DOSING OPTIMIZATION OF BETA-LACTAM ANTIBIOTICS USING PARAMETRIC AND NONPARAMETRIC POPULATION PHARMACOKINETIC MODELS

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ISBN 978-94-6332-620-9
Layout by Loes van de Kraats - Kema
Cover design by Floris te Boekhorst
Printed by GVO Drukkers & Vormgevers, Ede

Printing of this thesis was financially supported by Stichting KNMP fondsen and Erasmus University Rotterdam.

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Optimalisatie van doseringen van beta-lactam antibiotica
met behulp van parametrische en non-parametrische
populatie farmacokinetische modellen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. R.C.M.E. Engels

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

woensdag 14 oktober 2020 om 11:30 uur

door

Femke de Velde
geboren te Zwolle

Erasmus University Rotterdam

Promotiecommissie

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Contents

Chapter 1

Introduction and outline of this thesis

Partly based on: Clinical applications of population pharmacokinetic models of antibiotics: challenges and perspectives

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Pharmacological Research. 2018; 134: 280-288

Bacterial infections, antibiotics and resistance

Infectious diseases are caused by pathogenic organisms, such as bacteria, viruses, fungi or parasites. Many of these organisms live in and on our bodies and are normally harmless, but may induce illness under certain conditions. Antibiotics can be used to treat infections caused by bacteria. Unfortunately, bacteria may become resistant to antibiotics that once could successfully treat the microbe. In case of antibiotic resistance, susceptible bacteria are killed while the resistant bacteria survive and selective pressure on resistance genes emerges [2]. Due a high selective pressure via overuse and misuse of antibiotics, antibiotic resistance is a growing problem worldwide [3]. The estimated number of deaths in Europe due to resistant bacteria was 33,000 in 2015 and this has more than doubled since 2007 [4]. Resistant bacteria are more difficult to treat than non-resistant bacteria and require alternative antibiotics or higher doses [5]. There is an urgent need for novel antibiotic treatments to combat these resistant bacteria, but few new drugs make it to the market due to clinical trial hurdles such as an insufficient number of patients infected with the resistant bacterial species [6-8]. Another reason is that it is more difficult to bear the costs of antibacterial drug development compared to, for example, anticancer or orphan drugs, due to the low-price generic market of antibiotics [9]. Importantly, suboptimal dosing of antibiotics has an important part in several failures of antibiotic drug development programs, even in late phases of these programs [10]. Underdosing decreases clinical outcomes of infections [11-14] and promotes emergence of resistance [15]. A "one dose fits all" strategy in highly variable populations is one of the reasons for inadequate antibiotic therapy [16].

The increasing global resistance and difficulties experienced during antibiotic development emphasize the importance of optimizing dosing regimens of old and new antibiotics in order to maximize clinical outcomes of infections and minimize emergence of resistance and toxicity [15]. Pharmacometric modelling of the pharmacokinetic and pharmacodynamic (PK/PD) characteristics of antibiotics can support the optimization of dosing regimens in drug development programs and can also be used for evaluation of dosing strategies of old antibiotics and individualisation of therapy [15]. In short, PK studies focus on the processes that describe what the body does to a drug (i.e. absorption, distribution, metabolism and excretion processes) and PD studies enclose what the drug does to the body (i.e. concentration-effect relationships). Population PK models describe concentration-time profiles of a specified population and explain PK variability. During new drug development, population PK models of antibiotics are recommended for optimizing dosing regimens, for example in European [17] and American [18] guidelines. However, these regulatory guidelines did not yet exist for many currently used antibiotics which were developed and approved decades ago when PK/PD principles were largely unknown and sophisticated population PK modelling techniques did not exist [19]. Nowadays, some of these old antibiotics are studied again and an increasing number of population PK models are published with new dosing recommendations for specific populations [20-25]. Because a coordinated redevelopment procedure for old drugs such as antibiotics does not yet exist [19],

the methods used to establish these dosing strategies are highly variable and the evidence for clinical implementation can therefore be unclear.

Aims of this thesis

This thesis is focused on dosing evaluation of old antibiotics using population PK models. More specifically, the aims of this thesis are:

1. Developing population PK models for two old beta-lactam antibiotics and one old beta-lactamase inhibitor, and subsequent evaluate dosing using these models.
2. Comparing different population PK modelling approaches.
3. Identifying challenges in the process of antibiotic dosing evaluation using population PK models.
4. Establishing recommendations for the use of population PK models in clinical practice.

The introduction of this thesis continues with providing background information on PK/PD principles of antibiotics and PK modelling and simulation, followed by an outline of all chapters of the thesis.

PK/PD principles of antibiotics

PK/PD of antibiotics describe the relationship between efficacy, the *in vitro* susceptibility of a drug to the microorganism (usually expressed as MIC, minimal inhibitory concentration) and the *in vivo* exposure to the drug, which relies on the PK and the dose (**Figure 1**) [15]. From this relationship follows that if the MIC is known, the microbiological and clinical outcome of treatment is determined by the individual PK profile and dose. To predict that exposure, population PK models can and are being used. The quality of the model used will determine the value of the estimated exposure.

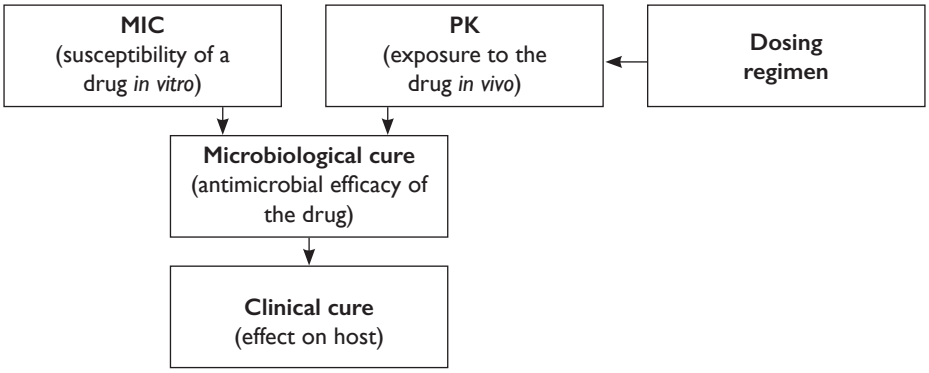

Figure 1. Relationship between MIC, PK, dose and drug effects.

PK/PD indices describe exposure-response relationships. A PK/PD index represents the relationship between a PK measure of exposure to the antibacterial agents (such as AUC, area under the concentration-time curve, or C_{max}, maximal concentration) and a PD measure of bacterial susceptibility to the drug (usually the MIC). Only the non-protein-bound fraction of an antibiotic is microbiologically active and can penetrate into the extravascular space [26]. Therefore, PK/PD indices are based on unbound concentrations.

For each antibiotic, different PK/PD indices (such as AUC/MIC, C_{max}/MIC and T>MIC, **Figure 2**) are tested in preclinical studies to identify which PK/PD index is most likely to be associated with efficacy. PK/PD indices are different for each antibacterial class [27]. For example, the PK/PD index of beta-lactams is the percentage of the dosing interval that the unbound (free) antibiotic concentration is above the MIC (%*f*T>MIC) [27] and the PK/PD index of vancomycin is *f*$AUC_{0\text{-}24}$/MIC [28].

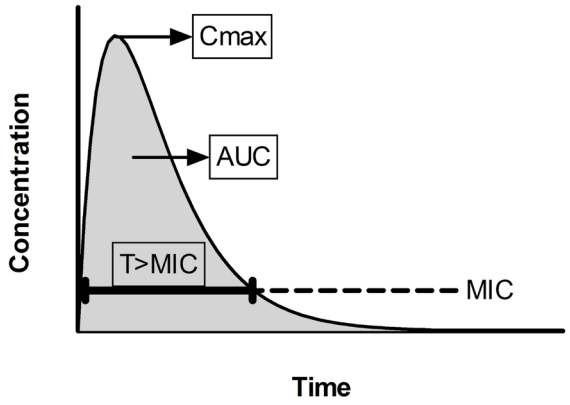

Figure 2. Concentration-time curve showing the pharmacokinetic parameters C_{max} (maximal concentration) and AUC (shaded area) and the PK/PD index Time>MIC.

The PK/PD target is the minimal PK/PD index value that ensures a high probability of successful treatment [15]. There is no unique PK/PD target value per antibiotic. PK/PD target values vary between the chosen endpoints such as stasis, maximal kill or resistance suppression (for preclinical studies) and microbiological or clinical cure (for clinical studies) [16, 29]. To attain a specific PK/PD target, the exposure of the microorganism to the antibacterial agent needs to be adequate. This exposure is dependent on the dose and PK properties of the drug.

The optimal PK/PD target value is still not clearly defined for all antibiotics [19, 30], in part because this depends on its clinical indication or use. For example, for beta-lactam antibiotics, used targets vary between 30-100% fT>MIC and 50-100% fT>4xMIC, dependent on several factors, such as the immune status/illness of the patient [16, 31, 32]. Small studies suggest that the required targets of the different beta-lactam subgroups are the highest in cephalosporines, followed by penicillins

and then carbapenems [16, 27]. Importantly, preclinical derived PK/PD target values differ from clinical derived values in critically ill patients [16]. Currently, there is a trend towards the use of more conservative targets for critically ill patients than for the less critically ill, for example the use of a target belonging to stasis or 1-$\log_{10}$ reduction in colony forming units for less severe infections or to a 2-$\log_{10}$ reduction for more severe infections [33].
Information about the PK/PD target, PK characteristics, exposure, variability and dosing regimens is needed to set clinical breakpoints. Clinical breakpoints are MICs that define microorganisms as susceptible or resistant to specific antibiotics [34]. Clinical breakpoints determine the antibacterial choice during empirical and culture-driven therapy. The epidemiological cut-off value (ECOFF) is the highest MIC for "wild type" organisms devoid of phenotypically detectable resistance [35].

PK analyses and simulations

PK describes the behaviour of drugs and their metabolites in the body in terms of absorption, distribution, metabolism and elimination. Concentration-time courses are related to the dose received and subject characteristics. PK analysis methods can be distinguished between individual and population approaches, which can be further classified as parametric, nonparametric, maximum likelihood and Bayesian methods (**Figure 3**). Simulations of population PK models are used to evaluate these models (internal or external validation) or to evaluate dosing regimens. For the latter purpose, the probability of target attainment (PTA) can be calculated.

Individual PK methods

Individual PK methods analyse concentration-time courses per individual subject. Examples of individual PK methods are the non-compartmental analysis and the standard-two-stage method. Non-compartmental analysis (NCA) is the simplest individual PK method. NCA applies no model to the data but connects the observed individual concentrations by linear interpolation. The standard two-stage (STS) method fits the data of each individual separately into a compartmental model equation and then combines individual parameter estimates to generate mean (population) parameters and standard deviations [36].
Individual PK methods are relatively simple techniques and useful to explore datasets and calculate PK measures as AUC and C_{max}. However, they don't provide detailed information (e.g. covariates) on variation of PK parameters in a population. Another disadvantage is that these methods require intensive sampling. Examples of individual PK software packages are Phoenix WinNonlin and PKSolver [37]. In addition to NCA and/or STS methods, some of the individual PK packages also offer population PK methods.

Population PK methods

Population PK methods analyse concentration-time courses of a population as a whole. During the modelling process, several models with different numbers of compartments, types of elimination and variability are evaluated. The final model

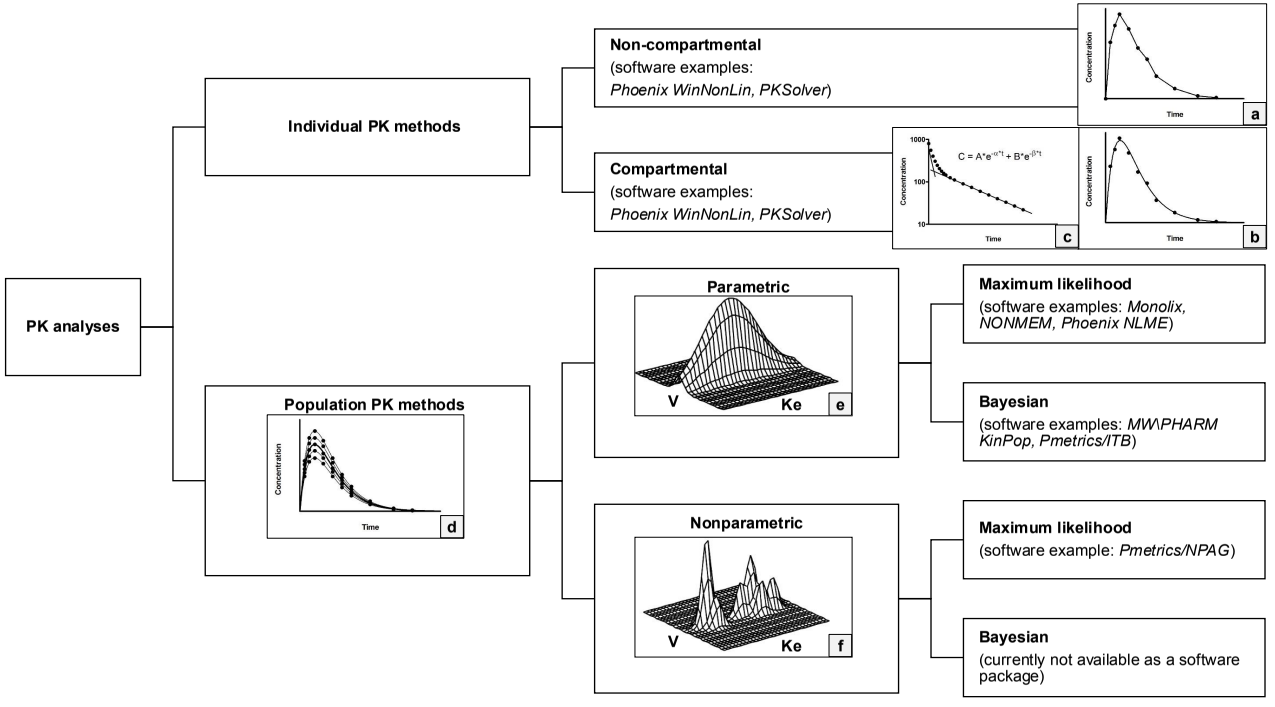

Figure 3. Overview of PK analysis methods. a) Non-compartmental analysis: connection of individual concentrations by linear interpolation; b) and c) Compartmental analysis: fitting of individual concentrations into a compartmental model equation (b: linear y-axis, oral administration, 1 distribution compartment. c: logarithmic y-axis, intravenous administration, 2 distribution compartments); d) Population PK data; e) Parametric and f) Nonparametric analysis of population PK data with 2 subpopulations of slow (low Ke, elimination constant) and fast (high Ke) metabolizers. The parametric method assumes that the data are normally distributed whereas the nonparametric method identifies the 2 subpopulations. e) and f) Reprinted with permission of the *Laboratory of Applied Pharmacokinetics* [1].

provides mean population PK parameters (e.g. volume of distribution, clearance) and describes variability between subjects (inter-individual or between-subject variability, BSV) and variability between the doses of an individual subject (intra-individual or between-occasion variability, BOV). The observed variability is explained by covariates (subject characteristics as body weight, renal function or age). Residual variability (e.g. assay variance or sampling uncertainties) is also taken in account [36, 38]. Thorough model evaluation and validation is important to deliver a robust and reliable model. Examples of model evaluation/validation methods and techniques are objective functions based on likelihood (e.g. AIC, Akaike Information Criterium), graphical plots, bootstrapping to estimate parameter precision, simulation-based diagnostics (e.g. VPC, Visual Predictive Check, or NPDE, Normalized Prediction Distribution Error) and external validation, when the developed model is applied to a new dataset [38, 39].

Traditionally, population PK parameters were estimated by the standard two-stage "individual" approach, which cannot describe and explain the types of variability. More sophisticated population PK methods involve the development of nonlinear mixed-effect models. These models are called "nonlinear" because PK equations are nonlinear. "Mixed-effect" implies the description of fixed effects (which are the same for each individual) and random (individual-specific) effects.

Population PK modelling methods can be statistically classified as either parametric or nonparametric. The parametric and nonparametric classifications can both be divided into maximum likelihood or Bayesian approaches [40, 41]. It is still unknown which population PK approach (e.g. parametric vs nonparametric) is most suitable for specific research questions. The different modelling approaches will be briefly described below.

Parametric maximum likelihood methods

Parametric maximum likelihood methods assume that the population parameter distribution is known with unknown population parameters [41]. These methods estimate the set of parameters that maximize the joint likelihood of observations. Most of the current software packages for population PK modelling are parametric maximum likelihood methods (e.g. Monolix, NONMEM and Phoenix NLME). Each package offers one or more mathematic algorithms to facilitate maximum likelihood modelling, such as FOCE, SAEM or QRPEM [42].

Nonparametric maximum likelihood methods

In contrast to parametric methods, nonparametric methods make no assumption about the shapes of the underlying parameter distributions, which is theoretically an advantage to detect subpopulations. Nonparametric methods use an exact likelihood function while most parametric methods use an approximation. A drawback of some nonparametric methods is that confidence intervals about parameter estimates are not easily determined [40, 41]. An example of a nonparametric maximum likelihood method is the NPAG algorithm in the software package Pmetrics (former MM-USCPACK / USC*PACK, previously based on the NPEM algorithm) [43].

Bayesian methods

The parametric iterative two-stage Bayesian (ITB) method uses mean parameter values and their standard deviations (obtained from a STS method or any reasonable initial guess) as Bayesian priors. Subsequently, individual patient data are examined to obtain Bayesian posterior parameter values based on the maximum a posteriori probability (MAP) Bayesian procedure. The mean parameter values can again be calculated and used as Bayesian priors to obtain new Bayesian posterior values. This iterative process is repeated until the difference between population and estimated values reaches a minimum value [44, 45]. Examples of software packages including the ITB method are the KinPop module in MW\PHARM [45] and the ITB algorithm in Pmetrics, which is mainly used to estimate parameter ranges to be passed to NPAG [43]. A nonparametric Bayesian approach is currently not available in a software package [40].

Simulations

For clinical applications, simulations using population PK models are generally performed for two purposes: 1) model evaluation and 2) dosing evaluation [36]. For model evaluation, concentration-time data are simulated and compared with a subset of the original dataset (internal validation) or new data (external validation). For dosing evaluation, concentration-time data are simulated for several dosing regimens to study the exposure and probability of target attainment, for example in specific subpopulations such as ICU-patients or patients with renal impairment [36, 46], or during the process of setting breakpoints [34].

Stochastic simulating from population PK models with fixed-effect and random-effect parameters is more complex than non-stochastic simulating from simple fixed-effect models. The stochastic Monte Carlo simulation (MCS) can handle random variability and is therefore the most used simulation type for population PK models [46, 47].

Probability of target attainment (PTA)

MCS based on population PK models can be used to calculate the PTA of specific PK/PD targets for several dosing regimens and a range of MICs [34, 46]. Different methods are used to present the PTA results. One option is to plot (**Figure 4a**) or tabulate the PTA of a specific PK/PD target as a function of the MIC. Several dosing regimens can be included in such a graph (or table). A disadvantage of this approach is that only one PK/PD target value can be included per graph. Since the optimal PK/PD target values are not defined for all antibiotics and indications, it may be useful to display several target values in one graph. The latter is possible in the graph shown in **Figure 4b**: the PK/PD target (here: %*f*T>MIC) is plotted as a function of the MIC for a specific dosing regimen. By including the mean (or median) of the population and the confidence interval (CI) estimations (percentiles) in the graph, the PTA's for several PK/PD target values can be read. The lower boundary of a CI of 95% corresponds to a PTA of 97.5%. For example, in **Figure 4b**, the PTA for 40% *f*T>MIC is 97.5% for 250mg q8h and an MIC of 0.25 mg/L. EUCAST (European committee on antimicrobial susceptibility testing) uses such graphs to determine breakpoints [34].

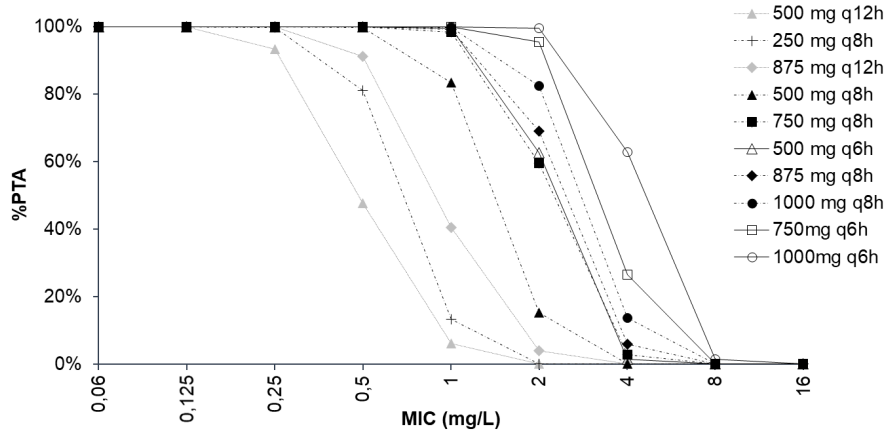

Figure 4a. PTA for various amoxicillin dosing regimens to reach the target 40% *f*T>MIC for a range of MICs. Modified from de Velde et al [48].

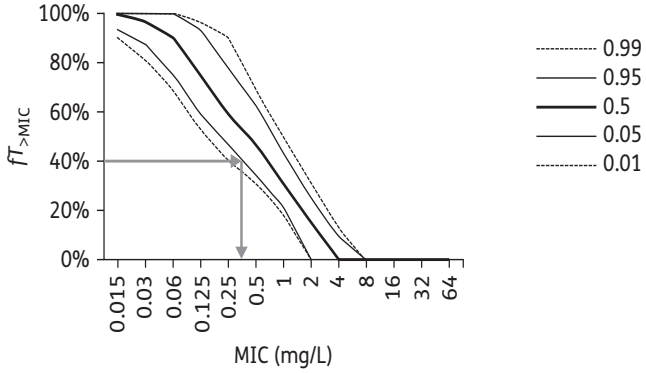

Figure 4b. %*f*T>MIC displayed as a function of the MIC for amoxicillin 250 mg q8h. The middle line represents the values for the median of the population and the surrounding lines indicate the 1st, 5th, 95th and 99th percentiles, obtained by 5000 Monte Carlo simulations. The arrows indicate the breakpoint that correspond to the 95% CI (97.5% PTA) for 40% *f*T>MIC. Modified from de Velde et al [48].

Current antibacterial drug development guidelines

Until 1995, new medicinal products were approved by national authorities without centralized European guidelines, leading to different summary of product characteristics (SmPCs) in several European countries [49]. Since the start of the European Agency for the Evaluation of Medicinal Products (EMEA, currently known as the European Medicines Agency, EMA) in 1995, drugs are evaluated and authorized at a European level. The first EMEA notes to guidance on antibacterial drug

development [50] and on the role of pharmacodynamics in antibacterial SmPCs [51] were approved in 1997. These notes to guidance [50, 51] were further developed in the following years [52, 53] and subsequently evolved to EMA guidelines [17, 54]. The role of PK/PD in antibacterial development has been increased during the last two decades. The current guidelines [17, 54] state that PK/PD analyses may be used to select dose regimens and that it may be possible to omit clinical dose-finding studies in case of a clear PK/PD relationship and convincing PK/PD analyses. In short, the PK/PD characteristics of the antibiotic may be investigated during the different phases of drug development as follows [8, 17, 33]:

1. **Nonclinical in vitro PK/PD studies.** *In vitro* models (static concentration time-kill assays, dynamic one-compartment models and dynamic two-compartment models such as a hollow fiber infection model) may be used to investigate PK/PD relationships, to assess multiple different PK profiles and to study the relationship between rates of emergent resistance, drug exposure and duration of therapy.
2. **Nonclinical in vivo PK/PD studies.** Animal studies (such as the murine thigh model or murine lung infection model) are used to further characterize the PK/PD *in vivo*. Population PK modelling and simulation may be used to calculate PTA's for several dosing regimens.
3. **Clinical phase I studies.** The PK is explored in healthy volunteers for several dosing regimens. Population PK modelling and simulation may be used to select one or two candidate dosing regimens with high PTA's.
4. **Clinical phase II and III studies.** The population PK models are further developed using PK data of the target population to confirm adequate drug exposure and to identify subpopulations with increased risk of toxicity or failure.

Outline of this thesis

The majority of the research in this thesis was performed as part of the STAT-Net group of the Combatting Bacterial Resistance in Europe (COMBACTE) consortium (www.combacte.com), which was established by the European Innovative Medicines Initiative (IMI). The general objective of STAT-Net was to deliver strategies that may yield more efficient clinical trial programs while focusing on 3 pillars: improved PK/PD modelling of old and new antibiotics, enhanced end points and innovative trial designs.

Chapter 2 focuses on PK/PD of amoxicillin and amoxicillin/clavulanic acid. Both amoxicillin alone and amoxicillin/clavulanic acid were developed and approved more than 30 years ago when PK/PD principles were largely unknown. Amoxicillin is a beta-lactam antibiotic of the penicillin group which is frequently combined with the beta-lactamase inhibitor clavulanic acid to target beta-lactamase producing bacteria. Beta-lactamases are enzymes which make bacteria resistant against beta-lactam antibiotics. Population PK modelling and simulation of oral amoxicillin (**Chapter 2.1**) and oral clavulanic acid (**Chapter 2.2**) were performed using data of a phase I

trial in healthy volunteers. In the original study report of this phase I trial conducted in 1993, only individual (non-compartmental) PK methods were used to analyse the data. We reanalysed these data using population PK modelling and simulation methods with the objectives to estimate the exposure of amoxicillin and clavulanic acid and the variability in the population and to compare the probability of target attainment for several dosing regimens. **Chapter 2.3** describes amoxicillin PK in patients with and without renal impairment treated with intravenous amoxicillin/clavulanic acid. This analysis was conducted as an addendum to the EXPAT study, a prospective observational PK/PD study of several antibiotics in the Erasmus Medical Center. Our objectives were to determine if the predefined PK/PD target was achieved with local dosing guidelines in patients with and without renal impairment and to compare concentrations and half-life's.

Chapter 3 is focused on the antibiotic imipenem, an intravenous beta-lactam antibiotic approved in the 1980s. Population PK modelling and simulation of imipenem in critically ill patients was performed using data of a prospective cohort study conducted by a Swiss STAT-Net partner. A parametric and a nonparametric PK model were build using the same data with the objective to investigate the advantages and disadvantages of both population PK modelling methods in practice (**Chapter 3.1**). In the second study (**Chapter 3.2**), the external validation and simulation results were compared using both population PK models described in Chapter 3.1.

Chapter 4 includes STAT-Net's white paper with recommendations about design and analysis strategies to support the evaluation of new and old antibiotics. The first 2 recommendations were written by the author of this thesis.

Chapter 5 describes the challenges of population PK models in clinical practise, which were noticed during the research of this thesis about dosing evaluation of old antibiotics, but are also applicable for other clinical applications such as settings clinical breakpoints and therapeutic drug monitoring. Chapter 5 is based on the second part of our review about challenges and perspectives of population PK models of antibiotics in clinical practise, while the first part of this review is implemented in the introduction of this thesis. Finally, the results and conclusions from the studies described in this thesis are summarized and discussed in **Chapter 6** and the perspectives for future research and clinical recommendations are included in **Chapter 7**.

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Chapter 2

Amoxicillin and clavulanic acid

Chapter 2.1

Population pharmacokinetics of oral amoxicillin in healthy volunteers

Nonlinear absorption pharmacokinetics of amoxicillin: consequences for dosing regimens and clinical breakpoints

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Journal of Antimicrobial Chemotherapy. 2016;71:2909-17.

Abstract

Objectives
To describe the population pharmacokinetics of oral amoxicillin and to compare the probability of target attainment of current dosing regimens.

Methods
Two groups of each 14 healthy male volunteers received oral amoxicillin/clavulanic acid tablets on two separate days one week apart. One group received 875/125 mg twice daily and 500/125 mg three-times daily and the other group 500/125 mg twice daily and 250/125 mg three-times daily. 1428 amoxicillin blood samples were collected before and after administration. We analysed the concentration-time profiles using a non-compartmental pharmacokinetic method (PKSolver) and a population pharmacokinetic method (NONMEM). The probability of target attainment (PTA) was computed using Monte Carlo simulations for several dosing regimens.

Results
$AUC_{0\text{-}24}$ and C_{max} increased nonlinearly with dose. The final model included the following components: Savic's transit compartment model, Michaelis-Menten absorption, two distribution compartments and first-order elimination. The mean central volume of distribution was 27.7 L and mean clearance was 21.3 L/h. We included variability for the central volume of distribution (34.4%), clearance (25.8%), transit compartment model parameters and Michaelis-Menten absorption parameters. For 40% $fT_{>MIC}$ and >97.5% PTA, the breakpoints were 0.125 mg/L (500 mg twice daily), 0.25 mg/L (250 mg three-times daily, 875 mg twice daily), 0.5 mg/L (500 mg three-times daily), 1 mg/L (750 mg, 875 mg or 1000 mg three-times daily, 500 mg four-times daily).

Conclusions
The amoxicillin absorption rate appears to be saturable. The PTA's of high dose as well as twice daily regimens are less favourable than regimens with lower doses and higher frequency.

Introduction

Amoxicillin is an aminopenicillin that has been used to treat bacterial infections since the 1970s. It is frequently combined with the β-lactamase inhibitor clavulanic acid to target β-lactamase-producing strains. Either alone or in combination with clavulanic acid, amoxicillin was the most consumed antibacterial agent in primary care in two-third of the EU/EEA countries in 2012 [1].

In an era of increasing antimicrobial resistance and with few new drugs making it to the market, antibiotic use must be optimized in order to improve clinical outcomes of infections [2]. These clinical outcomes are dependent on the relationship between MIC, efficacy and exposure [2]. For β-lactams, the pharmacokinetic-pharmacodynamic (PK/PD) index that best correlates with efficacy is the duration that the unbound concentration exceeds the MIC, expressed as a percentage of the dosing interval ($\%fT_{>MIC}$) [3]. The minimal PK/PD index value that ensures a high probability of successful treatment is the pharmacodynamic target [4]. The pharmacodynamic target appears to be different for each β-lactam group (penicillins, cephalosporins and carbapenems) [5]. For penicillins, the pharmacodynamic target is >30-50% $fT_{>MIC}$, dependent on the microbial species and the choice of the antibacterial endpoint (i.e. the necessary $\%fT_{>MIC}$ is larger for a 1 or 2-log bacterial kill than for a static effect) [3, 5-8]. While a high $\%fT_{>MIC}$ is related to increased bacterial efficacy, an inadequate $\%fT_{>MIC}$ is associated with emergence of resistance and selection of resistant strains [5, 8]. To attain a specific pharmacodynamic target, the exposure of the microorganism to the antibacterial agent needs to be adequate. This exposure is dependent on the dose and pharmacokinetic properties of the drug.

Despite the abundant use of oral amoxicillin, the drug's pharmacokinetics has been described in only a few studies. While small-scale pharmacokinetic studies have shown amoxicillin to have a nonlinear absorption profile [9-14], it remains unclear how such nonlinear absorption might influence the exposure of the various dosing regimens. At present, standard dosing regimens of oral amoxicillin in adults and children ≥ 40 kg vary between 750 and 3000 mg/day, divided into 2 to 4 doses (e.g. 250 mg, 500 mg or 1000 mg three-times daily, 500 mg twice daily, 500 mg four-times daily). For oral amoxicillin/clavulanic acid, standard dosing regimens are 500/125 mg three-times daily or 875/125 mg twice or three-times daily.

A population pharmacokinetic model can be used to estimate the exposure of various dosing regimens and variability of the antibiotic in the population. Yet, such a model is currently not available for oral amoxicillin in the literature. Monte Carlo simulations based on a population pharmacokinetic model can support recommendations for more appropriate dosing regimens with a reduced likelihood of ineffectiveness and resistance (with too low doses) and adverse events (with too high doses).

Information about the pharmacodynamic target, pharmacokinetics, exposure, variability and dosing regimens is needed to set clinical breakpoints. Clinical breakpoints are MICs that define microorganisms as susceptible, intermediate or resistant to specific antibiotics [4].

The purposes of this study were to estimate the exposure of various oral amoxicillin dosing regimens and the variability in the population, to compare the probability of

pharmacodynamic target attainment of these dosing regimens, and to suggest which clinical breakpoints would be justified for oral dosing. We therefore developed a population pharmacokinetic model using NONMEM and performed Monte Carlo simulations.

Methods

Study design and population

The study was designed as an open label, randomized two part-crossover investigation to study the pharmacokinetics of oral amoxicillin/clavulanic acid. Male volunteers were enrolled into the study if they were aged between 18 and 50 years and in good general health. Exclusion criteria were more than 20% deviation from ideal weight for height, use of prescribed medication in the 2 weeks prior to the study (antibiotics: 4 weeks), use of any medication during the study without consent, alcohol intake more than 3 units/day, participation in a trial within 2 months prior to the start of this study, prior hypersensitivity to the trial drug or to drugs with a similar chemical structure, diseases known to interfere with the drug pharmacokinetics, or blood donation of more than 1500 mL within the previous year.

Ethics

The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Freiburger Ethics Committee (Freiburg, Germany). All volunteers gave written informed consent prior to the study. The study (reference number 25000/360) [15] was conducted in 1993 at FOCUS Clinical Drug Development GmbH (Neuss, Germany) in commission of SmithKline Beecham Pharmaceuticals (Harlow, UK).

Study procedures

The study consisted of two parts, each with two dosing regimens given for 1 day. The order of the dosing regimens was randomized and treatment days were separated by 6 or 7 days. In part 1, 16 subjects were allocated to amoxicillin/clavulanic acid 875/125 mg twice daily and 500/125 mg three-times daily. In part 2, 16 other subjects were assigned to 500/125 mg twice daily and 250/125 mg three-times daily. Each dose was provided as a single tablet amoxicillin/clavulanic acid (Augmentin®, SmithKline Beecham Pharmaceuticals, Bristol, TN, USA). Doses were administered with 200 mL water at the start of a standard meal. The first dose of each day was administered at 8:00h after having fasted from food and fluids from 22:00h the night before.

Blood samples were collected just before administration and after 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12h (three-times daily regimens until 8h). Samples were frozen at -70 °C within 1h of sampling and were assayed within 6 weeks of collection. Amoxicillin plasma concentrations were determined by Hazleton Laboratories (UK) using the ASTED (Automated Sequential Trace Enrichment of Dialysates) system coupled to high pressure liquid chromatography (HPLC) with ultraviolet absorbance detection. The lower limit of quantification was 0.1 mg/L.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis of the plasma concentration-time data was performed using PKSolver [16] (version 2.0, China Pharmaceutical University, Nanjing, China). Population pharmacokinetic analysis was performed using nonlinear mixed effects modelling (NONMEM version 7.2, ICON Development Solutions, Ellicott City, MD, USA). The Intel Visual Fortran Compiler XE 14.0 (Santa Clara, CA, USA) was used. The first-order conditional estimation method with interaction was used throughout the model building process. Tools used to evaluate and visualise the model were RStudio (version 0.98.1028), R (version 3.1.1), XPose (version 4.5.0) and PsN (version 4.2.0), all with the graphical interface Pirana [17] (version 2.9.0). Different absorption models (first-order, zero-order, Michaelis-Menten) with and without lag-time or Savic's transit compartment model [18] were evaluated in combination with one- and two-compartment distribution models and an absorption storage compartment. Model selection criteria were decrease in the NONMEM objective function value (OFV), goodness-of-fit plots and visual predictive checks (VPC). A decrease in the OFV of 3.84 units was considered statistically significant ($p<0.05$) in a nested model [19]. For each VPC, a set of 200 simulated datasets was created to compare the observed concentrations with the distribution of the simulated concentrations (using the final model and parameter estimates). Between-subject variability was tested using an exponential variance model. Residual variability was evaluated with a combined (additive and proportional) error model.

From the available subject characteristics (age, height and weight), weight was selected to evaluate as a covariate. Dose was also evaluated as covariate. One covariate at a time was included using the likelihood ratio test. The covariate effect was considered significant at $p<0.05$ (decrease in the OFV of 3.84 units).

The 95% CI of each parameter in the final model was determined from a nonparametric bootstrap analysis, in which the dataset was resampled 500 times.

Monte Carlo simulations

Monte Carlo simulations were performed using the final model in NONMEM. Eight amoxicillin dosing regimens were evaluated: 250 mg three-times daily, 500 mg twice daily, 500 mg three-times daily, 500 mg four-times daily, 750 mg three-times daily, 875 mg twice daily, 875 mg three-times daily and 1000 mg three-times daily. 5000 subjects were simulated for each dosing regimen. For each simulated concentration-time profile, $\%fT_{>MIC}$ was calculated for MICs from 0.015 to 64 mg/L. The unbound amoxicillin concentration was calculated from the total concentration using a fixed value for protein binding of 20% [20-22]. The probability of target attainment (PTA) was assessed for MICs from 0.015 to 64 mg/L.

Results

Study population

32 healthy male volunteers (16 per part) entered the study. After 2 withdrawals (one because of diarrhoea and one due to personal reasons), amoxicillin plasma

concentrations were determined in 30 volunteers (15 per part) completing both dosing regimens. Pharmacokinetics could not be evaluated in one subject in part 1 due to very low plasma concentrations and in one subject in part 2 because of analytical interference. The characteristics of the 28 pharmacokinetic evaluable subjects are shown in **Table 1.**

Table 1. Characteristics of study population (values expressed as mean ± s.d.)

Characteristic	Total *(n=28)*	Part 1 *(n=14)*	Part 2 *(n=14)*
Age (years)	33 ± 7	35 ± 8	31 ± 6
Height (cm)	179 ± 6	179 ± 6	179 ± 7
Weight (kg)	77 ± 8	78 ± 5	77 ± 10
BMI (kg/m^2)	24 ± 2	24 ± 2	24 ± 2

Adverse events

Mild to moderate diarrhoea was the most frequently reported drug related adverse event. The number of these events increased with total daily doses (1750/250 mg/day: 5 events, 1500/375 mg/day: 4, 1000/250 mg/day and 750/375 mg/day: 2 each). In addition, mild abdominal pain and mild nausea were both once reported.

Pharmacokinetic analysis

140 amoxicillin concentration-time profiles (5 profiles per subject) with in total 1428 samples were analysed. The results of the non-compartmental pharmacokinetic analysis are shown in **Table 2.**

The increase in mean Cmax and $AUC_{0\text{-}24}$ was proportional to the daily dose for 750, 1000 and 1500 mg/day amoxicillin, but the mean $AUC_{0\text{-}24}$ of 1500 mg/day was equal to 1750 mg/day and the mean C_{max} of 875 mg was lower than expected. T_{max} increased with rising doses. $t_{1/2}$ was comparable for the 4 doses.

Population pharmacokinetic analysis showed that amoxicillin pharmacokinetics was best described by Savic's transit compartment model [18] followed by Michaelis-Menten absorption, two distribution compartments and first-order elimination (NONMEM subroutine ADVAN6 and TOL=5). A literature-based [20] fixed value of 70% for bioavailability was used because no pharmacokinetic data with intravenous administration were collected in this study. With each concentration-time profile analysed separately, variability was included for central volume of distribution, clearance, the parameters belonging to the transit compartment model and the Michaelis-Menten absorption parameters. Weight and dose didn't improve the model as a covariate and, therefore, was not included in the final model. A schematic representation of the final model is shown in **Figure 1.**

As shown in **Table 3**, the model-based parameter estimates were similar to the values computed from the bootstrap analysis, indicating the stability of the model.

The goodness-of-fit plots in **Figure 2** show that the model adequately described the observed concentrations. The VPC plot, presented in **Figure 3**, indicate a good predictive performance for each of the used doses of 250, 500 and 875 mg amoxicillin.

Table 2. Results of the non-compartmental pharmacokinetic analysis of amoxicillin (values expressed as mean ± s.d.)

Dosing regimen amoxicillin/ clavulanic acid	C_{max} (mg/L)	Dose normalised C_{max} (mg/L)/g	T_{max} (h)	AUC_{0-24} (mg.h/L)	Dose normalised AUC_{0-24} (mg.h/L)/g	$t_{1/2}$ (h)
250/125 mg three-times daily	3.93 ± 1.13	15.74 ± 4.53	1.31 ± 0.33	27.29 ± 4.72	36.39 ± 6.29	1.13 ± 0.38
500/125 mg twice daily	7.17 ± 1.63	14.34 ± 3.26	1.40 ± 0.44	34.33 ± 7.12	34.33 ± 7.12	1.23 ± 0.33
500/125 mg three-times daily	8.12 ± 2.71	16.25 ± 5.43	1.33 ± 0.38	54.67 ± 8.98	36.44 ± 5.99	1.11 ± 0.22
875/125 mg twice daily	11.21 ± 3.42	12.81 ± 3.91	1.52 ± 0.40	55.04 ± 12.68	31.45 ± 7.24	1.14 ± 0.21

Table 3. Model-based population pharmacokinetic parameter estimates and values obtained after bootstrap analysis

	Final model estimate	Bootstrap median	Bootstrap 95%CI
Fixed effects			
BIO (-)	0.7 (fixed)	0.7 (fixed)	–
MTT (h)	0.524	0.521	0.455 – 0.591
N (-)	4.41	4.43	3.29 – 8.20
Vm (mg/h)	1220	1222	960 – 2036
Km (mg)	287	289	191 – 572
Vc (L)	27.7	27.2	25.0 – 29.6
CL (L/h)	21.3	21.2	20.1 – 22.2
Q (L/h)	1.70	1.75	1.07 – 3.03
Vp (L)	3.02	3.12	2.48 – 3.89
Between-subject variability (% CV)			
BIO	35.1	39.0	29.2 – 71.6
MTT	46.8	46.2	38.1 – 55.9
N	113	107	73.5 – 130
Vm	31.9	44.9	21.5 – 120
Km	98.7	110	78.5 – 164
Vc	34.4	36.5	26.4 – 70.9
CL	25.8	29.4	17.0 – 66.7
Residual variability			
Additive	0.0524	0.0525	0.0406 – 0.700
Proportional	0.0824	0.0809	0.0680 – 0.0931

BIO, bioavailability; MTT, mean transit time to depot; N, number of transit compartments; Vm, maximal absorption rate; Km, amount corresponding to 50% Vm; Vc, central volume of distribution; CL, clearance; Q, intercompartmental clearance; Vp, peripheral volume of distribution

Monte Carlo simulations

The results of the simulations, presented in **Figure 4,** showed that 100% of the population reached the pharmacodynamic target 40% $fT_{>MIC}$ for MICs up to 0.125 mg/L with all dosing regimens. For >95% PTA, breakpoints were 0.125 mg/L (500 mg twice daily), 0.25 mg/L (250 mg three-times daily and 875 mg twice daily), 0.5 mg/L (500 mg three-times daily), 1 mg/L (750 mg, 875 mg or 1000 mg three-times daily and 500 mg four-times daily). **Figure 5** shows the $\%fT_{>MIC}$ as a function of the MIC for several dosing regimens. The breakpoints that correspond to the 95% CI (= 97.5% PTA) are the same as the aforementioned breakpoints for >95% PTA.

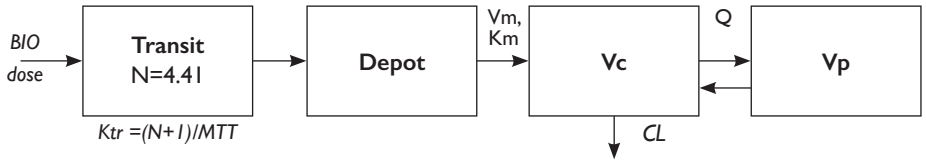

Figure 1. Schematic representation of the final model
BIO, bioavailability; MTT, mean transit time to depot; N, number of transit compartments; Ktr, transit rate constant; Vm, maximal absorption rate; Km, amount corresponding to 50% Vm; Vc, central volume of distribution; CL, clearance; Q, intercompartmental clearance; Vp, peripheral volume of distribution

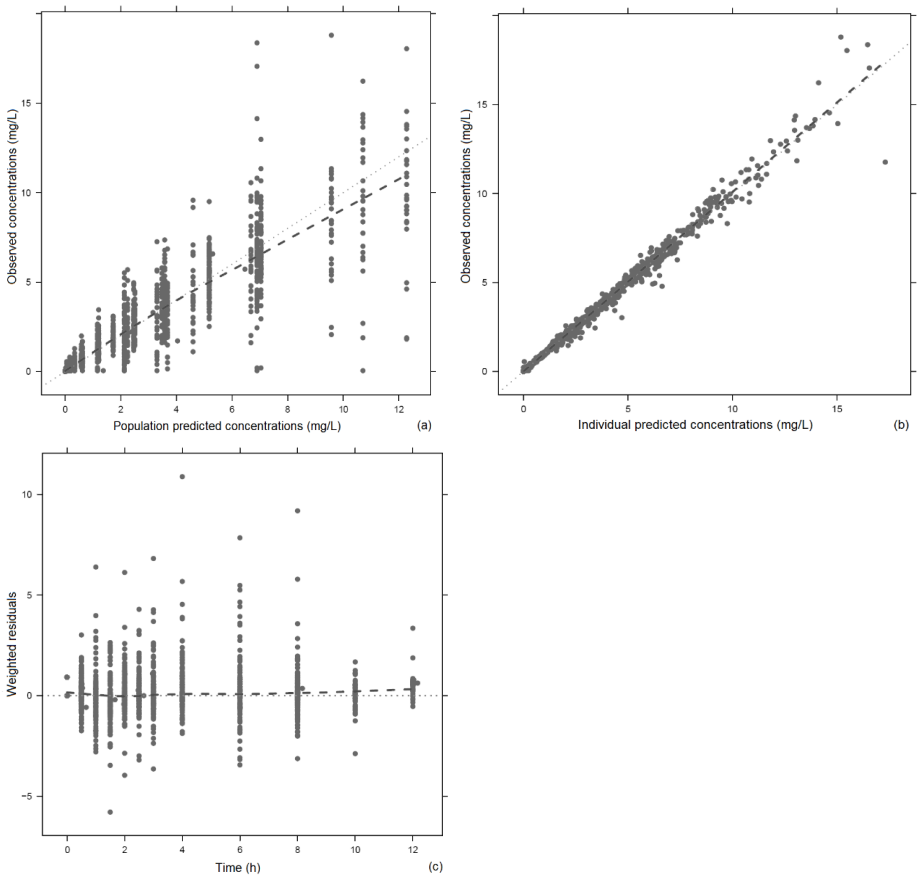

Figure 2. Goodness-of-fit plots for the final model. (a) Observed versus population predicted amoxicillin concentrations. (b) Observed versus individual predicted amoxicillin concentrations. (c) Weighted residuals versus time.

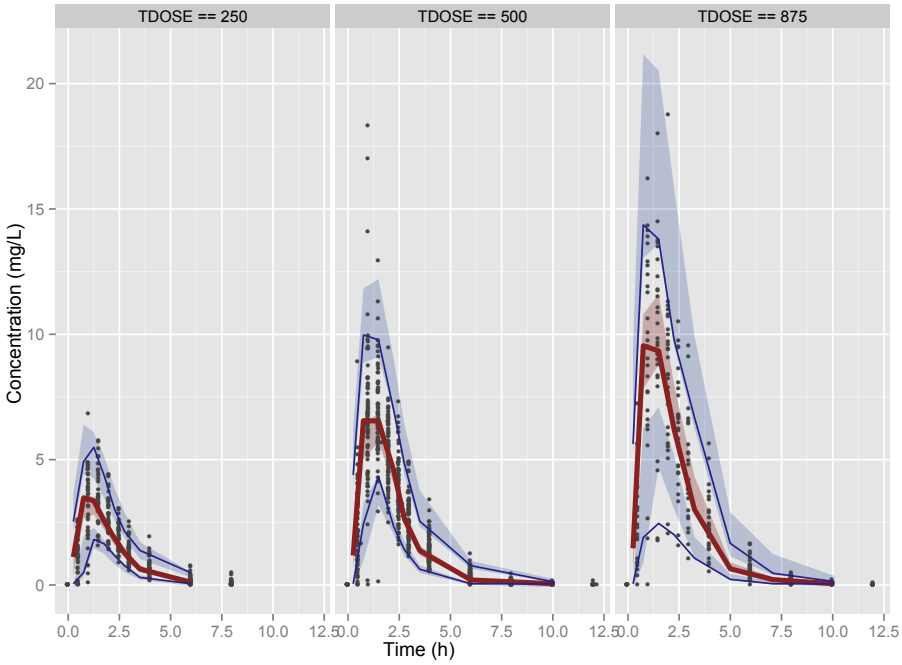

Figure 3. Visual predictive check stratified on dose (TDOSE). The solid circles are observed concentrations. The upper, middle and lower lines indicate the 95th, 50th and 5th percentile of observations, respectively. The shaded areas represent the 95%CI of the corresponding percentiles of predictions.

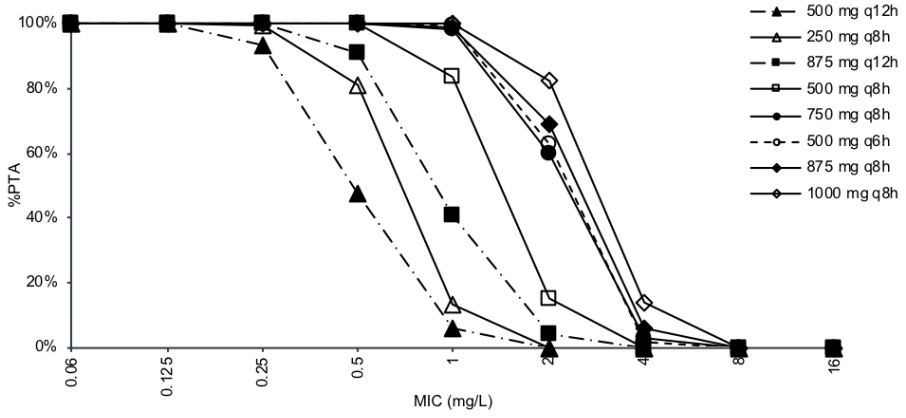

Figure 4. Probability of target attainment (PTA) for various amoxicillin dosing regimens to reach the pharmacodynamic target 40% $fT_{>MIC}$ for a range of MICs.

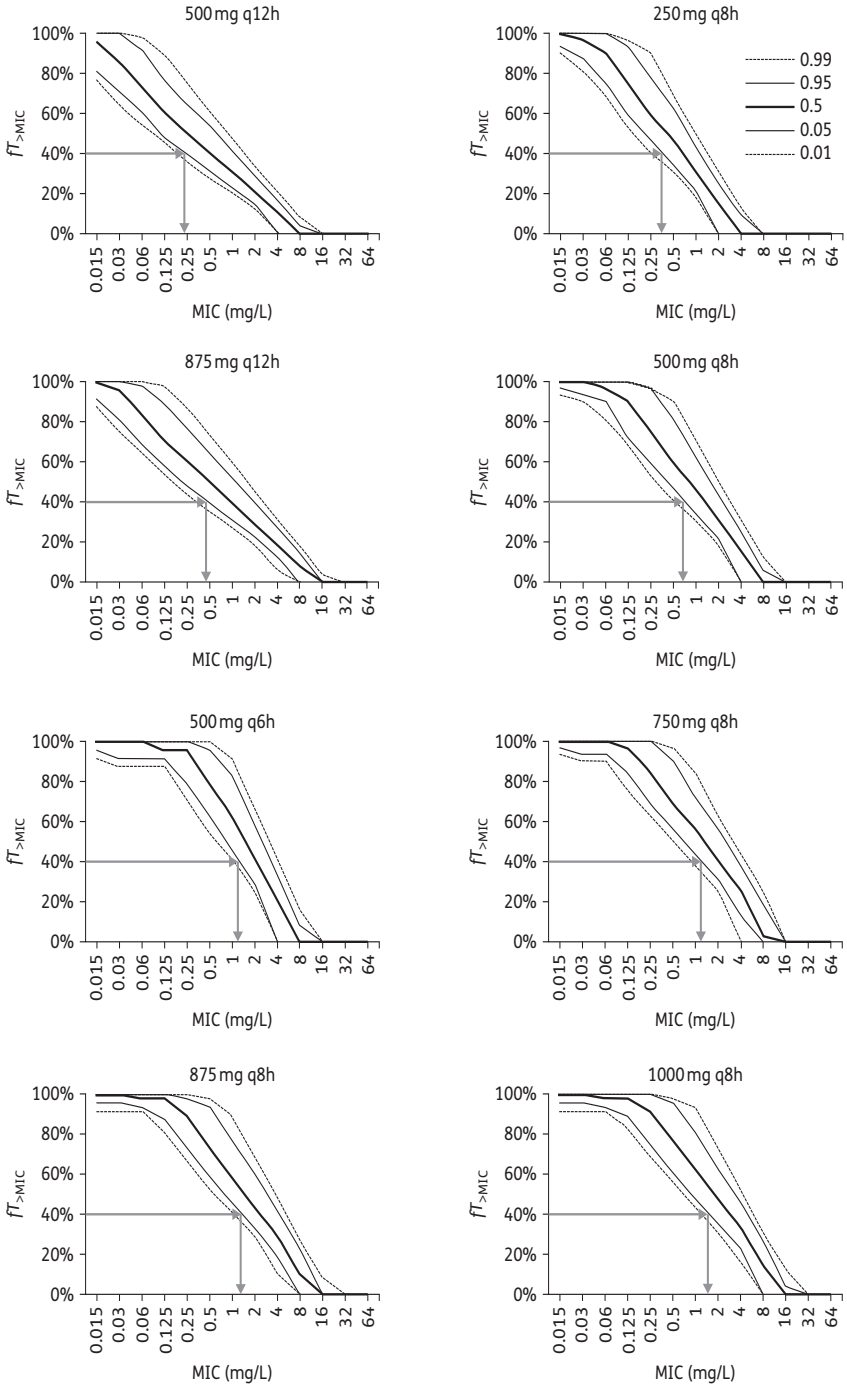

Figure 5. %$fT_{>MIC}$ displayed as a function of the MIC for several dosing regimens.

Discussion

Our population pharmacokinetic model includes a saturable absorption rate for amoxicillin, which corresponds to the nonlinear and delayed absorption found in the non-compartmental analysis.

The findings of our non-compartmental and population pharmacokinetic analyses with rich data are similar to the smaller studies that previously reported the absorption pharmacokinetics of amoxicillin to be nonlinear [9-14], mostly based on non-compartmental analysis and standard-two-stage methods [9-13]. Evidence for nonlinear pharmacokinetics was provided by the fact that C_{max} and AUC were relatively low and T_{max} was later for increasing single doses up to 3000 mg [9, 10, 12]. Spyker and colleagues showed a reduction in absorption rate for increasing single doses up to 1000 mg [9]. In two studies with single doses up to 3100 mg, Michaelis-Menten parameters were used to describe the nonlinear relationship between dose and the amount absorbed [11, 12]. Two analyses that studied oral suspension data resulted in a time-constrained Michaelis-Menten absorption model with lag time followed by a storage compartment and two disposition compartments [13, 14]. In our population pharmacokinetic analysis, models with Michaelis-Menten absorption described the data better than models with first-order or zero-order absorption, as expected given the previous studies [11-14]. Similar to the two oral suspension studies [13, 14], our model became better when we added a storage compartment. The absorption phase of our model further improved when we replaced the lag time with Savic's transit compartment model, which describes the absorption delay as a multiple step process represented by a chain of presystemic compartments [18]. Because the model with a combination of Savic's transit compartment model and a storage compartment was similar to the model with the transit compartment model alone, the final model only included the transit compartment model.

The nonlinearity in C_{max} and AUC that we found in our study and those of others may potentially be explained by several factors other than absorption, such as disposition or clearance. However, a proportional increase in AUC for increasing intravenous doses of 250 to 1000 mg [9, 23] excludes the probability of nonlinear disposition. Dose-dependent renal drug clearance is unlikely because of the finding in our study that $t_{1/2}$ was stable throughout the dose range of our study as well as in another study with oral doses of 375 to 3000 mg [12].

Regarding the influence of fasting/non-fasting on amoxicillin C_{max} and AUC, previous studies have shown results that contradict our results. SmithKline Beecham performed a study similar to ours in which oral amoxicillin/clavulanic acid was administered to fasting subjects [24], and which also demonstrated a nonlinear increase in C_{max} and AUC_{0-24}. In this study with fasting subjects [24], the amoxicillin C_{max} and AUC_{0-24} were lower than those in the here presented SmithKline Beecham study in which the subjects were non-fasting. Indeed, the current instructions for use recommend that amoxicillin/clavulanic acid be taken at the start of a meal [20]. In contrast, Welling and colleagues revealed that fasting subjects had higher amoxicillin serum levels than non-fasting subjects [25]. These contrary results may be explained by the differences in the amounts of water with which the drug was administered, which could affect

the dissolution of amoxicillin. The here presented SmithKline Beecham study used 200 mL water for 250-875 mg amoxicillin, compared with 120 mL for 250-875 mg in the other SmithKline Beecham study [24], and 25-250 mL for 500 mg in the study of Welling et al [25]. This is evidenced by the fact that fasting subjects given a 500 mg dose had significantly lower amoxicillin serum levels when the water volume was reduced from 250 to 25 mL [25]. An in vitro study showed that the solubility curve of amoxicillin is U-shaped with a minimum of 5.5 mg/L at pH 5 and 37 °C [26]. Since in our study the highest concentration was below 5.5 mg/L (875 mg amoxicillin administered with 200 mL water results in a concentration of 4.4 mg/L), we do not expect that the amoxicillin solubility influenced drug absorption.

Nonlinear absorption in human subjects has also been demonstrated for other aminopenicillins, such as ampicillin and bacampicillin [12, 27]. Penicillins can be regarded as a dipeptide derived from cysteine and valine [12] and a rat model suggest that aminopenicillins are absorbed via intestinal dipeptide carrier-mediated transport [28]. The amoxicillin absorption percentage from rat small intestine decreased with increasing concentrations, which indicates a saturable rate-limiting step in the absorption process [29].

Our final population pharmacokinetic model includes a Michaelis-Menten equation for absorption, which indicates that the absorption rate is saturable. This finding corresponds to the nonlinear and delayed absorption shown in the non-compartmental analysis. Despite the non-proportional increase in C_{max}, the dosing simulations demonstrate that increasing the dose results in a larger $\%fT_{>MIC}$, which is explained by the delayed absorption. However, high doses may increase the risk of adverse events and of disturbances in the intestinal microflora [30]. In our study, the frequency of diarrhoea tend to increase at higher daily doses of amoxicillin, which may be explained by the higher amount of unabsorbed antibiotic [30]. Sjövall and colleagues described an increased number of adverse events at higher single doses of amoxicillin [12]. In another study [31], high doses of clavulanic acid were related to upper digestive adverse events, but the amounts used (750 mg/day) were much higher than currently used (maximal 500 mg/day). The daily clavulanic dose in our study seems to be such low (250-375 mg/day) that it is not likely to be the cause of the diarrhoea. We did not found any correlation between diarrhoea and daily clavulanic acid amounts.

The $\%fT_{>MIC}$ becomes also larger when the frequency of dosing increases. On the other side, less frequent dosing can lead to a too low $\%fT_{>MIC}$ which reduces the probability of antimicrobial efficacy and may contribute to the development of resistance. The balance between dose and frequency should be optimal to maximize the antimicrobial efficacy and to minimize the risk of adverse events. For example, the breakpoint of 250 mg three-times daily is similar (0.25 mg/L) to that of 875 mg twice daily, based on a 95% CI and 40% $fT_{>MIC}$. The first regimen with a lower dose and a higher frequency is preferred to the second regimen with a higher dose and a lower frequency, because both the daily dose and the dose taken at one time of the first regimen are lower which appears to reduce the risk of adverse events. In case of regimens with a high dose and a low frequency which have the same breakpoint as regimens with a lower dose and higher frequency, high doses are wasteful. It is not

possible to recommend a maximal dose using the current data of 1750 mg/day and 875 mg/dose, because the non-proportional increase in C_{max} didn't reach a plateau.
The view that amoxicillin/clavulanic acid 500/125 mg three-times daily is interchangeable with 875/125 mg twice daily should be reconsidered, because the target attainment and therefore the breakpoint of the twice daily regimen is lower than the three-times daily regimen (0.25 mg/L vs 0.5 mg/L based on a 95% CI and 40% $fT_{>MIC}$).
The current EUCAST PK/PD non-species related breakpoints for amoxicillin are based on a $fT_{>MIC}$ target of 30-40% and a PTA >90% which resulted in S ≤2 mg/L (500 mg three-times daily) and R >8 mg/L (1000 mg three-times daily) [6]. These amoxicillin breakpoints are based on intravenous administration. The acceptance level of PTA is still under debate and PTA values of 99%, 95% and 90% have all been used. In the majority of the present rationale documents for EUCAST breakpoints, the $\%fT_{>MIC}$ as a function of the MIC is displayed with CIs. CIs of 99%, 95%, 90% and 80% correspond to PTAs of 99.5%, 97.5%, 95% and 90%, respectively [4]. Most current EUCAST PK/PD breakpoints for β-lactams (e.g. piperacillin/tazobactam, ceftazidime, meropenem) are based on the 95% CI (= 97.5% PTA). In this paper we use a 40% $fT_{>MIC}$ target and a 95% CI to simplify the comparison of dosing regimens. Obviously, the PK/PD breakpoint for oral amoxicillin is lower than that for intravenous administration due to a bioavailability of approximately 70% [20]. A 95% CI of 500 mg three-times daily results in a breakpoint of 0.5 mg/L for 40% $fT_{>MIC}$.
While our study describes the population pharmacokinetics of amoxicillin administered with clavulanic acid, we expect that the results of amoxicillin alone are similar, as others have shown [32].
This paper has a number of limitations. First, only a few covariates were available and creatinine data were lacking. However, the participants were healthy volunteers with normal renal function. Since oral amoxicillin or amoxicillin/clavulanic acid is mostly prescribed to patients with only relatively mild infections and normal renal function, the results of our study can be extrapolated to such patients. A second limitation is that the current model is only suitable for single doses, because we analysed each concentration-time profile separately. Incorporation of between occasion variability was not successful. If a multiple dose model will be developed, an output from the depot compartment should be considered to prevent possible accumulation of non-absorbed amoxicillin. Our population pharmacokinetic model is based on single doses up to 875 mg for which concentrations are measured 12 hours after administration. Because the maximum simulated dose of 1000 mg is just slightly higher than 875 mg and the interval between doses never exceed 12 hours, we assume that the extrapolation is justified.

In conclusion, the amoxicillin absorption rate appears to be saturable which results in a nonlinear increase in C_{max} and a later T_{max} for higher doses. Increasing the dose results in a larger $\%fT_{>MIC}$ due to this delayed absorption, despite the non-proportional increase in C_{max}. However, a higher dose increases the risk of adverse events. A smaller interval between doses leads to a larger $fT_{>MIC}$ as well. The balance between dose and frequency should be optimal to maximize the antimicrobial efficacy ($fT_{>MIC}$) and

to minimize the risk of adverse events. Clinicians should take care when prescribing oral amoxicillin regimens with high doses as well as those involving twice daily doses.

Funding

This work was supported by the Innovative Medicines Initiative Joint Undertaking under grant agreement n° [115523], resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.
The research leading to these results was conducted as part of the COMBACTE-NET consortium. For further information please refer to http://www.combacte.com/.

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Chapter 2.2

Population pharmacokinetics of oral clavulanic acid in healthy volunteers

Highly variable absorption of clavulanic acid during the day: a population pharmacokinetic analysis

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Journal of Antimicrobial Chemotherapy. 2018;73:469-76.

Abstract

Objectives
To calculate the clavulanic acid exposure of oral amoxicillin/clavulanic acid dosing regimens, to investigate variability using a population pharmacokinetic model and to explore target attainment using Monte Carlo simulations.

Methods
Two groups of healthy male volunteers received amoxicillin/clavulanic acid tablets at the start of a standard meal on two separate days one week apart. One group (n=14) received 875/125 mg q12h and 500/125 mg q8h and the other group (n=15) 500/125 mg q12h and 250/125 mg q8h. 1479 blood samples were collected until 8-12h after administration. Concentrations were analysed using non-compartmental (WinNonLin) and population pharmacokinetic methods (NONMEM).

Results
Median C_{max} and AUC_{0-8} were 2.21 mg/L (0.21-4.35) and 4.99 mg*h/L (0.44-8.31), respectively. In 40/58 daily concentration-time profiles, C_{max} and AUC_{0-8} of the morning dose were higher than later doses. The final population model included a lag time (0.447 h), first-order absorption (3.99 h^{-1} at 8:00h, Between-Subject Variability 52.8%, Between-Occasion Variability 48.5%), one distribution compartment (33.0 L, BSV 23.9%) and first-order elimination (24.6 L/h, BSV 26.7%). Bioavailability (fixed at 1 at 8:00h, BOV 28.2%) and absorption rate decreased over the day. For 97.5% of the simulated population after 125 mg q12h or q8h, %*f*T>Ct at 0.5 mg/L was 8.33% (q12h) and 15.2% (q8h), at 1 mg/L 0% (q12h+q8h) and *f*AUC_{0-24} 3.61 (q12h) and 5.56 (q8h) mg*h/L.

Conclusions
Clavulanic acid absorption in healthy volunteers is highly variable. Bioavailability and absorption rate decrease over the day. The model developed may serve to suggest clavulanic acid dosing regimens to optimize efficacy and prevent under-dosing.

Introduction

Clavulanic acid is a beta-lactamase inhibitor that is combined with beta-lactam antibiotics such as amoxicillin to target beta-lactamase-producing strains. Amoxicillin alone or in combination with clavulanic acid was the most consumed antibacterial agent in primary care in two-third of the EU/EEA countries in 2012 [1]. However, despite its ample use since for 30 years, the pharmacokinetics of oral clavulanic acid have received little attention.

Several non-compartmental pharmacokinetic (PK) studies showed highly variable pharmacokinetics [2-4] but it still remains unclear how the variable pharmacokinetics influences the exposure and thereby the efficacy of the drug. It is plausible that activity against beta-lactamase-producing strains will not be attained if the exposure of clavulanic acid is inadequate. A population PK model can be used to further investigate variability and exposure in the population. Currently, only 1 population PK model for oral amoxicillin/clavulanic acid suspension is available in a thesis [5].

The exposure of an antimicrobial to the microorganism in vivo (dependent on dose and PK) and the potency of a drug in vitro (usually expressed as a MIC) determine the antimicrobial efficacy [6]. For antimicrobials, pharmacokinetic-pharmacodynamic (PK/PD) indices, such as $AUC_{0\text{-}24}$/MIC, C_{max}/MIC and $T_{>MIC}$, describe exposure-response relationships of antimicrobial agents [6]. The pharmacodynamic target is the minimal PK/PD index value that ensures a high probability of successful treatment [6]. However, clavulanic acid has very weak antimicrobial activity when used alone [7]. For beta-lactamase inhibitors the time that the free concentration exceeds a threshold concentration (%*f*T>Ct) and the total daily dose and *f*AUC have been described to be important for inhibitory activity [8-12]. For tazobactam, sulbactam and avibactam, the PK/PD index seems to be %*f*T>Ct [8-11]. In contrast, for relebactam (MK-7655), the total daily dose and *f*AUC were linked to effect [12]. Clavulanic acid is an irreversible suicide inhibitor similar to tazobactam and sulbactam, but has a unique chemical structure and different affinities for beta-lactamase enzymes than the other inhibitors [7]. Its pharmacodynamic properties can therefore not be extrapolated. For lack of preclinical data, the PKPD index best describing its activity is currently not known.

The purposes of this study were therefore twofold. The first was to build a population pharmacokinetic model using NONMEM to determine the clavulanic acid exposure and variability of various oral amoxicillin/clavulanic acid dosing regimens and to investigate whether PK interactions occur between the two agents [13]. A population pharmacokinetic model for oral amoxicillin using the same amoxicillin/clavulanic acid data was previously published by the authors [14]. The second purpose was to explore the variability of clavulanic acid exposure and its effects on target attainment for standard dosing regimens of 125 mg twice daily (q12h) and 125 mg three times daily (q8h) clavulanic acid. Because the PK/PD index and PD target of clavulanic acid are still unknown, both %*f*T>Ct and *f*AUC were calculated.

Methods

Study design and population

The study was designed as an open label, randomized two part crossover investigation to study the pharmacokinetics of oral amoxicillin/clavulanic acid. Male volunteers were enrolled into the study if they were aged between 18 and 50 years and in good general health. Exclusion criteria were more than 20% deviation from ideal weight for height, use of prescribed medication in the 2 weeks prior to the study (antibiotics: 4 weeks), use of any medication during the study without consent, alcohol intake more than 3 units/day, participation in a trial within 2 months prior to the start of this study, prior hypersensitivity to the trial drug or to drugs with a similar chemical structure, diseases known to interfere with the drug pharmacokinetics, or blood donation of more than 1500 mL within the previous year.

The study (reference number 25000/360) [15] was conducted in 1993 at FOCUS Clinical Drug Development GmbH (Neuss, Germany) in commission of SmithKline Beecham Pharmaceuticals (Harlow, UK).

Ethics

The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Freiburger Ethics Committee (Freiburg, Germany). All volunteers gave written informed consent prior to the study.

Study procedures

The study consisted of 2 parts. Each part included a separate group of subjects that each received 2 dosing regimens of one day. The order of the dosing regimens was randomized and treatment days were separated by a washout period of 6 or 7 days. In part 1, 16 subjects were allocated to amoxicillin/clavulanic acid 875/125 mg q12h (2 doses) and 500/125 mg q8h (3 doses). In part 2, 16 other subjects were assigned to 500/125 mg q12h (2 doses) and 250/125 mg q8h (3 doses). Each dose was provided as a single tablet amoxicillin as trihydrate and clavulanic acid as potassium clavulanate (Augmentin®, SmithKline Beecham Pharmaceuticals, Bristol, TN, USA). Doses were administered with 200 mL water at the start of a standard meal. Each meal (approximately 800 kcal and 30% fat content) consisted of 4 slices of pork, slices of cucumber, 250 g of pasta-salad and a half slice of coarse wholemeal bread. Dosing times were 8:00h, 16:00h and 24:00h for three times daily regimens and 8:00h and 20:00h for twice daily regimens. For twice daily regimens, a second standard meal was provided at 12:00h. The first dose of each day was administered at 8:00h after having fasted from food and fluids from 22:00h the night before.

Blood samples were collected just before administration and after 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 h (three times daily regimens until 8 h). Samples were frozen at -70 °C within 1 h of sampling and were assayed within 6 weeks of collection. Clavulanic acid plasma concentrations were determined by Hazleton Laboratories (UK) using the ASTED (Automated Sequential Trace Enrichment of Dialysates) system coupled to high pressure liquid chromatography (HPLC) with ultraviolet absorbance detection. The lower limit of quantification was 0.05 mg/L [15].

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis of the plasma concentration-time data was performed using WinNonLin (version 7.0, Certara, Princeton, NJ, USA). Population pharmacokinetic analysis was performed using nonlinear mixed effects modelling (NONMEM version 7.2, ICON Development Solutions, Ellicott City, MD, USA). The Intel Visual Fortran Compiler XE 14.0 (Santa Clara, CA, USA) was used. The first-order conditional estimation method with interaction was used throughout the model building process. Tools used to evaluate and visualise the model were RStudio (version 0.98.1028), R (version 3.1.1), XPose (version 4.5.0) and PsN (version 4.6.0), all with the graphical interface Pirana [16] (version 2.9.4).

First-order, zero-order and Michaelis–Menten absorption models with and without lag time were evaluated in combination with one- and two-compartment distribution models. Between-subject variability (BSV, variability between individual subjects) and between-occasion variability (BOV, variability between the doses of an individual subject) were tested using an exponential variance model. Residual unexplained variability (RUV) was evaluated with a combined (additive and proportional) error model. A stepwise covariate model building was performed with forward addition at $p<0.05$ (decrease in the Objective Function Value, OFV, of 3.84 units) followed by backward elimination at $p<0.001$ (decrease in the OFV of 10.88 units). Evaluated covariates were body weight, dosing time, amoxicillin time dose and amoxicillin daily dose (dosing time and dose were evaluated as continuous and categorical covariates). Model selection criteria were decrease in OFV, goodness-of-fit plots and visual predictive checks (VPC). A decrease in the OFV of 3.84 units was considered statistically significant ($p<0.05$) in a nested model [17]. For each VPC, a set of 1000 simulated datasets was created to compare the observed concentrations with the distribution of the simulated concentrations.

The 95% CI of each parameter in the final model was determined from a nonparametric bootstrap analysis, in which the dataset was resampled 1000 times.

Monte Carlo simulations

Monte Carlo simulations were performed using the final model in NONMEM. Two clavulanic acid dosing regimens were evaluated: 125 mg q12h (at 8:00h and 20:00h) and 125 mg q8h (at 8:00h, 16:00h and 24:00h). Five thousand subjects were simulated for each dosing regimen. For each simulated concentration-time profile, the $fAUC_{0\text{-}24}$ was calculated as well as the $\%fT>Ct$ (the percentage of the dosing period 24 h that the free clavulanic acid concentration exceeds the threshold concentration Ct) for threshold concentrations of 0.015–64 mg/L. The unbound clavulanic acid concentration was calculated from the total concentration using a fixed value for protein binding of 25% [18, 19].

Results

Study population

32 healthy male volunteers (16 per part) entered the study. After 2 withdrawals

(one because of diarrhoea and one due to personal reasons), clavulanic acid plasma concentrations were determined in 30 volunteers (15 per part) completing both dosing regimens. Pharmacokinetics could not be evaluated in one subject in part 1 due to very low plasma clavulanic acid and amoxicillin concentrations. The characteristics of the 29 pharmacokinetic evaluable subjects are shown in **Supplementary Table 1.** The average ± SD values were: age 33 ± 7 years, height 179 ± 6 cm, weight 78 ± 9 kg and BMI 24 ± 2 kg/m^2.

Pharmacokinetic analysis

One hundred and forty-five clavulanic acid concentration-time profiles (5 profiles per subject) with in total 1479 samples were analysed. The concentrations in twice daily regimens with sampling until 12 h after administration were detectable for maximal 8 h.

A summary of the results of the non-compartmental pharmacokinetic analysis is shown in **Table 1**. Considering the ranges of C_{max} and AUC_{0-8}, the ratio between the maximum and minimum values is circa 20 for both parameters (C_{max} 4.35/0.21 and AUC_{0-8} 8.31/0.44). The individual concentration-time profiles illustrate that this high variability in C_{max} and AUC_{0-8} not only exists between the individual subjects but also between the different doses in one dosing regimen of a subject. In 40/58 daily concentration-time profiles, the C_{max} and AUC_{0-8} of the first dose were higher than the C_{max} and AUC_{0-8} of the second or third dose. A summary of the individual concentration-time profiles is displayed in **Figure 1**.

Population PK analysis showed that the clavulanic acid data were best described by a model with a lag time and first-order absorption, one distribution compartment and first-order elimination. A second distribution compartment didn't further improve the model. Implementation of BSV was significant for volume of distribution (Vd), clearance (CL) and first-order absorption rate constant (Ka). Implementation of BOV was significant for bioavailability (F) and Ka. BSV for F and lag time and BOV for Vd, CL and lag time was not significant and were therefore not implemented.

The covariate analysis resulted in two significant covariates: dosing time (8:00h, 16:00h, 20:00h, 24:00h) was proportionally correlated with F and Ka. The effect of dosing time was further studied by implementation of a cosine function [20]. Replacing the covariate dosing time with cosine functions on Ka and F didn't improve the model and therefore the proportional effects of dosing time on Ka and F were implemented in the model. For example, at dosing time 8:00, the population value of Ka was 3.99 h^{-1} (fixed proportional effect of 1) and at 16:00 the population value was 3.60 h^{-1} (estimated proportional effect of 0.903). A model with combined dosing times 20:00h and 24:00h was also tested because the proportional effects on Ka and F looked similar at these dosing times, but this combination didn't improve the model further and was therefore not implemented.

The population PK parameter estimates of the final model are displayed in **Table 2**. Since no data with intravenous administration were collected in this study, F could not be quantified and was fixed to 1. Consequently, Vd/F and CL/F values are displayed instead of Vd and CL values.

As shown in **Table 2**, the model-based parameter estimates were similar to the

values computed from the bootstrap analysis, indicating the stability of the model. The goodness-of-fit plots in **Figure 2** and **Supplementary Figure 1** show that the model adequately described the observed concentrations. The VPC plots, presented in **Supplementary Figure 2**, indicate a good predictive performance.

Monte Carlo simulations

The results of the simulations with 125 mg q12h and 125 mg q8h are presented in **Table 3** ($fAUC_{0-24}$) and **Figure 3** (%*f*T>Ct). **Table 3** shows that 97.5% of the population reached an $fAUC_{0-24}$ of 3.61 mg*h/L with 125 mg q12h and 5.56 mg*h/L with 125 mg q8h. For 97.5% of the population, the %*f*T>Ct at 1 mg/L was 0% for both regimens and the %*f*T>Ct at 0.5 mg/L was 8.33% (125 mg q12h) and 15.2% (125 mg q8h). Half of the population (q12h: 46%, q8h: 53%) attained a concentration of 2 mg/L, but the average %*f*T>Ct values at 2 mg/L were low: 2.09% with 125 mg q12h and 3.05% with 125 mg q8h.

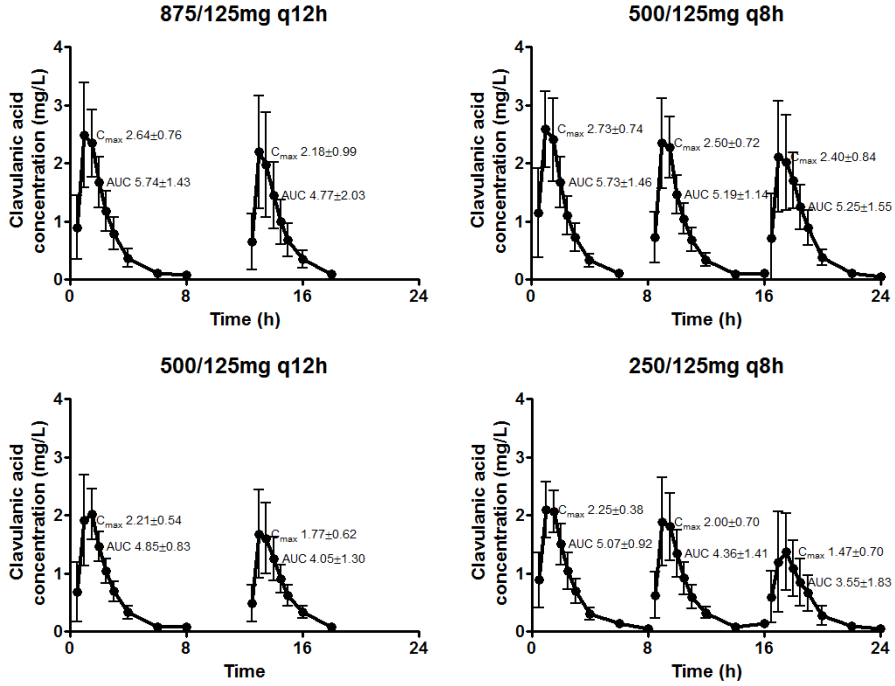

Figure 1. Clavulanic acid concentration-time curves for 4 dosing regimens: amoxicillin/clavulanic acid 875/125 mg q12h (n=14), 500/125 mg q8h (n=14), 500/125 mg q12h (n=15) and 250/125 mg q8h (n=15). The average concentration and standard deviation at each time point is displayed as a filled circle with error bars.

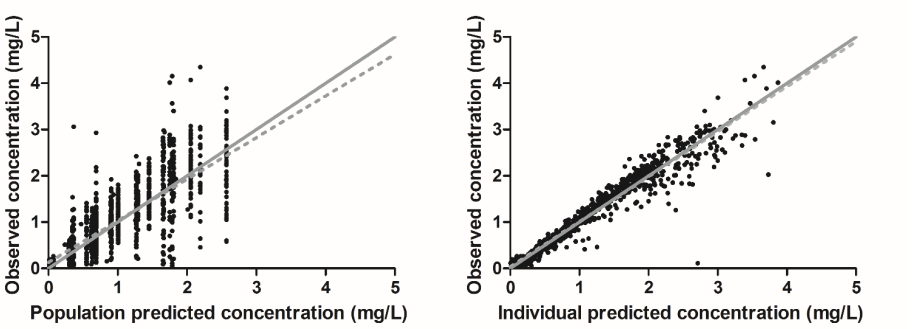

Figure 2. Observed versus population predicted concentrations **(a)** and observed versus individual predicted concentrations **(b)**.

Table 1. Results of the non-compartmental pharmacokinetic analysis of clavulanic acid (values expressed as mean ± SD and median [minimum – maximum])

Dosing regimen amoxicillin/ clavulanic acid	Pharmacokinetic parameters of clavulanic acid				
	C_{max} (mg/L)	T_{max} (h)	AUC_{0-8} (mg*h/L)	AUC_{0-24} (mg.h/L)	$t_{1/2}$ (h)
250/125 mg q8h					
mean ± SD	1.91 ± 0.68	1.30 ± 0.31	4.33 ± 1.54	12.98 ± 3.33	1.07 ± 0.28
median [range]	1.99 [0.21-2.97]	1.50 [1.00-2.00]	4.60 [0.44-6.76]	13.45 [4.63-18.26]	0.98 [0.46-1.69]
500/125 mg q12h					
mean ± SD	1.99 ± 0.61	1.33 ± 0.40	4.45 ± 1.15	8.90 ± 1.92	1.05 ± 0.19
median [range]	1.91 [0.56-3.00]	1.25 [1.00-2.50]	4.63 [1.08-6.41]	9.26 [4.86-11.55]	0.99 [0.72-1.41]
500/125 mg q8h					
mean ± SD	2.54 ± 0.76	1.26 ± 0.28	5.39 ± 1.38	16.17 ± 3.89	1.09 ± 0.41
median [range]	2.59 [1.09-4.35]	1.25 [1.00-2.00]	5.39 [2.58-8.31]	17.17 [8.23-21.75]	0.99 [0.66-3.37[a]]
875/125 mg q12h					
mean ± SD	2.41 ± 0.90	1.29 ± 0.32	5.25 ± 1.79	10.51 ± 3.07	1.02 ± 0.17
median [range]	2.62 [0.23-4.02]	1.25 [1.00-2.00]	5.63 [0.50-7.78]	10.85 [3.51-13.87]	1.00 [0.73-1.47]
All regimens					
mean ± SD	2.21 ± 0.78	1.29 ± 0.32	4.82 ± 1.53	12.10 ± 4.10	1.06 ± 0.30
median [range]	2.21 [0.21-4.35]	1.50 [1.00-2.50]	4.99 [0.44-8.31]	11.95 [3.51-21.75]	0.99 [0.46-3.37[a]]

q12h, every 12 h; q8h, every 8 h; range, minimum-maximum; C_{max}, maximum concentration; T_{max}, time to maximum concentration; AUC_{0-8}, Area Under the concentration-time Curve for 0-8 h, AUC_{0-24}, Area Under the concentration-time Curve for 0-24 h (q12h: 2 doses, q8h: 3 doses); $t_{1/2}$, half-life.

[a] Outlier (penultimate $t_{1/2}$ was 1.53 for 500/125 mg q8h).

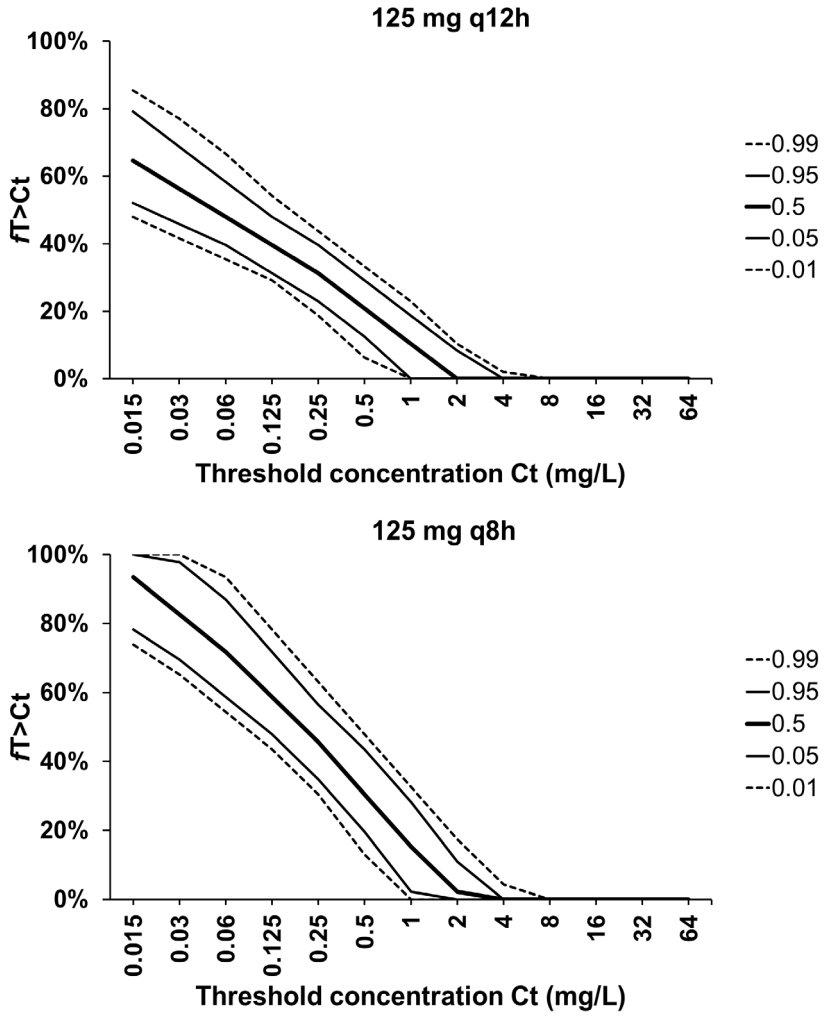

Figure 3. %*f*T>Ct (the percentage of the dosing period 24 h that the free clavulanic acid concentration exceeds the threshold concentration Ct) displayed as a function of Ct for two dosing regimens 125 mg q12h **(a)** and 125 mg q8h **(b)**. The middle line represents the values for the median of the population and the surrounding lines indicate the 1st, 5th, 95th and 99th percentiles, obtained by 5000 Monte Carlo simulations.

Table 2. Model-based population PK parameter estimates and values obtained after bootstrap analysis.

Parameter	Final model estimate	RSE (%)	Bootstrap median	Bootstrap 95% CI
Fixed effects				
Vd/F (L)	33.0	3.8	33.0	30.3 – 35.6
CL/F (L/h)	24.6	3.8	24.7	22.6 – 26.6
F (-)	1 (fixed)	-	1 (fixed)	-
Ka (h^{-1})	3.99	14.1	3.95	3.07 – 5.20
Lag time (h)	0.447	1.3	0.447	0.436 – 0.456
Covariate: proportional effect on Ka				
Dosing time 8:00	1 (fixed)	-	1 (fixed)	-
Dosing time 16:00	0.903	9.9	0.904	0.737 – 1.08
Dosing time 20:00	0.610	15.1	0.617	0.442 – 0.801
Dosing time 24:00	0.636	14.8	0.638	0.476 – 0.843
Covariate: proportional effect on F				
Dosing time 8:00	1 (fixed)	-	1 (fixed)	-
Dosing time 16:00	0.873	5.6	0.876	0.765 – 0.949
Dosing time 20:00	0.799	7.4	0.802	0.688 – 0.904
Dosing time 24:00	0.801	8.8	0.806	0.663 – 0.944
Between-subject variability (BSV)				
Vd (%CV)	23.9	23.2	23.4	12.9 – 33.3
CL (%CV)	26.7	17.1	26.1	17.8 – 34.8
Ka (%CV)	52.8	15.8	51.7	34.4 – 67.8
Between-occasion variability (BOV)				
Ka (%CV)	48.5	12.8	47.3	37.8 – 60.2
F (%CV)	28.2	21.4	27.2	16.7 – 38.6
Residual unexplained variability (RUV)				
Additive (mg/L)	0.0533	8.2	0.0530	0.0450 – 0.0625
Proportional	0.142	8.2	0.141	0.119 – 0.165

Vd, volume of distribution; F, bioavailability; CL, clearance; Ka, first-order absorption rate constant; RSE, relative standard error; CV, coefficient of variation

Table 3. *f*AUC_{0-24} distribution obtained by 5000 Monte Carlo simulations for two dosing regimens 125 mg q12h and 125 mg q8h.

	***f*AUC$_{0-24}$ (mg*h/L)**	
	125 mg q12h	**125 mg q8h**
Minimum	2.10	3.43
1st percentile	3.17	4.86
2.5th percentile	3.61	5.56
5th percentile	4.07	6.15
50th percentile	6.94	10.4
95th percentile	12.2	17.5
97.5th percentile	13.7	19.2
99th percentile	15.4	21.2
Maximum	28.0	36.1

Discussion

Our non-compartmental pharmacokinetic analysis showed that clavulanic acid C_{max} and AUC_{0-8} in healthy volunteers were highly variable, whereas $t_{1/2}$ had a limited variability. In two-third of the subjects, the C_{max} and AUC_{0-8} of the morning dose were higher than later doses. Our population PK model indicated that most variability was present on the first-order absorption rate constant (Ka). Ka and bioavailability (F) were estimated to be higher in the morning than in the afternoon and evening.

Similar to the present study, other non-compartmental PK studies with oral clavulanic acid demonstrated a more variable C_{max} and AUC than $t_{1/2}$ [2-4]. A population PK model for oral amoxicillin/clavulanic suspension [5] also included a high between-subject variability and between-occasion variability of absorption parameters rather than on Vd and CL. Another study in 10 volunteers receiving a single oral and a single intravenous dose of clavulanic acid also found a wide bioavailability range (31.4-98.8%) [2].

In our population PK model, we could explain part of the observed variability by the effect of dosing time on Ka and F. To our knowledge, time-varying absorption and bioavailability of oral clavulanic acid has not previously been described. For two other beta-lactams, meropenem and ceftazidime, it has been shown that morning concentrations were higher than afternoon concentrations [21-23]. However, since those antibiotics were both given intravenously, the varying concentrations were explained by renal clearance variation rather than absorption differences as in our study. Our finding of the inversely proportional effect of dosing time on Ka may be caused by nonlinear processes, such as saturation of Ka. However, this seems to be unlikely since implementation of Michaelis-Menten absorption didn't improve the model. Unfortunately, it is impossible to predict the results for second and later dosing days, because our data only included dosing regimens of 24 hours. Differences in meal composition can be excluded as a reason for variation in Ka and F, because each dose was taken at the start of a standardized meal which was the same for each moment of the day. Administration without food does not eliminate

the variable pharmacokinetics, since fasting studies with oral clavulanic acid also showed a high variation in C_{max} and AUC [2, 4]. In a chronopharmacokinetic study with oral midazolam [20], the daily variation in Ka was described by a time-varying covariate and F was parameterized as a cosine function. The Ka and F differences were explained by 24-hour variation in gastric emptying, gastrointestinal mobility and splanchnic blood flow [20], which may be the most reliable explanation for the findings in our study as well. Possible clinical implications of time-varying Ka and F for dosing regimens, such as higher afternoon and evening doses than morning doses, should be further studied.

The results of our non-compartmental analysis seem to suggest that the amoxicillin dose influences the clavulanic acid PK. However, we tested several covariate types of amoxicillin dose (e.g. time dose, daily dose, categorical covariate, continuous covariate) during the modelling process and none was significant. The literature is not conclusive about the effect of amoxicillin on clavulanic acid PK. It has been reported that the C_{max} and AUC of oral clavulanic acid in presence of amoxicillin were higher than clavulanic acid alone and that the AUC ratio of amoxicillin/clavulanic acid was lower (2.55) than expected (500/125=4) [24]. These findings suggest an interaction between the absorption of amoxicillin and clavulanic acid. However, these AUC ratios differ enormously between studies. For oral 500/125 mg, the AUC ratios in our study were 3.4 (500/125 mg q12h) and 3.9 (500/125 mg q8h) whereas other studies found ratios of 2.0 [25] and 4.3 [4]. We found an AUC ratio of 2.1 for oral 250/125 mg which was the same as found by another study [4]. However, a third study found a ratio of 1.4 [26]. The AUC ratios for oral 875/125 mg were 5.3 (our study) and 5.7 [4]. It is possible that the saturable absorption rate of amoxicillin influences the AUC ratio [14]. However, for intravenous amoxicillin/clavulanic acid too, the AUC ratios were not as expected: 2.8 (500/100 mg) [27], 2.7 (1000/200 mg) [27], 3.2 (500/100 mg) [25], 7.1 (625/125 mg) [2] and 6.5 (2000/200 mg) [27]. These findings indicate that also other factors than absorption influence the interaction between the two drugs. Although we were not able to find a significant effect of the amoxicillin dose on clavulanic acid pharmacokinetics, the influence of the interaction between both compounds is not yet clear. The EMA guideline on the use of PK/PD in the development of antimicrobials recommends to study the PK interaction of beta-lactams and beta-lactam inhibitors [13]. Future research should elucidate the influence of amoxicillin on clavulanic acid PK.

Similar to a study with oral amoxicillin/clavulanic suspension in healthy volunteers [5], the between-subject variability and between-occasion variability magnitude of the absorption parameter was comparable. We hypothesize that in a patient population the BSV will be higher than the BOV. Due to highly variable clavulanic acid concentrations, the risk of ineffectiveness (with too low concentrations) and adverse events (with too high concentrations) should be attended.

When the PD target is known, dosing regimens can be optimized to attain a high probability of successful treatment. However, it is still unknown which PK/PD index and PD target should be taken into account for clavulanic acid. Since clavulanic acid has a unique structure in the group of beta-lactamase inhibitors [7], it is difficult to simply extrapolate a PK/PD index from another inhibitor. Clavulanic acid is a

clavam isolated from *Streptomyces clavuligeres* whereas tazobactam and sulbactam are synthetic penicillinate sulfones [7, 28]. The different chemical structures possibly explain the differences in enzyme activities of these inhibitors [7]. The beta-lactamase inhibitors avibactam and relebactam are diazabicyclooctane derivatives that don't have structural similarity with beta-lactams [28]. These two compounds have different pharmacodynamic properties, although they are both from the same group. Avibactam activity is primarily dependent on %*f*T>Ct [10, 11] and relibactam on *f*AUC [12].

Since the PD target for clavulanic acid is unclear, optimization of treatment by ensuring a high probability of attainment is not well possible. However, we do present simulations and attainment for different targets and these will be useful once the PD target of clavulanic acid becomes available. Current EUCAST guidelines use a fixed clavulanic acid concentration of 2 mg/L for susceptibility testing purposes [29]. Our Monte Carlo simulations show that with 125 mg q12h or q8h half of the population attains concentrations of 2 mg/L and the average %*f*T>Ct is only 2-3%. Similarly, assuming a $fAUC_{0\text{-}18}$ of 36 mg*h/L in vitro (based on using the same 2 mg/L concentration and an incubation time of 18 hours) the probability of target attainment is 0% with 125 mg q12h or q8h. However, the in vivo effect of clavulanic acid is thereby far underestimated. Ultimately, the susceptibility in vitro has to be correlated to efficacy in vivo and there is at present - in contrast to antimicrobials - no clear consensus for inhibitors. For example, the EUCAST fixed concentration for tazobactam is 4 mg/L [29], which is much higher than the PD target %*f*T>Ct at 0.5 mg/L [8]. Pharmacodynamic studies providing data on the clavulanic acid target are clearly required.

A limitation of this study is that only a few covariates were available and unfortunately, creatinine data were lacking. This prohibited an analysis of the impact of renal function on clavulanic acid exposure. However, the participants were healthy volunteers with normal renal function and a clear relationship would likely not have been found in this population. Since oral amoxicillin/clavulanic acid is mostly prescribed to patients with only relatively mild infections and normal renal function, the results of our study can therefore be extrapolated to such patients. Second, it was not possible to evaluate different clavulanic acid doses in the population pharmacokinetic analysis, because only one dose of clavulanic acid (125 mg) was included in this study. However, 125 mg is the dose most generally used for oral dosing. This study included 3 different tablets and 4 dosing regimens for amoxicillin/clavulanic acid. It is impossible to extrapolate these results to other oral formulations (e.g. the extended release (XR) tablet or the suspension). However, because the clavulanic acid formulation and the dosing frequency of the XR tablet are the same as used in our study, we expect that the timing problem may also exist with the XR tablet. A third limitation is that our data only included dosing regimens of 24 hours, which makes it impossible to predict the results for second and later dosing days.

In conclusion, clavulanic acid concentrations in healthy volunteers are highly variable after oral administration. Bioavailability and absorption rate decrease over the day. The consequences of the variable concentrations for underdosing and adverse

events should be further studied for multiple day dosing and dosing regimens should be optimized. Studies on the PK/PD index and PD target are needed.

Acknowledgements

We thank Peter de Bruijn for assistance with WinNonLin.

Funding

This work was supported by the Innovative Medicines Initiative Joint Undertaking under grant agreement n° [115523], resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.
The research leading to these results was conducted as part of the COMBACTE-NET consortium. For further information please refer to http://www.combacte.com/.

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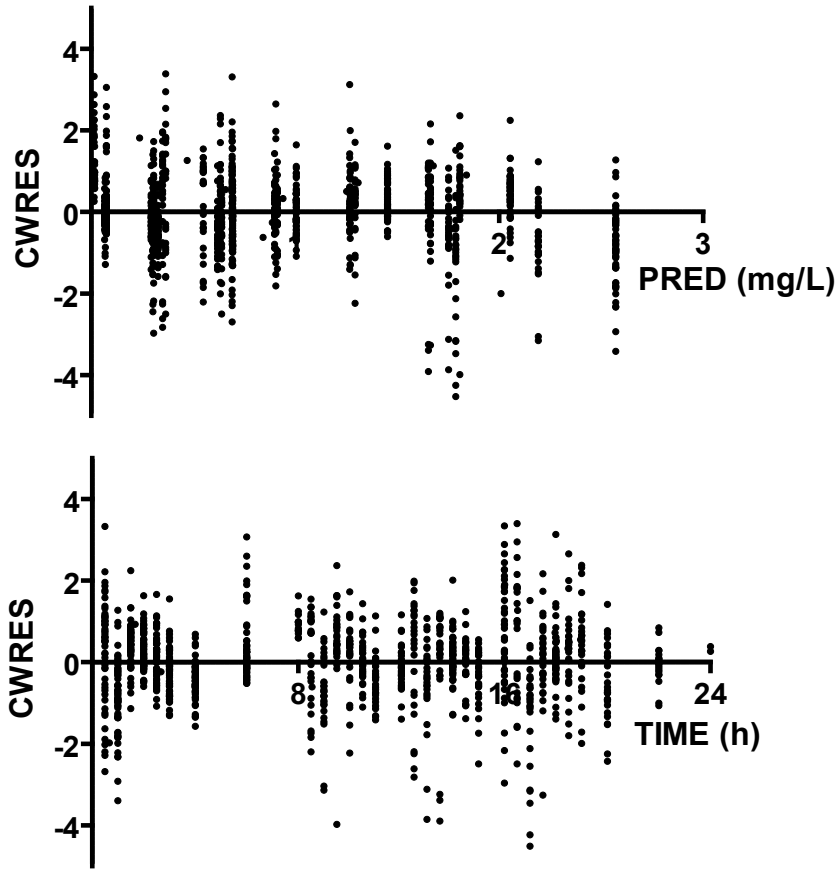
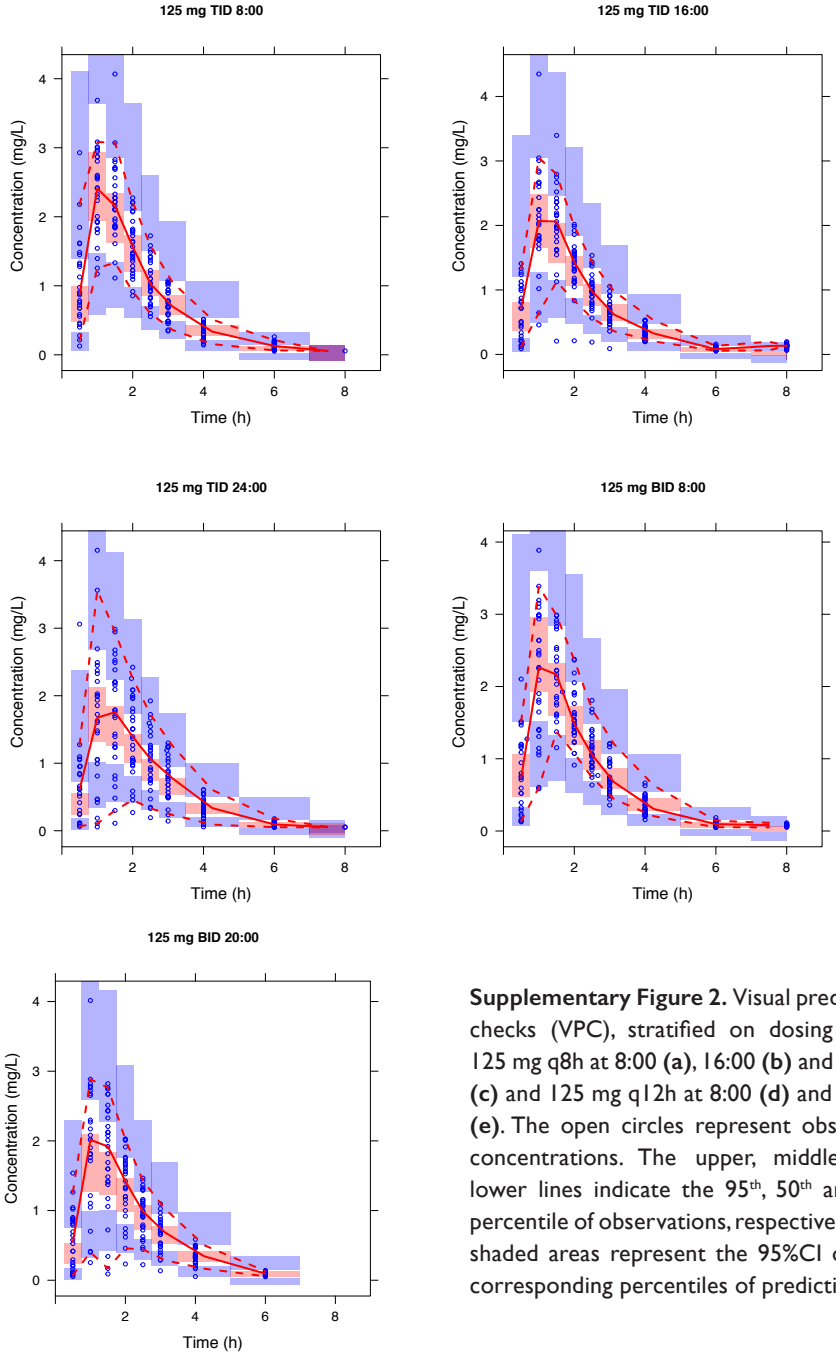
Supplementary Table 1. Characteristics of study population (values expressed as mean ± SD)

Characteristic	*Total (n=29)*	*Part 1 (n=14)*	*Part 2 (n=15)*
Age (years)	33 ± 7	35 ± 8	30 ± 5
Height (cm)	179 ± 6	179 ± 6	179 ± 7
Weight (kg)	78 ± 9	78 ± 5	78 ± 11
BMI (kg/m^2)	24 ± 2	24 ± 2	24 ± 3



Supplementary Figure 1. CWRES (Conditional Weighted Residuals) plots: CWRES versus PRED (population predicted concentrations) **(a)** and CWRES versus time **(b)**.



Supplementary Figure 2. Visual predictive checks (VPC), stratified on dosing time: 125 mg q8h at 8:00 **(a)**, 16:00 **(b)** and 24:00 **(c)** and 125 mg q12h at 8:00 **(d)** and 20:00 **(e)**. The open circles represent observed concentrations. The upper, middle and lower lines indicate the 95th, 50th and 5th percentile of observations, respectively. The shaded areas represent the 95%CI of the corresponding percentiles of predictions.

Chapter 2.3

Pharmacokinetics of intravenous amoxicillin in patients with and without renal impairment

Intravenous amoxicillin in patients with various degrees of renal function: are we dosing adequately?

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Submitted

Chapter 3

Imipenem

Chapter 3.1

Population pharmacokinetics of imipenem in critically ill patients

Population pharmacokinetics of imipenem in critically ill patients: a parametric and a nonparametric model converge on CKD-EPI eGFR as impactful covariate

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Clinical Pharmacokinetics, 2020;59:885-898

Abstract

Background
Population pharmacokinetic (popPK) models for antibiotics are used to improve dosing strategies and individualize dosing by therapeutic drug monitoring. Little is known about the differences in results of parametric versus nonparametric popPK models and their potential consequences in clinical practice. We developed both parametric and nonparametric models of imipenem using data from critically ill patients and compared their results.

Methods
Twenty-six critically ill patients treated with intravenous imipenem/cilastatin were included. Median estimated glomerular filtration rate (eGFR) measured by the CKD-EPI equation was 116 ml/min/1.73 m^2 (interquartile range, 104-124) at inclusion. The usual dosing regimen was 500mg/500mg four times daily. On average, 5 imipenem levels per patient (138 levels in total) were drawn as peak, intermediate and trough levels. Imipenem concentration-time profiles were analyzed using parametric (NONMEM 7.2) and nonparametric (Pmetrics 1.5.2) popPK software.

Results
For both methods, data were best described by a model with 2 distribution compartments and the CKD-EPI eGFR equation unadjusted for body surface area as a covariate on the elimination rate constant (K_e). The parametric population parameter estimates were: K_e 0.637 h^{-1} (between-subject variability [BSV]: 19.0% CV) and central distribution volume (V_c) 29.6 L (without BSV). The nonparametric values were: K_e 0.681 h^{-1} (34.0% CV) and V_c 31.1 L (42.6% CV).

Conclusions
Both models described imipenem popPK well, the parameter estimates were comparable and the included covariate was identical. Estimated BSV was higher, however, in the nonparametric model. This may have consequences for estimated exposure during dosing simulations and should be further investigated in simulation studies.

Introduction

Because of increased antimicrobial resistance and few new antibiotics making it to market, optimization of antibiotic dosing regimens remains an important challenge to improve clinical outcomes of infections. Antimicrobial efficacy is determined by the susceptibility of a drug *in vitro* (usually expressed as the minimal inhibitory concentration, MIC) and exposure to the drug *in vivo*, which relies on the pharmacokinetics and the dose [1]. Population pharmacokinetic (popPK) models describe the variability of exposure to a drug and are therefore used to support the optimization of dosing regimens with the objective to improve antimicrobial efficacy. During the development of new antibiotics, popPK models are recommended to support dose regimen identification and selection [2]. For already marketed antibiotics, popPK models are used in different ways to improve antibiotic dosing: individualization of dosing via therapeutic drug monitoring (TDM) software by Bayesian estimation and control, optimization of dosing regimens described in the package insert (especially for specific subpopulations), and setting clinical breakpoints [3]. Clinical breakpoints are MICs that define microorganisms as susceptible, intermediate or resistant to specific antibiotics [4].

While individual PK methods analyze the concentration-time profiles per individual subject, popPK methods analyze these profiles of a population as a whole. PopPK models describe and explain different types of PK variability, such as between-subject variability (BSV) and residual variability. PopPK modelling methods are classified as either parametric or nonparametric methods, which can each be divided in maximum likelihood or Bayesian approaches [3]. Bayesian popPK methods are used much less often than maximum likelihood popPK methods. Most published popPK models are based on parametric maximum likelihood methods (e.g. Monolix, NONMEM and Phoenix NLME), which estimate the set of PK parameters that maximize the joint likelihood of observations. Parametric methods assume that the population parameter distribution is known with population parameters to be estimated [5]. An example of nonparametric maximum likelihood software is the NPAG algorithm in the R package Pmetrics [6]. Nonparametric methods make no assumption about the shapes of the underlying parameter distributions. Another difference is that nonparametric methods use an exact likelihood function while most parametric methods use an approximation. A disadvantage of nonparametric methods in the past was that confidence intervals about parameter estimates could not be easily determined [5, 7]. However, Pmetrics can estimate credibility intervals around median parameter estimates using a bootstrap method.

Some studies comparing parametric and nonparametric models are available in the literature. Precluding studies with currently outdated modelling software [8-11], we found eight comparison studies [12-19]. Four of these studies showed comparable parameter estimates of both models [12-15], one study showed different estimates [16], for two studies the estimates were incomparable due to a different model structure [17, 18] and in one study no parameter estimates were reported [19]. However, less similarity among methods was noticed for the BSV of parameters. The BSV is defined as the percent coefficient of variation (CV%), which is the standard

deviation (SD) divided by the parameter mean. Three studies showed a higher BSV for the nonparametric model [12, 13, 16], one study showed similar BSV [14] and, for another study, the BSV of the nonparametric model was not reported [15]. Only one comparison study [18] presented goodness-of-fit (GOF) plots and visual predictive checks (VPCs) of both models. The other studies [12-17, 19] showed either GOF or VPC plots of both models, displayed the plots of only one method or did not show any GOF or VPC plots.

Many parametric and nonparametric popPK models have been published in the literature and are often accompanied by dosing recommendations based on simulations of the model [3]. Differences in modelling results between these methods may have consequences for simulation findings and may therefore also influence dosing recommendations. Different parameter value probability distributions may influence dosing recommendations based on popPK models in TDM software.

To investigate the advantages and disadvantages of both modelling methods in practice, we developed both parametric and nonparametric popPK models using the same data and compared the results. We used imipenem PK data from critically ill patients given this population's known PK variability for several antibiotics [20]. Imipenem is a carbapenem antibiotic administered in combination with cilastatin to prevent degradation by dehydropeptidase-I in the kidneys. When combined with cilastatin, approximately 70% of imipenem is recovered in the urine within 10 hours and the rest is excreted as inactive metabolites via the urine [21]. The half-life is 1 hour in patients with normal renal function but is extended in patients with renal dysfunction [21]. Protein binding is reported as 10 to 20% [22].

Although it is known that the correlation between measured creatinine clearance (CL_{cr}) and estimated glomerular filtration rate (eGFR) equations is weak [23], these equations are used in daily practice in many intensive care units (ICU). In our study population, measured CL_{cr} was unavailable. Therefore, we decided to test several eGFR equations during covariate model building, to find the most suitable one in our population.

Methods

Study population

Imipenem PK data from a previously published prospective cohort study [24] conducted between 2010 and 2013 in the ICU of the Geneva University Hospitals (Geneva, Switzerland) were used for this popPK study. The usual dosing regimen for imipenem/cilastatin was 500mg/500mg four times daily, administered by intermittent intravenous infusion of 30 minutes. Inclusion criteria were suspected or documented severe bacterial infection and age 18-60 years. Exclusion criteria were estimated glomerular filtration rate (eGFR) <60 ml/min (measured by the Cockcroft-Gault equation [25]), Body Mass Index <18 or >30 kg/m^2 and pregnancy. The study protocol was approved by the University Hospital's Ethics Committee (NAC 09-117). Given its observational nature, the Committee waived the requirement for informed consent from patients who were unconscious or otherwise unable to understand the study protocol.

Among the 54 critically ill patients from the Swiss study who were receiving imipenem therapy, the last 27 patients could be included because exact dosing and blood sampling times were known, in contrast to the first 27 patients, for whom levels were labeled only as trough, intermediate or peak. After excluding one subject because of missing height [26], data from the remaining 26 patients were included in the popPK study. None of these patients received probenecid, the only drug known to influence imipenem concentrations [21].

Study procedures

Patients were included on their first or second day of imipenem therapy. Blood samples were planned on days 1, 2, 3, 4 and 6 of therapy, although in some patients not all planned samples were realized, e.g. due to discontinuation of therapy or problems with blood drawing. Imipenem TDM included peak (approximately 15-30 minutes after end of infusion), intermediate (midway between two sequential administrations, approximately 30 minutes) and trough (approximately 15 minutes before the next dose) concentrations. Creatinine was monitored daily.

Imipenem blood samples were drawn and immediately placed on ice and transported to the laboratory for centrifugation. MOPS [3-(N-morpholino)propanesulfonic acid], a stabilizing buffer that protects imipenem from degradation [27], was added to an equivalent volume of plasma. Stabilized imipenem samples were subsequently stored at −80 °C for maximally 1 month.

Imipenem plasma concentrations were analysed by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 298 nm. Ceftazidime was used as an internal standard in the HPLC-UV analysis. Acetonitrile was added to the stabilized plasma for deproteinisation. The calibration curve was linear from 0.5 to 80 mg/L. Limit of detection (LOD) and limit of quantification (LOQ) were 0.2 mg/L and 0.5 mg/L, respectively [24].

Parametric population PK analysis (NONMEM)

Parametric population PK analyses were performed using nonlinear mixed effects modelling (NONMEM version 7.2, ICON Development Solutions, Ellicott City, MD, USA). The Intel Visual Fortran Compiler XE 14.0 (Santa Clara, CA, USA) was used. The first-order conditional estimation method with interaction (FOCE-I) was used throughout the model building process. Tools used to evaluate and visualize the model were RStudio (version 1.1.456), R (version 3.5.1), XPose (version 4.6.1) and PsN (version 4.6.0), all with the graphical interface Pirana [28] (version 2.9.4).

General model selection criteria were decrease in objective function value (ΔOFV), GOF plots and VPCs. A decrease in the OFV of 3.84 units was considered statistically significant ($p<0.05$, $df=1$) in a nested model [29]. For each VPC, a set of 1000 simulated datasets was created to compare the observed concentrations with the distribution of the simulated concentrations. A numerical predictive check (NPC) of the final model was created to compare with the NPC of the final nonparametric model.

During modelling, only lower bounds (of 0) and no upper bounds were set for each parameter [29]. One-, two- and three-compartment distribution models were evaluated [30]. Databases with untransformed and logarithmic transformed

concentrations were compared by assessing GOF plots and parameter estimates. For both databases, residual unexplained variability was tested with proportional and combined (additive and proportional) error models [31]. The proportional (exponential) error model of the final NONMEM model with log-transformed data is shown in equation 1. The observed concentration (OBS) consists of the individually predicted concentration IPRED with added residual unexplained variability ε (epsilon, fixed to 1 in our model) weighted by an estimated error parameter.

$$OBS = IPRED + \sqrt{(error^2)} * \varepsilon \quad (1)$$

Variability of a population PK parameter was estimated using an exponential variance model (individual popPK parameter = population popPK value * e^{η}). Eta (η) is a random variable drawn from a normal distribution with a mean of 0 and a variance of omega (ω^2) [32]. The BSV (CV%) of a population parameter is calculated by equation 2 [33]. The SD is subsequently calculated by multiplying the CV% with the population parameter estimate.

$$CV\% (e^{\eta}) = \sqrt{(e^{\omega 2} - 1)} * 100\% \quad (2)$$

First, models with BSV on K_e and BSV on V were compared by assessing ΔOFV and GOF plots. Subsequently, one-by-one addition of BSV on the other parameters was studied. A stepwise covariate model building was performed with forward addition at $p<0.05$ (ΔOFV of 3.84 units, df=1) followed by backward elimination at $p<0.001$ (ΔOFV of 10.83 units, df=1) [34]. Covariates were tested on parameters with BSV. The tested covariates are described in the Methods section "Covariates".
The 95% CI of each parameter in the final model was determined from a nonparametric bootstrap analysis, in which the dataset was resampled 1000 times.

Nonparametric population PK analysis (Pmetrics)

Nonparametric population PK analysis was performed using Pmetrics version 1.5.2 (Laboratory of Applied Pharmacokinetics and Bioinformatics, Los Angeles, CA, USA) [6] in RStudio (version 1.1.456) as a wrapper for R (version 3.5.1). The Intel Visual Fortran Compiler XE 14.0 (Santa Clara, CA, USA) was used. The Non-Parametric Adaptive Grid (NPAG) program was used throughout the model building process. The Iterative 2-stage Bayesian (IT2B) program was used to estimate parameter ranges to pass to NPAG. An NPAG will create a nonparametric popPK model consisting of discrete support points, each with a set of estimates for all parameters in the model plus an associated probability of that set of estimates (see **Figure 1** for an illustration of the distribution of a parameter) [6]. The sum of all probabilities is 1. There can be a maximum of 1 point for each subject in the study population [6]. Besides an overview of support points with corresponding parameter estimates, the NPAG output contains also the mean, SD and CV% of each parameter. The reported means are weighted means which are calculated by multiplying the estimate of each support point by the probability of that point and then summing up the resulting numbers. The SD is calculated from the parameter distribution. The BSV (CV%) of each parameter estimate is calculated by

dividing the SD by the weighted mean.
One-, two- and three-compartment distribution models were evaluated. A stepwise covariate model building was performed with forward addition at $p<0.05$ (decrease in the -2 times the log-likelihood, Δ-2LL, of 3.84 units, df=1) followed by backward elimination at $p<0.001$ (Δ-2LL of 10.83 units, df=1) [34]. Covariates were tested on parameters selected after a graphical examination of possible covariate-parameter relationships. The tested covariates are described in the Methods section "Covariates". Each observation in Pmetrics is weighted by $1/error^2$. Both gamma and lambda error models were tested (see equation 3 and 4). The SD of an observation is based on the assay error polynomial (see equation 5) [6]. However, because the assay error polynomial was unavailable in our study, we estimated the error coefficients with $C_0 = 0.5 * LOQ$, $C_1 = 0.1$, $C_2=0$ and $C_3=0$ as a starting point.

$$error = SD * gamma \quad (3)$$

$$error = \sqrt{(SD^2 + lambda^2)} \quad (4)$$

$$SD = C_0 + C_1 * OBS + C_2 * OBS^2 + C_3 * OBS^3 \quad (5)$$

Model selection criteria were decrease in -2LL, bias, imprecision, GOF plots and VPCs. A decrease in the -2LL of 3.84 units was considered statistically significant ($p<0.05$, df=1) in a nested model. For each VPC, a set of 1000 simulated datasets was created to compare the observed concentrations with the distribution of the simulated concentrations. An NPC of the final model was created to compare with the NPC of the final parametric model. The raw VPC and NPC data were imported into PsN (version 4.6.0) using the Pirana interface [28] to generate plots with a similar layout as the parametric plots. VPC and NPC plots were created using XPose (version 4.6.1) within RStudio (version 1.1.456). Bias (mean weighted prediction error) and imprecision (bias-adjusted mean weighted squared prediction error) are automatically calculated by Pmetrics according to equations 6 and 7, both population and posterior predictions.

$$Bias = \frac{\sum \left((PRED_1 - OBS_1)/(error^2)_1 + \ldots + (PRED_n - OBS_n)/(error^2)_n \right)}{n} \quad (6)$$

$$Imprecision = \frac{\sum \left(\frac{(PRED_1 - OBS_1)^2}{((error^2)_1)^2} + \ldots + \frac{(PRED_n - OBS_n)^2}{((error^2)_n)^2} \right)}{n} - bias^2 \quad (7)$$

The 95% CI of each parameter in the final model was determined using a Monte Carlo simulation approach by creating 1000 samples with replacement for each support point [35], resulting in the 2.5th, 50th and 97.5th percentiles of the weighed median and the median absolute weighted deviation (MAWD). The SD was estimated by multiplying MAWD by 1.4826 [36]. The CV% was calculated by dividing the SD by the 50th percentile of the weighted median.

Covariates

The tested covariates for both modelling approaches were: total body weight (TBW), ideal body weight (IBW) [37], lean body weight (LBW) [37], Cockcroft-Gault (CG)

eGFR [25], 4-variable Modification of Diet in Renal Disease (MDRD) Study eGFR [38], Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) eGFR [39], and Jelliffe's eGFR equation for patients with unstable renal function [40]. Per patient, one body weight measure was available, while a median of 3 creatinine samples per patient was drawn (see also **Table 1**). The MDRD, CKD-EPI and Jelliffe's equations provide eGFR values adjusted for body surface area, BSA, (ml/min/1.73 m^2). The BSA-unadjusted (absolute) values (ml/min) were also calculated by multiplying the original eGFR by the individual BSA [41] and evaluated as covariates: MDRD-abs, CKD-EPI-abs and Jelliffe-abs. The CG eGFR equation is unadjusted for BSA (ml/min) and no adjustment was made.

All covariates were evaluated by power models with normalized covariates where the median covariate value was taken as the reference value (see equation 8). Because multiple creatinine samples per patient were collected, which are each used to calculate CG, MDRD and CKD-EPI eGFR values, eGFR was tested as a time-varying covariate. We also tested the possible situation of reaching a maximum value of the elimination constant K_e for high (adjusted or unadjusted) eGFR values from 150, 120 and 90. For total, lean and ideal body weight, both fixed (-0.25 for K_e and 1 for V) and estimated values of the power exponent were evaluated [42].

$$Par_{ind} = Par * \left(\frac{Cov_{ind}}{Cov_{median}} \right)^{power} * e^{n} \quad (8)$$

Par_{ind}: individual PK parameter estimate, Par: population PK parameter estimate (for NONMEM) or weighted median value of the Bayesian posterior distribution (for Pmetrics), Cov_{ind}: individual covariate value, Cov_{median}: median covariate value, power: covariate effect, e^{η}: individual variability (e^{η} only for NONMEM). See equation 9 and 10 in the Results section for further clarification of the differences between equations in the final parametric and nonparametric models.

Default covariate settings were used for each modelling approach. In NONMEM, by default, the next observation is carried backward (NOCB) until the time point of the previous covariate observation. For Pmetrics, covariates are applied at each dose event. By default, for missing covariate values, the last observation is carried forward (LOCF) until the last dose before the next covariate observation, when the last observation is linearly interpolated to the next observation.

Model development and comparison

Both models were developed by a medium-experienced NONMEM and Pmetrics modeler (FdV) supervised by highly experienced NONMEM (BdW) and Pmetrics (WY, MN) modelers. The development of both models occurred independently from each other according to a predefined study procedure, where the modelling workflow (e.g. model selection criteria, building of the structural model and covariate evaluation) was described. During model development, the GOF plots and VPCs were assessed in the layout of the separate programmes. During writing of the manuscript, the raw data of the GOF plots were transferred to GraphPad Prism (version 8.1.1) and the raw VPC and NPC data of Pmetrics were transferred to PsN (see Methods

section about Pmetrics) to create plots with the same layout. The R^2 of nonlinear regression was calculated within GraphPad Prism by the 4th equation of Willett and Singer [43] for the GOF plots as a description of the graphical fit. R^2 was not used during model selection.

Results

Study population

Demographic and clinical characteristics of the 26 included patients are summarized in **Table 1**. None of the patients received continuous renal replacement therapy (CRRT).

Table 1. Demographic and clinical characteristics of study population (n=26).

Parameter	Value
Male, n (%)	18 (69)
APACHE II score, median (IQR)	22 (17-27)
Age (years), median (IQR)	51 (39-54)
Creatinine at inclusion (µmol/L), median (IQR)	59 (46-70)
Creatinine samples per patient, median (IQR)	3.0 (2.0-4.0)
Creatinine samples per patient per day, median (IQR)	1.5 (1.2-1.8)
eGFR at inclusion	
CG (ml/min), median (IQR)	146 (123-170)
CKD-EPI (ml/min/1.73 m^2), median (IQR)	116 (104-124)
CKD-EPI-abs (ml/min), median (IQR)	119 (110-139)
MDRD (ml/min/1.73 m^2), median (IQR)	121 (104-159)
MDRD-abs (ml/min), median (IQR)	127 (118-162)
Jelliffe (ml/min/1.73 m^2), median (IQR)	156 (132-183)
Jelliffe-abs (ml/min), median (IQR)	168 (141-202)
Height (cm), median (IQR)	175 (168-179)
Total bodyweight (kg), median (IQR)	75 (66-85)
Ideal bodyweight (kg), median (IQR)	70 (59-73)
Lean bodyweight (kg), median (IQR)	58 (46-64)
BMI (kg/m^2), median (IQR)	25 (22-27)
BSA (m^2), median (IQR)	1.89 (1.72-2.04)
Presumed infection, n (%)	
Lower respiratory tract infection	16 (62)
Intra-abdominal infection	4 (15)
Bloodstream infection	3 (12)
Surgical site infection	1 (4)
Meningitis	1 (4)
Gynecological infection	1 (4)

APACHE, Acute Physiology and Chronic Health Evaluation; IQR, Interquartile Range, eGFR, estimated Glomerular Filtration Range; CG, Cockcroft-Gault; CKD-EPI, Chronic Kidney

Disease Epidemiology Collaboration; CKD-EPI-abs, absolute CKD-EPI (= CKD-EPI multiplied by BSA); MDRD, 4-variable Modification of Diet in Renal Disease; MDRD-abs, absolute MDRD (= MDRD multiplied by BSA); Jelliffe-abs, absolute Jelliffe (= Jelliffe multiplied by BSA); BMI, Body Mass Index; BSA, Body Surface Area.

Imipenem samples

In total, 138 imipenem blood samples were collected from 26 patients and were subsequently analyzed. Fewer than 10% [30] of all concentrations (13/138 = 9.4%) were below the limit of quantification (0.5 mg/L) and were excluded from the popPK analysis. The average number of levels per patient was 5 (range, 1-11). Almost half of all samples (65/138 = 47.1%) were drawn on the second day of therapy.

A graph of the analyzed imipenem concentrations (n=125) plotted against the time after dose is shown in **Supplementary Figure 1.** These 125 concentrations were drawn after 84 individual doses. Following 33 of these doses two or three concentrations were taken and after the other 51 doses one concentration was drawn. For all PK samples, a creatinine concentration was available in the 24h before PK sampling.

Parametric population pharmacokinetic model

The parametric popPK analysis using NONMEM showed that the data were best described by a model with 2 distribution compartments, BSV on K_e and CKD-EPI-abs as a covariate on K_e. The parameter estimates of the final model are displayed in **Table 2**. Only BSV on K_e was included (see also **Figure 1**), because BSV on central distribution volume (V_c), rate constant from central to peripheral compartment (K_{cp}) and rate constant from peripheral to central compartment (K_{pc}) did not significantly improve the model (ΔOFV <3.84 and no improvement of GOF plots). V_c, K_{cp} and K_{pc} are the same for each subject (no BSV was included and therefore no CV% is shown in **Table 2**). Eta-shrinkage was low (14%). No large correlation (>0.95) between the parameters was detected [29]. The GOF and VPC plots, displayed in **Figure 2a**, **Figure 3a** and **Supplementary Figure 2a**, show a good predictive performance for all concentrations, for the time range after dose and for the CKD-EPI-abs range of 18-190 ml/min (except from an outlier for the 124-141 ml/min bin). The NPC did not show points outside boundaries (data not shown). As shown in **Table 2**, the model-based parameter estimates were similar to the bootstrap values, indicating stability of the model.

A two-compartment model described the data better than one- or three-compartment models, according to GOF-plots and OFV increase of 22.99 for a one-compartment model and 0 for a three-compartment model. The popPK parameters of a two-compartment model with logarithmic transformed data were comparable to the same model with untransformed data. The GOF plots were improved by logarithmic transformation and therefore the following analyses were performed with transformed data. An exponential (proportional) error model (see equation 1) was preferred to a combined error model due to estimation problems with the combined model. This confirmed the findings of the untransformed data, where the proportional error model had a better performance than the combined error model.

Several covariates (as described in Methods section "Covariates") were tested on K_e, the only parameter with BSV. All tested eGFR covariates on K_e resulted in a significant OFV decrease (ΔOFV 24.4 until 38.3, $p<0.05$) compared to the two-compartment model without covariates. However, the OFV of the model with CKD-EPI-abs as a covariate on K_e was significantly better than the other eGFR models (for example, the second best eGFR was CKD-EPI on K_e with an OFV increase of 4.3 compared with CKD-EPI-abs, $p<0.05$). Due to the observation of a maximum of eta in the eta-eGFR plots, implementation of a maximum K_e value for eGFR values from 150, 120 and 90 was tested, but this did not further improve the model. None of the tested measures of body weight improved the model as a covariate on K_e (ΔOFV<3.84) during the univariate analysis.

Equation 9 describes the calculation of individual Ke (Ke_i) values in the final NONMEM model, using the population parameter estimates for K_e and K_e(cov) from **Table 2**, the individual CKD-EPI-abs value at a certain time point and e^{η} (individual variability). Eta (η) is drawn from a normal distribution with a mean of 0 (note equation 9: then $e^0 = 1$) and variance ω^2 (estimated from the data as 0.0354). The corresponding BSV for K_e (CV% 19.0%) was calculated from equation 2.

$$Ke_i = 0.637 * \left(\frac{CKD - EPI - abs_i}{119} \right)^{0.655} * e^{\eta} \quad (9)$$

A plot of the individual Ke against the individual CKD-EPI-abs (n=86) is shown in **Supplementary Figure 3a.** Simulated concentration-time profiles for CKD-EPI-abs of 150, 120 and 90 ml/min (n=1000 for each eGFR) are shown in **Supplementary Figure 4a.**

The median and interquartile range of the untransformed residuals (observed minus predicted concentrations) was -0.159 (-0.960 - 1.267) mg/L for the population predictions and -0.027 (-0.649 – 0.698) mg/L for the individual predictions. Residual plots are shown in **Supplementary Figure 5a**.

Nonparametric population pharmacokinetic model

The nonparametric popPK analysis using Pmetrics resulted in the same model structure as the parametric analysis: a model with 2 distribution compartments and CKD-EPI-abs as a covariate on the elimination constant K_e. The mean parameter estimates of the final model are displayed in **Table 2**. For example, the mean Ke_{pop} value is the mean of the support points weighted by population probabilities. This is illustrated in **Figure 1**. Each individual has a Bayesian posterior (i.e. personal or individual) set of support points weighted by individual probabilities [6]. No large correlation (>0.95) between the parameters was detected. The GOF and VPC plots, displayed in **Figure 2b, Figure 3b** and **Supplementary Figure 2b**, show a good predictive performance for all concentrations, for the time range after dose and for the CKD-EPI-abs range of 18-190 ml/min (except from an outlier for the 124-141 ml/min bin, similar to the parametric model). Similar to the parametric model, the NPC did not show points outside boundaries (data not shown). As shown in **Table 2**, the model-based parameter estimates were similar to the bootstrap values, indicating stability of the model.

A two-compartment model described the data better than one- or three-compartment models, according to GOF-plots and -2LL increase of 26.5 for a one-compartment model and 0.8 for a three-compartment model. The gamma error model was preferred to the lambda model (see equations 3 and 4). The final values for C_0 and C_1 in the assay error polynomial were both 0.05 and C_2 and C_3 were both 0 (see equation 5). Parameter boundaries (K_e 0-1.5, V 1-70, K_{cp} and K_{pc} 0-1) were set based on an IT2B run.

We evaluated several covariates (as described in Methods section "Covariates") on K_e and V. All tested eGFR covariates on K_e resulted in a significant -2LL decrease (Δ-2LL 53.3 until 59.9, $p<0.05$) compared to the two-compartment model without covariates. The -2LL value of the 4 models with the largest -2LL decrease (MDRD, MDRD-abs, Jelliffe-abs and CKD-EPI-abs) did not differ significantly from each other. The model with CKD-EPI-abs had the lowest bias and imprecision compared to the 3 other models. Implementation of a maximum K_e value for eGFR values from 150, 120 or 90 did not improve the model. The univariate analysis resulted in a further six significant covariates ($p<0.05$): TBW, IBW and LBW on K_e (Δ-2LL 4.0 until 8.8) and CG, Jelliffe and Jelliffe-abs on V (Δ-2LL 4.6 until 5.5). After backward elimination at $p<0.001$, none of these six covariates remained in the final model.

Equation 10 describes the calculation of individual K_e values (Ke_i) in the final Pmetrics model. Contrary to the parametric model with equal K_e and K_e(cov) values for each individual subject (see equation 9), these values are different for each individual subject in the nonparametric model. $Ke_{i,med}$ is the weighted median value of the Bayesian posterior distribution for K_e in the i^{th} individual, $K_e(cov)_{i,med}$ is the weighted median value of the Bayesian posterior distribution for K_e(cov) in the i^{th} individual and CKD-EPI-abs_i is the individual CKD-EPI-abs value at a certain time point.

$$Ke_i = Ke_{i,med} * \left(\frac{CKD\text{ - }EPI\text{ - }abs_i}{119} \right)^{Ke(cov)i,med} \quad (10)$$

A plot of the individual Ke against the individual CKD-EPI-abs (n=86) is shown in **Supplementary Figure 3b.** This plot shows a similar Ke_i versus CKD-EPI-abs relationship as the parametric model, although the Ke_i distribution is wider for the nonparametric model. A wider distribution is also shown in the simulated concentration-time profiles for CKD-EPI-abs of 150, 120 and 90 ml/min (**Supplementary Figure 4b**).

The median and interquartile range of the residuals (observed minus predicted concentrations) was -0.045 (-0.498 – 1.506) mg/L for the population predictions and 0.011 (-0.295 – 0.533) mg/L for the individual predictions. Residual plots are shown in **Supplementary Figure 5b**. These are similar to the parametric ones.

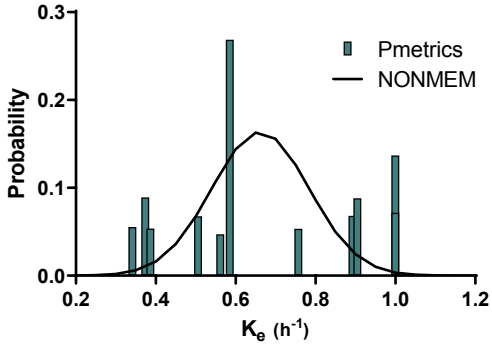

Figure 1. Distribution of K_e in the NONMEM and Pmetrics popPK models. NONMEM: normal distribution (mean 0.637 h^{-1} and SD 0.121 h^{-1} (CV 19.0%)). Pmetrics: marginal distribution of 16 support points with 11 unique values for K_e (weighted mean 0.681 h^{-1} and SD 0.232 h^{-1} (CV 34.0%))

Table 2. Population parameter estimates.

	NONMEM					
	Final model		**Bootstrap**			
Parameter	**Parameter estimate**	**CV (%)**	**Median parameter estimate**	**95%CI parameter estimate**	**Median CV (%)**	**95%CI CV (%)**
V_c (L)	29.6	-	29.4	22.9 – 34.4	-	-
K_{cp} (h^{-1})	0.166	-	0.169	0.092 – 0.436	-	-
K_{pc} (h^{-1})	0.195	-	0.192	0.079 – 0.604	-	-
K_e (h^{-1})	0.637	19.0	0.634	0.543 – 0.805	18.6	10.5 – 27.4
K_e(cov)	0.655	-	0.665	0.474 – 1.184	-	-
Exponential error (mg/L)	0.348	-	0.340	0.281 – 0.413	-	-
	Pmetrics					
	Final model		**Bootstrap**			
Parameter	**Mean parameter estimate**	**CV (%)**	**Median parameter estimate**	**95%CI parameter estimate**	**Median CV (%)**	**95%CI CV (%)**
V_c (L)	31.1	42.6	35.1	20.1 – 38.3	36.3	3.8 - 62.8
K_{cp} (h^{-1})	0.374	81.2	0.347	0.122 – 0.563	75.2	6.5 - 169.1
K_{pc} (h^{-1})	0.495	72.0	0.387	0.278 – 0.846	90.1	6.2 - 157.6
K_e (h^{-1})	0.681	34.0	0.586	0.533 – 0.905	47.0	3.1 - 66.1
K_e(cov)	0.658	55.2	0.791	0.516 – 1.000	38.3	0.0 - 82.6
Gamma (error model)	3.40	-	-	-	-	-

V_c: central distribution volume, K_{cp}: rate constant from central to peripheral compartment, K_{pc}: rate constant from peripheral to central compartment, K_e: elimination rate constant, $K_e$cov: covariate effect on K_e, CV: coefficient of variation, CI: confidence interval.

Figure 2. Goodness-of-fit plots with the observed against the predicted concentrations of both models. Solid line: identity (1:1) line. Dotted line: regression line.

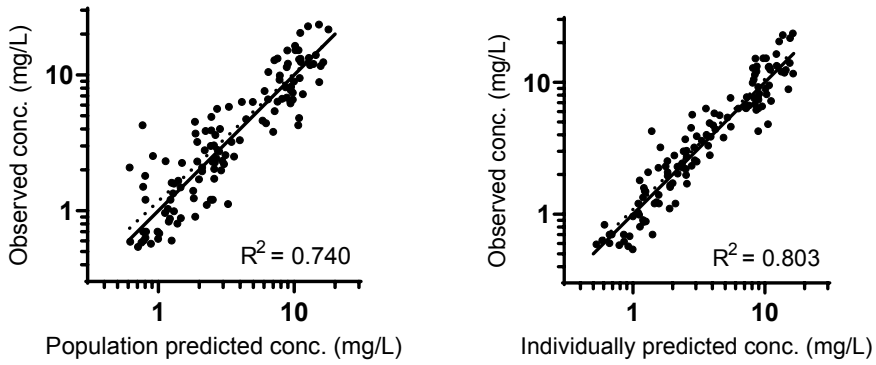

Figure 2a. Goodness-of-fit plots of the final parametric model. The log-transformed concentrations are back transformed for an easier comparison with the untransformed concentrations in **Figure 2b**

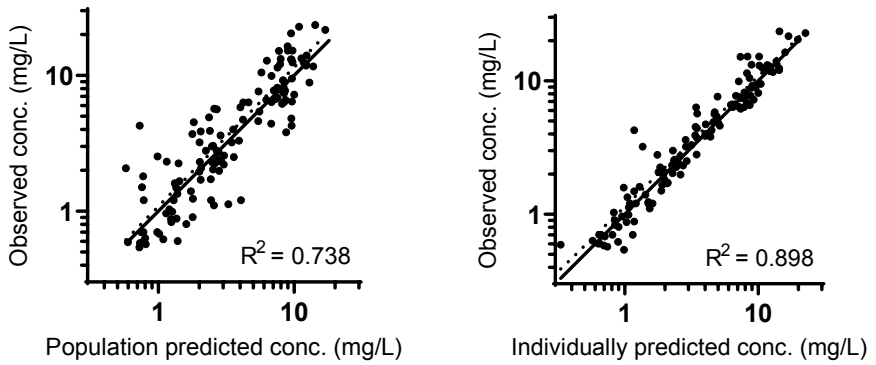

Figure 2b. Goodness-of-fit plots of the final nonparametric model

Figure 3. Visual Predictive Checks (VPCs) of both models
Circles: observed concentrations. Upper, middle and lower lines: 95th, 50th and 5th percentile of observations. Shaded areas: 95%CI of the corresponding percentiles of predictions.

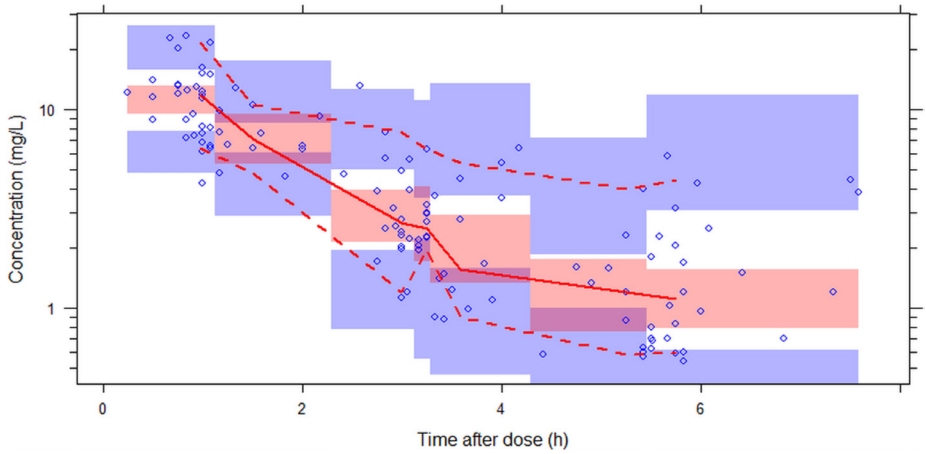

Figure 3a. VPC of the final parametric model. The log-transformed concentrations are back transformed for an easier comparison with the untransformed concentrations in **Figure 3b**

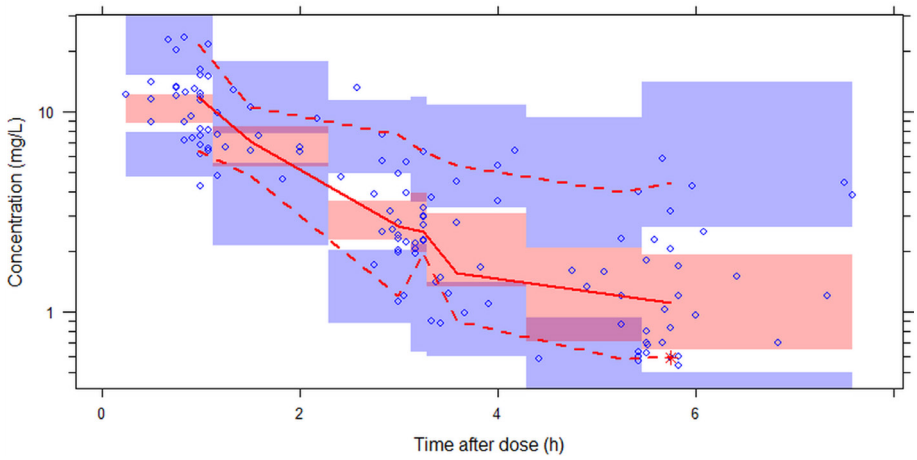

Figure 3b. VPC of the final nonparametric model

Discussion

The structure and parameter estimates of our two independently developed parametric and nonparametric popPK models of imipenem in critically ill patients treated with imipenem/cilastatin were similar: both included 2 distribution compartments and CKD-EPI-abs as a covariate on K_e. Body weight as a covariate

was not found to be a significant covariate in either model. Both models described imipenem PK well. Two main differences between the models emerged. First, the parametric model included between-subject variability (BSV) for K_e only, while the nonparametric model included such variability on all popPK parameters. Second, the estimated BSV (defined as CV%) for K_e was higher for the nonparametric model (34.0% vs 19.0%). The findings of similar parameter estimates but higher BSV for the nonparametric model are in line with two previously published studies comparing parametric and nonparametric models of other drugs [12, 13]. These BSV differences could be explained by the statistics behind the modelling methods. Both models have fixed (no BSV) and random (including BSV) parameters. However, while during parametric modelling the inclusion of BSV is examined for each popPK parameter, all popPK parameters for nonparametric models are in principle random parameters while the residual error model is fixed. In addition, while parametric methods assume a normal or lognormal distribution of parameters, nonparametric methods make no assumption about the parameter distributions, which can cause a wider CI of the parameter estimates (e.g. see **Figure 1** for K_e, which also clearly shows the differences between the two CV% measures, and **Supplementary Figure 3**). The simulated concentration-time profiles in **Supplementary Figure 4** indicate that the concentrations of the 2.5th percentile are approximately 2-fold lower for the nonparametric model. The consequences of this finding (e.g. higher or more frequent dosing following the nonparametric model) should be further explored by probability of target attainment calculations based on extensive Monte Carlo simulations for several dosing regimens [44].

Our finding of 2 distribution compartments is in accordance with other published popPK models of imipenem in critically ill patients (all with pneumoniae) [45-47]. However, our V_c and clearance ($CL = V_c * K_e$) values were higher than previously described [45-47]. This could be explained by a higher creatinine clearance in our population, which could be attributed to augmented renal clearance (ARC) [48, 49]. ARC is defined as increased renal elimination of circulating solutes and drugs as compared with normal baseline [23] and has been reported in approximately 30-65% of critically ill patients [48]. Our K_{cp} (0.2-0.4) and K_{pc} (0.2-0.5) values of both models were similar to the previously published parametric model [45], but remarkably different from the two nonparametric models (K_{cp} 3-8 and K_{pc} approximately 9) [46, 47]. Possibly, these differences could be explained by (unpublished) wider parameter boundaries of their nonparametric models. The parameter ranges in nonparametric software are strict boundaries wherein the optimal values are sought. To assist with optimal setting of parameter ranges in nonparametric popPK, we used the parametric IT2B module in Pmetrics to estimate the parameter ranges to pass to the nonparametric NPAG module. During parametric modelling with NONMEM, the parameter initial estimate is a starting point to search for the optimal value and setting limits is not always necessary [29]. This was underlined by the fact that the results of our final NONMEM model with only lower boundaries were the same as a model with the same upper and lower boundaries as the final Pmetrics model.

Many types of standard goodness-of-fit plots used for model evaluation are applied both in parametric and nonparametric modelling: observed versus population

predicted concentrations, observed versus individually predicted concentrations and weighted residual (WRES) plots. The observed versus predicted concentration plots of our popPK models are comparable. In Pmetrics, the standard layout of these plots includes the R^2, intercept, slope, bias (mean weighted residual) and imprecision. In NONMEM, these measures are not automatically calculated. A residual is the difference between an observed and a predicted concentration. Weighted residuals are used in WRES plots as well as for the calculation of bias and imprecision. We did not show WRES plots nor calculated weighted bias and imprecision for both methods because of two reasons. First, the weighting differs between the two modelling methods. In NONMEM, the Conditional WRES (CWRES) is the residual weighted by the square root of the covariance of a FOCE model [50], while in Pmetrics WRES is the residual weighted by the squared error [35]. Second, a weighted residual of logarithmic transformed data (in our NONMEM model) is not the same as a weighted residual of untransformed data (in our Pmetrics model). We used weighted bias and imprecision in Pmetrics only to differentiate between 4 covariate models with a -2LL decrease which did not differ significantly from each other. As an alternative to weighted bias, we calculated unweighted bias (the unweighted residuals of untransformed concentrations), of which the median and interquartile range were comparable for both methods.

The VPCs of both popPK models indicate a sufficient predictive performance. The means of the 95th, 50th and 5th percentiles of predictions are comparable for both models, but the 95%CI of these percentiles differ for some bins (timeframes) of the VPCs. Most remarkably, the 95%CI of the 95th and 50th percentile of the last 2 bins (4.3-5.8 h after dose) is wider for the nonparametric model. This could be explained by a higher estimated BSV of K_e in the nonparametric model, leading to a wider distribution of concentrations in the elimination phase. We did not show prediction-corrected VPCs (pcVPCs) because this option has been developed for parametric methods [51] and has not yet been tested for nonparametric methods. In a pcVPC, the variability coming from binning across the independent variable, e.g. due to different doses or influential covariates, is removed [51]. The pcVPCs of the NONMEM model did not show important differences from the traditional VPCs (data not shown).

Both models include CKD-EPI-abs as a covariate on K_e. Two of the three previous mentioned published popPK models of imipenem also included renal function as a covariate, but other measures were used: 4h creatinine clearance (4h-CL_{cr}) in urine [45] and the Cockcroft-Gault equation [47]. Sakka et al. did not find 12h-CL_{cr} in urine as a significant covariate [46]. Besides eGFR, also other covariates were included in the published models: body weight [45-47], height [46, 47], BSA [46, 47], age [46, 47], sex [47] and albumin [45]. In our covariate screening plots, sex, age and height did not show a relationship with any PK parameters. Body weight did show a relationship in the covariate plots, but inclusion as a covariate did not improve the model. Albumin data were not available. BSA was taken into account during eGFR covariate testing. We tested both BSA-unadjusted (ml/min) and BSA-adjusted (ml/min/1.73 m^2) eGFR equations, because the European Medicines Agency [52] and the Kidney Disease Improving Global Outcomes (KDIGO) guideline [53] recommends to base dosing on

absolute instead of BSA-normalized eGFR. The KDIGO [53] recommends using the CKD-EPI eGFR equation. This guideline, however, is based on chronic kidney disease, while our study population consisted of critically ill patients with a high median CKD-EPI eGFR of 116 ml/min/1.73 m^2. The correlation between measured CL_{cr} and eGFR equations is known to be weak in critically ill patients [23, 54]. Nonetheless, the measurement of CL_{cr} (as a surrogate for GFR) is time consuming and not standard practice in many ICUs. In daily practice eGFR is also used for dosing drugs with renal clearance, although many patients have a renal function that is not in steady state. Therefore, we decided to test several eGFR equations to find the most suitable one in our population. Minichmayr et al. performed a similar eGFR covariate analysis for meropenem in critically ill patients. They found that the Cockcroft-Gault equation best described meropenem clearance [55]. We observed a maximum in the BSV-eGFR plots. However, implementation of a maximum K_e value for eGFR values from 150, 120 and 90 did not further improve the model. As already mentioned, the correlation between measured CL_{cr} and eGFR equations is weak, but it is also shown that this correlation varies over the eGFR range [23]. For example, in a previous study was shown that the CKD-EPI equation performed better for measured CL_{cr} <120 ml/min than for CL_{cr} > 120 ml/min [23]. We did not confirm this finding in our study (see **Supplementary Figure 2**). This could be explained by a different study population. For both modelling approaches, eGFR was implemented as a time-varying covariate using a stepwise (discontinuous) approach. The default covariate settings of both methods were slightly different (i.e. NOCB without interpolation or LOCF with linear interpolation from the last dose before the next covariate value). However, the parameter estimates of both final models, developed with the default settings, were very similar to the same models using LOCF without interpolation. This is explained by the majority (85%) of the patients having reasonably stable CKD-EPI-abs eGFR values around PK sampling, according to the KDIGO definition of an eGFR drop or rise of less than 25% to the previous value [53]. This stability statement is supported by frequent creatinine monitoring. A creatinine concentration in the 24h before TDM was available for all PK samples. Due to the stable eGFR values, a continuous covariate approach was not necessary.

We performed our study using the most used parametric (NONMEM) and nonparametric (Pmetrics) software. For approximately a decade, a nonparametric method also exists in NONMEM and some publications [19, 56-58] regarding the evaluation and optimization of this approach are available. However, this method is seldom used in clinical practice. The parametric module IT2B in Pmetrics is used to estimate parameter ranges to pass to NPAG, but underperformed compared with other parametric algorithms [12].

One of the limitations of our study is that we used eGFR equations for a critically ill population with a high frequency of augmented clearance, while these equations are developed for more stable patients with chronic kidney disease. Nonetheless, as measured CL_{cr} was unavailable and is also not standard practice in many ICUs, we aimed to find the eGFR equation that would best describe imipenem clearance. Another drawback is that we could not compare weighted residuals, because the weighting is different for both methods and the residuals of untransformed data

(used for the nonparametric model) are different from transformed data (used for the parametric model). Other limitations are the small number of subjects and the absence of an external validation. It would be interesting to compare the predictions of both models.

Conclusions

The general structure and the parameter estimates of both models were comparable. The identical covariate results (CKD-EPI-abs on K_e) of the two different modelling methods strongly support the findings in this population. The nonparametric model included BSV for all parameters while the parametric model only included BSV on K_e, and the estimated BSV of K_e was higher in the nonparametric model. The consequences of the BSV differences may affect estimated exposure during dosing simulations, and this should be further investigated in simulation studies.

Funding

This work was supported by the Innovative Medicines Initiative Joint Undertaking under grant agreement no. [115523], resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies' in-kind contribution. The research leading to these results was conducted as part of the COMBACTE-NET consortium. For further information please refer to http://www.combacte.com/.

The initial cohort study was funded by a Research and Development Grant awarded by the Geneva University Hospitals in 2009 [PRD 09-II-025]. AH and EvD were partially supported by the EU-funded project AIDA [grant Health-F3-2011-278348].

Ethical approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee (Geneva University Hospital's Ethics Committee, NAC 09-117) and with the 1964 Helsinki declaration and its later amendments.

Informed consent

Informed consent was obtained from individual participants included in the study, unless from patients who were unconscious or otherwise unable to understand the study protocol. This was approved by the above mentioned ethics committee, given the observational nature of the study.

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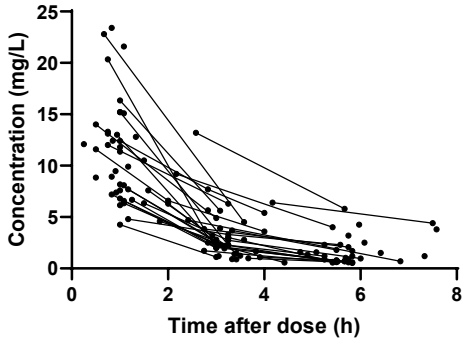
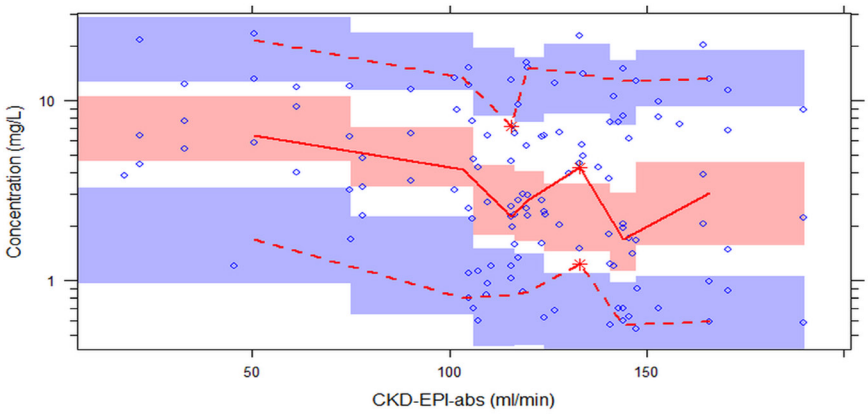
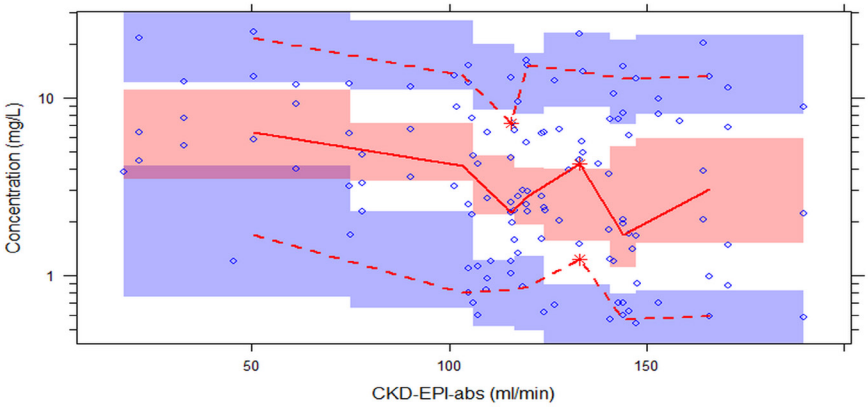


Supplementary Figure 1. Observed imipenem concentrations (n=125) plotted against the time after dose.

Supplementary Figure 2. Visual Predictive Checks (VPCs) of both models with CKD-EPI-abs as the independent variable.
Circles: observed concentrations. Upper, middle and lower lines: 95th, 50th and 5th percentile of observations. Shaded areas: 95%CI of the corresponding percentiles of predictions.

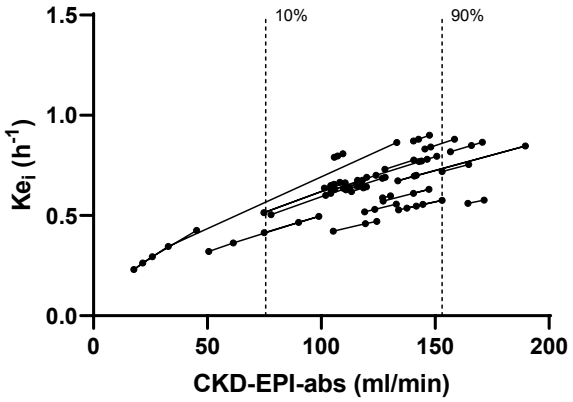


Supplementary Figure 2a. VPC of the final parametric model. The log-transformed concentrations are back transformed for an easier comparison with the untransformed concentrations in **Supplementary Figure 2b.**

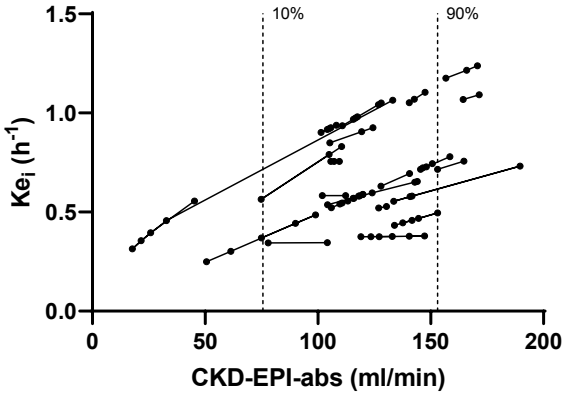


Supplementary Figure 2b. VPC of the final nonparametric model.

Supplementary Figure 3. Individual K_e values plotted against CKD-EPI-abs (n=86) for the final parametric (a) and nonparametric models (b). The values of each individual subject (n=26) are connected with a solid line. The dotted lines indicate the 10th and 90th percentile of the CKD-EPI-abs values.

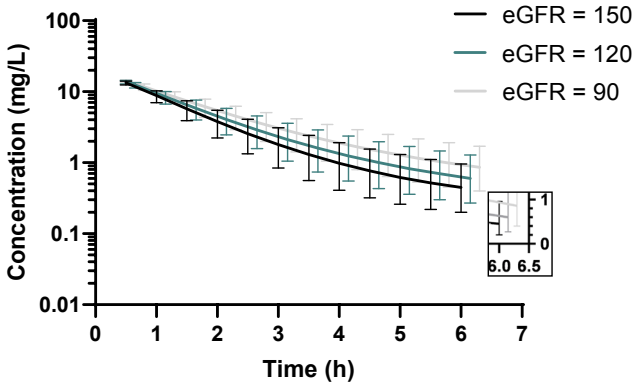


Supplementary Figure 3a

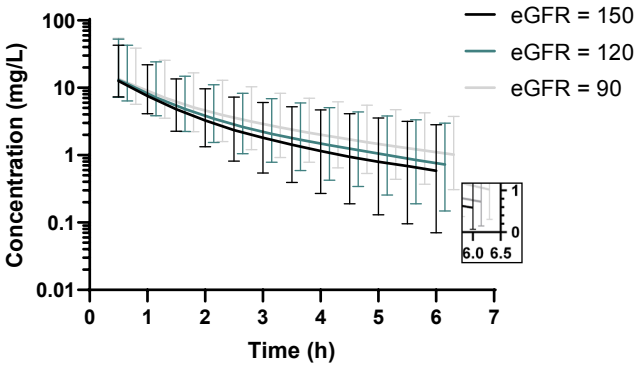


Supplementary Figure 3b

Supplementary Figure 4. Simulated concentration-time profiles for eGFR (CKD-EPI-abs) of 150, 120 and 90 ml/min (n=1000 for each eGFR), displayed as the median, 97th and 2.5th percentile, for the final parametric (a) and nonparametric models (b). The inserts show the simulations at 6h on a linear scale.

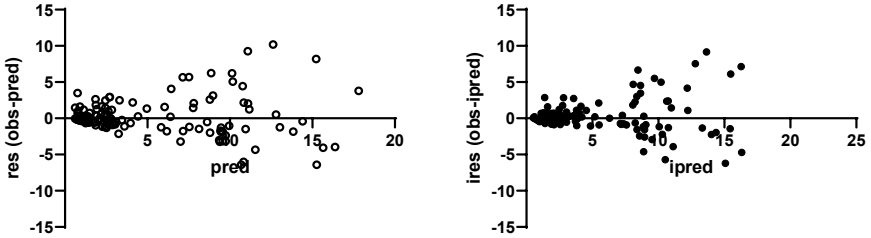


Supplementary Figure 4a

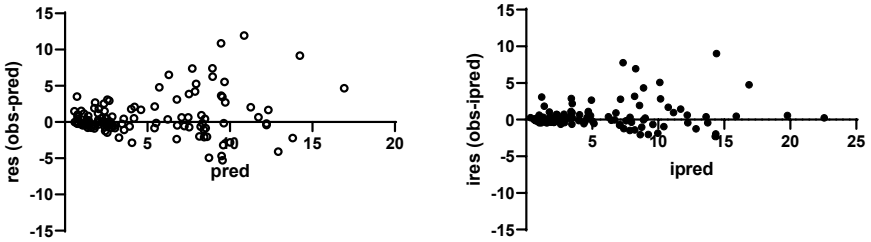


Supplementary Figure 4b

Supplementary Figure 5. Residual plots
Obs: observed concentration
Pred: population predicted concentration
Ipred: individual predicted concentration
Res: residual (observed minus population predicted concentration)
Ires: individual residual (observed minus individual predicted concentration)



Supplementary Figure 5a. Residual plots of the final parametric model. The log-transformed concentrations are back transformed for an easier comparison with the untransformed concentrations in **Supplementary Figure 5b**.



Supplementary Figure 5b. Residuals plot of the final nonparametric model.

Chapter 3.2

Validation and simulation of two population pharmacokinetic models of imipenem

Parametric and nonparametric population pharmacokinetic models to assess probability of target attainment of imipenem concentrations in critically ill patients

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Chapter 4

Optimising the design and analysis of clinical trials for antibiotics

Optimising the design and analysis of clinical trials for antibacterials against multidrug-resistant organisms: a white paper from COMBACTE's STAT-Net

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Clinical Infectious Diseases. 2018;67:1922-31.

Abstract

Innovations are urgently required for clinical development of antibacterials against multidrug-resistant organisms (MDROs). Therefore, COMBACTE STAT-Net (2013-2017), a European, public-private working group, has reviewed and tested several innovative trials designs and analytical methods for randomised clinical trials, which has resulted in eight recommendations. The first three focus on pharmacokinetic and pharmacodynamic (PK/PD) modelling, emphasizing the pertinence of population-based PK models, regulatory procedures for the re-assessment of old antibiotics, and rigorous quality improvement. Recommendations 4 and 5 address the need for more sensitive primary endpoints through the use of rank-based, or time-dependent, composite endpoints. Recommendation 6 relates to the applicability of hierarchical nested trial designs, while the last two propose the incorporation of historical or concomitant trial data through Bayesian methods and/or platform trials. Although not all of these recommendations are directly applicable, they provide a solid, evidence-based approach to develop new, and established, antibacterials and address this public health challenge.

Introduction

There is a gap between the number of new antibacterials in research and development (R&D) and the medical need caused by the increasing prevalence of infections by multidrug-resistant organisms (MDROs) [1]. In a 2014 survey [2], pharmaceutical industry professionals provided their opinion on the main challenges underlying this discrepancy. Most frequently, they indicated the low return on investment for antibacterials, followed by the lack of new regulatory pathways for antibacterial medicines that address a high unmet medical need, such as novel treatment options against MDROs. Importantly, innovative trial designs were seen as an important tool to promote R&D efforts for new antibacterials. Since this survey was conducted, political awareness of the need to encourage R&D for new antibacterials has risen enormously, with regulatory guidance being updated and international harmonization efforts underway [3-5]. However, critical to these processes is the need to advance and optimize trial design and make more effective use of the available data, so as to accelerate antibacterial approval and ensure appropriate use of established antibiotics active against MDROs.

Indeed, in the area of MDRO therapeutics where the number of patients with resistant infections for each specific indication is generally still rare, large studies are impossible or impractical [6]. Within traditional randomized controlled trials (RCTs), outcomes for patients with susceptible and resistant infections are combined, and although the subset of infections with resistant pathogens is presented separately, sample size is often insufficient. As a consequence, assessment of safety and effectiveness of new agents against MDROs is challenging, and without novel trial designs, which make better use of the available data, progress is difficult. Rex *et al.* have proposed a 'totality-of-evidence' approach to resolve the impasse [7]; where instead of 2-3 large Phase II and III trials, multiple sources of data could contribute to the evidence base of the clinical benefit of a new drug, including smaller RCTs. However, this approach needs to be supported by robust pharmacokinetic and pharmacodynamic (PK/PD) data on optimal dosing of patients and the ability to design, analyze and interpret clinical trials as efficiently as possible.

In 2013, the Innovative Medicines Initiative (IMI; www.imi.europa.eu) established the Combatting Bacterial Resistance in Europe consortium (COMBACTE; www.combacte.com) to conduct prospective clinical trials and refine clinical trial design for new treatments against MDROs [8]. The specific objective for STAT-Net (Workpackage 4; WP4) was to deliver strategies that may yield more efficient Phase II and III clinical trial programs, and to focus on three pillars: improved PK/PD modelling, enhanced endpoints, and innovative trial designs.

In this white paper, we propose several innovative design and analysis strategies for regulatory and pragmatic clinical trials to support the evaluation of new and established antibacterials against MDRO infections, and discuss their scientific robustness and feasibility.

Methods

STAT-Net was initiated in response to the 6th Call for proposals issued by IMI in 2012 [9]. Subtopic 1A, WP4, specified three research pillars as described above, which were aligned with regulatory and scientific challenges at the time of writing. SH assembled, through open invitation, a group of experts with strong track records in PK/PD, biostatistics, Bayesian statistics, infectious diseases, intensive care medicine, epidemiology, and clinical development. Aligned with the IMI public-private partnership, pharmaceutical company partners joined to provide additional expertise. This group proposed a description of work in line with the original call, which was granted by IMI in autumn 2012.
Between January 2013 and July 2017, systematic reviews, re-analyses of existing clinical trial data, and simulations have been carried out. Some of this work has already been peer-reviewed and published [10-19]. The final recommendations presented here are based on this work, extensive discussions via teleconferences, and STAT-Net-specific and COMBACTE-wide meetings, and have been approved by all authors. We created an objective scoring system for each recommendation, which was approved by all partners (**Table 1**), and was used to score 1 – alignment with the current regulatory framework, 2 – feasibility of technical implementation, like the need for specific biostatistical or PK/PD skills 3 – ease of data interpretation, 4 – ease of practical implementation at the clinical site, and 5 – the strength of the evidence base for each recommendation (**Table 2**). Whenever disagreement concerning a recommendation arose, recommendations and scoring was adapted until consensus was reached. Based on the average score, recommendations are presented as: "strongly recommend" (++++), "recommend" (+++), "strongly suggest" (++), or "suggest" (+).

Results

Innovative biostatistical methods for pharmacokinetic and pharmacodynamic modelling

Recommendation 1 (study design): We recommend that Phase II and III clinical trials of new antibiotics, particularly those active against MDROs, always apply population PK (popPK) models to describe and explain PK variability, optimize dose finding and evaluate outcome data relative to exposure.

Antibiotic efficacy, which determines microbiological cure, depends on the *in vitro* potency of the drug (usually expressed as minimal inhibitory concentration, MIC) and the *in vivo* exposure of the microorganism to the drug which relies on the concentration-time profile (pharmacokinetics, PK) and the dose. Due to MIC and PK variability, dosing optimization for certain patient subgroups is essential to avoid poor outcomes from ineffective treatment or resistance selection with too low doses, or adverse events with too high doses [20].

Identification of efficacious dosing regimens based on preclinical PK/PD analyses and Phase I and II studies support the dosing rationale in Phase II and III clinical studies [21]. An important aspect of preclinical PK/PD analyses is the identification of a dominant drug-specific PK/PD index which describes the exposure-response relationship (e.g., the percentage of the dosing interval that the free drug concentration remains above the MIC, fT>MIC) and the minimal index value that ensures a high probability of efficacy (the PK/PD target, e.g., 50% fT>MIC). In Phase I studies, the PK of several dosing regimens is explored in healthy volunteers. Subsequently, in Phase II and III studies, popPK models can be further developed to describe and explain PK variability in the heterogeneous target population [21]. Simulations based on popPK models can be used to determine the probability of target attainment (PTA) of various dosing regimens in specific subpopulations, and thereby improve efficiency of Phase II and III studies. PopPK data, combined with MICs and outcome data, can also be used to refine pre-clinical exposure-response relationships and identify clinical-relevant PK/PD indices and targets.
This recommendation is highly evidence based and in alignment with the current regulatory framework (**Table 2**) [21, 22], however, not yet fully applied for all recently approved antibiotics. Another drawback is that evaluation of data is often restricted to blood levels, while data for other relevant body sites is important as well, although interpretation for these body sites still remains uncertain. This recommendation is applicable for all infections including those caused by MDROs (**Table 3**).

Recommendation 2 (regulatory procedures): We recommend an EU-coordinated regulatory procedure for re-assessment of old antibiotics and their licensing, particularly those active against MDROs, which addresses justification of dosing regimens and exposure-response data according to modern PK/PD principles. This should include description of PK/PD targets, development of popPK models, and re-assessment of antibacterial spectra.

Many currently used antibiotics have been available clinically for many years, and long before current PK/PD principles and popPK modelling programs were employed in drug evaluation [20]. PK analyses were based on non-compartmental or simple compartmental methods without covariate investigation. PK/PD targets were mostly unknown and PTA simulations were not performed. The optimal dosing regimens of many old antibiotics therefore remain unknown which makes the probability of efficacy attainment uncertain. Also the antibacterial spectrum of old antibiotics is often poorly defined as changes in resistance epidemiology have not been systematically studied.
Re-analysis of old PK data may support dosing optimization of old antibiotics, provided the PD target is or will be established. For example, within the framework of STAT-Net, a popPK model using 45-year-old amoxicillin drug concentration data was developed and new dosing recommendations were published based on PTA simulations [18]. Unfortunately, the retrieval of such old data can be cumbersome. Alternatively, new PK studies of old antibiotics can be performed, for example, in the EU-project AIDA (www.aida-project.eu). Unfortunately, even though new popPK models are being developed, PK/PD targets are still lacking for many old antibiotics

[20]. Thus, developing rational dosing recommendations for all old antibiotics can be complex.
This recommendation is evidence based (**Table 2**), but doesn't align with current regulatory approaches as a coordinated redevelopment procedure including updating of Summary of Product Characteristics (SmPCs) is currently unavailable [20]. However, some studies are performed in EU- and NIH projects (COMBACTEwww.nih.gov/news-events/news-releases/nih-funds-four-clinical-trials-fight-antibiotic-resistance), which may provide a suitable basis. This recommendation is applicable for all infections including those caused by MDROs (**Table 3**).

Recommendation 3 (study design): We recommend that future clinical PK/PD studies provide more robust results by a priori determination of the sample size, adjustment for known confounders of the exposure-response relationship, assessment of both microbiological and patient orientated outcomes, and application of appropriate statistical techniques.

Although clinical PK/PD studies of antibiotics are always performed, their design and analysis have had limitations. Consequently, guidance on dosing regimens does not always have a strong evidence base, and to maximize the possibility of successful clinical outcomes and pathogen eradication, especially in the case of MDROs, more robust data are needed.
We conducted a systematic review of clinical PK/PD studies published since 1980 and which related a calculated PK/PD index to the probability of a clinical or microbiological response [17]. After de-duplication, 6,096 records were reviewed resulting in 85 articles containing 97 PK/PD analyses being finally included. Only three out of 97 studies included a sample size calculation, and as such it cannot be determined whether clinical meaningful results would have been detected if present. Less than half of the studies included adjusted analysis for known confounders, including physiological patient characteristics, infection characteristics, and concurrent treatments. About half of the studies focused on clinical response, while the other half reported bacteriological response, while in most cases both would be important. Clinical response is the most important outcome to the patient, but bacteriological response measures the direct effect of the antibiotic. From an analytical perspective, only 61 out of 97 studies reported some form of regression, time to event analysis or used the Hill/Emax equation to look at the association between PK/PD index and outcome. In order to prevent inappropriate interpretation and multiplicity errors from analyzing a number of factors in a data dependent way, we recommend that pre-approved analysis plans should be implemented. These should ideally be based on the recently published regulatory guidance document EMA/CHMP/594085/2015 [21], which took into consideration the above described work and emphasizes application of the most appropriate methods. Further, presentation of confidence intervals around stated effects will guard against inappropriate interpretation of underpowered analyses.
This recommendation is based on expert opinion, moderately easy to apply (**Table 2**), and is applicable for all infections including those caused by MDROs (**Table 3**).

Selection of novel and more sensitive primary outcomes for clinical trials

Recommendation 4 (study design/analysis): We recommend using rank-based, composite endpoints combining patient-oriented and disease-related endpoints to assess new therapies against MDROs.

At present, an ideal endpoint that would allow assessment of the efficacy of new therapies against MDRO is still lacking [15]. Patient-oriented endpoints such as mortality or quality of life are robust and directly matter to patients [23]. However, they also rely on several other non-infectious, confounding factors. Moreover, they require large sample sizes for non-inferiority (NI) testing, with clinically unacceptable NI margins [15]. On the other hand, disease-oriented primary endpoints such as clinical cure or organ failure free days are more sensitive and require smaller sample size, but these are not always unequivocally linked to true patient benefit [15].
In this context, composite endpoints appear worthy of further study [15], especially if they are easy to use and give appropriate priority to the more clinically important events (e.g. mortality). New methods for analyzing composite endpoints have already been reported [24, 25], and applied [26, 27]. In a recent Delphi process, 26 experts in the field of severe nosocomial pneumonia confirmed that such a composite endpoint combining patient-oriented endpoints and disease-related endpoints was expected to be the best method for assessing antibacterial efficacy in future clinical trials. This could be extended by applying multistate models (recommendation 5a), or a hierarchical nested trail design (HNTD) (recommendation 6).
The development of a rank-based, composite endpoint needs to be planned before RCTs start and discussed with regulatory authorities. It is technically feasible to apply, but interpretation is complex (**Table 2** and **Table 3**).

Recommendation 5a (study design/analysis): We strongly suggest, in RCTs dealing with MDROs, to apply multistate models to examine a range of time-dependent clinical outcomes in the primary analysis to better characterize patients' disease course.

FDA and EMA guidelines have suggested the use of different primary endpoints in clinical trials evaluating treatment for patients with hospital- or ventilator-associated pneumonia (HAP/VAP) that include either a clinical outcome at the TOC visit or all-cause mortality at a specific point in time [28]. As discussed above, composite endpoints combining these clinical important events could be more informative. On top of that, validity could be improved by considering the occurrence of events over time, instead of assessing them at a predefined time interval.
If cure and death endpoints are of particular interest, both measures of clinical benefit can be simultaneously accounted for in a multistate framework using the co-primary endpoint "*get cured and stay alive over time*". The application of this type of analysis has been illustrated by using data from a recently published trial on HAP/VAP patients [14] and can be adapted to more complex disease histories as, for example, patients with *Clostridium difficile* infection [13]. The application of such multistate models has the advantage of avoiding common survival biases (which occur if deleting or

censoring death outcomes when studying cure), since ignoring time dependency may lead to over- or underestimated efficacy. Furthermore, it provides patient-relevant information about getting cured and staying alive, instead of providing cure rates and mortality rates separately.
This recommendation requires specific statistical expertise, but can be applied to many potential MDRO treatment indications both in the design and the analytical phase of RCTs (**Table 2** and **Table 3**).

Recommendation 5b (study design/analysis): We strongly suggest, when applying multistate models, to perform statistical significance testing for the probability of being cured and alive over the entire treatment process rather than at the end of follow-up.

Traditionally, the hypothesis tested in RCTs uses the data obtained at the end of follow-up (e.g. at the TOC visit). So far, no method has been validated which statistically tests NI or superiority for a multistate endpoint demonstrating a treatment effect over the complete treatment process instead of merely at the end of follow-up.
We applied an innovative resampling technique to construct one-sided simultaneous confidence bands to test the difference in probabilities of being cured and alive between study arms [29, 30], which performed well [12]. This provides a comprehensive picture of the time-dynamic effect of the entire treatment process while preserving the desired alpha level for statistical testing, resulting in a much stronger NI or superiority statement.
This recommendation is promising for the analysis of future RCTs, although it involves statistical expertise for implementation. Moreover, NI margins are difficult to establish given their reliance on historical data of the effects without treatment. Discussions with regulatory authorities would be required to agree upon an acceptable NI margin for this novel outcome measure (**Table 2**).

Innovative trial design in the absence of rapid diagnostic tests

Recommendation 6 (study design/analysis): We strongly suggest that trials aiming to assess the clinical benefit of a new therapy against MDRO pathogens should apply a hierarchical nested trial design if a priori power calculations indicate feasibility.

Superiority trials for new antibacterials targeting MDROs are, in general, considered infeasible [6]. It is usually impossible to select a MDRO subgroup at the time point of randomization, since this usually occurs before standard organism susceptibility testing is available. Rapid diagnostics would be very useful in this perspective, but unfortunately rapid antibiotic susceptibility testing has not yet developed to a level which would make application for RCTs feasible. If a new drug against MDROs were to be tested among a mixed patient group, with susceptible and MDRO infections, the chances of showing superiority is likely low, especially if the proportion of patients infected with MDROs is low. Therefore, NI trials have become the standard in this area, with limited data about clinical benefit for the MDRO patient subgroup.

The hierarchical nested trial design (HNTD) [31] is an innovative approach of addressing clinical benefit for patients with susceptible infections and patients with MDRO infections within one RCT. The HNTD originally suggests power calculations to be aligned with inference hierarchy and thus sample size calculations will be conducted based on the overall NI testing. In our simulations we observed that demonstration of superiority in the MDRO subgroup can become practically infeasible if the sample size of this subgroup is small. The power implications of designing the trial on the basis of the superiority test in the MDRO subgroup should therefore be explored in advance. This is especially important from an ethical point of view, as it will indicate the likelihood of success of a RCT for the targeted MDRO subgroup, which should be a criterion for patient participation, just like expected beneficence for the subgroup of patients with susceptible infection. Nevertheless, hierarchical approaches should be considered whenever feasible, as it can provide valuable information for both the susceptible and MDRO subgroups. Possibly, a combination with rank-based, composite endpoints (recommendation 4) could make this approach more powerful.
Application of this recommendation is moderately complex, should be discussed with regulatory authorities before application, and requires a large sample of MDRO patients (**Table 2** and **Table 3**).

Methods to incorporate historical clinical trial data

Recommendation 7 (study design/analysis): We strongly suggest that clinical trial investigators make use of the multitude of available historical clinical trial data in the design and analysis of novel MDRO treatment trials.

As discussed earlier, in most trials it is difficult to prospectively identify a large number of patients with MDRO infections and thus adequately power RCTs. Historical data from previous studies could be more effectively used to make the preparation and conduct of clinical trials more feasible and efficient.
Historical data are already used to help justify NI margins, but they can also be used in a Bayesian approach by using pre-existing data to incorporate knowledge about possible trial results. Where several trials have been conducted with a similar or identical treatment to the control arm of the new trial, a meta-analysis based on these data can be performed and a mathematical expression of the prior knowledge regarding control efficacy can be constructed. These priors can be interpreted as historical control patients, and when used alongside clinical trial patients can reduce the number of patients needed in the RCT, or increase the power of a given comparison [32, 33]. The new trial can be conducted using a traditional design [34, 35], or an adaptive design, where the sample size or randomization ratio can be adjusted based on interim analyses to optimize power [33, 36].
This methodology highly depends on the quality of historical data, detail of publicly available data, and possible time-dependent changes in medical care. It also requires extensive communication between the sponsor and regulatory agency to agree on the prior distribution and acceptable type I and II error rates in the context of

the efficiencies gained with such a design. This recommended methodology is not limited to specific endpoints or infection types, so it can be applied in any trial where relevant data are available (**Table 2** and **Table 3**).

Recommendation 8 (study design/analysis): We suggest the use of platform trials to study new antibacterial treatments against MDROs.

If no historical data exist (recommendation 7), there are other ways to more efficiently improve sample size. In a platform trial investigators focus on the disease rather than any particular experimental therapy, and can simultaneously, or subsequently, investigate multiple experimental and control treatments, as a way to handle patient involvement as effectively as possible.
A key recommendation in the report issued by the President's Council of Advisors on Science and Technology (PCAST) in the United States [37] is the establishment of a clinical trials' infrastructure that would in turn support a "platform trial" for antibacterial agents [38', 39, 40]. While there are notable operational hurdles (for example, finding/identifying patients), many of them are germane to the larger complexities of implementing clinical trials for antibacterials [37]. Initiating a single platform trial in this setting could therefore aid in managing some of these barriers, similar to what has already been accomplished in therapy areas such as Oncology and Alzheimer's [41]. The efficiency of such a platform trial is driven by key design features. These include use of a shared control and incorporation of Bayesian methods to allow use of information across sites of infection and/or from historical data (recommendation 7) in one analysis, bearing in mind the general pre-requisites for using Bayesian approaches, such as assuring similarity of historical to contemporary data [38, 42, 43].
This recommendation requires specific statistical expertise, and there is no real world experience yet for antibacterial development, but based on experiences in other medical fields it seems a promising alternative (**Table 2** and **Table 3**).

Discussion

Innovations are urgently required in the field of antibacterial development, especially for treatments against MDROs. The recommendations provided here could be instrumental to advances in this field. Although the proposed recommendations would not always be applicable within the same trial, not all of them align with current regulatory guidelines, and they differ regarding ease of implementation or interpretation, and evidence base, they are all relevant to the debate supporting change.
In **Table 2**, the different recommendations were scored, showing that, in general, recommendations for PK/PD studies have the strongest evidence base, and our view is that they should be implemented as soon as possible to improve drug dosing in RCTs. Bayesian methods, incorporating data from historical controls in new RCTs, can be successfully used to reduce the number of patients required for RCTs. They

have already been applied to re-analyze clinical trial data, and regulatory applicability is promising, provided the historical and contemporary data can be shown to be comparable. Rank-based, composite or time-dependent endpoints are another way to improve statistical efficiency, and provide more meaningful data at the same time. However, technical and practical implementation still present some challenges and a regulatory framework to support this approach is still lacking. Our recommendations related to HNTDs and platform trials are the least pertinent. Although the HNTD approach has clear merit, it generally faces the huge challenge of recruiting sufficient patients with MDROs to support a meaningful superiority assessment. Platform trials could efficiently provide data for multiple, concurrent or subsequent, control arms, by establishment of a common clinical trials infrastructure, but the practical implementation is challenging, particularly in gaining commitment at the initiation of the platform trial. By increasing RCT efficiency, superiority trials could become more feasible, which would be preferable considering the ethical issues associated with NI trials [44].

STAT-Net partners are already working on the evaluation and refinement of some of the proposed solutions. First, as an extension of recommendation 6, a novel HNTD is being evaluated, where an alternative, more sensitive endpoint (statistically) is introduced for the subgroup of patients infected by resistant pathogens. Such an approach could curtail the required sample size for superiority testing in the subgroup. In order to maintain confidence in the clinical benefit, the point estimate for the clinical endpoint of interest, as used in the NI assessment for the whole sample (i.e., cure rate), should be similar as well. An example of a more statistically sensitive endpoint could be the, regulatory approved endpoint of, absolute reduction of skin lesions in skin and soft tissue infections. This approach could be especially valuable for very rare MDROs.

Secondly, the application of multistate models (recommendation 5) is being studied for combined endpoints other than cured and alive, e.g., to be alive and not under mechanical ventilation in patients with acute respiratory distress. Study planning for more specific multistate endpoints will typically be simulation based [45]. Previous studies can be used to define the assumptions needed for these types of simulation studies, possibly using only published graphs of outcome probability over time for control groups as explained in Allignol *et al.* [46].

In addition, novel statistical methods will be tested that allow historical control data from one single study (as compared to multiple studies, recommendation 7) to be utilized in future RCTs [11]. Outcome estimates from a single, previous trial can be weighted depending on the similarity to the outcome in the new trial, thereby increasing sample size and power with only a limited increase in type I error. This may help to demonstrate the totality of evidence to reviewers, by including the relevant historical data in a secondary analysis. This new approach can be extended to multiple historical studies as well [10], and as such is a promising alternative to the methods listed in recommendation 7 since it is more flexible and does not require the strong assumptions about heterogeneity and exchangeability.

Finally, efforts to initiate an antibacterial platform trial (recommendation 8) are underway in both the European Union, through COMBACTE-Net and PREPARE, and

in the United States, through the Antibiotics Resistance Leadership Group (www.arlg.org), and also globally, through the Wellcome Trust. Additional simulation work to understand operating characteristics and further discussions with regulatory agencies on a case-by-case basis will be needed in order to continue to progress and embed these innovative trials.

The work presented here was bound by the IMI Call text and the subsequent description of work. Since 2012, the landscape has changed, and a multitude of methodological approaches for accelerated antibacterial development have been proposed. Rex *et al.* have discussed the four-tiered approach to registration, whereby required strength of the evidence depends on the severity of the unmet medical need, ranging from disease focused double Phase III RCTs to pathogen-focused observational studies [7]. Although the current regulatory framework seems more open for these alternative routes, acceptance still requires alignment and assessment of unmet medical need. For PK/PD data, EMA has recently updated their guidance document [21], and sponsors are now encouraged to include and utilize PK/PD data in their application for regulatory approval of new antibiotics, although this is – unfortunately- not yet fully adopted in all current application dossiers. Clearly, these dossiers would need a re-assessment. Additionally a proper framework for the re-assessment of old antibiotics is urgently required. Multiple Bayesian applications have been proposed as well, of which some would fit logically within the current regulatory framework; Bayesian-based meta-analyses could be used to determine more appropriate NI margins, while balancing the degree of unmet need and the feasibility of the RCT [47].

From an ethical point of view, the possible benefit for society should be subordinate to the individual risks of participation in research, and should include assessment of operational risks of RCTs (e.g. too complex trials stopped due to poor recruitment), and scientific rigor (e.g. data validity and power issues). To quote Ruberg *et al.* '*Our professional challenge is to implement adaptive approaches while maintaining sufficient rigor in the design and analysis of clinical development trials and programs without inhibiting innovation or delaying the access of needed medications to patients who are waiting.*' [48]. Hopefully, these recommendations and their continued evaluation and evolution will accelerate antibacterial approval and ensure appropriate use of established antibiotics to help those in need as soon and best as possible.

Acknowledgements

We would like to acknowledge Eric Burroughs, René Eijkemans, Fabricio Da Liberdade Jantarada, Esther Oomen - de Hoop, Kit Roes, Nicola Little, Martin Schumacher, and Jeffrey Wetheringthon for their valuable contributions to the work of STAT-Net.

Financial support

This work was supported by the Innovative Medicines Initiative Joint Undertaking (IMI JU) [grant number 115523], Combatting Bacterial Resistance in Europe [COMBACTE], resources of which are composed of financial contribution from the European Union's 7th Framework Programme (FP7/2007±2013) and the European Federation of Pharmaceutical Industries and Associations (EFPIA) companies' in kind contribution, and the German Research Foundation (Deutsche Forschungsgemeinschaft) [grant number WO 1746/1-2 to MW]. O.A., T.A., A.D. and D.W. belong to an EFPIA member company in the IMI JU and costs related to their part in the research was carried by the respective company as in kind contribution under the IMI JU scheme. The funders had no role in study design, conduct, analysis, and interpretation of the data; drafting of the manuscript; and the decision to submit the manuscript for publication.

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Table 1. Classification table for the recommendations.

Classification	Alignment with current regulatory framework	Ease of technical implementation	Ease of interpretation	Ease of practical implementation	Evidence base	Formulation
+	Adaptations in design AND analytical methods needed, currently not supported by regulatory authorities	Only by statistical or PK/PD experts familiar with the methodology	Very complex; requires statistical expertise and experience with the methodology	Design and analytical methods require significant adaptation of standard clinical trial protocols and extra data is required	This recommendation is based on expert opinion, and/or external panel consensus.	"We suggest .."
++	Adaptations in design OR analytical methods needed, currently not supported by regulatory authorities	Only applicable by statisticians or PK/PD experts	Moderately complex; statistical expertise required	Design and analytical methods require moderate adaptations of standard clinical trial protocols or extra data is required	This recommendation is based on encouraging results from simulations.	"We strongly suggest ..."
+++	Adaptations in endpoints, consultation with regulatory authorities required	Applicable by those experienced in applied statistical or PK/PD analysis	Moderately easy; clinical trial background required, but no need for a statistical background	Design and analytical methods require small adaptations to specific parts of a standard clinical trial protocol and no extra data is required	There is fair research-based evidence to support the recommendation; re-analysis of clinical trial data has provided encouraging results.	"We recommend …"
++++	No obstacles for implementation identified, in line with current regulatory guidelines	Only basic statistical or PK/PD expertise required	Easy; no need for a statistical/clinical trial background	Standard clinical trial protocols can be applied and no extra data is required	There is good research-based evidence to support the recommendation: it has already been applied and tested in clinical trials.	"We strongly recommend ..."

PK, pharmacokinetics; PD, pharmacodynamics

Table 2. Recommendations and their classification regarding current regulations, implementation, interpretation, and evidence base in line with **Table 1**.

Recommendations	Alignment with current regulatory framework	Ease of technical implement-tation	Ease of inter-pretation	Ease of practical implement-tation	Evidence base
Innovative biostatistical methods for pharmacokinetic and pharmacodynamic modelling					
1: We recommend that Phase II and III clinical trials of new antibiotics, particularly those active against MDROs, always apply population PK models to describe and explain PK variability, optimize dose finding and evaluate outcome data relative to exposure.	++++	+++	++	+++	++++
2: We recommend an EU-coordinated regulatory procedure for re-assessment of old antibiotics and their licensing, particularly those active against MDROs, which addresses justification of dosing regimens and exposure-response data according to modern PK/PD principles. This should include description of PK/PD targets, development of popPK models, and re-assessment of antibacterial spectra.	++	+++	++	+++	+++
3: We recommend that future clinical PK/PD studies provide more robust results by *a priori* determination of the sample size, adjustment for known confounders of the exposure-response relationship, assessment of both microbiological and patient orientated outcomes, and application of appropriate statistical techniques.	++++	+++	+++	+++	+
Selection of novel and more sensitive primary outcomes for clinical trials					
4: We recommend using rank-based, composite endpoints combining patient-oriented and disease-related endpoints to assess new antibacterial therapies against MDROs.	+++	+++	+++	++	++
5a: We strongly suggest, in trials dealing with MDROs, to apply multistate models to examine a range of time-dependent clinical outcomes in one primary analysis to better characterize the patient's disease course.	++	+	++	++	+++

table continues

Recommendations	Alignment with current regulatory framework	Ease of technical implement-tation	Ease of inter-pretation	Ease of practical implement-tation	Evidence base
5b: We strongly suggest, when applying multistate models, to perform statistical significance testing for the probability of being cured and alive over the entire treatment process rather than at the end of follow-up.	+	+	++	+	+++
Innovative trial design in the absence of rapid diagnostics					
6: We strongly suggest that trials aiming to assess the clinical benefit of a new therapy against MDRO pathogens should apply a hierarchical nested trial design if a priori power calculations indicate feasibility.	++	++	++	++	++
Methods to incorporate historical clinical trial data					
7: We strongly suggest that clinical trial investigators make use of the multitude of historical clinical trial data in the design and analysis of novel MDRO treatment trials.	++	++	+++	++	+++
8: We suggest the use of platform trials to study new antibacterial treatments against MDROs	+	+	++	++	+

MDRO, multi-drug resistant organisms; PK, pharmacokinetics; PD, pharmacodynamics

Table 3. Application and possible benefits and disadvantages for each recommendation

Recommendation	Type of trial	Indications	Population	Benefits (+) and disadvantages (-)
Innovative biostatistical methods for pharmacokinetic and pharmacodynamic modelling				
1: We recommend that Phase II and III clinical trials of new antibiotics, particularly those active against MDROs, always apply population PK (popPK) models to describe and explain PK variability, optimize dose finding and evaluate outcome data relative to exposure.	PK/PD analyses with PK and PK/PD data from Phase I, II, and III studies. PK/PD target based on preclinical and clinical data.	All infections	All	+ Optimized dosing, increasing the likelihood of detecting true efficacy in RCTs, decreasing the likelihood of emergence of resistance - Additional patient sampling required - Extra costs for RCT sponsors
2: We recommend an EU-coordinated regulatory procedure for re-assessment of old antibiotics and their licensing, particularly those active against MDROs, which addresses justification of dosing regimens and exposure-response data according to modern PK/PD principles. This should include description of PK/PD targets, development of popPK models, and re-assessment of antibacterial spectra.	PK/PD analyses with PK and PK/PD data from Phase I, II, and III studies. PK/PD target based on preclinical and clinical data.	All infections	All	+ Optimized dosing and indications of old antibiotics, increasing the likelihood of detecting true efficacy in RCTs, decreasing the likelihood of emergence of resistance - Need for alignment with regulatory authorities - New licensing required - Public funding for re-assessment studies required
3: We recommend that future clinical PK/PD studies provide more robust results by a priori determination of the sample size, adjustment for known confounders of the exposure-response relationship, assessment of both microbiological and patient orientated outcomes, and application of appropriate statistical techniques.	PK/PD analyses with PK and PK/PD data from Phase I, II, and III studies. PK/PD target based on preclinical and clinical data.	All infections	All	+ More reliable PK/PD data, optimized dosing, increasing the likelihood of detecting true efficacy in RCTs, decreasing the likelihood of emergence of resistance - Additional patient sampling required - Extra costs for RCT sponsors

table continues

Recommendation	Type of trial	Indications	Population	Benefits (+) and disadvantages (-)
Selection of novel and more sensitive primary outcomes for clinical trials				
4: We recommend using rank-based, composite endpoints combining patient-oriented and disease-related endpoints to assess new therapies against MDROs.	All	All infections, especially those with low mortality	All	+ More meaningful, and sensitive endpoints, increasing the likelihood of true positive findings in RCTs - Endpoints could become more subjective - Endpoints may be more difficult to interpret - It can be difficult to establish an acceptable NI or superiority margin
5a: We strongly suggest, in trials dealing with MDROs, to apply multistate models to examine a range of time-dependent clinical outcomes in one primary analysis to better characterize the patient's disease course.	All	Those with moderate to high mortality rates	Populations with moderate to high mortality rates	+ More meaningful, and sensitive endpoints, increasing the likelihood of true positive findings in RCTs - Composite endpoints may be more difficult to interpret - It can be difficult to establish an acceptable NI or superiority margin
Innovative trial design in the absence of rapid diagnostics				
6: We strongly suggest that trials aiming to assess the clinical benefit of a new therapy against MDRO pathogens should apply a hierarchical nested trial design if a priori power calculations indicate feasibility.	Non-inferiority trials	All	MDROs	+ Statistically sound results for treatment efficacy in the MDRO subgroup without the need for rapid diagnostics -Large sample of non-MDRO patients required
Methods to incorporate historical clinical trial data				
7: We strongly suggest that clinical trial investigators make use of the multitude of available historical clinical trial data in the design and analysis of novel MDRO treatment trials.	All	All	All	+ RCTs can include a lower number of patients, but remain powered, and existing evidence is efficiently used - Increased type I error - Need for historical RCT data, which may be difficult to obtain

table continues

Recommendation	Type of trial	Indications	Population	Benefits (+) and disadvantages (-)
8: We suggest the use of platform trials to study new antibacterial treatments against MDROs.	All	All	All	+ Increasing the efficiency of RCTs by using the same patients for multiple RCTs - Large database and high workload, which may not be utilized eventually - Potential conflicts between different study sponsors and/or companies

Chapter 5

Clinical applications of population pharmacokinetic models of antibiotics: challenges and examples

Based on: Clinical applications of population pharmacokinetic models of antibiotics: challenges and perspectives

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Pharmacological Research. 2018; 134: 280-288

Clinical applications of population PK models

Three main clinical applications of population pharmacokinetic (popPK) models can be specified for antibiotics: 1) dosing evaluation of old antibiotics, 2) setting clinical breakpoints and 3) therapeutic drug monitoring (TDM). This thesis is mainly focused on dosing evaluation of old antibiotics. However, many challenges regarding the interpretation of popPK models used for dosing evaluation are also applicable for the other clinical applications.
The most important challenges are summarized in **Table 1** and further clarified in the text below the table. At first, the role of popPK models in setting clinical breakpoints and in TDM are explained in the two paragraphs below.

Setting clinical breakpoints

The European committee on antimicrobial susceptibility testing (EUCAST) provides species-related and PK/PD (non-species related) clinical breakpoints. The EUCAST procedure for setting PK/PD breakpoints includes Monte Carlo simulations using population PK models to estimate exposure of an antimicrobial agent in the target patient population for commonly used dosing regimens [1]. Following the simulations, the probability of target attainment (PTA) is determined for different PK/PD targets. Subsequently, the PK/PD target is plotted as a function of the minimal inhibitory concentration (MIC) for the mean of the population and 95% and 99% CI estimates (corresponding to 97.5% and 99.5% PTA). EUCAST uses the MIC values resulting from both PTAs to determine a PK/PD breakpoint [1].

Therapeutic drug monitoring (TDM)

Therapeutic drug monitoring is the measurement of drug concentrations to optimize dosing regimens for individual patients with the objective to maximize efficacy and minimize toxicity [2]. Criteria for a drug to be appropriate for TDM are: large between-subject variability, small between-occasion variability, defined concentration-effect relationship, small therapeutic range, available analysis method and no clearly defined clinical parameter that allows dose adjustments (e.g. glucose or INR levels) [3].
TDM can be applied by evaluating if the drug concentrations are in the therapeutic range, but in case of deviating concentrations, or when the therapeutic target is not just a concentration but e.g. an AUC, it is difficult to provide a dosing recommendation manually. A more robust approach to individualize dosing by TDM is the maximum a posteriori probability (MAP) Bayesian fitting procedure [4], which is implemented in various TDM software programs [5, 6]. The library of these TDM software tools include population parameters, SD's and covariates based on population PK models. Several TDM computer tools are available. In 2012, a review of 12 software tools was published [5]; they differ in the number of drugs offered in the library (from 2

to 180). Eight of these programs provide the option to add new drug models. Other differences are the availability of MAP Bayesian dosage adaptation (10/12 tools) and the proposal of a priori dosage regimens based on certain covariates (9/12 tools). The authors of the review recommend that most TDM software tools can be improved, more specifically the interface, user friendliness, data storage capability and report generation.

TDM is applied to many antibiotic classes. For aminoglycosides (e.g. gentamicin, tobramycin and amikacin) and glycopeptides (e.g. vancomycin and teicoplanin), TDM has become standard clinical practice to balance efficacy and toxicity [7, 8]. For other groups, such as beta-lactams and fluoroquinolones, TDM is not yet commonly employed [7, 8]. The most important reasons that TDM is not commonly used for all antibiotic classes are unclear therapeutic targets, the lack of clinical outcome studies and the unavailability of an assay in the hospital [7-13].

Table 1. Challenges per clinical application of population PK models. The degree of importance is scored with ++ (highly important), + (important), - (not applicable). The most important challenges are further clarified in the text.

Challenges		Clinical applications of population PK models		
		Dosing evaluation of old antibiotics	Setting clinical breakpoints	Therapeutic drug monitoring
1.	PK/PD targets	++	++	++
2.	Protein binding	+	++	++
3.	Site of measurement	+	+	+
4.	Clinical validation	++	+	++
5.	PTA interpretation	++	++	-
6.	Population PK model selection	+	+	++
7.	Assay availability	+	+	++
8.	MIC accuracy and variation	+	+	++

Challenge 1: PK/PD targets

The optimal PK/PD target value is still not clearly defined for all antibiotics [14, 15], in part because this depends on its clinical indication or use. Preclinical derived PK/PD target values differ from clinical derived values in critically ill patients [6]. Currently, there is a trend towards the use of more conservative targets for critically ill patients than for the less critically ill. However, it may be possible that this assumption is the consequence of variation in MIC measurements [16]. More research in this area is clearly required.

For dosing evaluation of old antibiotics, some authors use two or more targets and give different dosing recommendations depending on the target [17]. For example, a review about gentamicin [18] showed that the chosen targets in 7 simulation studies in adults varied between $C_{max} \geq 8$ mg/L, $C_{max} > 10$ mg/L, $C_{max} = 22$ mg/L, $C_{max}/MIC > 8$, $C_{max}/MIC \geq 10$, AUC_{24} 70-120 mg*h/L and $AUC_{48} > 140$ mg*h/L.

Challenge 2: protein binding

PK/PD indices and targets are (almost) always defined as free (unbound) concentrations whereas many assays measure total (unbound and protein bound) concentrations [19, 20]. However, protein binding is often highly variable and hypoalbuminemia occurs frequently in critically ill patients [21, 22], which might lead to unreliable outcomes if a free concentration is calculated using a literature value for protein binding. In addition, protein binding can be concentration dependent and even nonlinear [23-25].

Challenge 3: site of measurement

Most PK/PD targets are based on blood levels. However, other body sites may be important as well, although the interpretation for these body sites still remains uncertain [26]. If there is a good correlation between plasma levels and body site levels this is not a major problem, as this is just a shift in target values. If the correlation is less predictable this may become a major issue [27]. For instance in the very obese patients, tissue concentrations can be much lower than expected [28].

Challenge 4: clinical validation of new dosing recommendations

Clinical validation of new dosing recommendations is often lacking. It is desirable that future clinical validation studies not only focus on target achievement, but also relate the exposure to clinical outcomes.

A recent review about the PK/PD of gentamicin and other aminoglycosides [18] found that only 1 study prospectively evaluated model-based dosing recommendations to see what exposure actually was achieved in clinically practice [29].

Another example of a dosing recommendation which was hardly prospectively validated, is ciprofloxacin. Ciprofloxacin is a frequently prescribed fluoroquinolone. The pharmacodynamic target of AUC0-24/MIC >125 (or fAUC0-24/MIC >100) for *Pseudomonas aeruginosa* is well established in *in vitro*, animal and clinical studies [30-32]. According to the manufacturer, for most indications, the recommended dosing regimen of intravenous ciprofloxacin is 400 mg twice or three times daily in patients with normal renal function [33, 34], which implies that the dosing frequency can be chosen by the prescribing physician. However, an increasing number of simulation studies using population PK models show that a dosing regimen of 1200 mg/day is necessary to attain the pharmacodynamic target of AUC0-24/MIC >125 (or 100 for unbound drug) for Gram-negative pathogens with an MIC ≥ 0.5 mg/L [32, 35-38]. A major drawback of these studies is that the dosing recommendations of 400 mg q8h for MICs ≥ 0.5 mg/L were based on simulations and not prospectively validated. The majority of the study population received a daily dose of maximally 800 mg/day. Despite the fact that these studies resulted in the same conclusion, the study designs were remarkable different. Some study populations were only ICU patients [35, 37] while other study populations also included general ward patients [32, 36, 38]. Some studies reduced the dose in patients with impaired renal function, each using a different dosing algorithm [32, 36, 38], while in other studies dose reduction was not applied [35, 37]. Translation of these recommendations to clinical practice is therefore difficult. Even the EMA and FDA provide different dosing recommendations for ciprofloxacin in patients with impaired renal function [33, 39].

Challenge 4: clinical validation of TDM

Despite the fact that TDM is used extensively in clinical practice, there is a paucity of prospective studies on the influence of TDM on clinical outcomes [7, 8, 11-13]. Also for antibiotics which are already in TDM programs, prospective studies are sparse [2]. Two prospective controlled studies about aminoglycoside TDM using Bayesian software showed that TDM significantly reduced nephrotoxicity, hospitalization and costs [40, 41]. However, these two studies were performed before the introduction of extended-interval dosing of aminoglycosides and might not reflect the current practice.

For vancomycin, two prospective controlled studies showed that TDM significantly reduced nephrotoxicity [42, 43].

A prospective controlled study including amikacin, ciprofloxacin, levofloxacin, ceftazidime and cefotaxime showed that TDM improved the probability of a good clinical outcome and pathogen eradication [44]. In this study, Bayesian software was used only for amikacin and the two fluoroquinolones, but not for the two beta-lactams [44]. Another prospective beta-lactam TDM study didn't use Bayesian software to adjust dosing, but manually adjusted the frequency or infusion time [45]. Dosing was adjusted if the trough or steady state unbound concentration was below 4-5x MIC or above 10x MIC, which happened in 74.2% of the patients [45]. A main drawback of this study was the calculation of free concentrations using measured total concentrations and literature values for protein binding, while protein binding is often highly variable in critically ill patients [21]. Another limitation was the unavailability of MICs for a major part of the study population. By using ECOFFs (EUCAST epidemiological cut-off value) when a pathogen was isolated but no MIC available, or a MIC based on local epidemiology information of a potential pathogen (when no pathogen was isolated), a worst case scenario was applied and dosing adjustments might have been unnecessary [16]. In 2018, a prospective Dutch study evaluating a Bayesian TDM software program of beta-lactams has begun (the DOLPHIN study [46]).

Challenge 5: PTA interpretation

PTA interpretation is not standardized. Used PTA acceptance levels vary between 90-100% and are sometimes not even mentioned. However, it is important to realize that a PTA of 90% means that 10% of the patients do not attain the target for a specific MIC, which implicates that the probability for successful treatment is diminished. For new antimicrobials, the EMA indicates a PTA of 90% for dose selection [26].

The chosen MICs for the dosing recommendations should always be weighed against the internationally published MIC clinical breakpoints. The EUCAST uses the MIC values based on PTA's of 97.5% and 99% for setting breakpoints [1].

Challenge 6: population PK model selection

An important aspect of reliable TDM programs is the choice of a population PK model which must be suitable for the patient population for which TDM is performed. Neef and colleagues presented a case of vancomycin MAP Bayesian adjustment where 4 different population PK models resulted in 4 strikingly different dosing schemes recommendations [47], see the vancomycin example below. Another study evaluated

different population PK models for amikacin and also showed significant differences in model performance (results not shown here) [48].

Challenge 6: example

This case [47] describes a 3-week-old neonate (3.6 kg, 50 cm, serum creatinine 25 µmol/L) receiving vancomycin 70 mg every 12 h with an infusion duration of 2 h. Two levels were drawn before and after the third dose. **Figure 1** shows the TDM performance of 4 models (presented in **Table 2**) for 4 tested dosing regimens. It is clear that model D has the worst fit of the measured levels. The other models have better fits, but model A predicts very high levels, probably due to absence of clearance in the model parameters. The conclusion of the authors is that model B best fit the data.

Challenge 7: assay availability

Obviously, the availability of an assay is a limiting condition for TDM. However, the availability of assays differs per antibiotic and also per hospital. Most hospitals have assays for aminoglycosides and vancomycin [47], but assays for beta-lactams and fluoroquinolones are less common [19, 49]. Possible reasons preventing institutions to provide TDM could be the absence of a prospective clinical outcome study or the requirement of a chromatographic method instead of an immunoassay [49].

Challenge 8: MIC accuracy and variation

For dose adjustment of antibiotics based on TDM, both the measure of the concentration of the drug itself and the MIC of the pathogen responsible for the infection are necessary. However, most institutions use a single MIC determination which is inappropriate and can potentially cause underdosing of patients [16]. The accuracy and variation of MIC measurements must be carefully considered during this process [16].

Table 2. PK models of vancomycin, used in the TDM software presented in **Figure 1** [47]. Kelm: metabolic elimination rate constant, Kelr: renal elimination rate constant, V1: volume of distribution central compartment, K12: distribution rate constant from central to peripheral compartment, K21: distribution rate constant from peripheral to central compartment, PNA: postnatal age (days), KKGT: Dutch Association for Quality Assessment in TDM and Clinical Toxicology, AHZ: Central Pharmacy of The Hague Hospitals. Reprinted with permission from *Therapeutic Drug Monitoring* [47].

Model	Kelm (h^{-1})	Kelr (h^{-1} (ml/min)$^{-1}$)	V1 (L/kg)	K12 (h^{-1})	K21 (h^{-1})
A: Neonate < 15 PNA	0.457 ± 0.022	-	0.46 ± 0.23	1.5 ± 0.75	2.6 ± 1.3
B: Neonate > 15 PNA	0.053 ± 0.025	0.003 ± 0.021	0.41 ± 0.21	1.88 ± 1.21	3.6 ± 2.0
C: KKGT pop model	0.01429 ± 0.00286	0.00327 ± 0.00109	0.21 ± 0.042	1.12 ± 0.028	0.48 ± 0.12
D: Neonate AHZ	0.0086 ± 0.0009	0.0022 ± 0.0002	0.633 ± 0.060	1.4357 ± 0.1400	2.5619 ± 0.2500

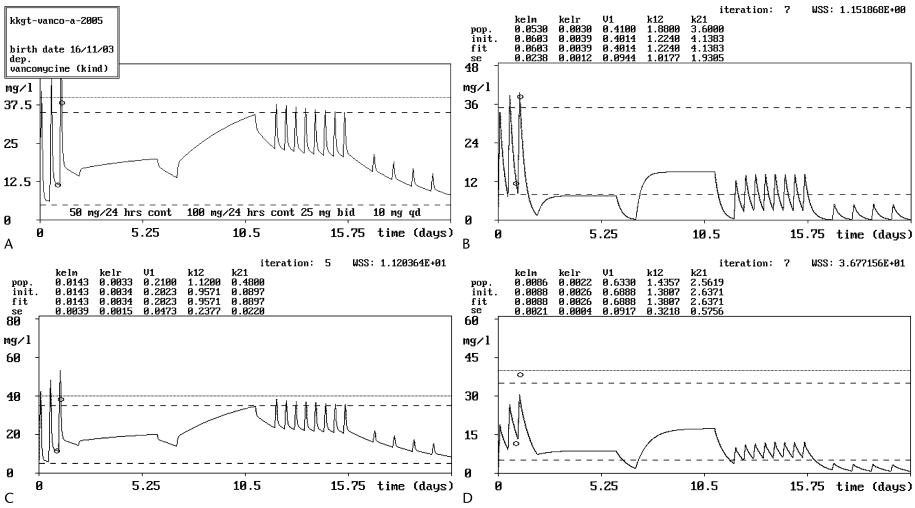

Figure 1. TDM performance of 4 population models A-D (details displayed in **Table 2**) for 4 tested dosing regimens for each model: 1) 50 mg/24 h continuous infusion, 2) 100 mg/24 h continuous infusion, 3) 25 mg/12h, 4) 10 mg/24h. Reprinted with permission from *Therapeutic Drug Monitoring* [47].

Summary and perspectives

There is an abundance of published population PK models which are used to evaluate dosing regimens, implemented in TDM software programs or used to set clinical breakpoints. These applications can be helpful to optimize the efficacy-toxicity balance of antibiotics, but have some limitations and knowledge gaps for which future research is needed.

PK/PD targets

More clarity about the optimal PK/PD target value of some antibiotics for specific clinical indications is required. Without a clear PK/PD target value, dosing recommendations from modelling and simulation studies are difficult to interpret and individualised dosing using TDM is hard to implement.

Assays

Obviously, an assay to measure antibiotic concentrations is essential to provide input for population PK models and to use them in clinical practice. It is important to measure unbound concentrations of antibiotics with a large variability in protein binding. Currently, assays for plasma concentrations are sufficient for clinical use because most current PK/PD target values are based on the concentrations in the central compartment, although the concentration at the infection site might also be important. Research on this topic is ongoing.

Population PK modelling and simulation

For reliable individual dosing recommendations in TDM programs as well as general dosing recommendations for specific patient groups, the choice of a population PK model suitable for that population is crucial. The used PTA acceptance levels in published dosing recommendations should be carefully considered.
PBPK (Physiologically Based PK) modelling methods [13] and joined clinical PK and PD modelling methods [11] are newer modelling methods which need to be further explored.

Clinical validation

It is imperative that the beneficial effects of dosing individualisation using TDM software be more prospectively studied. MIC accuracy and variation should be carefully considered during this process [16].

Interpretation of population PK modelling studies

Little knowledge of PK/PD and modelling prevents a good understanding of dosing recommendations resulting from modelling and simulation studies, clinical breakpoints and TDM. Therefore, good education on these topics is essential to improve antibiotic dosing in clinical practice.

Conclusions

Population PK models are extensively used in clinical practice to optimize antibiotic dosing. However, more clarity about PK/PD targets values, more clinical evaluation studies of model-based dosing recommendations and more clinical outcome studies of TDM are required.

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Chapter 6

Summarizing discussion

Optimizing antibiotic therapy is necessary in a world of increasing antimicrobial resistance and few new antibiotics coming into the market. Population pharmacokinetic (PK) models describe the behaviour of a drug in a body and can be used to improve dosing with the objective to maximize antibiotic efficacy and minimize resistance and toxicity. **Figure 1** gives an overview of the different roles of population PK models in optimizing antibiotic therapy: by dosing (posology) evaluation, setting breakpoints and therapeutic drug monitoring.

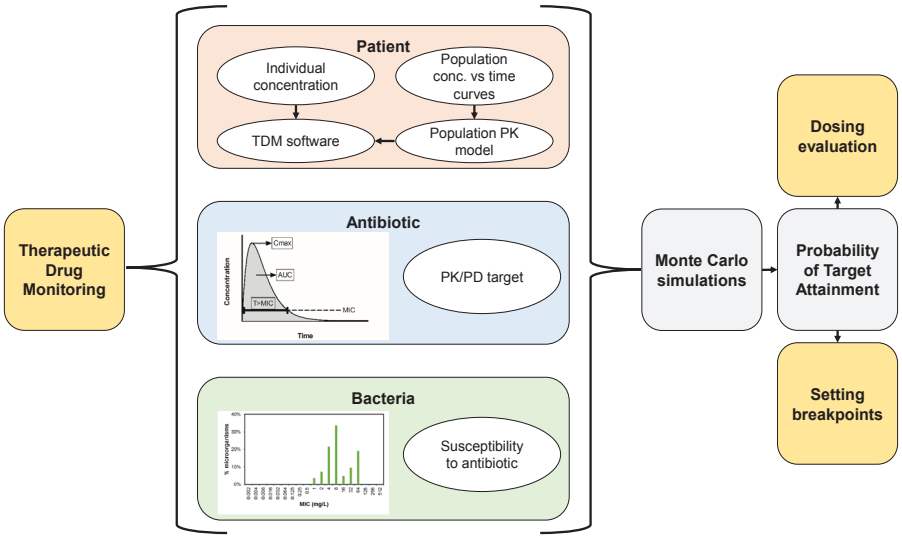

Figure 1. The roles of population PK models in optimizing antibiotic therapy. Reprinted from De Velde et al [1].

This thesis is mainly focused on dosing evaluation of old antibiotics, as their dosing was developed in a period when PK/PD principles were largely unknown with less sophisticated PK methods than currently available. Several examples of dosing evaluation of old antibiotics using population PK modelling are included in this thesis. Additionally, the population PK modelling itself was evaluated by comparing population PK approaches and assessing modelling-related challenges. Recommendations on the (re)development of old and new antibiotics, following from the work conducted in this thesis, were included in a white paper of the STAT-Net group of the COMBACTE consortium.

This chapter summarizes and discusses the findings of the research performed in this thesis.

Amoxicillin and clavulanic acid

In **Chapter 2.1**, the population pharmacokinetics of oral amoxicillin in healthy volunteers was described. Simulations using the population PK model were performed to compare the probability of target attainment (PTA) of current dosing regimens for a target of 40% *f*T>MIC (percentage of the dosing interval that the free concentration remains above the minimal inhibitory concentration). The main finding was a saturable (non-linear) absorption rate with increasing amoxicillin doses. The simulations showed that high doses as well as twice daily regimens are less favourable than regimens with lower doses and higher frequency. For example, the PTA and therefore the breakpoint of amoxicillin 875 mg twice daily was lower than the 500 mg three-times daily regimen. The main conclusion was that the current view of interchangeability of these regimens should therefore be reconsidered, because they are now considered to be equally effective in the summary of product characteristics (SmPC) [2].

A population PK model of oral clavulanic acid was described in **Chapter 2.2** using the same phase I trial data as in **Chapter 2.1**. The population PK analysis revealed that clavulanic acid concentrations were not only highly variable between patients, but also varied between the three daily doses per patient, despite that the food intake was the same around each dose. The population PK model showed that the bioavailability and absorption rate decreased over the day. For the simulations using the population PK model, both %*f*T>Ct and *f*AUC were calculated because the PK/PD index and target were (and are) still unknown for clavulanic acid combined with amoxicillin. A recent paper about clavulanic acid combined with ceftibuten in a murine model reported that the PK/PD index %*f*T>Ct with a threshold concentration (Ct) of 0.5 mg/L best correlated with efficacy and that 20% *f*T>0.5mg/L was correlated with the static endpoint [3]. Our simulations in **Chapter 2.2** showed that 20% *f*T>0,5mg/L was achieved with 125 mg three times daily by 95% of the population. However, as few information about the PK/PD targets of beta-lactamase inhibitors is available, it is unknown if the clavulanic acid-ceftibuten results can be extrapolated to clavulanic acid-amoxicillin. When the new evidence about the target becomes available, our population PK model can be used to further evaluate the current dosing regimens to optimize efficacy and prevent underdosing. More research regarding the target of clavulanic acid is clearly required. We did not find any significant effect of the amoxicillin dose on clavulanic acid PK during population PK modelling, but the literature of the interaction between both compounds is not conclusive. Recent reviews [4, 5] indicate that also for other combinations of beta-lactams and beta-lactamase inhibitors, the interactions are not clear. Investigating these interactions becomes even more important because new combinations with old beta-lactams and new beta-lactamase inhibitors are increasingly studied [6]. Studying these interactions is also included in the EMA guideline on PK/PD of antimicrobials [7].

Chapter 2.3 included a smaller PK study of intravenous amoxicillin/clavulanic acid in patients with and without renal impairment. In all patients, the amoxicillin target of 40% *f*T>MIC for MIC 8 mg/L was achieved, concluding that amoxicillin 1000 mg four times daily is sufficient in both populations. However, the %*f*T>MIC was significantly higher

in the renally impaired patients due to prolonged half-lives, which raised the question whether their dosing is not unnecessary high. More research is necessary to determine the upper amoxicillin concentration limit. Additionally, after the clavulanic acid target becomes clear, the clavulanic acid dosing in renal impairment should be reassessed. We used a PK/PD target of 40% *f*T>MIC in both amoxicillin studies, similar to the target in the amoxicillin EUCAST rationale document [8] and in the EMA background documentation on harmonizing the amoxicillin/clavulanic acid SmPC [9]. Importantly, this target is primarily based on *Streptococcus pneumoniae* and *Haemophilus influenzae* in patients with otitis media and on preclinical studies with *Enterobacterales* [8, 10]. Amoxicillin (or another penicillin) exposure-response relationships for *Enterococcus* species, as well as clinical studies on *Enterobacterales*, are currently unknown. In absence of a better alternative, the 40% *f*T>MIC target is also used for *Enterococcus* species and *Enterobacterales* [11]. It is important to realize that this is an extrapolation from other microbiological species and primarily derived from one indication. More research on exposure-response relationships of amoxicillin is clearly required to further optimize dosing: not only for the monotherapy, but also for the combination with clavulanic acid.

Imipenem

In **Chapter 3.1**, a parametric and a nonparametric population PK model were described for imipenem in critically ill patients. Both models had the same structure and the included covariate (absolute CKD-EPI eGFR on K_e) was identical. The parameter estimates were comparable except for the estimated between-subject variability, which was higher in the nonparametric model. The consequences of this finding were further explored by simulations using both population PK models, which were performed for three dosing regimens and three eGFRs (90, 120 and 150 ml/min). The simulations and PTA's for 50% *f*T>MIC and 100% *f*T>MIC were described in **Chapter 3.2**. Half of the PTA results were similar for both models, while for the other half those of the nonparametric model resulted in lower MICs. These different PTA results may lead to different dosing advices, e.g. decreased dosing following the parametric model and increased dosing following the nonparametric model. This finding was explained by the higher estimated between-subject variability of the nonparametric model. The simulations of both models indicated that 1000 mg four times daily is suitable to reach MICs of 2 mg/L in critically ill patients with eGFRs of 90-120 ml/min, using the 50% *f*T>MIC target. However, for MICs of 2 mg/L and an eGFR of 150 ml/min, and for MICs of 4 mg/L, dosing recommendations could not be given due to largely different PTA values per model (e.g. 1000 mg q6h, eGFR 90 ml/min, MIC 4 mg/L: PTA 90% using the parametric model and 63% using the nonparametric model). More research on the differences between both modelling methods and associated simulations is clearly needed. Besides the simulations, **Chapter 3.2** also included an external validation of both population PK models. The external predictive performance of both models was adequate for subjects with high eGFRs but insufficient for low eGFRs. This was explained by a lack of subjects

with renal impairment in the modelling population. Due to this finding, the previous mentioned simulations were only performed for eGFRs higher than 90 ml/min. This finding emphasizes the importance of external validation of population PK models to confirm or preclude the use of a certain population PK model with the objective to optimize dosing for a specific population.
The two PK/PD targets (50% and 100% *f*T>MIC) for the imipenem simulations were based on the findings of a large prospective study on beta-lactam exposure-relationships in an ICU population [12]. The dosing recommendations following from our imipenem study were based on the 50% *f*T>MIC target because the regression analysis of the original study indicated that an elevated target of 100% *f*T>MIC might not be necessary in this particular ICU population. The imipenem PK/PD targets in EUCAST and EMA documents vary between 30-40% *f*T>MIC (EMA background documentation on harmonizing the imipenem SmPC [13]) and 40-50% *f*T>MIC (EUCAST imipenem rationale document [14]), although these are not developed for ICU patients which might need higher targets [15]. Other imipenem simulation studies in ICU patients used targets of 20-100% *f*T>MIC [16-18], but they did not analyse exposure-relationships. Similar to our conclusions of the amoxicillin study, we recommend that more research on imipenem exposure-relationships is needed to further optimize dosing.

White paper on (re)development of new and old antibiotics

As a member of the STAT-Net group of the European COMBACTE consortium, we contributed to two recommendations on evaluation of new and old antibiotics in STAT-Net's white paper (included in **Chapter 4**). A third recommendation of the white paper was written by other STAT-Net members than the Dutch team, but is also PK/PD related and therefore highly relevant for this thesis.

Recommendation 1: We recommend that phase II and III clinical trials of new antibiotics, particularly those active against MDROs, always apply population PK models to describe and explain PK variability, optimize dose finding, and evaluate outcome data relative to exposure.

The first recommendation is in alignment with current EMA guidelines [7] and FDA guidance's [19]. However, a complete pharmacometric analysis with population PK modelling, evaluation of exposure-response relationships and PTA simulations is not yet fully applied for all recently approved antibiotics. This was again mentioned in a recent minireview resulting from an NIH workshop [20] and very clearly illustrated by Paul Ambrose with 4 examples of antibiotic development failures due to PK/PD shortcomings [21].

Recommendation 2: We recommend a EU–coordinated regulatory procedure for reassessment of old antibiotics and their licensing, particularly those active against MDROs,

which addresses justification of dosing regimens and exposure-response data according to modern PK/PD principles. This should include description of PK/PD targets, development of population PK models, and reassessment of antibacterial spectra.

Many antibiotics were approved long before PK/PD principles were known and sophisticated population PK modelling approaches existed, and their dosing regimens may therefore not be optimal. Reanalysis of old PK data (such as our work on amoxicillin in **Chapter 2.1** and clavulanic acid in **Chapter 2.2**) or organizing new PK studies for old drugs can be performed to investigate PK variability and exposure. However, as PK/PD targets for old drugs are often still lacking (such as for clavulanic acid), it can be cumbersome to formulate dosing recommendations. Future research should focus on this knowledge gap. A second problem is that a coordinated redevelopment programme is currently unavailable, although some EU and NIH projects are set up [22]. The Committee for Medicinal Products for Human Use (CHMP) of the EMA performs "article 30 referrals" to harmonise indications, posology and contra-indications in European SmPC's [9, 13, 23]. For amoxicillin, clavulanic acid and imipenem, referrals are available, but these were not included in the respective chapters in this thesis. Therefore, the posology and PK/PD information in the referrals of amoxicillin, amoxicillin/clavulanic acid and imipenem are discussed below.

For **amoxicillin** (both oral and intravenous), the assessment report of the article 30 referral procedure was published in 2015 [23]. Their recommendation to dose three times daily (TID) matches with the conclusion of our amoxicillin study in **Chapter 2.1**. However, despite that the vast majority of the clinical trials was conducted with the TID regimen, the referral document states that twice daily (BID) dosing can be still an option in patient groups in which TID dosing may give compliance problems. The BID regimen as an alternative for TID dosing remained therefore in the SmPC. Based on our results in **Chapter 2.1**, we would recommend to remove BID dosing as an alternative for TID dosing from the SmPC. We are specifically concerned about BID dosing in patients with compliance problems, because the effect of one missed dose has even more effect with BID dosing compared to more frequent regimens. It was defined that the harmonised dosing recommendations were based on the doses studied in clinical trials and supported by PK/PD data, but, unfortunately, very few details about the PK/PD data are given (e.g. no PK/PD targets and unclear if population PK methods were used). Remarkably, section 5.2 about PK properties in the SmPC only includes Cmax, Tmax, AUC, t1/2 and urine recovery, while trough levels and *f*T>MIC values are missing but clearly more relevant. The posology for renally impaired patients was only based on small studies from 1975-1979 [24-26] without assessing trough levels or %*f*T>MIC.

The article 30 referral documents for **amoxicillin/clavulanic acid** (both oral and intravenous) were published in 2009 [9]. The amoxicillin PK/PD target (T>MIC 40%) is mentioned in the combination referral, although it is unclear if free or total concentrations are meant. The breakpoints for oral amoxicillin are higher than those calculated with our population PK model: 250/125mg TID 1 mg/L versus 0.25 mg/L, 500/125mg TID 2 mg/L versus 0.5 mg/L, 875/125 mg TID 2-4 mg/L versus

1 mg/L, possibly due to a lower PTA cut-off value (not specified in the referral). For renally impaired patients, the 4:1 (500/125 mg) regimen is recommended based on therapeutic clavulanic acid concentrations, although it is unclear which target concentrations are meant as they were not mentioned.
For **imipenem**, the referral documents were published in 2011 [13]. Dosing was evaluated using data of clinical trials as well as by Monte Carlo simulations based on a population PK model (NONMEM). The described PTA's were higher than our findings due to a lower PK/PD target (*f*T>MIC 40% vs. *f*T>MIC 50%) and because no ICU patients were included, who have often a higher distribution and thus lower antibiotic concentrations. However, the referral's conclusions were comparable to our NONMEM model for patients with normal renal function: 500 mg four times daily (QID) and 1000 mg TID have similar drug exposure and 1000 mg QID is recommended for very severe infections and less susceptible pathogens (i.e. MIC 4 mg/L). For renally impaired patients, the posology in the referral was based on a study with 17 patients, but it is unclear if population PK methods were used. In 2018, the marketing authorisation holder performed another population PK analysis (NONMEM) for imipenem using data of the new drug imipenem/cilastatin/relebactam [27]. Dosing regimens in renal insufficiency were updated in the SmPC of imipenem/cilastatin based on model simulations and a lower target of *f*T>MIC 30%.
The main conclusion about these referrals is that population PK analyses are occasionally performed to optimize dosing and the PK/PD evidence in the underlying documents is often unclear. Of course, the objective of article 30 referral procedures is to harmonise SmPC's across Europe, and not to redevelop the old drugs. A coordinated redevelopment procedure for old antibiotics is still necessary.

Recommendation 3: We recommend that future clinical PK/PD studies provide more robust results by a priori determination of the sample size, adjustment for known confounders of the exposure-response relationship, assessment of both microbiological and patient-oriented outcomes, and application of appropriate statistical techniques.

The third recommendation of the white paper was based on a draft review about PK/PD targets, both prepared by STAT-Net members from Bristol (UK). Recently, this review has been published [28]. For beta-lactams, they included 20 clinical PK/PD studies, precluding all studies that did not measure MICs for all patients. Of note, half of these 20 studies did not perform or report statistical analysis. The majority (7) of the remaining 10 studies investigated cephalosporines, while penicillins (1) and carbapenems (2) were much less frequently reported. The current EMA guideline on PK/PD of antibiotics [7] "does not attempt to provide detailed guidance on issues such as methodologies for modelling and simulation". However, the results of this review [28] indicate again that coordinated reassessment of PK/PD targets is needed to justify antibiotic dosing.

Challenges of population PK models in clinical practise

While this thesis was mainly focused on dosing evaluation of old antibiotics using population PK models, these models are also used to set clinical breakpoints and in therapeutic drug monitoring (TDM) software. We summarized challenges of population PK models in clinical practise in a review (**Chapter 5**). Main challenges were knowledge gaps regarding PK/PD targets for all antibiotics, clinical evaluation studies of model-based dosing recommendations and clinical outcome studies of TDM.

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Chapter 7

Perspectives, recommendations and conclusions

Introduction

In this chapter, general perspectives for future research and practical recommendations for clinicians to assess population PK models are described.

General perspectives for future research

The following 4 general perspectives for future research were established, following from the work performed in this thesis and supported by recently published literature.

1. Reassessment of PK/PD targets for effectivity, toxicity and resistance

PK/PD targets of old antibiotics should be reassessed because proper exposure-response studies are lacking for many of these drugs [1, 2].There should not only be a focus on targets for effectivity, but also for toxicity (see **Chapter 2.3**) and resistance. Special attention should be given on the differences between beta-lactam groups (i.e. penicillins, cephalosporins, carbapenems [3]), targets of beta-lactamase inhibitors [4, 5] and special populations such as ICU patients [6]. Additionally, the differences in targets between prophylactic and therapeutic indications should be further examined.

A coordinated regulatory procedure for reassessment of the dosing of old antibiotics, including refining of PK/PD targets, is highly needed. Preferably, private funding at a European level should be used to set up such a project [7].

2. Development of population PK models for beta-lactam/beta-lactamase inhibitor (BL/BLI) combinations

Although we could not find evidence for an interaction between amoxicillin and clavulanic acid (**Chapter 2.2**), the influence of the interaction between the two compounds is not yet clear for this and other combinations [4, 5]. Given the redevelopment of old beta-lactams as a combination with new beta-lactamase inhibitors [8], this topic is increasingly important and should be further investigated. The EMA guideline on PK/PD of antimicrobials [9] instructs to develop population PK models for BL/BLI combinations to support dosing simulations.

A recent review on semi-mechanistic PK/PD models of antibiotic drug combinations, based on *in vitro* experiments, identified 13 publications of which 1 BL/BLI combination [10]. No publications of PK/PD antibiotic interaction models based on clinical data could be found [10].

3. Further exploring PK/PD modelling approaches

Chapter 3 illustrated the similarities and differences between a parametric and a nonparametric model developed with the same data. Main difference was a higher between-subject variability in the nonparametric model. Currently, most population PK models are developed with parametric modelling approaches. One should be aware that the between-subject variability could be lower than estimated with a nonparametric model. The differences between parametric and nonparametric modelling needs to be further investigated.

Although population PK modelling is currently the cornerstone of the pharmacometric

framework, other pharmacometric approaches are also increasingly used during drug (re)development. Quantitative Systems Pharmacology (QSP) and Physiologically Based PK (PBPK) models integrate drug physiochemical and human physiological parameters into mathematical models [11, 12]. Key challenge of these models is the estimation of numerous physiological parameters. Recently, EMA and FDA guidelines on PBPK modelling have been published [13, 14].

4. Improvement of validation and evaluation of population PK models

The importance of an external validation of a population PK model has been shown in the imipenem study described in **Chapter 3.2**. Additionally, a model should be prospectively clinically evaluated [15]. Preferably, a prospective evaluation is not only focused on target achievement, but also on clinical outcome. However, there are few prospective studies evaluating the effects of TDM (using population PK models) or dosing strategies (based on these models) on clinical outcomes, as described in **Chapter 5** and in other recent papers [16]. An important aspect in these studies is the use of measured MICs. In the often referenced DALI-study [17], for only 34% of the patients an actual MIC was available and for the rest of the patients an assumption was necessary (which is often a worst case scenario). Fortunately, more recent studies do include actual MICs [18]. However, the use of a MIC obtained by a single determination is considered inappropriate and MIC variation should be taken in account [19, 20].

Practical recommendations for clinicians to assess population PK models

There is an abundance of published population PK models which are used to evaluate dosing regimens, implemented in TDM software programs or used to set clinical breakpoints. These applications can be helpful to optimize the efficacy-toxicity balance of antibiotics, but have some limitations and knowledge gaps. For reliable individual dosing recommendations in TDM programs as well as safe implementation of general dosing recommendations for specific patient groups, the choice of a population PK model suitable for that population is crucial. Therefore, the following recommendations to assess population PK models were composed for clinicians. When one is reading a paper about a population PK model and considering to implement this model in the local TDM software and/or to use the dosing recommendations in the hospital, the following aspects are recommended to assess:

1. The **modelling population** should be as similar as possible to the local population. Models based on ICU patients should not be used for non-ICU patients, and vice versa.
2. An **external validation** of the population PK model and a **prospective evaluation** of dosing strategies or TDM should be performed to make sure that model-based dosing recommendations are robust.
3. The **sample size** of the populations used for modelling, validation and evaluation should be adequate. The sample size for modelling and validation depends on the number of subjects as well as the number of PK samples (per patient and

per time point) [21, 22]. Sparse PK sampling at few timepoints can be a problem when the number of patients is low. Sample size calculations of TDM evaluation studies result in general in a few hundreds of subjects [23-26].

4. The used **PK/PD target** should be suitable for population and indication.
5. The used **PTA acceptance level** should be considered, although unfortunately not always mentioned in publications. The EMA guideline on PK/PD in antimicrobial development recommends a PTA > 90% for dose selection [9], while the EUCAST uses PTA > 97.5% for breakpoint setting [27]. It is important to realize that with a 90% level, 1 of 10 patients will not achieve therapeutic concentrations.
6. The used **population PK approach** should be assessed. Results of nonparametric models can show higher between-subject variability compared to parametric models.
7. In case of eGFR covariates: the used **eGFR equation** should be assessed and one should be aware of differences because they are not completely interchangeable.
8. The use of **measured MICs** should be checked. When EUCAST cut-off values (ECOFFs) are included instead of measured MICs, this may be a worst case scenario with possibly low PTAs. In case of measured MICs, accuracy and variation should be carefully considered [19].
9. The **used assay** and the measurement of **total or free concentrations** should be assessed. Analysis of free concentrations may be more appropriate in case of high protein binding and/or a high variation in protein binding.

Overall, good education regarding interpretation of population PK studies and MIC-based dosing is essential to improve antibiotic dosing in clinical practice. Little knowledge of PK/PD and modelling prevents a good understanding of dosing recommendations resulting from modelling and simulation studies, clinical breakpoints and TDM.

Conclusions of this thesis

This thesis studied the dosing evaluation of old beta-lactam antibiotics using population PK models. The amoxicillin/clavulanic acid results revealed highly relevant PK issues that were not found during the original phase I studies. Amoxicillin high dose twice daily regimens are less favourable than regimens with lower doses and higher frequencies, contrary to the SmPC that qualifies these regimens as interchangeable. The imipenem results provided more insight to the similarities and differences between parametric and nonparametric modelling approaches. PTA's calculated from simulations using parametric population PK models might be higher than those from nonparametric models; the truth is probably somewhere in between. Practical recommendations for clinicians to assess population PK models were formulated.

The following general perspectives for future research were established:

1. Reassessment of antibacterial PK/PD targets for effectivity, toxicity and resistance
2. Development of population PK models for beta-lactam/beta-lactamase inhibitor

combinations
3. Further exploring PK/PD modelling approaches
4. Improvement of validation and evaluation of population PK models

In conclusion, given the global problem of increasing antibacterial resistance, a coordinated redevelopment procedure for dosing optimization of old antibiotics using PK/PD principles is urgently needed, with a particular priority to the reassessment of PK/PD targets.

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Appendices

Nederlandse samenvatting

Infectieziekten, bacteriën en resistentie

Infectieziekten worden veroorzaakt door ziekteverwekkers zoals bacteriën, virussen, schimmels of parasieten. Antibiotica worden gebruikt voor de behandeling van infectieziekten die door bacteriën worden veroorzaakt. Helaas kunnen bacteriën resistent worden voor antibiotica waardoor deze niet meer goed de infectie kunnen bestrijden. Antibacteriële resistentie is wereldwijd steeds groter wordend probleem door veelvuldig en onjuist gebruik van antibiotica. Voor de behandeling van resistente bacteriën zijn nieuwe antibiotica of hogere doseringen van oude antibiotica nodig. Er zijn echter weinig nieuwe antibiotica op de markt gekomen de afgelopen jaren. Achteraf gezien bleek dat voor sommige antibiotica in de onderzoeksfase te lage doseringen zijn gebruikt, waardoor deze niet effectief genoeg waren om de infectie te bestrijden. Daarnaast is het een bekend probleem dat er voor veel geneesmiddelen slechts één dosisadvies beschikbaar is voor een grote groep mensen terwijl er veel variatie is binnen die groep, waardoor deze vaste dosis bij sommige mensen leidt tot te lage geneesmiddelconcentraties in het lichaam en bij andere mensen juist tot te hoge concentraties.

Het toenemende resistentieprobleem en de problemen bij de ontwikkeling van nieuwe antibiotica laten zien dat het belangrijk is om de doseringen van oude en nieuwe antibiotica te optimaliseren. Voor de optimalisatie van doseringen kunnen farmacokinetische en farmacodynamische (PK/PD) studies worden gebruikt. Farmacokinetiek (PK) beschrijft wat het lichaam doet met een geneesmiddel en omvat de processen absorptie, verdeling, metabolisme en uitscheiding. Farmacodynamiek (PD) beschrijft wat het geneesmiddel doet met het lichaam; in het geval van antibiotica wordt beschreven wat dit middel doet met de bacteriën in het lichaam (bijvoorbeeld het remmen van bacteriële groei). Populatie PK modellen beschrijven de concentraties van het geneesmiddel in een bepaalde populatie en verklaren de verschillen tussen individuele concentraties. Dosisevaluatie van antibiotica kan plaatsvinden door simulaties van deze populatie PK modellen te combineren met PD effectmaten. De toepassing van deze modellen wordt in de huidige internationale richtlijnen aangeraden voor dosisoptimalisatie tijdens de ontwikkeling van nieuwe antibiotica, maar ze zijn niet of nauwelijks gebruikt tijdens de ontwikkeling van oude antibiotica decennia geleden omdat deze populatie PK methoden en de huidige PK/PD kennis toen nog niet bestonden.

Het voornaamste doel van dit proefschrift was de evaluatie van de juistheid van doseringen van oude antibiotica door het ontwikkelen van populatie PK modellen. Ook zijn twee methoden voor het maken van populatie PK modellen met elkaar vergeleken, namelijk parametrische en non-parametrische technieken. Daarnaast zijn uitdagingen bij het proces van dosisevaluatie met behulp van populatie PK modellen geïdentificeerd en aanbevelingen voor het gebruik van deze modellen tijdens geneesmiddelontwikkeling en in de klinische praktijk opgesteld.

Amoxicilline en clavulaanzuur

Hoofdstuk 2 bevat drie PK/PD studies naar amoxicilline en clavulaanzuur. Amoxicilline is een penicilline en behoort tot de groep van beta-lactam antibiotica. Clavulaanzuur

is een beta-lactamase remmer. Beta-lactamases zijn enzymen die bacteriën resistent maken tegen beta-lactam antibiotica. Amoxicilline wordt gecombineerd met clavulaanzuur bij infecties met beta-lactamase producerende bacteriën, maar kan ook alleen worden toegediend. Beide middelen zijn al meer dan 30 jaar beschikbaar en hun doseringen zijn destijds ontworpen zonder het gebruik van populatie PK modellen. In het kader van dit proefschrift zijn populatie PK modellen ontwikkeld voor orale amoxicilline (**Hoofdstuk 2.1**) en clavulaanzuur (**Hoofdstuk 2.2**) in gezonde proefpersonen die amoxicilline/clavulaanzuur tabletten kregen. Voor amoxicilline werd gevonden dat de absorptie verzadigbaar was met toenemende doseringen. Vervolgens zijn verschillende amoxicilline doseringen onderzocht met behulp van simulaties van het model, waarmee werd berekend wat de waarschijnlijkheid van het bereiken van de PK/PD streefwaarde was. Als PK/PD streefwaarde werd in deze studie 40% *f*T>MIC gebruikt, wat inhoudt dat de niet eiwitgebonden geneesmiddelconcentratie boven een bepaalde MIC (minimaal remmende concentratie van het antibioticum, bepaald in het microbiologisch laboratorium) moet liggen gedurende minimaal 40% van het doseerinterval. Bij het bereiken van deze PK/PD streefwaarde, die in eerdere studies is bepaald, is er een hoge waarschijnlijkheid van succesvolle behandeling. Voor een hoge effectiviteit van beta-lactam antibiotica, zoals amoxicilline, is het belangrijk om frequent te doseren gedurende de dag. Dit blijkt ook uit de belangrijkste conclusie van deze studie, namelijk dat twee maal daags 875 mg niet uitwisselbaar is met drie maal daags 500 mg, in tegenstelling tot wat in de bijsluiter van amoxicilline/clavulaanzuur wordt genoemd. Voor clavulaanzuur werd gevonden dat de clavulaanzuur absorptie gedurende de dag afnam, waardoor concentraties in de ochtend hoger waren dan in de avond. Er konden helaas geen doseeradviezen worden opgesteld voor clavulaanzuur, aangezien (nog steeds) onbekend is wat de PK/PD streefwaarde is voor een hoge waarschijnlijkheid van succesvolle behandeling.

Hoofdstuk 2.3 beschreef de farmacokinetiek van intraveneuze amoxicilline in patiënten die behandeld werden met amoxicilline/clavulaanzuur infusen. Dit onderzoek werd gedaan om de amoxicilline dosering te evalueren voor zowel patiënten met verminderde nierfunctie als patiënten met normale nierfunctie. Met vier maal daags 1000 mg amoxicilline werd de PK/PD streefwaarde van 40% *f*T>MIC behaald voor beide patiëntengroepen. In patiënten met een verminderde nierfunctie waren de amoxicilline concentraties echter veel hoger dan in patiënten met een normale nierfunctie. Op dit moment wordt alleen een ondergrens van amoxicilline concentraties (*f*T>MIC) gehanteerd, maar het is onbekend wat de bovengrens is. Hier dient meer onderzoek naar te worden verricht.

Imipenem

Hoofdstuk 3 gaat over het intraveneuze beta-lactam antibioticum imipenem, dat beschikbaar is sinds de jaren 80. In **Hoofdstuk 3.1** werd de ontwikkeling van en vergelijking tussen een parametrisch en een non-parametrisch populatie PK model van imipenem in kritisch zieke patiënten (opgenomen op de intensive care) beschreven. Deze vergelijking werd gedaan omdat er weinig bekend is over de verschillen tussen beide methoden. Parametrische populatie PK methoden gaan uit van een bekende verdeling van populatie parameters, terwijl non-parametrische methoden geen

aanname doen over deze verdeling. Bij de twee imipenem modellen zijn geen verschillen gevonden in structuur en covariaten. De parameter schattingen waren vergelijkbaar met uitzondering van de interindividuele variabiliteit, die hoger was voor het non-parametrische model. In **Hoofdstuk 3.2** zijn deze modellen vervolgens gevalideerd met een tweede database en tevens zijn ze gebruikt voor simulaties ter evaluatie van verschillende doseringen. De externe validatie liet zien dat de twee modellen geschikt waren voor patiënten met een goede nierfunctie, maar dat ze niet geschikt waren voor patiënten met een verminderde nierfunctie. Dit kon verklaard worden omdat de modellen waren gebouwd met weinig patiënten met een verminderde nierfunctie. De doseringssimulaties werden vervolgens alleen uitgevoerd voor patiënten met een goede nierfunctie. Vijftig procent van deze doseringssimulaties gaf dezelfde resultaten voor beide modellen, terwijl de andere helft verschillend was. Deze verschillende resultaten leidden tot andere doseeradviezen, waarbij volgens het non-parametrische model hoger gedoseerd moest worden dan voor het parametrische model. Dit werd verklaard door een hogere interindividuele variabiliteit van het non-parametrische model.

Aanbevelingen voor de (her)ontwikkeling van antibiotica

Als deelnemer aan het Europese consortium COMBACTE (Combatting Bacterial Resistance), heeft de auteur van dit proefschrift bijgedragen aan een publicatie met aanbevelingen voor ontwerp en analysestrategieën om nieuwe en oude antibiotica te evalueren (**Hoofdstuk 4**). De twee PK/PD aanbevelingen opgesteld door de auteur van dit proefschrift waren:

1) dat altijd populatie PK modellen moeten worden gebruikt tijdens klinisch onderzoek met antibiotica om PK variabiliteit te verklaren, doseringen te optimaliseren en de concentratie-effect relaties te evalueren, en
2) dat een Europese procedure moet worden opgezet voor de herontwikkeling van oude antibiotica met als doel de optimalisatie van doseringen met populatie PK modellen, de evaluatie van concentratie-effect relaties en voor het vaststellen van PK/PD streefwaarden.

In het kader van dit proefschrift is de onderbouwing van de doseringen in de bijsluiters van amoxicilline, amoxicilline/clavulaanzuur en imipenem nader bekeken. Ondanks dat deze bijsluiters recent nog zijn herzien, bleek dat deze doseringen niet altijd waren gebaseerd op populatie PK modellen en dat de gehanteerde PK/PD streefwaarden vaak onduidelijk waren.

Gebruik van populatie PK modellen in de klinische praktijk

Populatie PK modellen worden niet alleen gebruikt tijdens geneesmiddelenonderzoek, maar ook in de klinische praktijk. Naast evaluatie van algemene doseerstrategieën (wat dit proefschrift voornamelijk behandelt), worden de modellen ook gebruikt voor het vaststellen van klinische breekpunten (die worden gebruikt in microbiologische laboratoria) en voor het geven van individuele doseeradviezen op basis van gemeten concentraties in het bloed. In **Hoofdstuk 5** werden diverse uitdagingen bij het gebruik van deze modellen in de klinische praktijk beschreven. De belangrijkste uitdaging is

gebrek aan (duidelijke) PK/PD streefwaarden, klinische studies ter evaluatie van populatie PK model gebaseerde doseerstrategieën en klinische studies ter evaluatie van individuele doseeradviezen op basis van gemeten concentraties in het bloed.

Aanbevelingen voor het gebruik van populatie PK modellen in de klinische praktijk

Na een samenvattende discussie in **Hoofdstuk 6**, worden in **Hoofdstuk 7** aanbevelingen gegeven voor apothekers, artsen en anderen die populatie PK modellen uit de literatuur gebruiken voor dosisoptimalisatie van antibiotica in de klinische praktijk:

1) De populatie die gebruikt is voor het bouwen van het model dient zo veel mogelijk gelijk te zijn aan de lokale populatie waarvoor het model beoogd is.
2) Een externe validatie van het model en een prospectieve evaluatie van de dosisadviezen dienen te zijn uitgevoerd om te verifiëren of het model robuust is.
3) Het aantal patiënten en het aantal monsters per patiënt, gebruikt om het model te bouwen, moeten voldoende hoog zijn.
4) De gebruikte PK/PD streefwaarde moet geschikt zijn voor de populatie en de indicatie.
5) De gebruikte afkapwaarde voor de waarschijnlijkheid van het bereiken van deze PK/PD streefwaarde moet hoog genoeg zijn.
6) De gebruikte populatie PK methode moet worden beoordeeld. Non-parametrische modellen kunnen een hogere interindividuele variabiliteit laten zien dan parametrische modellen, wat kan leiden tot andere doseeradviezen.
7) In het geval dat nierfunctie een covariaat is, moet de gebruikte nierfunctie formule worden beoordeeld. Deze formules zijn niet altijd goed onderling uitwisselbaar.
8) De methode voor het vaststellen van MICs moet worden beoordeeld. Bij gemeten MICs moeten de juistheid en variatie worden bekeken. Bij populatie MICs moet rekening worden gehouden met een onderschatting van de waarschijnlijkheid van het bereiken van de PK/PD streefwaarde.
9) De gebruikte analysemethode en de meting van totale of alleen ongebonden concentraties moet worden beoordeeld.

Aanbevelingen voor vervolgonderzoek

De volgende aanbevelingen voor vervolgonderzoek werden opgesteld:

1) Herbeoordeling van PK/PD streefwaarden voor effectiviteit, toxiciteit en resistentie van antibiotica, bij voorkeur door middel van een Europees gecoördineerde procedure.
2) Ontwikkeling van populatie PK modellen voor combinaties van beta-lactam antibiotica met beta-lactamase remmers.
3) Meer onderzoek naar de verschillen tussen de diverse methoden voor het ontwikkelen van PK/PD modellen, zoals parametrische en nonparametrische populatie PK methoden.
4) Verbetering van validatie en evaluatie van populatie PK modellen.

Conclusies van dit proefschrift

In dit proefschrift zijn diverse voorbeelden van dosisevaluatie van oude beta-lactam antibiotica met behulp van populatie PK modellen opgenomen. De amoxicilline/clavulaanzuur studies lieten relevante PK informatie zien die niet tijdens de geneesmiddelontwikkeling waren opgemerkt. Ons onderzoek liet zien dat twee maal daags een hoge dosis amoxicilline minder gunstig is dan drie maal daags een lagere dosis, in tegenstelling tot de bijsluiter die beide als evenwaardig beschouwt. De imipenem studies hebben meer inzicht gegeven in de gelijkenissen en verschillen tussen parametrische en nonparametrische populatie PK methoden. Sommige verschillen leidden tot andere doseeradviezen, waarbij volgens het non-parametrische model hoger gedoseerd moest worden dan volgens het parametrische model.

Aanbevelingen voor het beoordelen van populatie PK modellen in de literatuur en voor vervolgonderzoek zijn opgesteld.

Gezien het wereldwijde probleem van antibacteriële resistentie en het gebrek aan nieuwe antibiotica, is een gecoördineerde procedure voor de herontwikkeling van oude antibiotica dringend nodig, waarbij de herbeoordeling van PK/PD streefwaarden prioriteit zou moeten hebben.

List of publications

Related to this thesis

de Velde F, de Winter BCM, Koch BCP, van Gelder T, Mouton JW, Combacte-Net consortium. Non-linear absorption pharmacokinetics of amoxicillin: consequences for dosing regimens and clinical breakpoints. Journal of Antimicrobial Chemotherapy. 2016;71:2909-17.

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de Kraker MEA, Sommer H, de Velde F, Gravestock I, Weiss E, McAleenan A, Nikolakopoulos S, Amit O, Ashton T, Beyersmann J, Held L, Lovering AM, MacGowan AP, Mouton JW, Timsit JF, Wilson D, Wolkewitz M, Bettiol E, Dane A, Harbarth S, Combacte-Net Consortium. Optimizing the Design and Analysis of Clinical Trials for Antibacterials Against Multidrug-resistant Organisms: A White Paper From COMBACTE's STAT-Net. Clinical Infectious Diseases. 2018;67:1922-31.

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de Velde F, de Winter BCM, Neely M, Strojil J, Yamada W, Harbarth S, Huttner A, van Gelder T, Koch BCP, Muller AE, Combacte-Net consortium. Parametric and nonparametric population pharmacokinetic models to assess probability of target attainment of imipenem concentrations in critically ill patients. Submitted.

de Velde F, Abdulla A, Muller AE, van Gelder T, Koch BCP. Intravenous amoxicillin in patients with various degrees of renal function: are we dosing adequately? Submitted.

Other publications

de Velde F, Alffenaar JW, Wessels AM, Greijdanus B, Uges DR. Simultaneous determination of clarithromycin, rifampicin and their main metabolites in human plasma by liquid chromatography-tandem mass spectrometry. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences. 2009;877:1771-7.

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Dankwoord

Ruim 6 jaar geleden startte ik met dit promotieonderzoek naast een baan als ziekenhuisapotheker. Uiteraard heeft het soms wat bloed, zweet en tranen gekost, maar bovenal kijk ik terug op een bijzonder plezierige en leerzame periode waarvan dit proefschrift het trotse resultaat is.
Dit proefschrift had ik niet kunnen voltooien zonder de hulp van een aantal mensen. Graag wil ik een aantal van hen in het bijzonder bedanken.

Prof.dr. Mouton, beste Johan, het is ontzettend verdrietig dat je de verdediging van dit proefschrift niet meer kunt meemaken. Je wordt enorm gemist door velen van ons. Ik kijk met veel plezier terug aan de vele gesprekken waarbij je me inspireerde tot nieuwe ideeën. Dankzij het Europese consortium hebben we vele reizen samen gemaakt: Freiburg, Toulouse, San Diego, Liverpool, Parijs, Sevilla, Brussel, Manchester - dat waren fantastische ervaringen! Bedankt voor het delen van je kennis en alle mogelijkheden die je me hebt gegeven.

Prof.dr. van Gelder, beste Teun, bedankt voor alle support die je hebt gegeven, eerst als tweede promotor en na het overlijden van Johan als eerste promotor. Ook voor de onderwijs coördinatie binnen de apotheek hebben we altijd prettig samengewerkt. Uiteraard ben ik zeer blij met je verhuizing naar het LUMC en vooral in de laatste fase van het proefschrift was dit een handige bijkomstigheid.

Dr. Koch, beste Birgit, begin 2014 spraken Johan en jij over dit promotieonderzoek en ik ben je zeer dankbaar dat je voor de uitvoering ervan aan mij hebt gedacht. Je hulp bij het koers houden van de voortgang van het onderzoek heb ik zeer gewaardeerd.

Dr. Muller, beste Anouk, terugdenkend aan onze kennismaking in het Maasstad ziekenhuis, hadden we niet kunnen indenken welke rol je bij de afronding van mijn proefschrift zou spelen. Ik ben je zeer erkentelijk voor het vervullen van deze rol en kan niemand anders bedenken die dit beter had kunnen doen.

Prof.dr. Knibbe, prof.dr. Spriet en prof.dr. Verbon, hartelijk dank voor het beoordelen van mijn proefschrift, wat ongetwijfeld een uitdaging was ten tijde van de pandemie.

Prof.dr. Guchelaar, dr. van Hest en dr. de Winter, veel dank voor het plaatsnemen in de grote commissie op 14 oktober.

Alle modellers van het wekelijkse nonmem overleg (Brenda, Louise, Linda, Rixt, Johan, Anouk, Birgit, Stijn, Sabine, Willem Jan, Sanne) wil ik bedanken voor hun hulp bij het bouwen van de modellen. Brenda: bedankt voor je geduld tijdens het beantwoorden van al mijn waarom vragen. Je hebt me samen met Linda en Rixt enorm verrast met een vrijgezellenfeestje in Lissabon tijdens het PAGE congres. Ook aan PAGE in Chersonissos en IATDMCT in Kyoto bewaar ik mooie herinneringen met jullie en anderen.

Prof.dr. Neely, dear Michael, it was such a pleasure to visit your lab in the spring of 2017. Thank you very much for the hospitality and all scientific discussions. Prof.dr. Jelliffe, dear Roger, unfortunately you passed away shortly ago. I greatly appreciated our lunch and museum meetings. Dr. Yamada, dear Walter, thank you so much for your patience to teach me all ins and outs of nonparametric modelling. I have good memories of our talks while enjoying the stunning Hollywood view from the lab. Dear Aida, many thanks for your help to organize my visit to LAPK. I still miss our brisket taco lunches!

All COMBACTE's STATnet members: it was a great experience to be a member of a European consortium and I enjoyed all meetings very much. Prof.dr. Stephan Harbarth, thank you very much for your contributions to the imipenem study.
Dr. Angela Huttner: thank you so much for your hospitality in Geneva and all your help with the imipenem study.

Alle collega's van de Apotheek, in het bijzonder de SKZ collega's (Heleen, Lidwien, Frederike en Robert) en de onderwijs collega's (Arnold, Patricia, Teun en Floor), en de afdeling Medische Microbiologie en Infectieziekten in het Erasmus MC: bedankt voor jullie interesse in het promotieonderzoek. Roomies (Monique, Zina, Floor): heel gezellig dat we nog steeds contact hebben. Alan en Soma: bedankt voor de samenwerking in het kader van het addendum van de EXPAT studie. Beide secretariaten: veel dank voor alle ondersteuning.

Sophie Ladage en Katia Pires: bedankt voor jullie hulp bij de amoxicilline/clavulaanzuur studies en veel succes met jullie carrières.

Alle collega's uit de apotheek van het LUMC: ik kan me geen betere collega's bedenken dan jullie! Het was de beste keuze ooit om te verhuizen naar deze warme organisatie. Veel dank voor alle betrokkenheid en interesse bij de afronding van mijn proefschrift.

Paranimfen: wat fijn dat jullie mij willen bijstaan tijdens de verdediging! Floor, we hebben veel samengewerkt voor het onderwijs, maar omdat we lang kamergenoten zijn geweest, heb je ook veel van de ups en downs van het promotieonderzoek meegemaakt. Dank voor je relativeringsvermogen en positiviteit! Daphne, na 6 jaar Farmacie in Groningen zijn we allebei naar het westen verhuisd. Afgelopen jaren waren extra bijzonder aangezien onze dochters slechts 6 weken in leeftijd verschillen. De sauna of shop uitjes zonder kinderen zijn nog wel noodzakelijk om goed bij te praten! Het geeft me rust en vertrouwen dat jullie straks achter me staan.

Lieve vrienden: allereerst natuurlijk het In-Side-Out genootschap (Mirte, Jenny, Daphne, Maeike, Marianne, Rosalie, Mariet, Mieke, Johanna, Evi: gelukkig hebben we nog steeds contact na 17 jaar en is de voorspelling na 7 jaar dus niet uitgekomen!), Annemarie, Yara, Jozien, Anneliene, Tanja, Marie-Claire, orkestvrienden en eenieder met onuitputtelijke interesse in het onderzoekstraject: dank hiervoor, maar vooral

ook voor de broodnodige ontspanning daarbuiten. Floris: mijn allerbeste cellomaatje, heel veel dank voor het ontwerp van de omslag en uitnodiging.

Lieve schoonfamilie: Jan, Maria en Marieke, wat heb ik het getroffen met jullie als schoonouders en schoonzusje. Bedankt voor jullie steun en liefde.
Lieve familie: papa en mama: bedankt voor de zorgeloze jeugd en vrijheid die jullie me altijd hebben gegeven. Lieve Menno en Dieuwke: wanneer ik terugdenk aan onze jeugd, denk ik uiteraard aan de ham-kaas croissants bij de lunch en de sinaasappelthee, maar vooral aan de heerlijk lange zomers in Italië. Mooi om te zien hoe jullie samen met Eva en Wout de traditie van Italiaanse zomers doen herleven. Ik hoop dat we over een aantal jaren weer zorgeloos met zijn allen dit prachtige land kunnen bezoeken.

Lieve Thijs en Hannah: jullie zijn het beste medicijn voor alles. Ik hou van jullie en kijk uit naar elke dag van onze toekomst samen! *Love is all you need.*

About the author

Femke de Velde was born on the 16th of October 1985 in Zwolle, the Netherlands. After graduating from secondary school at the Gymnasium Celeanum in Zwolle in 2003, she started to study Pharmacy at the University of Groningen. During the Master programme, she performed a research project of six months at the hospital pharmacy of the University Medical Center Groningen under supervision of prof. dr. Donald Uges and dr. Jan-Willem Alffenaar. This project was focused on the pharmacokinetics of clarithromycin and rifampicin in patients with a *Mycobacterium ulcerans* infection.

After having obtained her Master's degree in 2009, Femke travelled through South-America for three months and subsequently worked as a community pharmacist for three months. In April 2010, she started a residency in hospital pharmacy in the Maasstad hospital in Rotterdam (supervisor: drs. Henk Spijker) which she completed in March 2014. Subsequently, Femke started with a PhD project at the department of Medical Microbiology and Infectious Diseases in combination with a position as a hospital pharmacist at the department of Pharmacy, both in the Erasmus Medical Center in Rotterdam. As a hospital pharmacist, she worked at the pharmacy of the Sophia Children's Hospital and was also responsible for the pharmacological education of the Bachelor Medicine. The PhD project on beta-lactam antibiotics was supervised by prof. dr. Johan Mouton, prof. dr. Teun van Gelder and dr. Birgit Koch. After prof. dr. Johan Mouton sadly passed away in the summer of 2019, the microbiological supervision was continued by dr. Anouk Muller. As a part of her PhD, in the spring of 2017 Femke spent one month at the Laboratory of Applied Pharmacokinetics in Los Angeles (USA), where she was trained in nonparametric population PK modelling under the supervision of prof. dr. Michael Neely. As of August 2018, Femke works as a hospital pharmacist in the Leiden University Medical Center.

Femke is married to Thijs Giezen and they live in Leiden with their daughter Hannah.

PhD Portfolio

Name PhD student	Femke de Velde
Erasmus MC Department	Medical Microbiology and Infectious Diseases
Research School	Molecular Medicine postgraduate school
PhD period	April 2014 – June 2020
Promotors	prof. dr. J.W. Mouton † prof. dr. T. van Gelder
Copromotors	dr. B.C.P. Koch dr. A.E. Muller

PhD TRAINING	Year	ECTS
General courses		
Systematic Literature Retrieval in Pubmed [Erasmus MC]	2014	0.3
Systematic Literature Retrieval in other databases [Erasmus MC]	2014	0.2
Endnote [Erasmus MC]	2015	0.2
Biomedical English Writing and Communication [Erasmus MC]	2015	1.5
Research Integrity [Erasmus MC]	2016	0.3
BROK "Basiscursus Regelgeving Klinisch Onderzoek" [NFU]	2016	1.0
Specific courses and workshops		
Advanced Antimicrobial PK/PD Modelling and Simulation [ESCMID Postgraduate technical workshop, Liverpool, UK]	2014	0.7
Population PK and PK/PD modelling with NONMEM [Centre for Human Drug Research, Leiden]	2014	1.0
Application of PK/PD Models and PK/PD for Black Belts [ICAAC/ICC, San Diego, USA]	2015	0.3
Antimicrobials for children [Stichting Infecties bij Kinderen, Rotterdam]	2016	0.6
Time management [VVAA, Utrecht]	2016	0.5
Conferences and symposiums		
PAGE conference [Chersonissos, Greece], poster presentation	2015	1.0
ICAAC/ICC conference [San Diego, USA], poster presentation	2015	1.0
ISAP post-conference meeting [San Diego, USA], oral presentation	2015	0.3
IATDMCT conference [Rotterdam], oral presentation	2015	1.3
NVZA hospital pharmacy conference [Den Bosch], poster presentation	2015	0.3
SWAB symposium "Prevention of infectious diseases" [Utrecht]	2015	0.2
Lage landen symposium "Acute intoxications" [Gent, Belgium]	2015	0.3
ECCMID conference [Amsterdam]	2016	1.0
PAGE conference [Lisbon, Portugal]	2016	1.0
SWAB symposium "Extremes in antimicrobial treatment" [Utrecht]	2016	0.2
IATDMCT conference [Kyoto, Japan], poster presentation	2017	1.3

Research meetings		
PK/PD modelling team weekly meetings [Erasmus MC]	2014-2019	3.0
STAT-Net yearly meetings [Freiburg / Paris / Manchester]	2014-2016	2.0
COMBACTE general assembly yearly meetings [Toulouse / Seville / Brussels]	2015-2017	2.0

Other presentations		
PK/PD and antimicrobial resistance [EMA PRAC meeting, Utrecht]	2016	0.3
PK/PD of antibiotics [pharmacology meeting, Erasmus MC]	2016	0.1
TDM of antimicrobials in children [paediatric pharmacology meeting, Erasmus MC]	2016	0.1
Dosing and breakpoints of old antibiotics revisited [NCOH meeting, Rotterdam]	2017	0.1

TEACHING	**Year**	**ECTS**
Lecturing		
Therapeutic drug monitoring [master students Medicine, Erasmus MC]	2014-2017	0.8
Prescribing drugs [master students Medicine, Erasmus MC]	2014-2015	0.3
Complementary and alternative medicine [master students Medicine, Erasmus MC]	2015	0.3
TDM of antimicrobial therapy [research master infection and immunity, Erasmus MC]	2015	0.3
Administration of parenteral medication [paediatric nurses, Erasmus MC]	2015	0.1
Paediatric dosing [master students Medicine, Erasmus MC]	2015-2017	0.5
Psychopharmacology [master students Medicine, Erasmus MC]	2016-2017	0.5
Medication safety [bachelor students Medicine, Erasmus MC]	2016-2017	0.5
PK/PD and TDM of antimicrobials [paediatric residents, Rotterdam]	2016-2018	0.7
Population PK and TDM of antimicrobials [microbiology residents, Lunteren]	2017	0.3
Infectious diseases [pharmacy assistants, Erasmus MC]	2017	0.1
Online mini lectures "Amoxicillin/clavulanic acid" and "Oral anticonception" [master students Medicine, Erasmus MC]	2017	0.2
E-learnings "Benzodiazepines" and "Contraception" [bachelor students Medicine, Erasmus MC]	2017	2.0

Supervising Master thesis		
Katia Pires (6 month research thesis)	2017	2.0

Teaching courses, workshops and symposiums		
Teach the teacher course I [Erasmus MC]	2015	1.0
BKO workshop "Dealing with groups" [Erasmus MC]	2016	0.2
BKO workshop "Designing exams" [Erasmus MC]	2017	0.2
Student-teacher symposium "Diversity" [Erasmus MC]	2017	0.5