm)	Septic state	APACHE II score	SAPS II score <sup>a</sup>	SOFA score	Albumin (g/dL)	Hematocrit	CrCL <sup>a</sup> (mL/min)	f <sub>u</sub> (%)
1	43	M	87	100	173	Sepsis	10	26	3	2.7	25.6	220	66.6
2	55	M	80	75	175	Septic shock	32	55	11	2.7	26.5	128	67.7
3	70	F	55	62.3	167	Septic shock	30	73	12	2.4	28.9	60.8	67.7
4	25	M	85	87.8	185	Sepsis	18	30	6	2.7	26.7	280.7	66.5
5	52	F	56	62.9	172	Septic shock	19	61	11	2.4	27	163.4	75.6
6	60	M	55	60.1	173	Sepsis	28	78	6	2.5	24.2	113.1	66.3
7	24	M	122	129.6	193	Sepsis	37	62	9	2.6	21.1	261	68.5
8	73	M	63	67.9	175	Septic shock	35	32	11	2.2	26.2	57.4	73
9	44	M	115	134.2	192	Septic shock	17	31	8	2.3	24.7	48.4	72.2
10	68	M	75	76	170	Sepsis	32	69	11	3.2	32.8	70.9	63.5

APACHE Acute Physiology and Chronic Health Evaluation, CrCL creatinine clearance, F female, f<sub>u</sub> fraction unbound, ICU intensive care unit, M male, SAPS Simplified Acute Physiology, SOFA Sequential Organ Failure Assessment

<sup>a</sup> Creatinine clearance calculated according to the Cockcroft–Gault equation: male CrCL = [140 – age (years)] × weight (kg)/[72 × serum creatinine (mg/dL)]; female CrCL = 0.85 × male CrCL

$$\begin{aligned}
 &V(\text{organ})_{\text{water, interstitial, 'septic'}} \\
 &= V(\text{organ})_{\text{water, interstitial, 'healthy'}} \\
 &+ (OW_{\text{'septic'}} - OW_{\text{'healthy'}}) - (V(\text{organ})_{\text{water, plasma, 'septic'}} \\
 &- V(\text{organ})_{\text{water, plasma, 'healthy'}}) + (0.1 \times V(\text{organ})_{\text{water, intracellular, 'healthy'}})
 \end{aligned}
 \tag{11}$$

Because no individual information on TBP and TBF was available, volumes for fat and protein were kept constant to the default value of the ‘healthy’ subject. As described for model 1, the protein ratio between interstitial space and plasma was adjusted to a value of 1.

2.7.3.3 *Elimination* Total CL<sub>van</sub> was calculated as described for model 0 incorporating individual patient CrCL values from the study.

### 2.8 Model Evaluation

For model evaluation, a visual predictive check between simulated and observed data was carried out. In addition, goodness-of-fit (comparison of simulated and observed data) plots were generated for predictive performance analysis. Percentage error (PE) and absolute percentage error (APE) were calculated for every concentration point according to Eqs. (12 and 13):

$$PE [\%] = \frac{(c_{\text{pred}} - c_{\text{obs}})}{c_{\text{obs}}} \times 100
 \tag{12}$$

$$APE [\%] = \frac{|c_{\text{pred}} - c_{\text{obs}}|}{c_{\text{obs}}} \times 100
 \tag{13}$$

where c<sub>pred</sub> is the PBPK-simulated plasma concentration of vancomycin and c<sub>obs</sub> is the actual concentration of vancomycin. To finally describe model accuracy and precision, mean prediction error (MPE) and mean absolute prediction error (MAPE) were calculated. For model acceptance, values should be located around the line of identity with a maximum deviation of ±30% owing to the relative narrow therapeutic index described for vancomycin. Additionally, the median area under the systemic drug concentration–time curve from time 0–24 h (AUC<sub>0–24h</sub>) was calculated by PK-Sim<sup>®</sup> for the ten septic patients in each model by extending the treatment interval for every patient to 24 h by their given dosing regimen and normalising the calculated AUC<sub>0–24h</sub> values to fixed daily doses of vancomycin 2 g. Spearman correlation coefficient (r<sub>s</sub>) was used to analyse the correlation of the dose-normalised AUC<sub>0–24h</sub> values among the models.

## 3 Results

### 3.1 Patient Data

In total, ten septic patients (eight male, two female) treated with vancomycin could be enrolled during the study period,

**Table 3** Calculated physiological scaling factors associated with different septic states, based on a meta-analysis for septic patients and control values observed for healthy individuals

Parameter	Septic state			References
	Sepsis	Severe sepsis	Septic shock	
Albumin <sup>a</sup>	0.57	0.59	0.46	[36, 37, 91–112]
$\alpha_1$ -acid glycoprotein <sup>b</sup>	1.51	1.98	1.98	[91, 113–115]
Hematocrit <sup>c</sup>	0.77	0.80	0.74	[36, 37, 93, 95, 103, 108, 116–123]
Cardiac index <sup>d</sup>	0.94	1.12	1.20	[96, 101, 104, 124–162]
Total liver blood flow <sup>d</sup>	1.65	1.63	1.88	[96, 124, 129, 143, 152, 160, 163–171]
Creatinine clearance <sup>e</sup>	0.84	0.70	0.57	[94, 99, 100, 103, 147, 158, 161, 162, 172–186]
Total body water <sup>f</sup>	1.27	1.27	1.27	[46–50]
Extracellular water <sup>f</sup>	1.76	1.76	1.76	[46–48, 50, 53]
Intracellular water <sup>f</sup>	0.96	0.96	0.96	[46–48, 50]
Total body fat <sup>f</sup>	0.88	0.88	0.88	[47–49]
Total body protein <sup>g</sup>	0.96	0.96	0.96	[46–48]

<sup>a</sup> Control value is described as the mean value of the official reference range

<sup>b</sup> Control value depicted as mean value from Israili et al. [187]

<sup>c</sup> Control value was set at 0.43

<sup>d</sup> Control value was set at 0.75 L/min/m<sup>2</sup> as described by Zacho et al. [69] and Madsen et al. [70]

<sup>e</sup> Control value was set at 120 mL/min

<sup>f</sup> Control value taken as mean value from Ritz et al. [51]

<sup>g</sup> Control values from Cohn et al. [55], Wang et al. [57], Vartsky et al. [58], Burkinshaw et al. [59] and Lukaski et al. [60]

yielding a total of 69 blood samples that were available for analysis of vancomycin concentration. All patients were classified as having sepsis or septic shock. Detailed demographic data and patient characteristics are summarised in Table 2.

### 3.2 Literature Search

The results of the literature search are presented regarding the qualitative and quantitative physiological changes related to sepsis. The resultant key scaling factors that are applied in the PBPK models for septic patients are summarised in Table 3.

#### 3.2.1 Plasma Proteins

A marked decrease in serum albumin is associated with sepsis, compared with standard values of 3.5–5.0 g/dL in healthy subjects (sepsis  $2.43 \pm 0.58$  g/dL; severe sepsis  $2.49 \pm 0.49$  g/dL; septic shock  $1.95 \pm 0.67$  g/dL). Proinflammatory cytokines such as interleukin (IL)-1, IL-6 and tumour necrosis factor alpha (TNF- $\alpha$ ) mainly cause this alteration through suppression of albumin messenger RNA synthesis in the liver [10]. Moreover, capillary leakage leads to an augmentation of the transcapillary elimination rate and to an albumin shift from the intravascular to the interstitial space. Studies determined a 1.5- to 4-fold

increase of the normal value of transcapillary elimination rate during sepsis [36–38], which may lead to an equalisation of plasma and interstitial protein concentrations. In contrast to a reduction of albumin, sepsis causes an increase in acute phase protein concentration of  $\alpha_1$ -acid glycoprotein (healthy subjects: 55–140 g/dL; sepsis subjects:  $147.5 \pm 75.2$  g/dL; severe sepsis subjects:  $192.7 \pm 81.7$  g/dL; septic shock subjects:  $192.7 \pm 67.3$  g/dL) [39].

#### 3.2.2 Hematocrit

A multitude of causes leads to a fall in hemoglobin levels and thus hematocrit in septic patients. Infusion of fluids, the loss of blood and a decreased erythropoiesis all contribute to anemia, and consequently to a loss of the cellular fraction of blood during sepsis (healthy subjects: 37–50%; sepsis subjects:  $33 \pm 7.7\%$ ; severe sepsis subjects:  $34.2 \pm 7.7\%$ ; septic shock subjects:  $31.8 \pm 6.5\%$ ).

#### 3.2.3 Metabolic Enzyme Activity

Studies of cytochrome P450 (CYP) activity in human sepsis are scarce and described semi-quantitatively. All studies show a distinct decrease in hepatic CYP clearance [40–44]. Alterations of CYP activity and expression are mainly regulated through the influence of proinflammatory



mediators (e.g. IL-1, IL-6, TNF- $\alpha$ , NO) downregulating CYP messenger RNA biosynthesis [9, 45]. Moreover, binding of NO to the heme moiety of CYPs, as well as nitrosylation of tyrosine residues in CYP proteins are discussed as possible causative mechanisms [42]. As a result, drug metabolism in sepsis can be influenced by a reduced amount of CYP enzymes, a decreased maximal velocity and reduced drug-enzyme affinity expressed as Michaelis constant, with no quantitative data being available for any CYP enzyme in septic patients so far. Owing to the non-enzymatic elimination of vancomycin, CYP-enzyme related changes were not considered in this study.

### 3.2.4 Body Composition

Fundamental changes in fluid distribution and body composition are observed in septic patients. In general, total body water (TBW) of septic patients increases to a mean value of  $47.8 \pm 2.8$  L [46–50]. Compared to healthy individuals with a mean TBW of about  $37.7 \pm 8.0$  L for men and women [51], this means an average increase of 27% (Table 3). Large amounts of fluid are shifted from the intravascular to the interstitial space leading to tissue edema [52]. Overall, ECW increases from  $15.2 \pm 3.9$  L in healthy individuals [51] to  $26.8 \pm 2.6$  L in septic subjects [46–48, 50, 53] and thus resulting in an ECW/TBW ratio of 55%, with the normal ratio being around 40% [51].

In contrast to ECW and TBW, ICW is not affected in early sepsis but decreases over the course of critical illness. While the ICW volume is comparable to that of healthy individuals in early sepsis ( $21.4 \pm 1.7$  L in sepsis subjects vs.  $22.4 \pm 4.1$  L in healthy individuals), up to 10–15% of ICW is lost in late sepsis ( $18.8 \pm 1.6$  L) [46–48, 50]. This is accompanied with a decrease in TBP, as reported by Häussinger et al., who postulated a dependency of TBP and ICW with cytokines being one possible mediator [35]. Analogous to ICW, TBP is hardly affected in early sepsis but declines in late sepsis through increased catabolism ( $9.7 \pm 0.9$  kg in early sepsis subjects vs.  $8.5 \pm 0.6$  kg in late sepsis subjects vs.  $10.2 \pm 2.2$  kg in healthy individuals) [46–50, 53–60]. TBF changes only slightly with values of  $16.5 \pm 2.2$  kg at onset of sepsis and  $15.8 \pm 2.5$  kg in late sepsis ( $18.8 \pm 7.8$  kg in healthy individuals) [47–49, 51].

### 3.2.5 Cardiac Activity

Sepsis is often associated with cardiovascular dysfunction, which affects cardiac activity expressed as CI. Especially in early sepsis, the reduction in systemic vascular resistance can lead to a compensatory increase in CI to maintain tissue perfusion [61]. However, in later stages of sepsis, multifactorial mechanisms (e.g. IL-1, TNF- $\alpha$ , NO) can lead

to myocardial depression and a reduced ejection fraction [62]. Data analysis for septic patients show a severity-dependent change in CI. Whereas the reference range for healthy individuals in rest is around 2.5–4.0 L/min/m<sup>2</sup> with a mean value of  $3.2 \pm 0.5$  L/min/m<sup>2</sup> [63], CI increases with severity of sepsis (sepsis subjects:  $3.01 \pm 1.12$  L/min/m<sup>2</sup>; severe sepsis subjects:  $3.59 \pm 1.25$  L/min/m<sup>2</sup>; septic shock subjects:  $3.84 \pm 1.26$  L/min/m<sup>2</sup>).

### 3.2.6 Renal Perfusion

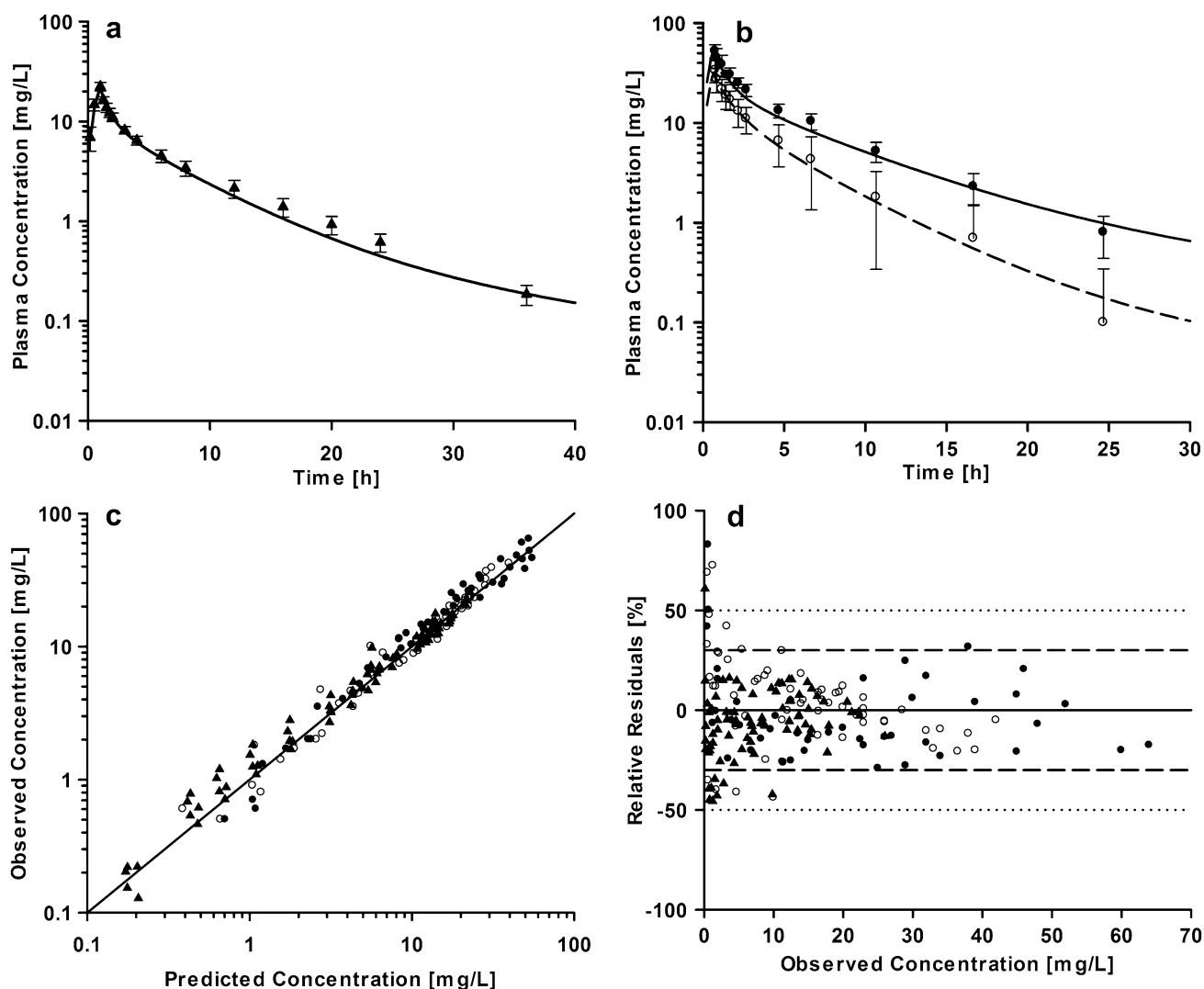
Limited data are available on changes of renal blood flow (RBF) in septic patients. Only five studies were identified, correlating RBF with cardiac output (CO). The RBF/CO-ratio is decreased in four studies ( $9.5 \pm 3.2\%$  [64],  $5.6 \pm 2.9\%$  [65],  $7.5 \pm 2\%$  [66],  $19.2 \pm 4.6\%$  [67]), whereas only Rector et al. determined a RBF/CO-ratio of  $21 \pm 4.4\%$  [68] being within the range of 20–25% for healthy individuals. Because the majority of studies did not fulfil any official sepsis criteria, the results were not considered for PBPK modelling.

### 3.2.7 Hepatic Perfusion

Numerous studies were undertaken to determine hepatic blood flow during sepsis. Absolute values for total splanchnic blood flow were higher in sepsis compared with a mean value of 0.75 L/min/m<sup>2</sup> reported for healthy people [69, 70] (sepsis subjects:  $1.24 \pm 0.51$  L/min/m<sup>2</sup>; severe sepsis subjects:  $1.22 \pm 0.23$  L/min/m<sup>2</sup>; septic shock subjects:  $1.41 \pm 0.87$  L/min/m<sup>2</sup>). Because cardiac activity was determined in all studies, authors also presented hepatic perfusion as a function of cardiac index. The mean values for all septic states were in accordance with the fractional perfusion indicated for healthy subjects (20–30% [71]), although a high variability for sepsis and septic shock was found (sepsis subjects:  $28.5 \pm 9.8\%$ ; severe sepsis subjects:  $24.3 \pm 4.3\%$ ; septic shock subjects:  $28.7 \pm 13.6\%$ ).

### 3.2.8 Creatinine Clearance

During sepsis, both acute kidney injury and augmented renal clearance can occur, resulting in changes in CrCL. Although the exact mechanism for altered kidney function is not clear, changes in vascular resistance, renal macro- and microvascular perfusion, cardiac activity and inflammatory mediators seem to play a pivotal role [72, 73]. Meta-analysis of the available data shows a constant fall of CrCL from  $100.3 \pm 56.4$  mL/min in sepsis to  $83.9 \pm 51$  mL/min and  $61.1 \pm 38.3$  mL/min in severe sepsis and septic shock, compared with a normal renal function of about 120 mL/min in healthy subjects.



**Fig. 2** **a** Predicted and observed mean ( $\pm$  SD) plasma concentration–time curve for vancomycin in healthy adults. The observed plasma concentrations were taken from Cutler et al. **b** Predicted and observed mean ( $\pm$ SD) plasma concentration–time–curve of vancomycin in healthy adults and healthy obese adults. The observed data were taken from Blouin et al. **c** Goodness-of-fit plot for model prediction in healthy adults. **d** Relative residuals vs. observed vancomycin

concentration for healthy adults. The *solid black line* indicates the line of identity, *dashed lines* indicate  $\pm 30\%$  range, *dotted lines* indicate  $\pm 50\%$  range, *filled triangles* indicate observed data from Cutler et al., *filled circles* indicate observed data from Blouin et al. for non-obese healthy adults and *open circles* indicate observed data from Blouin et al. for healthy obese adults. *SD* standard deviation

### 3.3 Vancomycin Model for Healthy Subjects

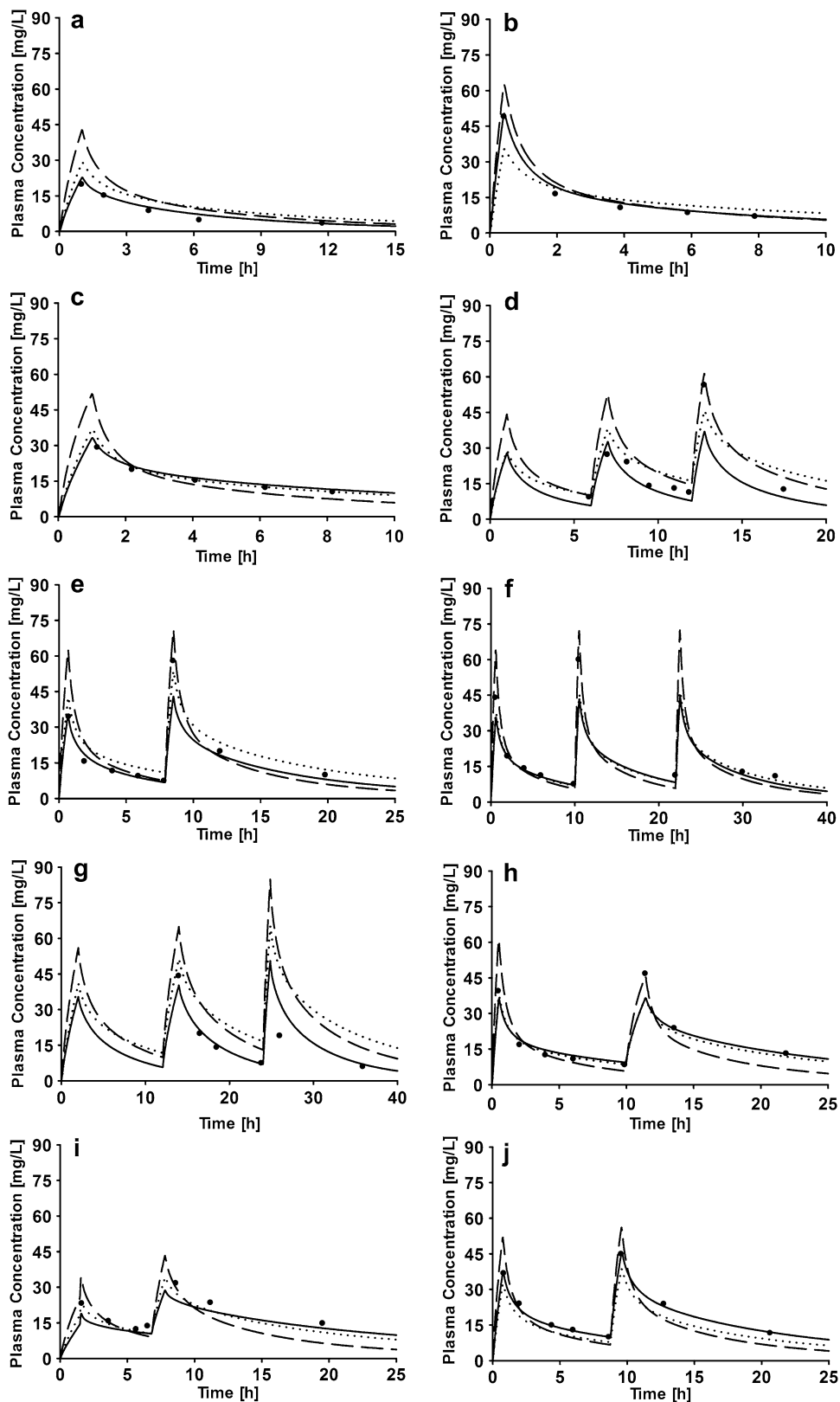
After optimisation of the mean logP to a value of 2.45, the model was able to accurately describe vancomycin exposure after intravenous administration for healthy volunteers (Fig. 2a, b) which is supported by the respective goodness-of-fit plots (Fig. 2c, d). About 10% of all values deviated from the target APE of 30% and less than 3% deviated more than 50% with the majority of these deviations being far below the therapeutic through level of 10–15 mg/L requested for vancomycin. In general, the model showed minimal bias (MPE =  $-1.94\%$ ) and a good precision

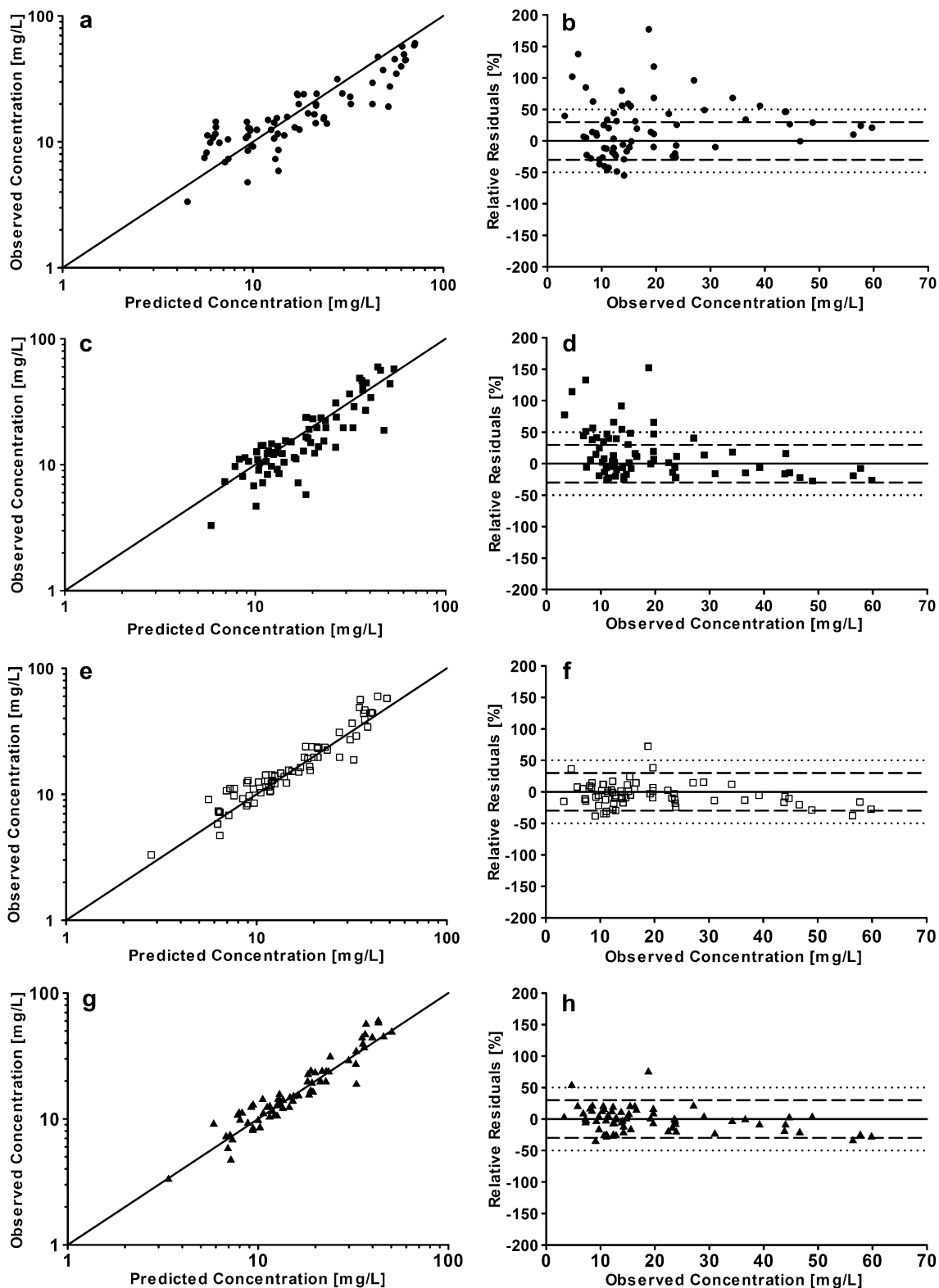
(MAPE = 15.7%). The model was able to predict the PK of vancomycin in healthy individuals and was therefore suitable for septic subgroup extrapolation.

### 3.4 Model Predictions for Septic Patients

Comparing model 1a with model 0, a distinct over-prediction of simulated plasma concentrations can be observed for both models (Figs. 3a–j, 4a–d), which is supported by positive MPE values (Table 4). APE values showed an improvement of model prediction for the literature-based model because 68 and 55% (model 1a vs. model 0) of the

**Fig. 3 a–j** Predicted plasma concentration–time-curves for ten septic patients. *Dashed black lines* indicate the PBPK model for healthy individuals considering no physiological alterations (model 0), *dotted black lines* indicate the PBPK model of healthy individuals adapted to physiological changes from the literature (model 1a), *solid black lines* indicate the PBPK model for healthy individuals adapted to individual physiological changes (model 2) and *filled circles* indicate observed values. *PBPK* physiologically based pharmacokinetic





**Fig. 4** a, c, e, g Goodness-of-fit plots for the model without physiological changes (circles [model 0]), the model accounting for literature values only (filled squares [model 1a]), the literature-based model accounting for individual creatinine clearance (open squares [model 1b]) and the model considering additional individual patient

characteristics (triangles [model 2]). b, d, f, h Relative residuals vs. observed concentrations for the data in a, c, e, g. solid black lines indicate lines of identity, dashed lines indicate lines of identity  $\pm 30\%$  range and dotted lines indicate lines of identity  $\pm 50\%$  range

**Table 4** Bias and precision for different model approaches depending on different vancomycin concentration ranges

	MPE (%)			MAPE (%)		
	Total range	<20 mg/L	≥20 mg/L	Total range	<20 mg/L	≥20 mg/L
Model 0	17.0	11.8	26.8	36.5	36.8	35.7
Model 1a	18.6	28.4	0.2	31.1	37.4	19.8
Model 1b	-4.9	-3.4	-7.7	14.9	14.7	15.2
Model 2	-1.6	1.9	-8.2	14.0	14.4	13.1

MAPE mean absolute prediction error, MPE mean prediction error

**Table 5** Dose-normalised  $AUC_{0-24h}$  values with inter-quartile ranges and Spearman correlations between AUC levels of the models

	Model 0	Model 1a	Model 1b	Model 2
Model 0	341 (320 – 349)	0.83	0.41	0.58
Model 1a		332 (309 – 378)	0.59	0.65
Model 1b			309 (234 – 339)	0.96
Model 2				315 (237 – 369)

$AUC$  area under the concentration–time curve,  $AUC_{0-24h}$  area under the concentration–time curve from time zero to 24 h

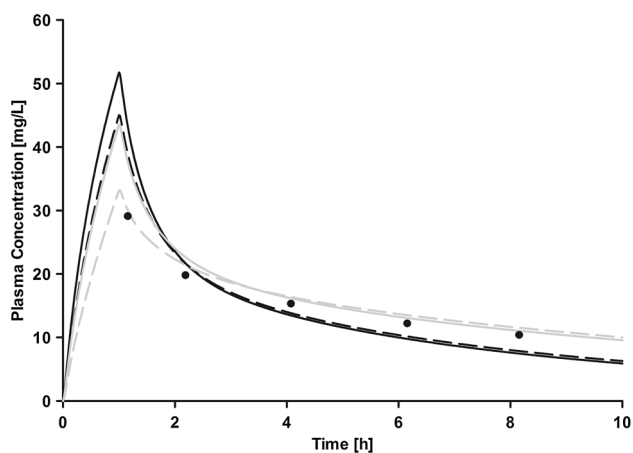
Light grey-filled areas indicate median dose-normalised  $AUC_{0-24h}$  (mg\*h/L) values and inter-quartile ranges for the ten septic patients in each model; non filled areas indicate Spearman correlation coefficients ( $r_s$ ) between the dose-normalised  $AUC_{0-24h}$  values of each model

simulated data were within the target range of 30%, whereas 84 and 77% of all data were within 50% of the observed values for both models, respectively. A trend for better precision can be observed for model 1a (MAPE = 36.5% for model 0 and MAPE = 31.3% for model 1a). Especially for concentrations greater or equal to 20 mg/L, a distinctly better prediction can be shown for model 1a, indicating that vancomycin distribution in the early phase of administration is predicted more accurately. In contrast, higher residuals at lower concentrations compared with model 0 can be observed, suggesting that clearance of model 1a is under-predicted. This in turn leads to an overestimation of concentrations in the elimination phase of the model. The respective values for MPE and MAPE support these observations (Table 4). Comparing both models in terms of dose-normalised  $AUC_{0-24h}$ , a low variability can be observed and a correlation between both models is observed ( $r_s = 0.83$ ) (Table 5). Refining model 1a through adjustment of individually observed CrCL values (model 1b) substantially improved model prediction (Fig. 4e, f) with 88% of the simulated data being within the target APE of 30% and 99% within the 50% APE range. Moreover, bias was minimised (MPE = -4.9%) and precision could be improved (MAPE = 14.9%) (Table 4).

Additionally, calculated dose-normalised  $AUC_{0-24h}$  was lower with a higher variability and a weak correlation compared with model 0 and model 1a ( $r_s = 0.41$  and  $r_s = 0.59$ , respectively) (Table 5).

Consideration of additional individual patient characteristics (model 2) was in good agreement with the observed data (Fig. 4g, h). Gradual implementation of physiological alterations gathered from patients medical records led to an optimisation of the model with the main determinant of improvement being the consideration of additional body weight as body water and CrCL as shown for patient 3 in Fig. 5. In comparison to model 0, the individualised model 2 showed a better prediction over the whole concentration range (Fig. 3a–j). Only peak concentrations after the second or third administration of vancomycin seem to be slightly underpredicted by model 2 (Fig. 3d–f, h). However, all peak levels were sufficiently described by model 2 because only 7% of all predicted peak concentrations deviated more than 30% from the observed value compared with 60% in model 0. In addition, both models show a weak comparability ( $r_s = 0.58$ ) in terms of  $AUC_{0-24h}$  (Table 5). In general, 94% of simulated concentrations were within the target APE of 30 and 97% within 50%, thus showing a better prediction





**Fig. 5** PBPK models for patient 3 considering gradual implementation of physiological changes. The *solid black line* indicates the PBPK model without any changes in physiology (model 0), the *dashed black line* indicates PBPK model considering changes in hematocrit, fraction unbound and protein ratio interstitial/plasma, the *solid grey line* indicates the PBPK model considering additional changes for clearance and organ blood flow, the *dashed grey line* indicates the final PBPK model (model 2) considering additional changes for body water and *black circles* indicate observed data for patient 3. *PBPK* physiologically based pharmacokinetic

compared with model 0 and model 1a and comparable results to model 1b. This is further supported by a minimal bias of  $MPE = -1.6\%$  and a precision of  $MAPE = 14.0\%$  (Table 4), as well as the good correlation between model 1b and model 2 in terms of the dose-normalised  $AUC_{0-24h}$ .

## 4 Discussion

In the present study, we investigated for the first time the influence of sepsis-related physiological changes on the PK of septic patients treated with vancomycin using a PBPK approach. As to our knowledge no database was available presenting physiological parameters being affected during sepsis, an extensive literature search was conducted to identify relevant physiological data to allow for PBPK modelling in this subpopulation.

In the past, changes in physiology and body composition in special populations were described in a time-dependent manner for PBPK models such as for pregnant women or children [20, 74]. For critically ill septic patients, alterations in physiology cannot be described as a function of time only because disease progression and also recovery are very heterogeneous owing to the influence of patient individual characteristics as well as therapy-specific factors. Moreover, time-dependent data are practically unavailable in literature. Consequently, we focused the literature search on the severity of sepsis according to the international guidelines, which has been successfully

conducted in the past for liver cirrhotic patients [17]. Exclusion criteria were only limited to children and dialysis-dependent renal failure to depict the variability and the clinically realistic situation of this patient population considering no further limitations in primary literature in terms of sepsis treatment (e.g. fluid resuscitation, choice of vasopressor/inotrope).

However, the literature search was complicated by the limited number of studies providing quantitative measurements of physiologic alterations in human sepsis. In some areas, valid quantitative data are almost entirely unavailable for humans, such as metabolic enzyme activity and renal perfusion, which are parameters with a significant impact on drug elimination. This is in part because of the high invasiveness of some procedures (e.g. blood flow determination) and is thus often prohibited because of ethical concerns. Therefore, most studies are conducted in animal septic models. However, the extrapolation of the results to humans is questionable because animal models differ in physiological response to septic insults and show too simple a depiction of human sepsis pathogenesis and pathophysiology [75–77]. An additional hurdle in the literature search was the necessary exclusion of many studies owing to a lack of distinction between septic states. Physiological and biochemical alterations in sepsis were often presented for a pooled population consisting of severe sepsis and septic shock patients, which might lead to distortion of results. Despite these limitations in our literature search, our database presents a majority of physiological parameters that are affected during sepsis, severe sepsis and septic shock and can thus be considered a starting point for PBPK modelling in this population.

During model development, the estimation of the lipophilicity input parameter  $\log P$ , which substantially contributes to the rate and extent of passive drug distribution in the model, proved to be difficult. In the literature, vancomycin is described as a mainly hydrophilic substance.  $\log P$  values of  $-4.4$  up to  $1.1$  could be found, supporting the hydrophilic to semi-polar characteristics of vancomycin [78]. However, the validity is questionable because these values are calculated based on chemical structure and experimental measurements are virtually not available. Moreover,  $\log P$  is only a surrogate for membrane affinity and cannot reflect a physiological membrane in its whole complexity. To optimize PBPK model predictions, parameterisation of  $\log P$  was conducted, resulting in a more lipophilic value of  $2.45$ . This can be explained by readily back-to-back dimer formation and even multimer complex binding of vancomycin in solution [79], resulting in a reduction of accessible hydrogen-binding partners and consequently a decreased hydrophilicity.

Transfer of the model for healthy adults to septic patients showed an improvement of model prediction when

physiological alterations from literature were implemented, as can be seen from the optimised MAPE values and the increased percentage of concentrations within the target APE range of 30%. Especially for concentrations exceeding 20 mg/L, a distinctly better prediction for model 1a can be observed. This demonstrates the importance of changes in body composition for PBPK modelling of septic patients. In addition, this supports the modification for individual generation conducted in the model through implementation of additional body weight in terms of TBW rather than body fat, which is the default procedure in the modelling software [21].

Optimisation through implementation of additional body weight appears reasonable owing to increased drug distribution into the augmented tissue water fraction. In fact, this represents a crucial step in PBPK modelling for septic patients treated with hydrophilic drugs because of its influence on the area under the curve, which can be a surrogate for drug effectiveness such as for antibiotics like vancomycin [80]. Unlike the good prediction for higher vancomycin concentrations, over-prediction of the model in terms of concentrations below 20 mg/L seem to be the main reason for the modest advance of the total model. Literature values for the respective septic state showed a clear tendency towards reduced renal function, which results in a reduced vancomycin clearance [32]. In contrast, the study group showed an inverse trend as CrCL of five septic patients exceeded mean standard value of 120 mL/min for healthy people. This phenomenon is known as augmented renal clearance and is often observed for critically ill patients [81]. However, quantitative studies on augmented renal clearance are scarce for septic patients, which may lead to a bias of CrCL under-prediction from mere literature data. Moreover, a larger study population would probably contribute to improve model prediction, as septic patients are also at high risk for development of acute kidney injury [82, 83], which would result in a general decrease of renal function.

Model improvement can be achieved when individual patient data are implemented in the PBPK model as shown in Fig. 5 and seen by minimisation of MPE and MAPE values for both, model 1b and model 2 (Table 4). Because CrCL was the only parameter individualised in model 1b, this clearly indicates the high influence of renal function on model optimisation as both, accuracy and precision, were substantially improved for this model compared with model 0 and model 1a (Table 4). These findings are in accordance with the results of population pharmacokinetic studies demonstrating a significant effect of CrCL on vancomycin clearance [84, 85].

Consequently, adjustment of CrCL for the PBPK model in septic patients is of great advantage for more precise model prediction and should be considered individually.

Referring to the small difference between MPE and MAPE values and the high correlation in terms of dose-normalised  $AUC_{0-24h}$  for model 1b and model 2, additional consideration of individual patient characteristics did not seem to result in further model improvement. This also includes body weight changes during intensive care stay. Nevertheless, adjustment of body composition as a result of sepsis-related influences, as described for model 1a and shown in Fig. 5, contributes to an improvement for model predictions in the distribution phase of vancomycin and thus clearly indicates the importance of this parameter for PBPK modelling in septic patients. However, as adjustment of body composition based on individual body weight data did not result in distinct optimisation of model performance compared with model 1b (Table 4), this demonstrates a reliable and accurate assessment of body composition changes based on the gathered literature data.

Although only a limited number of ten septic patients were included in this study, individual vancomycin PK are accurately (MPE) and precisely (MAPE) predicted when sepsis-related parameters are integrated into the model with the additional consideration of individual patient CrCL. Consequently, the predictive performance of the PBPK model should be tested in a larger septic population to show the clinical applicability.

Finally, it should be noted that all findings are based on the renally cleared vancomycin, where the effects of sepsis-induced changes on physiological parameters such as hematocrit, organ blood flow or protein binding are suggested to be minor for model prediction. However, these effects could be of major importance for model prediction of highly protein-bound drugs or those with a high hepatic-extraction-ratio, which should therefore encourage the further investigation of drugs with a more complex pharmacokinetic profile to show broader applicability of the septic PBPK model.

## 5 Conclusion

Variations of system parameters, including biochemical processes, body composition and organ function are important elements for PBPK simulations of drug PK in patients with a varying health status. In the current study, we provided essential qualitative and quantitative information on physiological changes of critically ill patients with sepsis of different degrees of severity that can be used as a starting point for further PBPK modelling in this subpopulation of patients. Incorporation of literature-based alterations as well as readily accessible individual patient information successfully predicted vancomycin PK in a respective study group of septic patients. Therefore, this study will allow for further extension and validation of

input parameters with the goal to optimise drug dosing in critically ill patients with sepsis and thus improve patient outcome. Additionally, the PBPK model for septic patients could be used to refine dosing recommendations in situations when plasma concentration measurements are not available.

### Compliance with Ethical Standards

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**Conflict of interest** Michaela Meyer and Thomas Eissing were employed by Bayer Technology Services GmbH during the preparation of the manuscript and are potential stock holders of Bayer AG, the holding owning Bayer Technology Services GmbH. Dagmar Horn, Christian Lanckohr and Björn Ellger have no potential conflicts of interest to declare.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all participants included in the study.

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