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# Using Pharmacokinetic and Pharmacodynamic Principles to Evaluate Individualisation of Antibiotic Dosing – Emphasis on Cefuroxime

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### Abstract

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Cefuroxime is a renally eliminated antibiotic used against a variety of different bacterial infections. The pharmacokinetics (PK) for cefuroxime was studied in 97 hospitalized patients using population analysis. To be able to measure cefuroxime in human serum a new sensitive analytical method was developed using mass spectrometry detection. The method was validated and shown to be sensitive and selective. Cystatin C was found to be a better covariate for cefuroxime clearance compared to the traditionally used creatinine clearance (CL<sub>Cr</sub>). This relation might be useful when designing dosing strategies for cefuroxime and other renally eliminated drugs.

The time-courses of the biomarkers C-reactive protein (CRP), serum amyloid A (SAA), interleukin-6 (IL-6) and body temperature were studied for the first 72 hours of cefuroxime treatment and was related to the duration of illness previous treatment with cefuroxime and to time to step-down of treatment. When duration of illness was short, CRP and SAA were showed increasing levels. None of the biomarkers could be used to differentiate between early or late step-down of therapy.

By use of known PK and pharmacodynamic (PD) principles, dosing strategies based on CL<sub>Cr</sub> for cefuroxime were estimated using minimization of a risk function. The risk function was constructed with the aim to expose patients to cefuroxime concentration above minimum inhibitory concentration (MIC) for 50 % of the dosing interval and to minimize the amount of drug administered in excess to reach the aim. Based on evaluation using wild type MIC distributions for *Escherichia coli* and *Streptococcus pneumoniae* improved dosing strategies were selected.

In vitro experiments were performed exposing *Streptococcus pyogenes* to constant concentration of benzylpenicillin, cefuroxime, erythromycin, moxifloxacin or vancomycin. A semi-mechanistic PK/PD model characterizing the time-course of the antibacterial effect was developed using all data simultaneously. Internal validation showed the model being predictive and robust.

**Keywords:** Cefuroxime, pharmacokinetics, pharmacodynamics, dosing individualisation, NONMEM

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To all patients contributing to this thesis



# Papers discussed

This thesis is based on the following papers, which will be referred to in the text by the Roman numerals assigned below.

- I     Determination of cefuroxime in human serum or plasma by liquid chromatography with electrospray tandem mass spectrometry.  
Anders Viberg , Marie Sandström, Britt Jansson  
Rapid Commun. Mass Spectrom. 2004; 18: 707–710
- II    A population pharmacokinetic model for cefuroxime with cystatin C as renal function marker.  
Anders Viberg, Anders Lannergård, Anders Larsson, Otto Cars, Mats O Karlsson, Marie Sandström.  
In press Br J Clin Pharmacol
- III   The time course of the biomarkers body temperature, serum amyloid A protein (SAA), C-reactive protein (CRP) and interleukin- (IL) 6 during initial treatment with cefuroxime in patients with bacterial infections.  
Anders Viberg, Anders Lannergård, Otto Cars, Mats O Karlsson, Marie Sandström, and Anders Larsson.  
Manuscript
- IV    Individualization of Cefuroxime Dosage using Pharmacodynamic targets, MIC distributions and Minimization of a Risk Function.  
Anders Viberg, Otto Cars, Mats O Karlsson, and Siv Jönsson  
Manuscript.
- V     A Semi-Mechanistic Pharmacokinetic-Pharmacodynamic Model for the Assessment of Antimicrobial Activity of Antibacterial Agents from Kill Curve Experiments.  
Elisabet Nielsen, Anders Viberg, Elisabeth Löwdin, Otto Cars, Mats O Karlsson and Marie Sandström  
Manuscript

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# Abbreviations

AUC	area under plasma concentration time curve
$B_{\max}$	maximum bacterial concentration
CDD	case deletion diagnostics
cfu	colony forming units
CL	clearance
CLcr	creatinine clearance
CRP	C-reactive protein
CysC	cystatin C
EC <sub>50</sub>	concentration giving one-half the maximum achievable effect
$E_{\max}$	maximum achievable effect
GFR	glomerular filtration rate
IIV	interindividual variability
IL-6	interleukin 6
IOV	interoccasion variability
$k_{\text{death}}$	rate constant for natural death of bacteria
$k_e$	rate constant for effect-delay
$k_{\text{growth}}$	rate constant for growth of bacteria
LC	liquid chromatography
LLOQ	lower limit of quantification
MIC	minimum inhibitory concentration
MS	mass spectrometry
OFV	objective function value
PD	pharmacodynamics
PK	pharmacokinetics
RF	risk function
SAA	serum amyloid A
Scr	serum creatinine
$T_{>\text{MIC}}$	time with concentration above minimum inhibitory concentration
% $T_{>\text{MIC}}$	percent of dosing interval with concentration above minimum inhibitory concentration
V	volume of distribution
V1	central volume of distribution
V2	peripheral volume of distribution
WT	body weight
XV	cross validation
$\gamma$	sigmoidicity factor



# Introduction

## Background

For more than half a century, antibiotics have been available for the treatment of bacterial infections. Although the use of antibiotics is well established in clinical practice according to data from the World Health Organisation infection was the second most common cause of death, causing 25 % of deaths worldwide in 1998 [1]. The use and development of antibiotics are far from uncomplicated. When performing clinical trials with antibiotics it can be difficult to measure a clinical endpoint. In some diagnoses it is possible to measure if the pathogen has been eradicated but often a subjective cure/not cure judgement is the only available endpoint measurement [2]. In addition, the development of bacterial resistance is an increasing problem that necessitates dosing strategies to be adapted to situations where the pathogen susceptibility has been altered.

In decision-making on treatment with antibiotics a number of different factors need to be taken into consideration. The first is whether antibiotics are needed at all. Most infections are viral and do not respond to antibiotics. However, the clinical differentiation between viral and bacterial infections can sometimes be difficult. Secondly, if a bacterial infection is suspected, an antibiotic with the appropriate antibacterial spectrum should be chosen. Thirdly, the dose and dosing interval needs to be considered. The dose size and dosing interval should result in an exposure of antibiotic concentration that is sufficient to eradicate the infecting pathogen. However, the level of side effects has to be tolerable. Due to variation between patients different individuals might have different shapes of the concentration-time exposure of the drug following administration. Some of the variability can often be explained by different patient factors and can therefore be accounted for in individualized dosing strategies [3-5]. Lastly, the treatment duration needs to be decided upon. For most infections, treatment guidelines include a standardized length of treatment. However, the evidence base for these recommendations is often weak. The optimal length of treatment will depend on many factors including the type of infection, the patient's condition and underlying diseases and especially the status of the host defence mechanisms. For example, a healthy young patient probably does not need the same treatment length as a neutropenic elderly patient. To decide the time point when the drug should be discontinued, the support of validated biomarkers associated with the bacterial infection would be of great value.

## PK-PD principles

Giving a patient a drug results in a concentration of the drug in the body. The shape of the concentration-time profile is dependent on both the given dose size and how the body absorbs, distributes, metabolizes and eliminates the drug. This is called pharmacokinetics (PK) and is routinely described by mathematical models. The study of the action or effects the drug has on the individual is called pharmacodynamics (PD) and is also routinely described by mathematical models. As one would expect, the PD of a drug are influenced by the PK of that drug and this relation (the PK/PD relation) is also often described mathematically. One benefit of using mathematical models to describe the PK, PD and PK/PD relationship of a drug is that it is not only possible to describe what happens in the studied patient population, but also possible to predict concentration and effect in new individuals during settings other than those studied.

## MIC distribution

Antibiotic PK/PD integrates the complex relationship between organism susceptibility and patient pharmacokinetics. The most routinely, as well as the clinically used method for determining pathogen susceptibility is minimum inhibitory concentration (MIC) testing [6]. This is performed in vitro where bacteria are exposed to a constant concentration of antibiotics for between 16-20 hours. The lowest concentration at which visible growth does not occur, usually determined in two-fold dilutions, is defined as the MIC. This is a point estimate that does not take the time course of the killing into consideration. One strain of bacteria might have different values of MIC depending on measurement methodology and measurement error, however, most differences will be due to the different susceptibility to the specified drug between individual strains belonging to the same species. By combining the result of large number of MIC determinations from individual strains it is possible to describe the distribution of MIC for the species. This is exemplified in Figure 1 where wild type MIC distributions for *Escherichia coli* and *Streptococcus pneumoniae* respectively are displayed. The distributions are obtained from the EUCAST database [7]. A micro-organism is defined as wild type for a species by the absence of acquired and mutational resistance mechanisms to the drug in question. The reference distributions from EUCAST is the result of aggregated MIC data where the individual MIC distributions are obtained from publications in international journals, national breakpoint committees, reference laboratories, international antimicrobial surveillance systems and antimicrobial susceptibility testing device manufacturers.

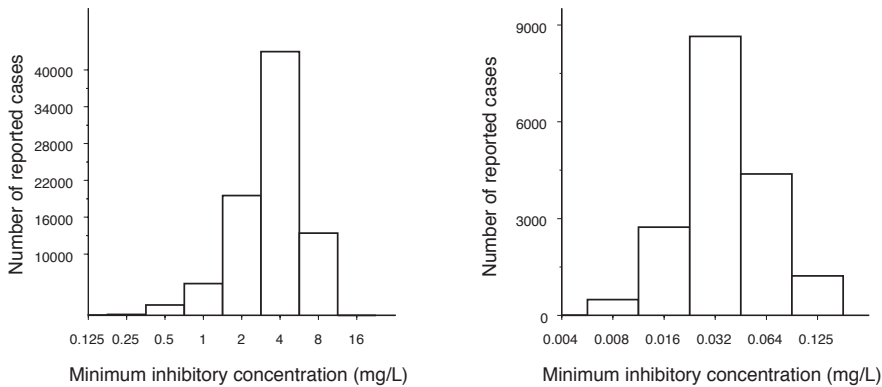


Figure 1. Wild type MIC distributions of *Escherichia coli* and *Streptococcus pneumoniae* obtained from the EUCAST database.

## PK/PD indices

During PK/PD studies of antibiotics it is common to investigate different relations between pharmacokinetic exposure of the drug and outcome. Commonly studied relations are to correlate area under the plasma concentration curve (AUC) normalized by MIC (AUC/MIC) to outcome, maximum concentration normalized by MIC (C<sub>max</sub>/MIC) and the time that the serum concentration of a given agent exceeds the MIC ( $T_{>MIC}$ ). These are called the pharmacodynamic indices, and the antibiotic drugs are often classified into one of these groups of relations [8]. These classifications are simplifications of the complex nature of a bacterial system. This can be exemplified by applying this train of thought to an example where a constant infusion of a drug is given where  $T_{>MIC}$  is important for outcome. If the infusion results in a concentration just below MIC this would have the consequence of giving no antibiotic effect. However, by increasing the infusion rate just slightly, resulting in a concentration above MIC, a full effect would suddenly appear. In this example, increasing the infusion rate further would not give any more treatment benefits. The biology in this example is certainly more complex, and our treatment of the system is a simplification; there might be several factors that are of importance for outcome. However, although they are simplifications, the pharmacodynamic indices provide useful tools when designing dosing strategies for antibiotics.

## In vitro experiments

Compared to many other drugs it is relatively easy to perform in vitro experiments for antibiotics [9]. In these experiments bacteria can be exposed to antibiotics of either constant concentration or different concentration time profiles. This provides the possibility of mimicking the human pharmacokinetics of a drug making it possible to compare different dosing strategies without using in vivo studies [10]. In vitro studies are easy to perform, allow more flexibility in the design of the studies and

the results are unaffected by factors that may contribute to the pharmacodynamic variability *in vivo*, such as drug disposition, disease burden and immune defence. The effect is usually recorded as the change in concentration of bacteria (colony forming units (cfu)/mL)

During PD studies of antibiotics it is common to measure the effect at one predefined time point during the experiment and relate this to the different PD indices. However, an attractive complement to this is studying the entire time course of the effect, which often is referred to as time kill curves [11-13]. These studies may support the development of more complex models that better can describe and predict the efficacy after drug exposure compared to only using PK/PD indices. Data from time kill curves has been used to support semi-mechanistic PK/PD models for describing the time course of the effect of different antibiotics *in vitro* [14-20]. The models can thereafter be used to explore the time course of effect following different dosing strategies with antibiotics using computer simulations [21].

## Estimation of dosing strategies

When developing drug dosing strategies the benefits and disadvantages of the treatment are considered, i.e. the desired effects have to be weighed against the potential side effects. There is usually an association between the magnitude of the drug exposure and the effect; a too low dosage may result in insufficient effect and too high dosage may lead to adverse effects. By weighing the effects, a target concentration associated with the greatest probability of treatment success (i.e. eradication of bacteria) – sufficient clinical effect with tolerable side effects - can be identified. Subsequently, dosing strategies can aim at reaching this target and the need for individualisation based on a patient characteristic such as body weight, sex or a biomarker can also be assessed. In addition to consideration of the drug effects/side effects, there may be further aspects to take into account in the establishment of a dosing strategy. For example, using a higher dosing rate than necessary to achieve a sufficient clinical response results in higher drug costs and, for parenterally administered drugs, it may also imply a logistic problem; the more frequent dosing, the more time and personnel is required for administration of the drug.

With the use of a PK and PD model it is possible to evaluate different dosing schedules by stochastic simulation and, based on a predefined criterion, judge which of the different schedules is preferred [22, 23]. However, to simulate all possible dosing schedules to find the optimal dosing schedule is not feasible. An alternative approach is to estimate an optimal dosing strategy by minimizing a risk function describing the seriousness of deviations from the target at which treatment aims. This method has been used when aiming at various PK and PD targets [24-30].

## Infectious disease biomarkers

During infectious diseases the levels of many endogenic markers are up or down-

regulated in the body. By correlating the time-course of the markers to the progression of a bacterial infection they might be able to be useful for monitoring the progress of bacterial infections. Body temperature and the changes in body temperature are two of the oldest observations used in relation to infectious diseases. The set-point is up-regulated during infections, probably mediated through increasing levels of prostaglandin E2 due to influence of pyrogens and cytokines [31-33].

Interleukin-6 (IL-6) is an important mediator of acute phase response, which increases rapidly as a result of infectious challenges [34]. Increases in cytokines can be detected one to two hours after a challenge with endotoxin in a sepsis model [35]. It has been shown that the IL-6 levels peak at 4-12 hours post surgical procedure [36-38] and that the half-life of IL-6 is approximately 1 hour in humans [39].

During bacterial infection, IL-6 stimulates the hepatocytes to synthesize the acute phase proteins serum amyloid A (SAA) and c-reactive protein (CRP) and as a result the levels of these two biomarkers increases 100-1000 fold [34]. The levels of these two biomarkers peak at 24-72 hours after surgery or challenge with steroid pyrogen etiocholanolone or influenza virus [40-42]. The elimination half-life for CRP in vivo has been estimated to be approximately 5 hours in mice [43] and 20 hours in humans [44, 45]. However, the actual half-life during bacterial infections in patients has been reported to be 3.3 days [46] since the inflammatory stimuli continue the production of CRP and the change of levels will be dependent on both the production and the elimination of the biomarker.

## Renal function

The kidneys are important for elimination of many drugs and metabolites and will therefore be a source of pharmacokinetic variability. Most antibiotics are removed by renal elimination. The elimination consists of glomerular filtration rate (GFR) and active secretion. When renal function is measured it is usually only the GFR that is considered. The golden standards for estimation of renal function are measurements of clearance of exogenous substances such as inulin, iothexol,  $^{51}\text{Cr}$ -EDTA,  $^{99\text{m}}\text{Tc}$ -labeled diethylenetriamine pentaaceticacid (DTPA) or  $^{125}\text{I}$ -labeled iothalamate [47, 48]. However, these measurements are both expensive and time-consuming and are often not applicable in clinical routine practice where endogenous biomarkers are preferred. The level in plasma of a biomarker will be the result of the production and the elimination of the compound. An optimal endogenous marker of glomerular filtration rate should be produced at a constant rate and be eliminated only by glomerular filtration [49].

## Creatinine

Creatinine is formed by non-enzymatic dehydration of creatine in muscle cell and the elimination is mainly renal filtration [49]. By collecting urine during a time period of 24 hours and measuring the content of creatinine in urine and plasma it

is possible to calculate creatinine clearance (CLcr) [49]. However, collecting urine for 24 hours is cumbersome. Since production of creatinine is dependent on muscle mass and elimination is almost exclusively dependent on renal function numerous different formulae's have been developed to be able to calculate the CLcr based on measurement of serum creatinine (Scr) without collecting urine [50]. These formulae's compensates for the fact that different body composition will result in different production rate of creatinine. The most widely use formulae is the developed by Cockcroft and Gault in the 1970's [51] and is displayed in Equation 1

$$CLcr(mL/min) = \frac{(140 - age) \cdot bodyweight(kg) \cdot k}{serum - creatinine(\mu mol / L)} \quad \text{Equation 1}$$

where k=1.23 for men and 1.04 for women However, Scr in particular is not an optimal renal function biomarker and tend, in particular for patients having strongly impaired kidneys, to over-estimate glomerular filtration rate [52].

## Cystatin C

Cystatin C (CysC) is an endogenous protease inhibitor that is produced in all nucleated cells [53] and seems to be freely filtered by the glomerulus [54]. The substance is thereafter reabsorbed and completely metabolized in the proximal renal tubular cells [55]. Therefore CysC levels can not be found in urine and it is not possible to measure CysC clearance using urine sampling. A meta-analysis has shown that CysC is superior to creatinine in prediction of renal function [56]. Furthermore, a few reports have appeared in the literature showing that plasma levels of drugs that are renally cleared may be better predicted using CysC than using either of creatinine and CLcr[57-59]. Formulae's exist that can convert plasma levels of CysC to an estimate of glomerular filtration rate [60].

## Cefuroxime

Cefuroxime has been used worldwide since the 1970's. It is a second generation cephalosporin that is active against a variety of bacterial infections. It is by far the most used parenteral administered antibiotic in Sweden (Figure 2) and is commercially available at 250, 750 and 1500 mg doses. It is also available as a 250 mg tablet and as an oral solution.



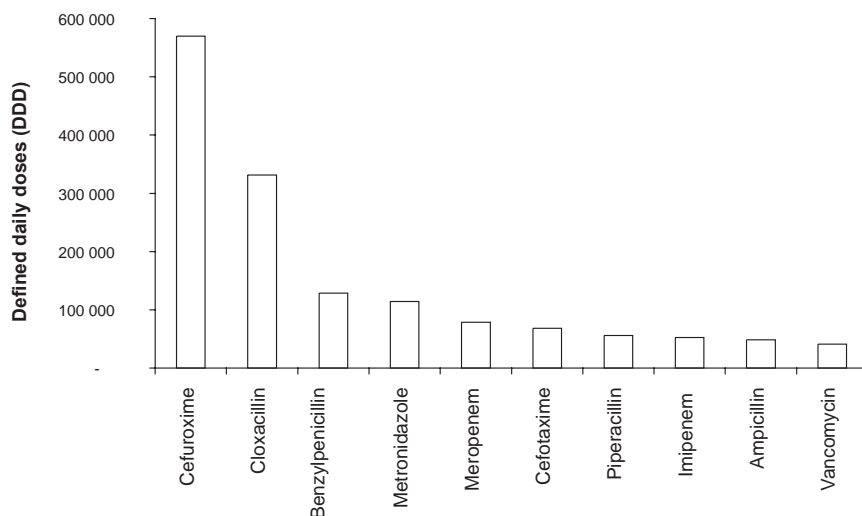


Figure 2. Defined daily doses for the ten most used intravenously administered antibiotics in Sweden during 2005. Sales statistics from Apoteket AB

## Chemical analysis

When investigating the pharmacokinetics of a drug it is necessary to have access to an accurate and precise analysis method. The method needs to be selective, especially when studying patients, since endogenic as well as exogenic compounds might result in erroneous results. Earlier publications describe methods for analysis of cefuroxime in serum or plasma samples using high performance liquid chromatography (LC) with UV-detection [61-67] or microbiological techniques [68-70]. Both UV-detection and microbiological techniques might give erroneous results depending on interaction with other compounds and the latter technique is also very time-consuming. In addition, two examples of qualitative mass spectrometric analysis of cefuroxime has been published [71, 72]

## Pharmacokinetics

The pharmacokinetics of cefuroxime has been shown in numerous publications. The half-life of cefuroxime has been reported to be 0.8-22.3 hours, the volume of distribution 11.6-29.6 L and clearance 0.9-10.1 L/h depending on renal function where the fraction of drug excreted unchanged in urine is > 90 % [61, 66, 68, 69, 73-77]. Approximately 35 % of the drug is bound to serum proteins [74, 78] and following an intravenous dose of cefuroxime the plasma concentration exhibits 2-compartment pharmacokinetics [68, 73, 75].

Pharmacodynamics

The pharmacodynamics of cefuroxime is not very well described in literature but the mechanism of actions has been suggested, as for other cephalosporins, inhibition of the transpeptidase and carboxypeptidase enzymes that are required for cell wall biosynthesis [79-81]. During treatment with cephalosporins, time above minimum inhibitory concentration is important for outcome [9]. For optimal outcome the concentration should be above MIC but there is no benefit with concentrations higher than MIC. In vivo as well as in vitro studies indicate that it is not necessary to attain unbound concentrations above MIC for 100 % of the dosing interval but instead 50 % of the dosing interval is suggested to be a breakpoint for full effect for cephalosporins [8, 82, 83].

Dosing strategies for cefuroxime

Due to the renal elimination of cefuroxime the recommended dosing individualisation is based on Scr or CLcr [78, 84]. Based on the pharmacodynamic principle that time above MIC is important for outcome, a number of authors have suggested dosing with continuous infusion for other beta-lactam antibiotics [85-87] as well as for cefuroxime [88]. The recommended dosing strategy at Uppsala University Hospital is based on CLcr and is presented in Table 1.

Table 1. Recommended dosing strategy for cefuroxime at Uppsala University Hospital.

CLcr (mL/min)	> 80	41-80	21-40	< 20
Dose (mg) non sepsis	750 x 3	750 x 3	750 x 2	750 x 1
Dose (mg) sepsis	1500 x 3	750 x 3	750 x 2	750 x 1

Nonlinear mixed effects modelling

PK-PD relations are very useful in drug product development [89]. This is often done using non-linear mixed effects modelling. The technique involves simultaneous estimation of mean and variance parameters using merged data from all studied individuals. This makes it possible to use sparse data from each individual but instead requires a number of individuals [90]. There are several software packages that provide nonlinear mixed effects modelling capabilities [91] but the most widely used is NONMEM [92].

Using mixed effects modelling the jth observation in individual i can be described by

$$y_{ij}=f(x_{ij},P_i)+\varepsilon_{ij}$$

where f(...) is the individual prediction described by a linear or nonlinear function with parameter vector  $P_i$  and independent variables  $x_{ij}$  (time, dose)  $\varepsilon_{ij}$  is the random

effect describing the discrepancy between individual prediction and the observation. The discrepancy reflects analytical measurement error, erroneous recorded sampling time and/or model mis-specification.  $\varepsilon_{ij}$  is assumed to be normally distributed with zero mean and an estimated variance  $\sigma^2$ .

The next level of variability explains differences in individual pharmacokinetic or pharmacodynamics parameters exemplified with

$$p_{ki} = \theta_k + \eta_{ki}$$

$$p_{ki} = \theta_k \cdot \eta_{ki}$$

where  $p_{ki}$  is the  $k_{th}$  individual parameter in  $P_i$  and  $\theta_k$  the typical value of  $p_k$ .  $\eta_{ki}$  denotes the difference between the individual and typical value of  $p_{ki}$  and is assumed to be normally distributed with a mean zero and a variance of  $\omega_k^2$ . Often parts of the difference between individual and typical value of  $p_{ki}$  can be described by patient factors, often called covariates. Then the individual parameter will be a function of both typical value of parameter and the covariate. When introducing an informative covariate into the model the inter individual variability (IIV) is usually decreased. Often a parameter is changing over time and if this not can be explained by a time dependent function of a covariate the intra-individual variability is estimated as the inter occasion variability (IOV) which can be introduced as an additional random effect [93].

NONMEM uses a parametric maximum likelihood method where the estimated parameters maximize the likelihood of the observations given the model. This is done by minimizing the extended least squares objective function value (OFV), which is proportional to  $-2 \log$  likelihood of the data. For hierarchical models an OFV drop of 3.83, 6.63 and 10.83 units designates an improved fit at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively, for a one-parameter difference [94].

## Aims

The aim of the present thesis was to characterize the pharmacokinetics and pharmacodynamics for the cephalosporin cefuroxime and, based on these pharmacokinetic and pharmacodynamic principles, develop a dosing strategy for cefuroxime.

The specific aims were to

- Develop a sensitive, accurate and fast chemical assay for determination of cefuroxime in human serum
- Characterize the pharmacokinetics of cefuroxime, including inter- and intra-individual variability, and to evaluate the influence of covariates on the pharmacokinetics
- Characterize the time course of the biomarkers interleukin-6, serum amyloid A and C-reactive protein during bacterial infection in relation to duration of illness and to correlate the time course of these biomarkers to the length of cefuroxime treatment
- To develop a dosing strategy using a risk function based on pharmacokinetic and pharmacodynamic principles
- To develop and validate a semi-mechanistic model, describing the time-course of the anti-infective effect of five different antibiotics, including cefuroxime, on *Streptococcus pyogenes* in vitro, that may aid in the development of improved dosing strategies

## Materials and methods

For more detailed information of the different methods and materials, consult the different papers.

### In vivo study

One in vivo study was conducted in the thesis project and the results from this study are presented in paper II and paper III. In the first paper the pharmacokinetics of cefuroxime is investigated and in the latter paper the time course of biomarkers associated with bacterial infections are investigated. Approval was obtained from the Swedish Medical Products Agency and the ethics committees, Faculties of Medicine, Uppsala University and Örebro University, Sweden. All patients signed an informed consent form prior to inclusion.

### Patient study

The patients were included from the Departments of Infectious Diseases and Nephrology, Uppsala University Hospital, Uppsala, Sweden and the Department of Nephrology, Karlstad Central Hospital, Karlstad, Sweden. In total, 97 patients were included with a variety of diagnoses such as sepsis, pneumonia, bronchitis, pyelonephritis, skin- and soft tissue infections and fever of other origin.

The patients were treated with cefuroxime or cefuroxime in combination with tobramycin. The dosing strategy for cefuroxime was based on the calculated plasma creatinine clearance and is presented in Table 1. Cefuroxime was administered as an intravenous injection over a period of 5-15 minutes. The tobramycin dose was also based on creatinine clearance and body weight. Patients with creatinine clearance > 80 mL/min, 41-80 mL/min and 20-40 mL/min were given 6.0 mg/kg, 4.5 mg/kg and 2.2 mg/kg tobramycin respectively once daily as an intravenous infusion. Most of the patients were only given one dose of tobramycin. The patients were not allowed to have had intravenous treatment of antibiotics within 2 weeks prior to inclusion or treatment with prednisolon at a dose of more than 10 mg daily.

### Sampling

Blood samples of 5 mL were withdrawn pre dose and at 5 different time points from 1 to 72 hours after start of treatment according to a flexible sparse data

sampling schedule (see Table 2). In paper II, three additional samples per patient were withdrawn 5 to 40 minutes after dose in a subgroup of 12 of patients (n=2, 2, 4 and 4 in the CLcr intervals <20, 21-40, 41-80 and >80 respectively). The samples were centrifuged at 2000g for 10 min and serum was stored at -20 °C in Ellerman tubes until analysis.

Table 2. Schedule for serum sampling during in vivo study

	Sampling time post dose (h)					
Patient n o 1	0	2	5	12	24	72
Patient n o 2	0	3	6	8	20	72
Patient n o 3	0	1	4	16	30	72
Patient n o 4	repeat from patient 1					

When studying the biomarkers associated with bacterial infection, the duration of the illness was defined as each patient’s estimation of the duration of symptoms related to the infection before admission (>24 h or <24 h). Patients who were included, but change was made to other intravenously administered antibiotic, were retrospectively removed from the final dataset. Patients whose intravenous treatment was stopped or changed to oral antibiotic therapy within 3 days were defined as early step-down, whilst the other patients were defined as late step-down.

Chemical assay

Different substances were chemically analyzed in the thesis project.

Cefuroxime (paper I)

The method for the analysis of cefuroxime in the in vivo study was developed using a Quattro Ultima triple-quadrupole mass spectrometer (Micromass Manchester, UK). Cefuroxime and cefotaxime were purchased from Sigma-Aldrich (St Louis, MO, USA). Acetonitrile (Lichrosolve) and formic acid (extra pure) were purchased from Merck (Darmstadt, Germany). Drug free human serum and plasma were obtained from University Hospital Blood Bank, Uppsala, Sweden. The water was purified by a Milli-Q Academic system (Millipore, Bedford, MA, USA).

The serum samples were precipitated using acetonitrile and cefotaxime was added as internal standard. After centrifugation the supernatant was diluted with a mobile phase consisting of 24 % acetonitrile in 5 mM formic acid and thereafter injected on a Zorbax SB-CN column (4.6 x 150mm, Agilent Technologies, Wilmington, DE, USA). Detection was performed in electrospray negative ion mode. MS control and spectral processing were performed using MassLynx software, version 4.0 (Micromass, Manchester, UK). The MS/MS transitions m/z 423.0 -> 317.9 for cefuroxime, and m/z 454.0 -> 238.9 for cefotaxime, were monitored. Calibration graphs were constructed using a linear regression of the test compound peak area / IS peak area ratio (Y) to nominal serum concentration of the test compound (X, µg/mL). The standard curve was forced through the origin.

Intra-day precision and accuracy were determined by analyzing quality control samples (n=6) at four concentrations during one day. The precision of the method was investigated by calculating the relative standard deviation (coefficient of variation, CV) at each concentration. Accuracy was determined as the percent deviation of analyzed concentration from the added concentration. The lower limit of quantification (LLOQ) was determined from the lowest concentration of samples (n=6) that could be analyzed with CV<20 % and accuracy <±20 %. The highest quality control sample was diluted 5 times with blank serum before sample preparation in order to fall within the standard curve range. The inter-day precision and accuracy were determined by analyzing, on three separate occasions, duplicate quality control samples interspersed with unknown clinical samples.

### **Biomarkers**

The biomarkers used in paper II (Scr and CysC) and paper III (IL-6, CRP and SAA) were analyzed using methods available at the Department of Clinical Chemistry, Uppsala University Hospital. The analysis of creatinine was performed on an Advia 1650 (Bayer Corp., Tarrytown, NY, USA). The total analytical imprecision of the method was 3 % at 89 and 167  $\mu\text{mol/L}$ . CysC and SAA measurements were performed by latex enhanced reagent (N Latex Cystatin C, Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec analyzer (Dade Behring). The total analytical imprecision of the method for CysC was 4.8 % at 0.56 mg/L and 3.7 % at 2.85 mg/L and for the SAA method it was 5.9 % at 12.8 mg/L and 3.2 % at 81.7 mg/L. Analysis of CRP was performed by turbidimetry on an Advia 1650 (Bayer HealthCare Diagnostics, Tarrytown, NY, USA). The total analytical imprecision of the CRP method was less than 9 % at 35 mg/L. IL-6 was measured using an enzyme-linked immunosorbent assay (ELISA) method (R&D Systems, Minneapolis, MN, USA). The total analytical imprecision of the IL-6 method was less than 7 %.

### **Pharmacokinetic data analysis (paper II)**

The pharmacokinetic modelling in paper II was performed using mixed effects modelling within the NONMEM version VI $\beta$  using first order conditional estimation with log-transformed data. The search for appropriate models was guided by the OFV as well as by graphical inspection within the Xpose program version 3.11 [95].

### **Structural and stochastic model**

Firstly the structural and stochastic models were developed. One-, two- and three-compartment models, using ADVANS 1, 3 and 11 and TRANS 2 and 4, were considered. IIV was assessed on all pharmacokinetic parameters and in addition correlations between those terms were evaluated. In order to accurately characterize the residual variability in the model combined additive and proportional error models were tested. Thereafter the significant covariates were included as described below.

Finally IOV was assessed as and tested on the parameters for which an IIV term was significant. Each evaluated parameter was kept in the model if the inclusion resulted in a drop of at least 10.83 in OFV.

### Covariate model

Covariates available in the analysis are specified in Table 5. In addition to use the recorded renal function biomarkers as such in the covariate analysis, different relationships of those were evaluated. Since the relationship between CysC and renal function has previously been suggested to be described as  $GFR = 77.237 \cdot CysC^{-1.2623}$ , this relationship was included in the analysis [60]. The following covariates for renal function were hence tested on CL: Scr, 1/Scr, CLcr, CysC, 1/CysC and  $77.237 \cdot CysC^{-1.2623}$ . The rationale for testing the inverse of Scr and CysC is that those markers are inversely correlated to the kidney function. The evaluated covariate relations are centered around the median value of the covariate, and are parameterized for continuous relations as

$$p_{ki} = \theta_k \cdot (1 + \text{covariate effect} \cdot (\text{covariate value} - \text{median covariate value}))$$

where  $p_{ki}$  is the individual parameter value of parameter  $k$ ,  $\theta_k$  is the typical population parameter value of  $k$ . The covariates were introduced one at a time. These linear relationships were initially tested and if such an inclusion resulted in a significantly improved model fit non-linear relationships, in terms of piecewise linear splines, were evaluated for the covariate in question. Biomarkers for renal function and functions of these were expected to be strongly correlated and therefore only the covariate giving the highest drop in OFV was used and the remaining covariates for renal function were not re-evaluated. Furthermore, since Scr and CysC were measured at two different occasions during the study period, they were considered in the model in different ways. The first alternative was to use the first measurement during the entire time period during which the patient was studied. The second alternative was to use the first measurement until the next measurement was done and thereafter use the second covariate value. The third alternative was to use a linear function between the two measurements and thereafter use the second measurement of the renal biomarker. In three of the patients no body weight (WT) was recorded and therefore the median WT for the population was used instead. In 11 of the patients the second measurement of CysC and Scr was missing and only the first measurement of the renal function biomarker was used.

The covariates age, WT and sex were first assessed on all pharmacokinetic parameters in the model, one at a time, and ranked according to the drop in OFV associated with their inclusion. Variables were then stepwise tested in the model, in descending order.

When no more covariates could be included creating a drop in OFV of more than 10.83, a backwards deletion was carried out retaining only covariates associated with an increase in the OFV of more than 10.83 on their exclusion.



### Biomarker data analysis (paper III)

The graphics were plotted and the statistics were calculated using S-PLUS® 7.0 for Windows (Insightful Corp., Seattle, USA). The smoothes in the plots are locally weighted linear regression curves as implemented in the S-PLUS function *loess*. ANOVA was used when comparing the different biomarker values at the baseline. Due to the flexible sample schedule it was not possible to statistically investigate differences in levels of the biomarkers at any other time point than the baseline.

### Estimation of dosing strategies (paper IV)

When the number of dose sizes available is limited, the dosing strategies estimated comprise a discrete number of dosing categories, i.e. different dose rates are assigned to subpopulations of the treated population. The treated population is then categorized in the dosing strategy using cut-off values (CO) of the patient characteristic used for individualisation.

The methodology used for estimation of dosing strategies has been previously described [27, 28] and involves several foundations; i) a population PK model and a description of covariate distributions in the target population, ii) the definition of the therapeutic target in terms of target variable and risk function, iii) the estimation procedures including constraints involved, and iv) an assessment of estimated dosing strategies. This section will describe the issues necessary to consider one by one.

### Description of the target population

This investigation aimed at establishing a dosing strategy for an adult population with bacterial infection treated with cefuroxime. The population pharmacokinetic model from paper II was used for the description of the pharmacokinetic fate of cefuroxime in the target population, however CysC was replaced with CLcr as covariate on CL and new parameter estimates were obtained. Based on empirical covariate distributions (WT and CLcr) descriptive of the target population and the modified PK model, individual PK estimates for one large population (N=5000) were simulated and used during the dose estimation. The pharmacokinetic parameters were constrained within  $\pm 3SD$  of the unexplained interindividual variability about the expected values based on CLcr, for CL, and WT, for V. Data for the empirical covariate distributions of WT and CLcr were obtained from 110 consecutively registered cefuroxime treated hospitalized adult patients.

### Definition of the therapeutic target

Aspects related to reaching sufficient efficacy, in terms of cefuroxime exposure, as well as the amount of cefuroxime administered, were taken into account by incorporation in the target and risk function defined as follows.

The dose estimation was performed using the underlying model

$$\text{Target} = \text{Pred}_i + \varepsilon_i \quad (\text{Equation 2})$$

where  $\text{Pred}_i$  is the individual prediction of Target based on individual pharmacokinetic parameters, covariates and the estimated dosing strategy.  $\varepsilon_i$  is the individual deviation from the target with variance of  $\sigma^2$  which is minimized during estimation. The optimal dosing strategy was defined as the one minimizing the deviations from the target overall, i.e. the one minimizing the risk function. Quadratic risk functions on the linear ( $R_{\text{LIN}}$ ) and log ( $R_{\text{LOG}}$ ) scale are described in equation 3.

$$R_{\text{LIN}} = \sum_{i=1}^N (\text{Target} - \text{Pred}_i)^2 \quad R_{\text{LOG}} = \sum_{i=1}^N [\ln(\text{Target} - \text{Pred}_i)]^2 \quad (\text{Equation 3})$$

Initially, the time of cefuroxime exposure above MIC (i.e. efficacy) in relation to the amount of cefuroxime administered was considered in the definition of the target variable and the risk function. The aim with treatment from an efficacy point of view was to expose the individuals to concentration above MIC for 50 % of the dosing interval. The MIC was set to a fixed value during the estimation. The time during which cefuroxime concentrations were above MIC ( $T_{>\text{MIC}}$ ) was calculated for each individual during the minimization, as a function of the individual pharmacokinetic parameters, the fixed MIC value and the estimated dosing strategy.

For each individual the percentage of the dosing interval with concentrations above MIC ( $\%T_{>\text{MIC}}$ ) was calculated as  $\%T_{>\text{MIC}} = 100 \cdot T_{>\text{MIC}} / \text{estimated dosing interval}$ . It was assumed that cefuroxime bound to serum proteins is inactive and that reported MIC values represents unbound concentrations. The fraction unbound cefuroxime was assumed to be 65 % [74, 78] in the estimations.

The second aspect; amount of drug administered; was taken into account when the efficacy target was reached, i.e. the individual prediction of  $\%T_{>\text{MIC}}$  was greater than 50 %. In that situation the target variable was switched to the relative amount of drug administered in excess to reach  $\%T_{>\text{MIC}} = 50\%$  on an individual level (hereafter called drug in excess) set to the value 0. Drug in excess was obtained according to Equation 4

$$\text{Drug in excess}_i = \left( \frac{1}{\tau} - \frac{1}{\tau_{\text{Dose},i}} \right) / \frac{1}{\tau_{\text{Dose},i}} \quad (\text{Equation 4})$$

where  $\tau_{\text{Dose},i}$  is the dosing interval resulting in  $\%T_{>\text{MIC}} = 50\%$  for each dose size for each individual in the simulated population and  $\tau$  the estimated dosing interval. Accordingly, a  $\tau$  shorter than  $\tau_{\text{Dose},i}$  would result in giving drug in excess.

Due to the low rate of serious adverse effect from cefuroxime, different penalties in the construction of the risk function was assigned, based on the reasonable assumption that giving too low dosing is worse than too high dosing. Dosing strategies resulting in predictions below the efficacy target, i.e.  $\%T_{>\text{MIC}} = 50\%$ , was penalized according to a quadratic function on the log scale. When  $\%T_{>\text{MIC}}$

> 50 % predictions with respect to drug in excess were penalized by a quadratic function on a linear scale. The two different penalties were weighed so that  $\%T_{>MIC} = 25\%$  gave the same penalty as the administration of 100 % drug in excess to reach  $\%T_{>MIC} = 50\%$ . The risk function (RF1) is described graphically in Figure 3. Even if the target  $T_{>MIC} = 50\%$  is reached,  $\tau$  can become very long (e.g. 48 hours) for the sub-populations of patients with low renal function, thereby increasing the risk for re-growth of bacteria as the time the patient is exposed to concentrations below MIC is considerable. Therefore, an alternative risk function (RF2) was developed in which a quadratic loss on the linear scale was added when the individual was exposed to a concentration below MIC for longer than 4 hours per dosing interval. The function was weighed so that six hours below MIC resulted in the same penalty as  $\%T_{>MIC} = 25\%$  (Figure 3).

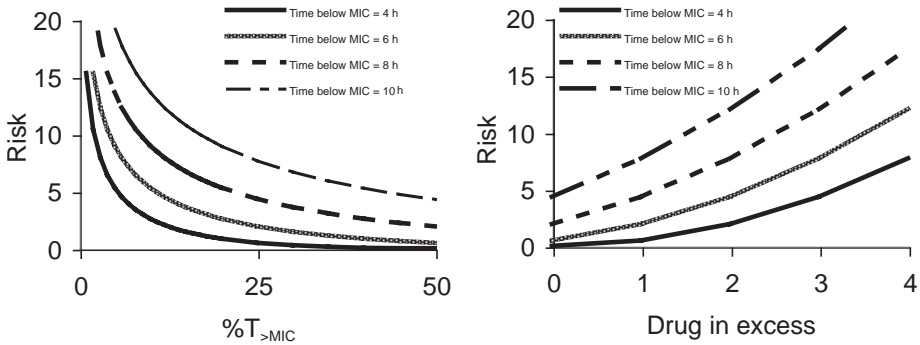


Figure 3. A graphical representation of the risk functions used in the estimations, where the solid black line (Time below MIC = 4 hours) represents RF1 and the other lines RF2.  $\%T_{>MIC}$  is the fraction of the dosing interval an individual is exposed above MIC, Drug in excess is the relative amount of drug given in excess to reach  $\%T_{>MIC} = 50\%$  and time below MIC is the hours of concentration below MIC in each dosing interval.

## Estimation of the dosing strategy

The dosing strategies estimated for cefuroxime assumes that only a discrete number of dose sizes are available. A dosing strategy consists of the dose size(s), the dosing interval(s) and the creatinine clearance COs at which the dose rate should be in- or decremented. In this study, a series of dosing strategies individualized on the basis of CL<sub>cr</sub>, comprising up to five dosing categories were estimated. In the estimations the dosing intervals and the COs were the dosing aspects estimated, while the dose sizes were fixed. All dosing strategies were estimated using the following fixed MIC values: 0.25, 1, 8 and 16 mg/L. The dose size was fixed to 250 or 750 mg for the 2 lower MIC values and to 750 or 1500 mg for the 2 higher MIC values. The estimation was a stepwise search in which the COs were restricted to take on values that were multiples of 10 mL/min as described previously [28]. For each stepwise

search the estimation resulting in the lowest OFV was considered as the best dosing strategy.

All estimations were performed for steady state conditions using NONMEM although the estimations do not require a nonlinear mixed effects computer program.

## Assessment of estimated dosing strategies

To assess whether an estimated dosing strategy was sufficient and to compare dosing strategies, evaluations related to the target definitions were performed as follows. The distribution of  $\%T_{>MIC}$  and, when estimated dosing intervals were long, the distribution of the time of drug exposure below MIC was obtained for the simulated population to evaluate the dosing strategies from the efficacy viewpoint. Similarly, the distribution of drug in excess was calculated to judge the non-beneficial side of cefuroxime treatment. The distributions of these three variables were calculated using wild type MIC distributions for two different species of bacteria representing typically infecting pathogens, *E.coli* and *S.pneumoniae*. In the calculations each individual in the simulated population was randomly assigned one MIC value from each of the MIC distributions. The wild type MIC distributions for *E.coli* and *S.pneumoniae* were obtained from the EUCAST database[7] and displayed in Figure 1.

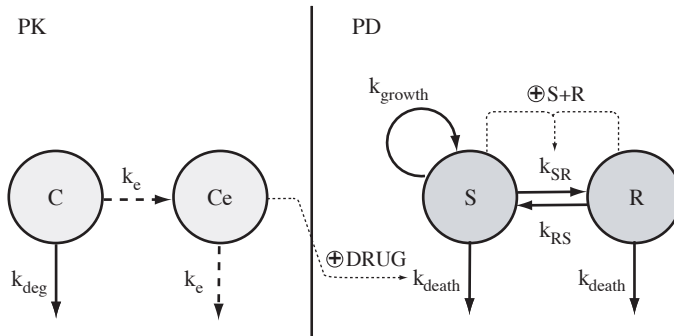
## In vitro study (paper V)

A total of 135 kill curve experiments were conducted during the study period. The experiments were performed in 10 mL glass tubes with 4 mL TH broth. *S.pyogenes* from a 6 hour logarithmic growth phase culture were added to obtain a start inoculum of  $10^6$  cfu/mL. Antibiotics were added to obtain concentrations corresponding to 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 16 and 64 x MIC for benzylpenicillin, cefuroxime and erythromycin, 0.25, 0.5, 1, 1.5, 2, 4, 16 and 64 x MIC for vancomycin and 0.25, 0.5, 1, 2, 4, 16 and 64 x MIC for moxifloxacin. Each time-kill experiment was carried out in duplicate or triplicate on separate occasions. At least one growth control experiment without addition of antibiotics was performed each day. For the growth control experiments start inoculums lower than  $10^6$  cfu/mL were also used. The stability of the antibiotics during the experiments was measured during separate experiments showing degradation for benzylpenicillin and cefuroxime which was accounted for in the PD model building. The effective concentration of the antibiotics benzylpenicillin, cefuroxime, erythromycin, moxifloxacin and vancomycin in the in vitro study were determined with conventional microbiological agar diffusion method [96].

## PD model building

The model building was performed using NONMEM (version VI $\beta$ ). The PD model consisted of 2 compartments where the bacteria are either in the drug sensitive compartment (S) or in the resting compartment (R). The concentration of bacteria in S over time without drug exposure can be described according using equations that describe growth rate ( $k_{\text{growth}}$ ), the rate constant for natural cell death ( $k_{\text{death}}$ ), the transfer from S to R and from R back to S. The transformation from the growing state into the resting state is triggered by the total amount of bacteria in the system and the transformation could be described using a linear function with the transfer rate ( $k_{\text{SR}}$ ) being equal to a proportionality constant times the total amount of bacteria in the system ( $S+R$ ). In the parameterization of the model however, it was chosen not to estimate this proportionality constant but the more easily comprehensible net result from this function i.e. the number of bacteria in the system when stationary phase is reached ( $B_{\text{max}}$ ). Thus, the transfer back to the susceptible state ( $k_{\text{RS}}$ ) was assumed to be negligible and was fixed to 0.

The antimicrobial effect of the drugs was incorporated into the bacterial system to increase the rate constant for bacterial death using an indirect response model for concentration. The antimicrobial effect was assumed to be nonlinearly dependent on the concentration of the antibacterial agent in the effect compartment and was modelled using an ordinary sigmoidal  $E_{\text{max}}$  model.  $E_{\text{max}}$  is the maximal achievable increase in  $k_{\text{death}}$  with a certain drug treatment,  $EC_{50}$  is the antibiotic concentration giving one-half the maximum achievable effect and  $\gamma$  is the sigmoidicity factor defining the shape of the concentration effect relationship. The semi-mechanistic model is presented schematically in Figure 4.



*Figure 4.* Schematic illustration of the PK/PD model. The PK model is a one compartment model (C) with first order elimination due to degradation of the drug ( $k_{\text{deg}}$ ) and a biophase compartment (Ce) with a first order rate constant ( $k_e$ ) accounting for a possible delay in the observed effect. The PD model include one proliferating and drug susceptible compartment (S) and one resting and drug insensitive compartment (R). The bacterial system is described with first order rate constants for multiplication of bacteria in the susceptible compartment ( $k_{\text{growth}}$ ), for degradation of bacteria in both compartments ( $k_{\text{death}}$ ), and for the transfer between the compartments ( $k_{\text{SR}}$  and  $k_{\text{RS}}$ ). The total bacterial content in the system ( $S+R$ ) stimulate transference from the normally growing state into the resting state ( $k_{\text{SR}}$ ). The antibiotic concentration in the biophase compartment is assumed to stimulate the rate of killing of bacteria in the susceptible state according to an  $E_{\text{max}}$  model (DRUG).

## Model validation

An internal model validation was performed using internal cross validation (XV) and case deletion diagnostics (CDD). During the XV, data from experiments with the same concentration were excluded and the model parameters were estimated from the remaining data. The excluded experiments were thereafter predicted by the model using the model parameter values from the result where data had been excluded. The procedure was repeated until data from each set of concentrations had been excluded. The observed values were plotted versus the predicted values and presented graphically.

The CDD was divided into two parts. During the first part, data from one experiment (one tube) at a time was excluded and the parameter values were re-estimated and compared with the estimates from the model developed using the full dataset. The procedure was repeated until data from all experiments had been excluded one time from the full dataset. During the second part of the CDD data from one day's experiments at a time were excluded from the full dataset. The parameter values were re-estimated and compared with the estimates from the model based on the full dataset. The procedure was repeated until data from each day had been excluded one time from the full dataset. The difference in percent between the CDD estimates and the full dataset estimates was calculated and presented graphically.

## Results

### Chemical analysis of cefuroxime

The analytical method for analysis of cefuroxime concentration in human serum was sensitive and selective. The method was able to sufficiently analyze samples in the range 0.025  $\mu\text{g/mL}$  to 50  $\mu\text{g/mL}$  with a retention time of 8 minutes (Figure 5). It was also shown that samples containing higher concentration could be diluted with blank serum and thereafter analyzed successfully. The precision and accuracy were never worse than  $\leq 9.1\%$  and  $\leq \pm 7.1\%$  (table 3 and table 4). No interfering peaks were detected and the internal standard response was constant.

Table 3. Intra-day precision and accuracy for serum samples..

	<b>Conc. added</b> ( $\mu\text{g/mL}$ )	<b>n</b>	<b>Conc. found</b> ( $\mu\text{g/mL}$ )	<b>CV</b> (%)	<b>Accuracy</b> (%)
QCL	0.207	6	0.222	9.4	7.1
QCML	4.15	6	4.13	2.6	-0.35
QCMH	41.5	6	39.8	2.9	-4.0
QCH	166	6	173	2.9	4.2

Table 4. Inter-day precision and accuracy for serum samples.

	<b>Conc. added</b> ( $\mu\text{g/mL}$ )	<b>n</b>	<b>Conc. found</b> ( $\mu\text{g/mL}$ )	<b>CV</b> (%)	<b>Accuracy</b> (%)
QCL	0.207	5*	0.217	7.5	4.6
QCML	4.15	6	4.20	5.0	1.3
QCMH	41.5	6	41.4	2.9	-0.050

\*One sample lost due to system failure.

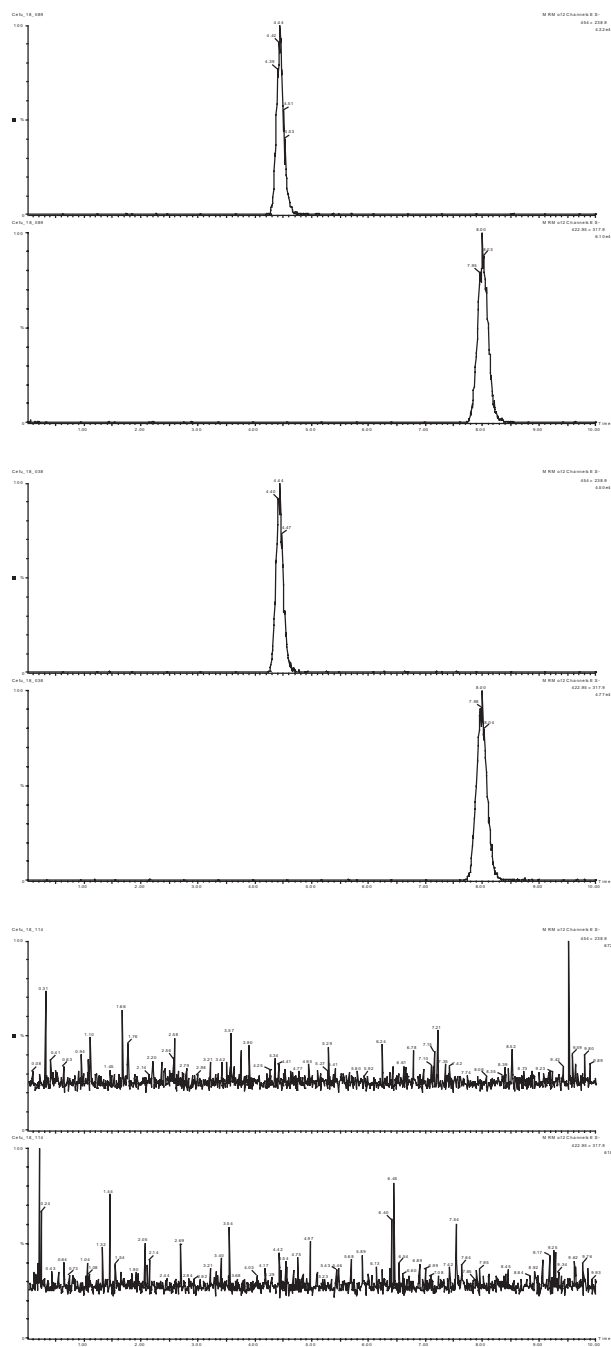


Figure 5. Typical chromatograms of standard serum sample, 10 mg/mL (top), patient sample with-drawn 3 h post dose (middle) and blank serum sample (bottom).



## The pharmacokinetics of cefuroxime

A total of 427 serum samples for determination of cefuroxime were collected after start of treatment and available for the pharmacokinetic analysis. None of the samples collected prior to start of treatment contained any traces of cefuroxime. Eighteen of the samples were excluded due to unrealistic concentrations or uncertainty in sampling times and therefore the final data set consisted of 409 cefuroxime concentrations from 97 individuals. The majority of the samples were collected within the first 24 hours after the start of therapy (n=321, Figure 6). Demographics of the studied patients are shown in Table 5.

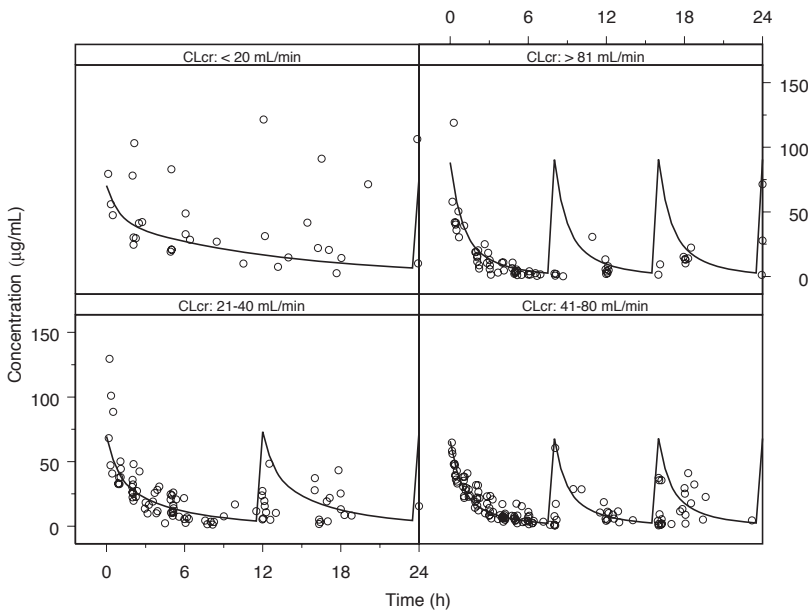


Figure 6. Observed cefuroxime concentration (circle) and model predicted (line) during the first 24 hours of treatment for each dosing group

Table 5. Demographics of the cefuroxime pharmacokinetics studied patients

CLcr (mL/min)	> 80		41-80		21-40		< 20	
	median	range	median	range	median	range	median	range
Creatinine (mmol/L)	97	69-131	101	61-177	127	97-350	295	110-1160
Cystatin C (mg/L)	1,12	0.743-1.5	1,18	0.727-3.78	2,05	0.978-6.06	4,51	2.15-6.18
Weight (kg)	85	60-115	74	54-107	70	50-100	68	35-137
Age (years)	56	24-75	74	35-90	82	44-95	78	67-94

A linear two-compartment model described the data well. Before the candidate covariates were considered in the model, the most favourable inter-individual variability structure was obtained when IIV was allowed on clearance, the central volume of distribution (V1) and the peripheral volume of distribution (V2). Allowing IIV on intercompartment clearance did not offer any further improvement. Different

variance/covariance structures of IIV were assessed but the model did not benefit from any block structure. The residual error was sufficiently described by only a proportional component.

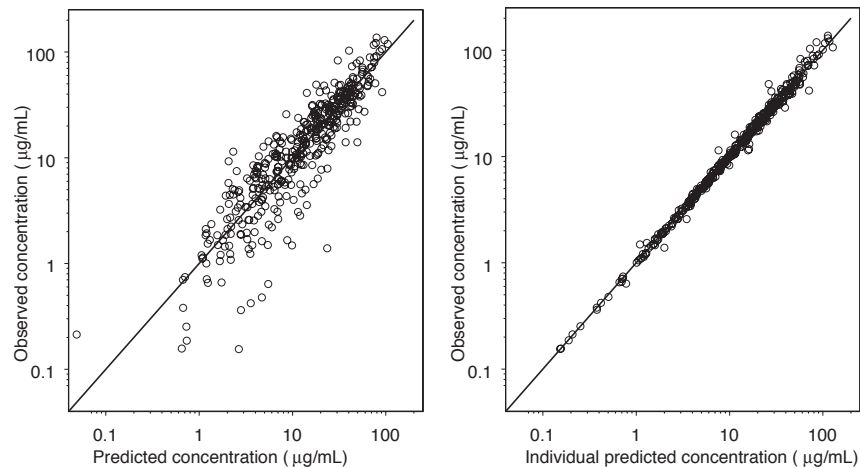


Figure 7. Observed cefuroxime concentration vs. model predicted concentration. Left panel population model prediction, right panel individual population model prediction.

Inclusion of the factor 1/CysC generated a drop in OFV of 154.0 units compared to the model without covariates. When CLcr or 1/Scr were integrated in the basic model a drop of 131.4 units and 75.4 units, respectively were obtained (Table 6). The IIV decreased from 70.2 % to 29.7 %, 33.8 % and 46.8 % respectively when 1/ CysC, CLcr or 1/Scr were included. Moreover, it was found that using only the first measurement of any of the renal function markers was sufficient and that no further information about the clearance of cefuroxime was obtained when taking also the second measurement into consideration. The model fit was further improved when CL and V1 were allowed to covary with WT. Finally, it was found to be beneficial to allow IOV on CL. The parameter estimates in the final model are specified in Table 7 and observed concentration versus model predicted concentration is presented in Figure 7.

Table 6. Change in OFV after inclusion of renal function covariate on CL

CL covariate	ΔOFV
Creatinine	-22.9
Creatinine Clearance	-131.4
1/Creatinine	-75.9
Cystatin C	-92.4
1/Cystatin C	-154.0
77.237·Cystatin C <sup>-1.2623</sup>	-153.3

\*Difference in objective function value compared to structural model without covariate.

Table 7. Parameter estimates in the final model<sup>a</sup>. Standard errors of the estimates within brackets.

Parameter	Estimate	IIV (%)	IOV (%)	Covariate effects	
				1/CYS (%/mg/L)	WT (%/kg)
CL (L/h)	6,00 (3.2)	27 (20)	16 (52)	1,43 (3.7)	1,08 (27)
V1 (L)	11,4 (5.3)	18 (60)	-	-	0,97 (23)
V2 (L)	5,11 (11)	48 (36)	-	-	-
Q (L/h)	3,65 (21)	-	-	-	-
Proportional error (%)	15,5 (15)	-	-	-	-

<sup>a</sup> In the final population model CL and V1 are implemented as  $CL (L/h) = 6.00 \cdot (1 + 1.43 \cdot [1/CysC (mg/L) - 0.758]) \cdot (1 + 1.08 \cdot [WT (kg) - 74])$  and  $V1 (L) = 11.4 \cdot (1 + 0.97 \cdot [WT (kg) - 74]) L$

## Time course of infectious disease biomarkers

The demographics of the biomarker studied patients are shown in Table 8. None of the baseline biomarker values differed statistically significantly between early and late step-down groups or between the 2 groups of duration of illness. Neither did the differences in baseline values for body temperature, SAA or CRP show statistical significance between the different groups of diagnoses, but IL-6 baseline values were significantly higher in the sepsis group ( $p < 0.05$ ) (Table 8). There were no correlations between any of the biomarkers and WT or age.

Table 8. Biomarker values at baseline divided into diagnosis group. Values represents median values (range within brackets). n DI is number of patients with duration of illness &gt;24 h or &lt;24h

Diagnose	n DI		Body temp (°C)	CRP (mg/L)	SAA (mg/L)	IL6 (ng/L)
	<24h	>24h				
Sepsis	1	3	39.3 (38.3-39.9)	150 (10-479)	436 (38-1870)	1294 (90-1970)
Pneumonia	3	15	38.8 (37.5-40.1)	170 (53-661)	650 (109-1720)	115 (7-3879)
Bronchitis	1	8	38.3 (36.5-39.8)	131 (41-231)	339 (125-865)	39 (18-338)
Pyelonephritis	8	15	38.8 (37.1-40.0)	176 (31-439)	501 (120-1510)	94 (9-276)
Skin- soft tissue	0	18	38.2 (36.5-40.5)	185 (5-579)	609 (28-1720)	128 (8-1200)
Fever other origin	3	6	38.4 (37.6-39.8)	147 (3-332)	550 (4.8-2650)	38 (9-536)

The body temperature of the patients declined during the first 24 hours, but thereafter no substantial change was observed (Figure 8). There was a weak trend for the temperature drop to be larger in the groups of patients who had early step-down in comparison to the patients who had late step-down (Figure 9). However, when the data points from the first 24 hours of treatment were used in a linear regression analysis, there was no statistical difference between patients with early step-down and late step-down. The duration of the illness had no impact of the time course of the body temperature (Figure 9).

The time course for SAA and CRP showed an increased trend up to approximately 24 hours after onset of treatment (Figure 8). After 24 hours, the levels of SAA and CRP showed a decreasing trend. The plot was divided into subplots according to time since start of symptoms, i.e. duration of illness < 24 hours or > 24 hours. The first group exhibited increasing levels during the first 24 hours whereas the patients in the second group did not, as displayed in Figure 10 and Figure 11. The change of levels showed a similar pattern when comparing patients with early and late step-down (Figure 10 and Figure 11). The changes over the time of observation were similar when comparing the two different biomarkers.

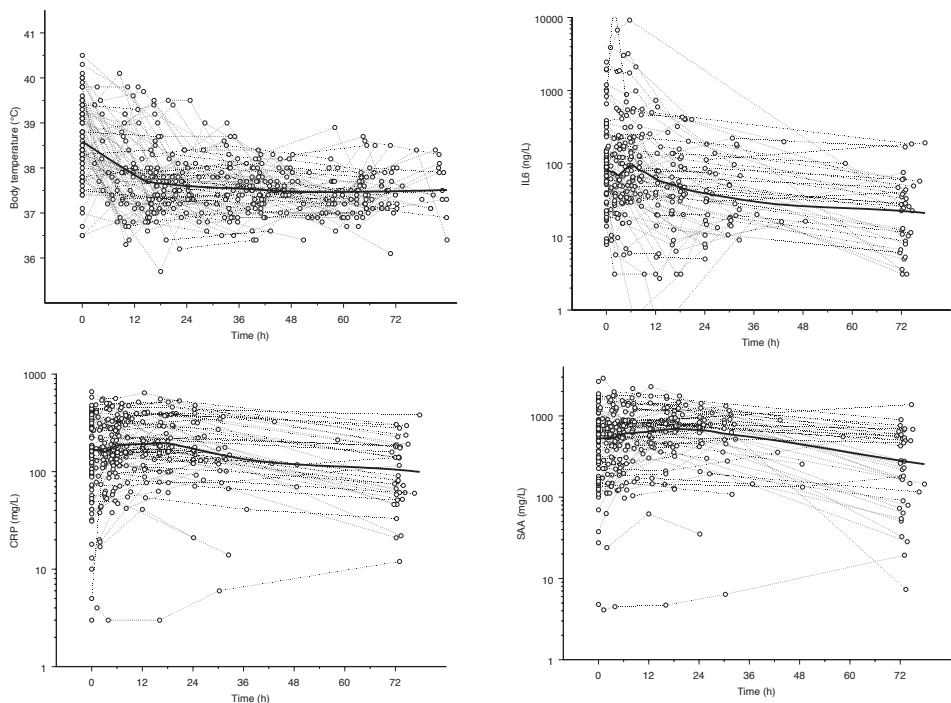


Figure 8. The biomarkers body temperature (top left), C-reactive protein (bottom left), Interleukin-6 (top right) and Serum Amyloid A (bottom right) versus time. ---- is individual values and — is loess smooth.

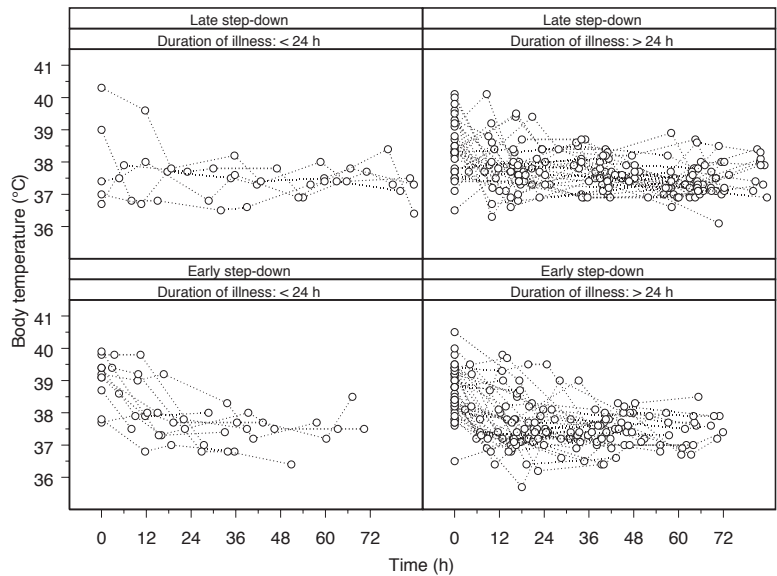


Figure 9. Body temperature vs time after start of treatment for patients with late step-down (top panels) and early step-down (bottom panels). Left panels are patients with duration of illness < 24 h before start of treatment and right panels are patients with duration of illness > 24h.

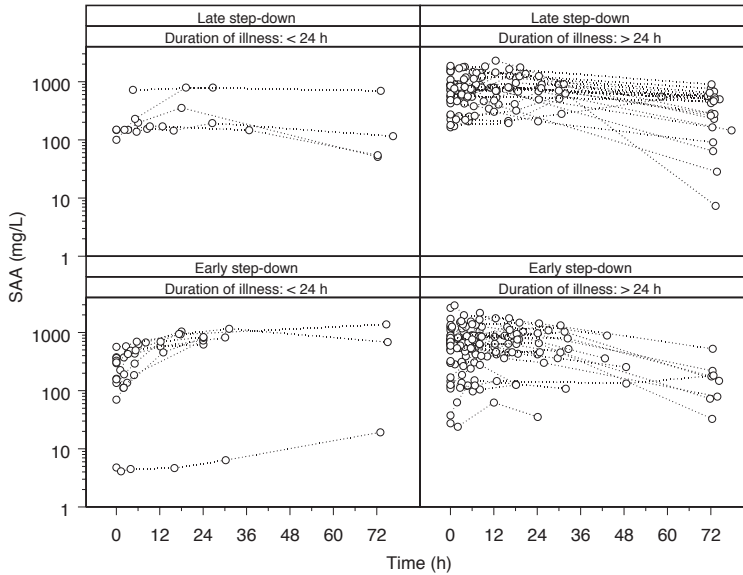


Figure 10. Serum amyloid A vs time after start of treatment for patients with late step-down (top panels) and early step-down (bottom panels). Left panels are patients with duration of illness < 24 h before start of treatment and right panels are patients with duration of illness > 24h.

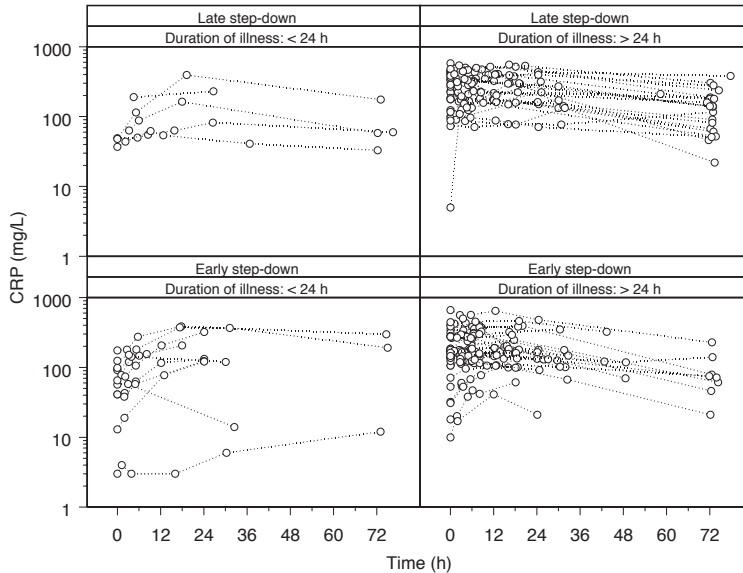


Figure 11. C-reactive protein vs time after start of treatment for patients with late step-down (top panels) and early step-down (bottom panels). Left panels are patients with duration of illness < 24 h before start of treatment and right panels are patients with time since start of illness > 24h.

There was a trend towards decreasing levels of IL-6 during the first 24 hours, but the inter-patient variability was great (Figure 8). There was no difference between the levels when the plot was divided into subplots according to duration of illness or early/late step-down.

## Estimation of dosing strategies

Generally, increasing the fixed MIC value in the dosing strategy estimation resulted in shorter estimated dosing intervals. Furthermore, the consequence of using the lower dose size compared with the higher dose size was shorter dosing intervals. It was possible to estimate dosing schedules for all tried settings but it was a clear difference in the assessment of the various strategies depending on the wild type MIC distribution used. Therefore, the result section is organized with respect to dosing strategies evaluated for each of the two distributions used in the assessment of the dosing strategies. The traditionally used dosing schedule resulted in  $\%T_{>MIC} < 50\%$  for 23 % of the *E.coli* infections but only 0.06 % of the treated *S.pneumoniae* infections.

### Dosing strategies with respect to *E.coli* infections

Using the fixed MIC value 8 mg/L and 750 mg dose size in the estimation resulted in a large proportion of individuals exposed to  $\%T_{>MIC} < 50\%$  Figure 12. This proportion diminished and the distribution of individuals below target was shrunken towards the target when the number of dosing categories was increased, i.e. fewer individuals were exposed to very low  $\%T_{>MIC}$  with increasing number of COs. Reversely, the proportion of individuals that were given drug in excess increased when the proportion of individuals below target decreased, i.e. with increasing the number of COs.

When the fixed MIC value was increased to 16 mg/L and 750 mg dose size was used, good target attainment was obtained but the estimated dosing intervals was very short (2 hours). When the 1500 mg dose size was used, only a small proportion of the individuals were exposed to  $\%T_{>MIC} < 50\%$  and, consequently, a large proportion was exposed to drug in excess (Figure 12). In accordance with the results for MIC 8 mg/L, by increasing the number of COs the width of the distribution of  $\%T_{>MIC}$  and drug in excess decreased. For all dosing strategies, and in particular when using 4 dosing categories (3 COs) the dosing interval for the patients with best renal function was very short. (Table 9)

To achieve acceptable efficacy one of the dosing strategies resulting from using MIC 16 mg/L in the minimization would be chosen (Table 9). When comparing efficacy and drug in excess among the different dosing strategies the benefits with an increasing number of dosing categories is limited and therefore 2 dosing categories would be considered sufficient. Setting the dosing intervals to practical numbers (6 and 12 hours) when using 2 dosing categories, and only estimating CO resulted in the same CO (50 mL/min) and similar distributions of efficacy and drug in excess was obtained.

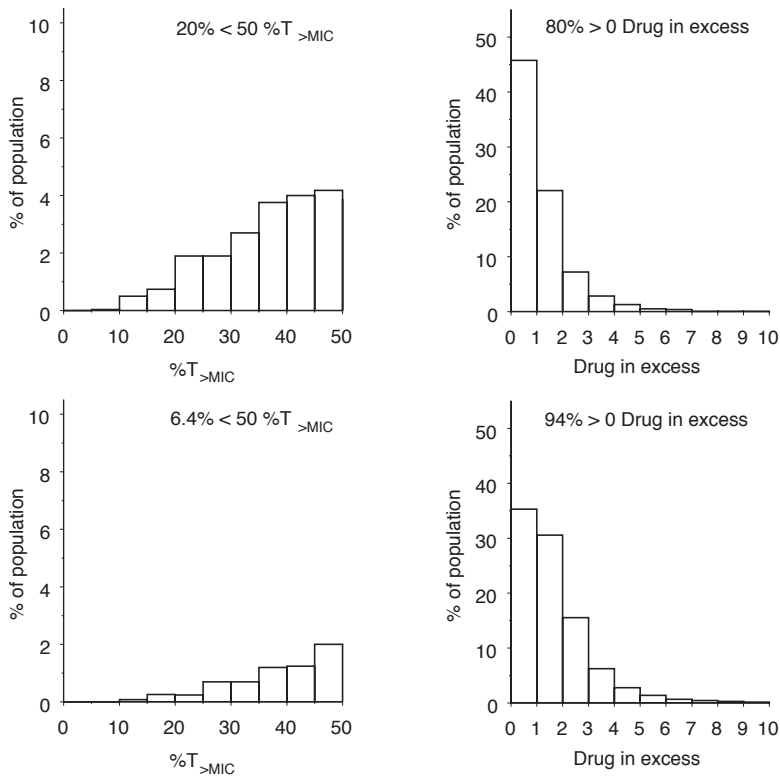


Figure 12. Distributions of the fraction of time with concentration above MIC per dosing interval ( $\%T_{>MIC}$ ) and drug in excess, assessed using the wild type distribution of *E.coli* for the dosing strategies using a fixed MIC value of 8 mg/L and the dose size 750 mg (upper panel) and using a fixed MIC value of 16 mg/L and the dose size 1500 mg (lower panel). Results are shown for dosing strategies with 2 dosing categories

Table 9. Estimated dosing strategies with 2, 3 and 4 dosing categories, respectively, using the dose size 1500 mg. A fixed MIC value of 16 mg/L was used in the estimation.

2 dosing categories		3 dosing categories		4 dosing categories	
CLcr (mL/min)	Dosing interval (h)	CLcr (mL/min)	Dosing interval (h)	CLcr (mL/min)	Dosing interval (h)
≤ 50	12.04	≤ 30	17.61	≤ 30	17.61
> 50	5.28	30-80	9.51	30-50	9.50
		> 80	5.29	50-70	6.23
				> 70	4.19

## Dosing strategies with respect to *S.pneumoniae* infections

Very long dosing intervals were estimated when using the fixed MIC value 0.25 mg/L and dose size 250 mg in the minimization, exemplified in Table 10. A large proportion of the individuals were exposed to  $T_{>MIC} < 50\%$  and, in addition, many of them were exposed to concentration below MIC for longer than 4 hours (Figure 13) but only a few individuals were exhibited high values of drug in excess. However, when incorporating the risk of being below MIC for more than 4 hours

in the estimation, i.e. using RF2 in the minimization, the resulting dosing intervals were shorter (Table 10), the proportion of individuals below target and number of individuals with concentration below MIC for more than 4 hours were considerably reduced, but the number of individuals over treated increased (Figure 13).

An overall assessment of efficacy ( $\%T_{>MIC}$  and the risk of being below MIC for more than 4 hours) and drug in excess resulted in only small benefits using more than 2 dosing categories. Hence, the estimated dosing strategy with two dosing categories using RF2 was re-estimated with the dosing intervals fixed to 12 and 24 hours. This resulted in a somewhat lower CO (30 mL/min) but similar efficacy and drug in excess was obtained.

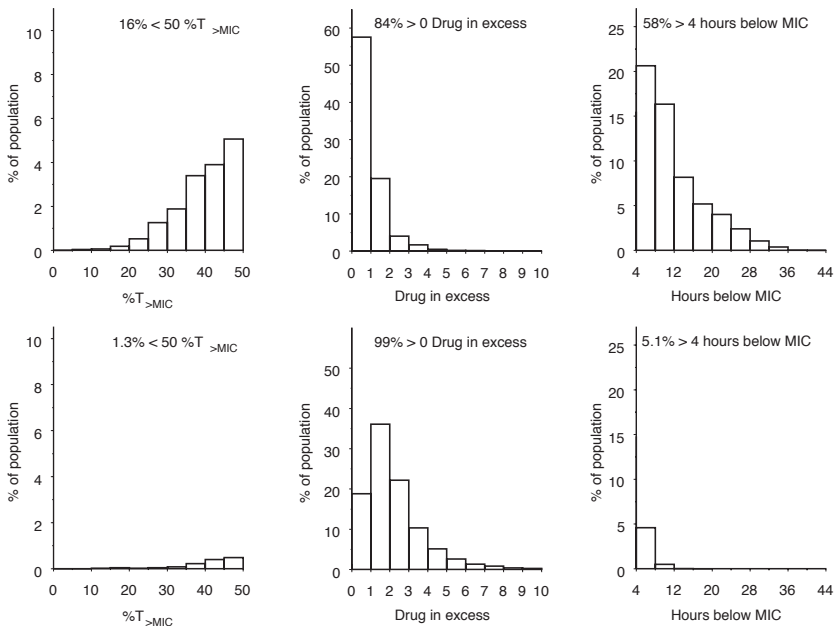


Figure 13. Distributions of the fraction of time with concentration above MIC per dosing interval ( $\%T_{>MIC}$ ), drug in excess and time below MIC per dosing interval assessed using the wild type distribution of *S.pneumoniae* for the dosing strategy estimated using a fixed MIC value of 0.25 mg/L, the dose size 250 mg and risk function 1 (upper panels) and risk function 2 (lower panels). Results are shown for dosing strategies with 2 dosing categories.

Table 10. Estimated dosing strategies using fixed MIC value 0.25 mg/L, the dose size 250 mg and risk function 1 (RF1) and risk function 2 (RF2), respectively.

RF1		RF2	
Clcr (mL/min)	Dosing interval (h)	Clcr (mL/min)	Dosing interval (h)
≤ 40	43.3	≤ 40	19.35
> 40	19.9	> 40	11.6



## In vitro PK/PD model

The final PK/PD model describes well the growth and killing of the studied bacterial system both without drug exposure and when exposed to a wide range of concentrations of the five antibacterial agents used in the study. All parameters were estimated simultaneously and parameter estimates with relative standard errors are presented in Table 11 for the bacterial specific parameters and in Table 12 for the drug specific parameters. A sigmoidal Emax model gave a significantly better fit than the ordinary Emax model (where the sigmoidicity factor,  $\gamma$ , is equal to 1) for all five antibiotics. For erythromycin,  $\gamma$  was estimated to be less than 1 (0.77), indicating a more shallow concentration effect relationship than for benzylpenicillin, cefuroxime and moxifloxacin. Vancomycin shows a very steep concentration effect relation and the sigmoidicity factor was estimated to very high value ( $>50$ ). Since such high values do not seem mechanistically plausible and might result in mathematical problems during minimization the sigmoidicity factor for vancomycin was fixed to the lowest value that did not have a detrimental effect on the fit, which in this case was found to be a value of 20.

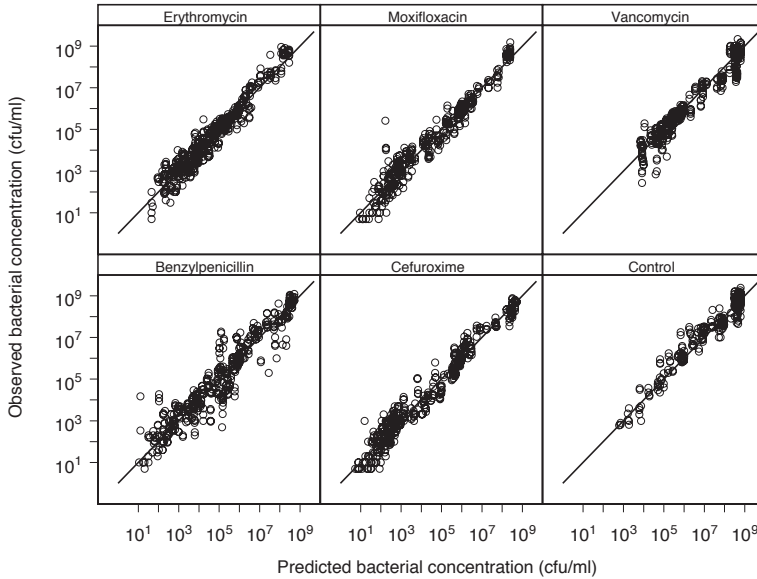


Figure 14. Goodness of fit plots with observed and model predicted bacterial concentrations. Included are lines of identity.

## Model validation

The cross validation shows that the model has good predictability (Figure 15). The first part of the CDD, where one experiment at the time was excluded from the dataset, revealed that one of the parameters,  $k_c$  for benzylpenicillin, was strongly influenced by one of the experiments (Figure 16). When the data from that single

experiment was excluded from the analysis,  $k_e$  increased drastically, hence indicating that no time-delay was evident from the data. The model was therefore re-fitted with  $k_e$  for benzylpenicillin fixed to a high value ( $100\text{ h}^{-1}$ ). This procedure resulted in an increase in the OFV of 18 units and no or only limited change of the remaining parameters ( $EC_{50}$  underwent the largest change, i.e. 11 %). For this reason the estimated  $k_e$  was kept in the final model. When experiments from one day were excluded, no parameter estimate changed substantially.

Table 11. Parameter estimates of the bacterial system specific parameters with the typical values and the relative standard error (RSE).

Parameter	Estimate	RSE (%)
$k_{\text{growth}} (\text{h}^{-1})$	1.35	5.4
$k_{\text{death}} (\text{h}^{-1})$	0.179	6.5
$B_{\text{max}} (\text{cfu/ml})$	$4.15 \cdot 10^8$	9.2
$f_{\text{mix1}} (-)$	0.747	16
$f_{\text{pers}} (-)$	0.0529	48
(%)	98	20
$\text{repl} (\%)$	47	9.3

Table 12. Parameter estimates of the drug specific parameters with the typical values and the relative standard error in parentheses.

Drug	$E_{\text{max}} (\text{h}^{-1})$	$EC_{50} (\text{mg/L})$	$\gamma (-)$	$k_e (\text{h}^{-1})$
benzylpenicillin	2.44 (8.6)	0.00438 (7.7)	1.29 (10)	1.00 (9.6)
cefuroxime	3.30 (6.1)	0.00829 (6.6)	1.69 (8.5)	0.861 (17)
erythromycin	2.03 (6.4)	0.0276 (15)	0.769 (19)	100 (-)
moxifloxacin	3.20 (4.6)	0.0747 (3.0)	1.59 (7.2)	0.644 (20)
vancomycin	1.36 (5.5)	0.384 (0.9)	20 (-)	100 (-)

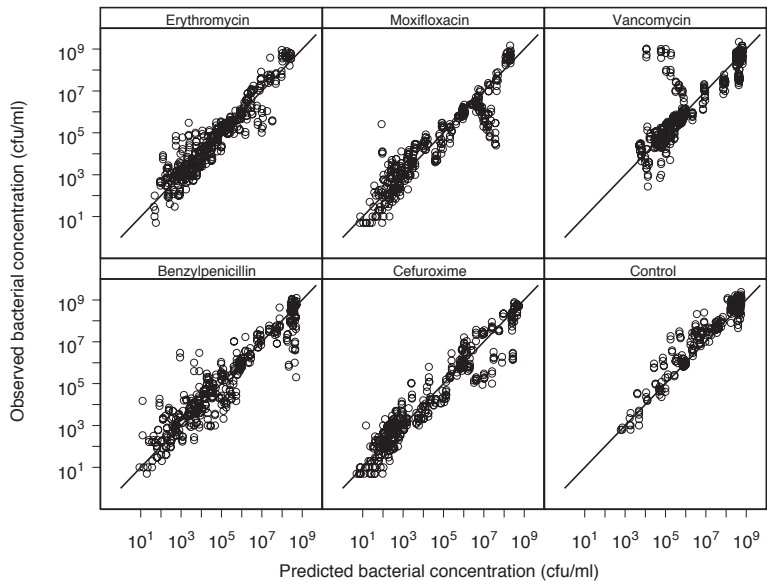


Figure 15. Results from cross validation. Goodness of fit plots with observed and model predicted bacterial concentrations.

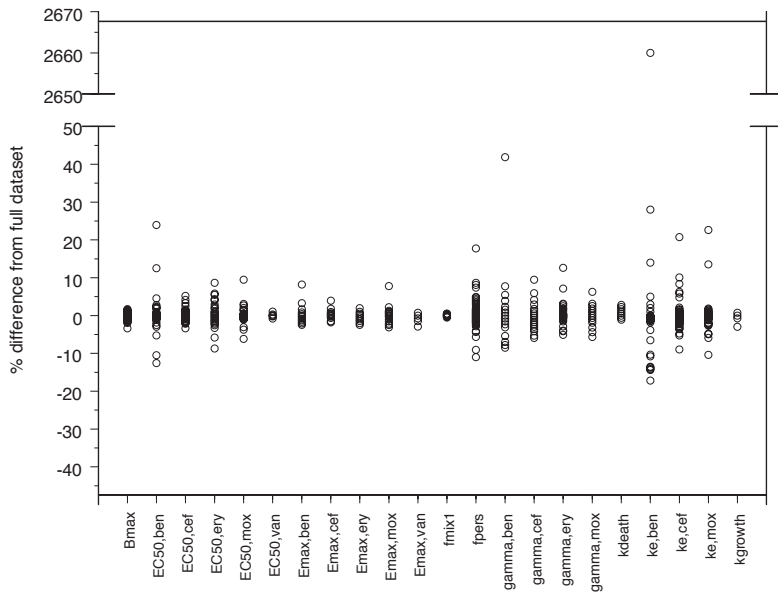


Figure 16. Results from the case deletion diagnostics (CDD), part 1. Data from one experiment at a time excluded and the parameter values are re-estimated and compared with the estimates from the full model. The meanings of the suffix used in the figure are as follows: mox, moxifloxacin; ben, benzylpenicillin; van, vancomycin; cef, cefuroxime; ery, erythromycin.

## Discussion

Although antibiotics have been established in clinical practice for more than 60 years, there are still large knowledge gaps regarding their dosing. The optimal situation would be to individualize both dose size and dosing interval for each patient. This is not feasible and therefore dosing strategies are developed for sub-populations of patients. By characterizing the pharmacokinetics and identifying patient characteristics that covary with the pharmacokinetics it is possible to recommend dosing that minimizes the variability in exposure of the drug within a group of individuals. By gaining more knowledge of the interaction between antibiotics and bacteria, the dosing may also be individualized based on the pathogen's susceptibility to the drug.

To be able to characterize the pharmacokinetics of drugs it is necessary to have access to a reliable method for analysis of the concentration of the substance. The method should not only be sensitive, it also has to be selective. By combining mass spectrometric detection with liquid chromatography it is possible achieve this for the analysis of exogenic (as well as endogenic) substances. Preferably, the method should also be easy to perform. The simpler work-up procedure that is required and the shorter retention time the better since then a large number of samples can be analyzed each day. The method presented for analysis of cefuroxime in this thesis is not only more sensitive compared to other published methods [61-70], it also consists of a simple work up procedure. This, in combination with short retention time, makes it possible to analyze up to 100 samples daily. Validation of this method showed that it had good precision and accuracy. Because no interfering peaks were detected when analyzing blank samples from patients, and because the variability in response of the internal standard cefotaxime was limited, it can be concluded that the method is stable and reliable and therefore useful for analyzing serum samples containing cefuroxime.

The pharmacokinetic analysis of cefuroxime was based on data from 97 hospitalized patients. The number of patients is larger compared to the previously published PK studies on cefuroxime and the range of renal function among the studied patients was wider [61, 66, 68, 69, 73-77]. The patients were included in a clinical setting and represent patients typically found at an infectious disease ward and the sampling schedule was designed to capture the complete dosing interval and repeated dosing. Since optimal treatment with cephalosporins is thought to be obtained when time above MIC is maximized makes it important to also characterize the distribution phase. This was obtained by adding samples withdrawn within the first 40 minutes post administration of dose and the data could satisfactory support

a 2-compartment model. This has only been performed in a few published studies previously [68, 73, 75] including a low number of patients. This was the first time a population PK model was published for cefuroxime.

The final model included CysC as covariate on CL. Compared to CL<sub>Cr</sub> this does not require measurement of body weight or any calculations and it is suggested that CysC is an attractive alternative instead of using CL<sub>Cr</sub>. This result could possibly be applicable to other renally eliminated drugs.

For common bacterial infections, treated with antibiotics in the community, guidelines include standard length of treatment. For more severe infections in hospitalized patients, the length of treatment is often individualized. Due to limitations in the availability of hospital beds, it is especially important to monitor the clinical progress of the bacterial infection in order to be able to determine when intravenous antibiotic treatment is no longer necessary. This decision is mostly based on clinical signs and symptoms, but might be supported by biomarkers. However, no surrogate marker has been validated for prediction of the time-course of bacterial infections following antibiotic treatment. In this study, the kinetics of several biomarkers (interleukin-6, serum amyloid A and C-reactive protein) were studied during bacterial infections treated with cefuroxime in an attempt to evaluate whether they could be useful to differentiate between responders/non responders and if they were correlated to the length of intravenous cefuroxime treatment. The data generated in this study did not make it possible to differentiate between responders and non responders to the antimicrobial treatment since most treated patients were considered to respond to treatment (the treatment was changed to other intravenously administered antibiotics in only a few individuals).

It was expected that there should be a difference in time course of SAA and CRP due to their different kinetics. However, a striking similarity was observed between the SAA and CRP time courses. The increase in levels immediately after start of antibacterial treatment was explained by the time of illness and was not associated with longer treatment times. However, it is of importance to take time of illness into account when evaluating these biomarkers. After 24 hours the levels decreased in a similar way regardless of the duration of illness. Due to the regulation of the biomarkers, it is not surprising that the levels increase during the initial treatment when the duration of illness is short. The levels of IL-6 decreased during the first 24 hours regardless of duration of illness, compared with SAA and CRP. None of the biomarkers helped in differentiating between patients having an early or late step-down; neither did they show any substantial change during the initial treatment with cefuroxime. A major difficulty in studies of antimicrobial treatment is the lack of established response endpoints. For some infections it is possible to measure bacterial eradication (i.e. urinary tract infection), but mostly the judgement of response is based on the clinician's opinion. There is a need for new objective and validated endpoint measurements for clinical trials of antibiotics to be able to further investigate the use of infectious disease biomarkers in relation to clinical treatment. However, further studies are needed to evaluate if the biomarkers studied in this thesis could be of use to early differentiate between responders/non-responders to

bacterial treatment or if they could be of use as surrogate endpoints during clinical trials.

By use of the previously suggested PK/PD principles regarding effect and side-effects for cefuroxime, and by combining this with knowledge regarding the pharmacokinetics of the drug, it was shown that individualized dosing strategies can be estimated based on a risk function. From an efficacy perspective the aim was to establish a dosing strategy resulting in exposing individuals to concentration above MIC for 50 % of the dosing interval. For cefuroxime, concentration dependent toxicity is limited and it can be argued that using a high enough dosing strategy resulting in all patients reaching the efficacy target would be appropriate. However, both from an economical as well as an ecological/resistance perspective it is also important to minimize the amount of drug administered in excess of reaching the efficacy target. A major difficulty in the construction of the risk function was to weigh these aspects against each other. The choices made should preferably have a scientific basis but will also contain value judgments, as exemplified in this study. However, it illustrates the approach of estimating optimal dosing strategies based on data-based models and decision-based risk functions. This was the first time estimations of dosing strategies using a risk function when time above a threshold concentration is important for outcome. Further, this was also the first time multi-dimensional risk functions were used.

The risk function was based on the knowledge of PK/PD for cefuroxime and MIC distributions of wild type bacteria, obtained from the EUCAST database [7]. Using the simplified PK/PD indices is of course not optimal in dosing strategy development. Arguing that  $\%T_{>MIC} = 50\%$  is optimal without any constraints would result in that multiple small doses can be considered equal to giving one extremely large dose per week (assuming that both dosing strategies results in  $T_{>MIC} = 50\%$  of dosing interval). This is of course not true. However, by applying the calculation of drug given in excess and introducing the absolute time below MIC in the risk function this problem was overcome.

The individualisation was based on CL<sub>cr</sub> instead of the previously proposed renal function marker CysC. This was done since no correct distribution of CysC values was available for cefuroxime treated patients. Further, using CL<sub>cr</sub> makes the results more applicable to a clinical situation since most hospitals do not have access to methods for analysis of CysC yet.

Depending on the MIC value for the pathogen intended to be treated, the estimated dosing strategies were very different and therefore different dosing strategies are presented for infections caused by either *E.coli* or *S.pneumoniae*. By knowing the species of the infected pathogen, and therefore the MIC distribution it originates from, the dosing can be individualized far beyond what is done today.

The PK/PD indices for beta-lactam antibiotics, like cefuroxime, are fairly well characterized. However, as described above, claiming that  $T_{>MIC}$  is the only important variable for efficacy for beta-lactam antibiotics is a simplification. When developing dosing strategies for new antibiotics it would be preferred to expand the PK/PD principles into more complex models. These models should not only

be able to describe the effect seen in the performed experiments that the model is based on, but also be able to predict effect during conditions different from those studied. In the semi-mechanistic model that is presented in this thesis, the concentration effect relation of several different drugs was estimated simultaneously and the model accurately describes the biphasic kill often observed during exposure of antibiotics. The model was validated using internal validation and was shown to be robust and predictive. By being able to characterize the complete time course of a bacterial system, the semi-mechanistic model might be very useful for *in silico* studies. By combining the PK/PD model with knowledge regarding drug toxicity, antibiotic resistance and human pharmacokinetics it should be possible to search for more optimal usage of the antibacterial agent. Since the drug and bacterial specific parameters were separated this gained knowledge of the bacterial system could be used when investigating new bacterial agents and therefore fewer experiments might be needed when examining new drugs.

The different aspects of individualisation of the dosing of antibiotics brought up in this thesis includes development of a method for chemical analysis and a covariate analysis of the pharmacokinetics of cefuroxime, studies of the time-course of biomarkers related to bacterial infections and how dosing strategies can be developed using risk functions based on pharmacokinetic and pharmacodynamic principles. Further, the thesis includes an example of how the time course of a bacterial system exposed to antibiotics can be described by a semi-mechanistic model.

## General conclusions

Characterisation of the pharmacokinetics of a drug requires access to a sensitive and accurate chemical assay for determination of the drug concentration in the relevant matrix. A new method for determination of cefuroxime in human serum was developed using liquid chromatography in combination with mass spectrometry detection and a simple work-up procedure. The final assay was shown to accurately, precisely and rapidly determine cefuroxime.

The identification of patient characteristics that significantly covary with pharmacokinetic parameters is important since this knowledge may aid in the development of dosing strategies. Most frequently used surrogates for renal function are serum creatinine or creatinine clearance and therefore dosing of renally cleared drugs are often based on those markers. For the renally eliminated drug cefuroxime it was shown within the presented work that cystatin C is markedly better than serum creatinine and at least as good as creatinine clearance for use as biomarker in the prediction of individual clearance. Furthermore, compared to creatinine clearance, the use of cystatin C offers the advantage of being easier to use in the clinical setting. The use of cystatin C instead of serum creatinine or creatinine clearance in the individualisation of drug treatment might be applicable also to other drugs that are renally eliminated.

Time courses of the concentration of infection biomarkers may reflect the progression of a bacterial infection already early after start of therapy and may thus provide a tool in the decision regarding the strategy therapy. By using a sparse sampling strategy, the time courses of interleukin-6, serum amyloid A and C-reactive protein during bacterial infection were within the present work successfully characterized in relation to the duration of bacterial infection in a hospitalized patient population receiving cefuroxime therapy. However, although the studied markers might be valuable at a later stage of treatment, from the present results it appears that they fail to reflect the early change in status of infectious disease in relation to treatment time. Further studies are needed to evaluate their usefulness in relation to therapeutic outcome.

Drug dosing strategies should preferably be based on the integrated relation between pharmacokinetics and pharmacodynamics. For cephalosporins the effect has been suggested to be optimised if the serum concentration exceeds the minimum inhibitory concentration for 50 % of the dosing interval. Using cefuroxime as an example it was shown how these PK-PD principles could be used to estimate dosing strategies considering both effect and risk of giving drug in excess. Furthermore, the possibility of using multiple aspects of the dosing by using a multi-dimensional



risk function in the estimation was shown. The estimated dosing strategies were evaluated using the PK-PD principles and MIC distribution of different strains of bacteria and thereafter improved dosing strategies could be suggested.

The relation between antibiotic exposure and effect is usually simplified into PK/PD indices. These have comprised important tools in the evolution of appropriate dosing approaches for antibiotics. However, more complex PK/PD models that perform well in describing the time-course of the antibiotic effect may be useful in the design of improved dosing strategies. By using all data simultaneously, collected from in vitro kill curve experiments where a bacterial system was exposed to five different classes of antibiotics, a semi-mechanistic model was developed. The final model differentiates between system and drug specific parameters and includes relevant components that are important factors for the rate of change of bacteria in the studied system. Furthermore, internal validation showed that the model was predictive and robust. The semi-mechanistic model may, after some refinement and external validation, be part of the future development of treatment strategies.

## Future perspectives

Cystatin C was shown to be a good biomarker for clearance of the renally eliminated drug cefuroxime. However, since nature never is simple there are probably situations when cystatin C is not a good biomarker. Future studies are needed to evaluate if and when cystatin C should not be used. There is also a need to develop dosing strategies based on cystatin C rather than creatinine and creatinine clearance.

There is a need to develop better models for concentrations effect relations for antibiotics. Both the complexity and variety of relations has to be explored. Although the semi mechanistic model presented in this thesis accurately describes the time course of a bacterial system exposed to constant concentration of antibiotics there is still a lack of knowledge of PK/PD relations describing what is happening when the model is applied to a kinetic system or when repeated dosing is performed. These models also need to be used in development of better dosing strategies. Often new models are only presented in the literature and never used to further improve the dosing of antibiotic agents.

The interaction between the immune system and antibiotics needs to be further investigated. The pre-clinical development of antibiotics is usually performed in vitro or in neutropenic animals. There is a need to investigate how the dosing strategies should be designed to optimally interact with the immune system. Further, the PK/PD relations might be changed when bacteria are developed into resistant strains. This requires further PK/PD studies.

The biomarkers studied in this thesis do not seem to provide any information about the clinical progression of a bacterial infection. The study did not evaluate the possibility to distinguish between responders and non-responder and further studies are needed to investigate their usefulness. Also, other biomarkers associated with the infectious disease progression needs to be evaluated. Such markers could not only provide tools for the clinician to monitor the treatment of a patient but also act as surrogate markers for outcome during clinical trials of new antimicrobial drugs.

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