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Antibiotic concentrations in the ICU

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Abstract

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Severe infections are life-threatening conditions and common cause of emergency admission to intensive care units (ICU). Initial adequate antibiotic treatment is known to be crucial for the outcome. However, mortality and morbidity remain high.

The overall aim of this thesis was to investigate strategies for optimising antibiotic concentrations, pharmacokinetic/pharmacodynamic (PK/PD) target fulfilment and dosing in ICU patients during the early phase of infection.

In a prospective multi-centre study on the first 72 h of treatment with one of three β-lactams, cefotaxime, piperacillin-tazobactam or meropenem, 138 ICU patients were included.

We found a high proportion of ICU patients not reaching the PK/PD targets suggested by European experts. Younger age, signs of augmented renal clearance, treatment with cefotaxime, and non-urinary tract infections were identified as risk factors for target failure where early therapeutic drug monitoring (TDM) could be encouraged.

When further investigating the impact of different minimum inhibitory concentration (MIC) parameters on the PK/PD target attainment, the current use of MIC_{WCS}, based on the bacterial worst case scenario (WCS), instead of the MIC_{ECOFF} (epidemiological cut-off (ECOFF)) based on the actual causative bacterial pathogen was found to overestimate target failure with risk of overdosing.

In predictions of target attainment using different infusion durations, we found that target attainment rates for primary pathogen scenarios were high regardless of infusion type, indicating that short infusion (SI) is sufficient in most community-acquired infections except for infections with *S. aureus* treated with cefotaxime, where a higher daily dose than 6 g is needed. In WCS-pathogens, reflecting infections with *P. aeruginosa*, SI was insufficient and routine use of extended (EI) or continuous (CI) infusions could be beneficial for piperacillin-tazobactam and meropenem. However, the risk of toxicity might increase and individualised TDM is warranted.

In a retrospective single-centre study of 255 ICU patients treated with gentamicin, the use of estimated gentamicin clearance CL derived from measured 8 h concentrations was found to be a potential exogenous marker of renal function in patients in an early phase of the severe infection which could improve dosing of renally eliminated drugs like the β-lactams.

Keywords: Intensive care unit, beta-lactams, minimum inhibitory concentration, pharmacokinetics, pharmacodynamics, target attainment, toxicity, prolonged infusion, therapeutic drug monitoring, glomerular filtration rate

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To patient N

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Smekal, AK., Furebring, M., Eliasson, E., Lipcsey, M. (2022) Low attainment to PK/PD-targets for β-lactams in a multi-center study on the first 72 h of treatment in ICU patients. *Scientific reports*, 12(1):21891

II. Smekal, AK., Furebring, M., Lipcsey, M., Giske, C. (2023) Swedish multi-centre study on target attainments with β-lactams in the intensive care unit: Which MIC-parameter should be used? *J Antimicrob Chemother*, 78:2895-901

III. Smekal, AK*., Swartling, M*., Furebring, M., Giske, C., Jönsson, S., Lipcsey, M., Nielsen, E.I. (2025) Short, extended and continuous infusion of β-lactams: predicted impact on target attainment and risk for toxicity in an ICU patient cohort. * Equal contribution. *J Antimicrob Chemother*, 2025; jan 23. Epub ahead of print

IV. Smekal, AK., Swartling, M., Nielsen, E.I., Furebring, M., Larsson, A.O. Can gentamicin concentrations be used to estimate glomerular filtration rate in intensive care unit patients? *Manuscript, in submission*

Other related publications by the author not included in this thesis

Jansson, AK., Enblad, P., Sjölin, J. Efficacy and safety of cefotaxime in combination with metronidazole for empirical treatment of brain abscess in clinical practice: a retrospective study of 66 consecutive cases. *Eur J Clin Microbiol Infect Dis.* 2004 Jan;23(1):7-14.

Swartling, M., Smekal, AK., Furebring, M., Lipcsey, M., Jönsson, S., Nielsen, E.I. Population pharmacokinetics of cefotaxime in intensive care patients. *Eur J Clin Pharmacol*. 2022 Feb;78(2):251-258.

Contents

Abbreviations

ACCIS	Antibiotic Concentrations in Critically ill ICU-patients in Sweden
ADR	Adverse drug reaction
AKI	Acute kidney injury
ARC	Augmented renal clearance
AST	Antimicrobial susceptibility testing
AUC	Area under the curve
BMD	Broth microdilution
BSA	Body surface area
C_{max}	Peak concentration
C_{min}	Minimum concentration (trough)
CI	Continuous infusion
CL	Clearance
CNS	Central nervous system
CPE	Carbapenemase-producing *Enterobacterales*
CRF	Case report form
eCL_{CR}	Estimated creatinine clearance
ECOFF	Epidemiological cut-off value
eGFR	Estimated glomerular filtration rate
$eGFR_{Creatinine}$	eGFR based on creatinine (LM-rev equation)
$eGFR_{CystatinC}$	eGFR based on cystatin C (CAPA-equation)
$eGFR_{Gentamicin}$	eGFR based on gentamicin clearance
EI	Extended infusion
EDL	EUCAST Development Laboratory
EMA	European Medicines Agency
ESICM	European Society of Intensive Care Medicine
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
f	Unbound (free) concentration of a drug
$fT_{>MIC}$	Fraction of time the free concentration exceeds the MIC
fC_{ss}	Free concentration at steady-state
IAI	Intra-abdominal infection
ICU	Intensive care unit

ID consultant	Infectious disease consultant
IQR	Interquartile range
ISO	International Organization for Standardisation
LC-MS	Liquid chromatography-mass spectrometry
LLOD	Lower limit of detection
LLOQ	Lower limit of quantification
LRTI	Lower respiratory tract infection
mGFR	Measured glomerular filtration rate
MRSA	Methicillin-resistant *Staphylococcus aureus*
MIC	Minimum inhibitory concentration
MIC_{ACTUAL}	Actual tested minimum inhibitory concentration
MIC_{ECOFF}	ECOFF-based minimum inhibitory concentration
MIC_{WCS}	Worst case scenario minimum inhibitory concentration
NONMEM	Non-linear mixed-effects modelling
NordicAST	Nordic committee on Antimicrobial Susceptibility Testing
PBP	Penicillin-binding proteins
PK	Pharmacokinetic
PD	Pharmacodynamic
RAF	Referensgruppen för antibiotikafrågor
ROC	Receiver operating characteristic
RRT	Renal replacement therapy
SAPS3	Simplified Acute Physiology Score 3
SI	Short infusion
SIRS	Systemic inflammatory response syndrome
SOP	Standard operating procedure
SSTI	Skin and soft tissue infection
TDM	Therapeutic drug monitoring
T½	Half-life
UTI	Urinary tract infection
V_D	Volume of distribution
WCS	Worst-case scenario
WT	Wild type

Introduction

Infections in the intensive care unit

Severe infections like sepsis and septic shock are life-threatening conditions and are common causes of emergency admissions to intensive care units (ICUs) globally (1, 2). In a recent point prevalence study on a worldwide sample of patients admitted to the ICU, more than 50% of the patients had a suspected or proven infection on the day of the study and the in-hospital mortality rate among this group was 30% (3). In another global study almost 70% of patients in the ICU were treated with antibiotics at some point of the ICU stay (4). Early treatment with efficient antibiotics has been proven to be lifesaving in septic shock (5-7). Among the antibiotics used in the ICU, the β-lactam antibiotics are considered the cornerstone for the treatment of infections because of their bactericidal effect and rapid killing of bacteria (8, 9). Despite efforts like the Surviving Sepsis Campaign, the mortality and morbidity caused by infections in ICUs remain high (1, 3).

Previous studies have shown that treatment with β-lactams often results in suboptimal antibiotic concentration levels (10-14). One reason could be that currently used antibiotic regimens are based on standard doses adopted from pharmacokinetic/pharmacodynamic-studies, (PK/PD-studies) in healthy volunteers and non-critically ill patients. Many clinical studies however have demonstrated that the pharmacokinetics in critically ill patients are very different since ICU treated patients have great inter- and intra-variation in total body water, plasma protein levels, volume of distribution, renal and liver function than healthy volunteers (10, 11, 15, 16). The reason for this is multi-factorial and includes both effects of the ICU treatment itself as well as fluctuating organ dysfunctions caused by the infection. The altered pharmacokinetics of antibiotics in ICU patients increases the risk of suboptimal antibiotic concentrations (under dosing) with a potential impact on outcome and the possible development of antimicrobial resistance in causative bacteria (17-19). Moreover, it also increases the risk of overdosing with possible toxic side effects in other patients (19).

The bacterial load in sepsis and septic shock is highest when the first doses of antibiotics are administered (20, 21). Therefore the first days of antibiotic treatment in the ICU and the antibiotic concentrations achieved during those days are of great importance to prevent mortality and morbidity among ICU

patients. It has been proposed that therapeutic drug monitoring (TDM) of antibiotics could help clinicians individualize dosing to overcome this problem (10, 22-25). However, because of economic and also practical issues TDM is not available outside the university hospitals in Sweden and elsewhere in many high-income countries, and a better understanding of which group of patients could benefit from TDM is warranted.

Several previous studies have investigated antibiotic plasma concentration in ICU patients (11-13, 26), but they did not study the first critical days of antibiotic treatment. Some of them also included a large proportion of patients on antibiotic prophylaxis instead of patients actually treated for an infection. In many previously published studies on target attainment for β-lactams, the causative pathogens were not taken into account and no MIC-testing was performed. Instead, a tentative worst case scenario MIC (WCS_{MIC}) has been used (11, 13). In a few studies, MIC testing of causative bacterial pathogens has been performed to some extent (12). However, the MIC testing was not performed with reference methods for MIC-testing, broth micro dilution (BMD) according to ISO 20776-2 (27). Accurate MIC values are of great importance in PK/PD- calculations for target attainment as well as the use of correct MIC-parameter (28). However, studies on the impact of using different MIC-parameters in the calculations are lacking.

β-lactam antibiotics

The most widely used class of antibiotics in the world are the β-lactams. In the US the β-lactams account for 65% of all intravenous antibiotics, in France 69% and in a Swedish ICU-study approximately 66% of the prescribed antibiotics (8, 29, 30). This class of antibiotics has many advantages like low toxicity, high efficacy and a variety of spectrum between different antibiotics within the class.

The fast bactericidal effect on dividing cells is caused by the interruption of the bacterial cell wall formation by the covalent binding between the β-lactam-ring of these agents and the penicillin-binding-proteins, PBPs of the bacteria. The PBPs are enzymes in the cell membrane and essential for the building of the bacterial cell wall after division in both gram-positive and gram-negative bacteria (29). Different bacterial species have different sets of PBPs that can range from three to eight enzymes per species (31). Their role is to create the terminal step of peptide-cross-linking between the peptide chains in the formation of peptidoglycan. When the PBPs are inhibited by the β-lactam antibiotics the cell wall building will stop and cause cell death.

The β-lactams can be divided into four major subclasses, the penicillins, the cephalosporins, the carbapenems and the monobactams, all with different

activity against different groups of bacteria. Some with broad-spectrum activity and some with narrow spectrum activity mainly due to their different affinity to bind to different PBPs of different bacterial species (29).

The β-lactams considered in this thesis, cefotaxime, piperacillin-tazobactam and meropenem belong to three of the four different subclasses of β-lactams.

Cefotaxime

Cefotaxime belongs to the 3rd generation cephalosporins, or the so called extended spectrum cephalosporins, and was introduced in the 1980s. Compared to the earlier generations of cephalosporins it was more stable against the most common penicillinases like the SHV and TEM β-lactamases which made the agents more active against gram-negative infections with the exceptions of those caused by *Pseudomonas aeruginosa* and *Acinetobacter* species. The activity against *Staphylococcus aureus* however was diminished compared to earlier cephalosporins (29). Despite this, the agent very quickly became a first line of choice in severe gram-negative infections in the ICU. It is still considered to be the empirical drug of choice in immunocompetent patients with community-acquired sepsis and septic shock in a low-resistance country like Sweden (32). However the agent has no effect on gram-negative bacteria harbouring ESBLs, the extended spectrum β-lactamases.

Cefotaxime is a small hydrophilic molecule with a protein binding in serum of approximately 25-40%. The compound undergoes hepatic metabolism to form desacetylcefotaxime which is the main metabolite (33). Desacetylcefotaxime has some antimicrobial effects that is about 2-16 times lower compared to cefotaxime and binds to the same PBPs as cefotaxime but weaker. On the other hand the half-time is longer than for cefotaxime (34, 35). However, the effect is also species dependent and earlier studies have reported the metabolite to have no antimicrobial effect on *Staphylococcus aureus*(36). About 80% of cefotaxime is excreted in urine by glomerular filtration and tubular secretion. About 50-60% as unchanged drug, 20% as desacetylcefotaxime and the rest as other metabolites. The elimination half-time is 1.2 hours for cefotaxime and 1.6 hours for the active metabolite in subjects with normal renal function (33). Dosage reduction is required in patients with severe renal insufficiency.

Piperacillin-tazobactam

Piperacillin-tazobactam belongs to the class of penicillins with a β-lactamase inhibitor. These agents were developed because of the increasing prevalence of extended β-lactamase producing gram-negative bacteria at the beginning of the 1990s. They consist of two parts, a β-lactam, in this case piperacillin, and a β-lactamase inhibitor, in this case tazobactam. Tazobactam has no antibacterial effect in monotherapy but instead serves as the substrate for β-lactam

enzymes leading to a restored sensitivity for piperacillin for bacteria producing among others classic ESBL. An advantage compared to cefotaxime is that the agent also has good activity against *P. aeruginosa*, anaerobic bacteria and *E. faecalis*. Because of this, the agent is considered one of the empirical choices for the treatment of severe infections in ICUs all over the world. In Sweden, it is recommended as empirical treatment in neutropenic immunocompromised patients, and empirical treatment in patients with intra-abdominal infections (IAI) or hospital-acquired pneumonia (37).

Piperacillin-tazobactam is a small hydrophilic molecule with a protein binding in serum of approximately 20-30%. About 50-60% of piperacillin is excreted unchanged by glomerular filtration and tubular secretion in the urine and < 2% by the biliary route. Tazobactam undergoes hepatic metabolism to an inactive metabolite and is excreted in the urine. The elimination half-time is 1 hour for piperacillin and 0.8 hours for tazobactam in subjects with normal renal function (38). Dosage reduction is required for patients with renal impairment

Meropenem

Meropenem belongs to the class of carbapenems which has the broadest spectrum of activity of the different classes of β-lactam antibiotics and is used in ICUs all over the world preferable in the most critically ill patients with hospital-acquired infections. They are stable against most β-lactamases and the use of this class increased during the 1990s and the 2000s when gram-negative ESBLs became more frequent, making the cephalosporins less attractive as empirical treatment in critically ill ICU patients (29, 39). During the last decades however, the emerging and increasing presence of carbapenemase-producing *Enterobacterales* (CPE) and carbapenemases in other gram-negative bacteria has made this class of antibiotics to some extent less reliable.

Meropenem was introduced in the late 1990s and has a very broad spectrum of activity covering gram-positive, gram-negative and anaerobic bacteria with the exception of *Enterococcus* spp, methicillin-resistant *S. aureus* (MRSA), *Stenotrophomonas maltophilia* and carbapenemase-producing gram-negative bacteria. In contrast to the other carbapenems, it can be used in infections of the central nervous system (CNS), like meningitis, because of its excellent penetration across the blood-brain barrier (29, 39). The indications for meropenem in Sweden is as an optional first line treatment in neutropenic sepsis in immunocompromised patients, sepsis with an IAI or lower respiratory tract infection (LRTI) in immunocompetent patients and meningitis (32, 37).

Meropenem is a small hydrophilic molecule with a protein binding of 2%. About 70% of the dose is excreted unchanged by glomerular filtration and tubular secretion in the urine within 12 hours. The elimination half-time is 1 hour in patients with normal renal function (40). Dosage reduction is required for patients with renal impairment

Resistance mechanisms

Since the discovery of penicillin G by Alexander Fleming in the 1920s there has been an intense race between the bacteria developing new resistance mechanisms to the β-lactams and the human development of new β-lactam agents with new functions to battle the development of resistance. The four major β-lactam resistance mechanisms in bacterial pathogens are PBP-mutations, production of β-lactamases and mechanisms leading to decreased uptake or increased efflux of the β-lactam agent from the cell (29).

β-lactam toxicity

β-lactams have traditionally been considered a very safe class of antibiotics with a wide therapeutic index and low risk of side effects (41, 42). However, adverse drug reactions (ADRs) occur and in recent years reports about serious ADRs of the CNS, like neurotoxicity, have been on the rise – especially for some cephalosporins like cefepime (43-48). This together with increased standard dosing of β-lactams in the ICU due to expanded knowledge about the significant changes in critically ill patients' pharmacokinetics (PK) of β-lactams, has led to discussions about the risk for toxicity associated with higher β-lactam exposure (8, 41, 46).

Apart from neurotoxicity, the β-lactams can also cause nephrotoxicity, and both of these ADRs have traditionally been considered to be linked to excessively high antimicrobial exposure and monitored by using trough concentrations in patients given intermittent dosing (42, 44). European guidelines regarding toxic threshold in intermittent dosing in the ICU were proposed in 2020 and set to total trough concentrations of 20 mg/L for cephalosporins (based on cefepime neurotoxicity), 44.5 mg/L for carbapenems (based on meropenem nephro- and neurotoxicity) and 361 mg/L for penicillins (based on piperacillin nephro- and neurotoxicity)(24). However, these recommendations are based on very limited data and should be used with caution since different antibiotics within each β-lactam group have different characteristics and safety profiles regarding for example neurotoxicity. The cephalosporin threshold is based exclusively on cefepime, but cefotaxime, for example, has a much lower ability to cause nephro- and neurotoxicity compared to other cephalosporins (49-53). The suggested threshold of 20 mg/L is most likely too strict for cefotaxime and the French national guideline recommended using an upper total trough concentration for cefotaxime of 60 mg/L (8). This threshold can also be supported by some case reports regarding neurotoxicity (43, 46, 54). Consensus guidelines regarding toxicity levels when using continuous infusions of β-lactams do not exist.

Neurotoxicity is especially challenging in the ICU because of the large panel of clinical signs and often sedated patients. Seizures, hallucinations, ab-

normal movements like myoclonus and tremor, disturbed vigilance, encephalopathy and confusion are the most often reported signs of neurotoxicity due to β-lactam therapy (51, 52). The main risk factors for developing neurological adverse reactions are renal insufficiency, higher age, underlying CNS disorders and use of excessive doses (52, 55). Some β-lactams are also known to be more prone to cause neurotoxicity for example cefepime, ceftazidime, piperacillin and imipenem (51, 52, 55).

Besides neuro- and nephrotoxicity β-lactams can also cause cytopenia, including leukopenia. The exact mechanism is not known but potentially via immunological reactions or direct toxicity to the cells (42, 56). High doses of benzylpenicillin or cefotaxime for longer than two weeks have been associated with the development of leukopenia in earlier studies (57, 58). This suggests a possible exposure-related toxicity from β-lactam degradation products that accumulate over time (42). Hepatotoxicity is mostly associated with the use of cloxacillin and amoxicillin-clavulanic acid and is usually described as immunogenic and associated with the presence of certain HLA-alleles. Thus, no clear concentration toxicity relationship has been established for hepatotoxicity and cytopenia (42).

Aminoglycosides

The aminoglycosides were introduced into clinical use in the 1940s after the discovery of streptomycin derived from *Streptomyces griceus*. Gentamicin, tobramycin and amikacin were introduced for parenteral use during the 1960s and the 1970s (59).

For many years this antimicrobial class was widespread as a first-line antibiotic and one of the cornerstones in antimicrobial treatment globally (60). However, the replacement as first-line antibiotic began in the 1980s with the introduction of extended-spectrum cephalosporins and later carbapenems and fluoroquinolones, all antibiotics with a more favourable safety profile compared to the aminoglycosides which are known to have considerable intrinsic toxicity, mainly ototoxicity and nephrotoxicity.

All aminoglycosides feature rapid bactericidal activity and their mechanism of action is by binding to the 16S rRNA in the 30S ribosomal subunit and causing interruption of the protein synthesis of the bacterial pathogens (59).

Gentamicin

Gentamicin was introduced into parenteral use in 1971 (61). Nowadays this aminoglycoside is considered active in monotherapy only against infections with *Enterobacterales* and *Acinetobacter* spp. originating from the urinary tract. Combined with another active agent it can also be used in combination

therapy in other infections for the species listed above and also in infections with *Staphylococcus* spp (37, 62).

As a consequence, gentamicin is mostly used in Sweden in combination therapy with a non-carbapenem β-lactam in cases of septic shock caused by gram-negative bacilli in the ICU. In some regions of Sweden, it is also used in combination therapy with benzylpenicillin as an initial empirical treatment in immunocompetent adult patients in cases with unknown focus of infection. This combination is also used in many neonatal ICUs in the early stages after birth with unknown focus of infection.

Antimicrobial susceptibility testing and breakpoints

Antimicrobial susceptibility testing (AST) of clinically relevant bacterial isolates causing infections is a cornerstone practice in clinical microbiology laboratories all over the world. The results of AST are used to guide treatment in a specific individual leading to targeted treatment for the patient. The goal of AST is to predict the likely outcome of treating an infection in a specific patient with a specific antibiotic agent. However, the aggregated epidemiological data obtained from retrospective analyses of results for pathogen/antimicrobial AST are the basis for international, national and regional treatment guidelines in for example the ICU and are also of great importance. Furthermore, AST and detection of acquired resistance mechanisms considered a threat to public health and the healthcare system, like MRSA or CPE, is important for the prevention of healthcare-associated outbreaks of multidrug-resistant (MDR) strains where AST results serve as a warning sign leading to infection control and /or public health measures.

AST is performed mainly by phenotypic methods, and they are all based on standardised measurements of the minimal inhibitory concentration (MIC) of antimicrobial agents for a specific microorganism and the availability of clinical breakpoints for that combination. The clinical breakpoints are cut-off values between the susceptibility categories, and are set in Europe by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

EUCAST was formed in 1997 by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) with the ambition of creating one European breakpoint committee and to harmonize breakpoints and AST methods in Europe and beyond since there were a plethora of methods and technical recommendations at that time mainly due to the six different national breakpoint committees in Europe (63, 64). Since 2005 EUCAST steering committee also collaborates with the European Medicines Agency (EMA) to set clinical breakpoints for existing and new antimicrobials using a standard operating procedure (SOP)(65).

The decision process establishing a clinical breakpoint is based on information on clinical results related to antimicrobial dosing and exposure, MIC-

values of targeted pathogens and aggregated MIC-distributions as well as PK/PD-data of the antimicrobial (64, 66). With the new EUCAST definition of susceptibility categories from 2019, this relationship between antimicrobial exposure (dosing) and effect has been emphasized.

- "S", means the organism is susceptible with standard dosing regimens.
- "I", means the organism is susceptible with high dosing regimens.
- "R" means that there is a high likelihood of therapeutic failure even with high dosing regimens.

In summary, an "S" result in the antibiogram indicates that the organism should respond to therapy with the tested antibiotic agent provided that you use the dosage regimen that the clinical breakpoint is based on (64). The dosing regimens the clinical breakpoints and susceptibility categories are based on can be found in the EUCAST breakpoint table for all antibiotics (62).

A key tool in breakpoint setting for EUCAST is the aggregated MIC-distributions for each species/agent combination that are also available on EUCAST website (64). These MIC-distributions are the basis for identifying epidemiological cut-off values (ECOFFs) for each combination. The ECOFF is the MIC-value that distinguishes between isolates with and without acquired resistance mechanisms. The part of the population with a MIC-value below the ECOFF is called the wild-type (WT) population (Figure 1).

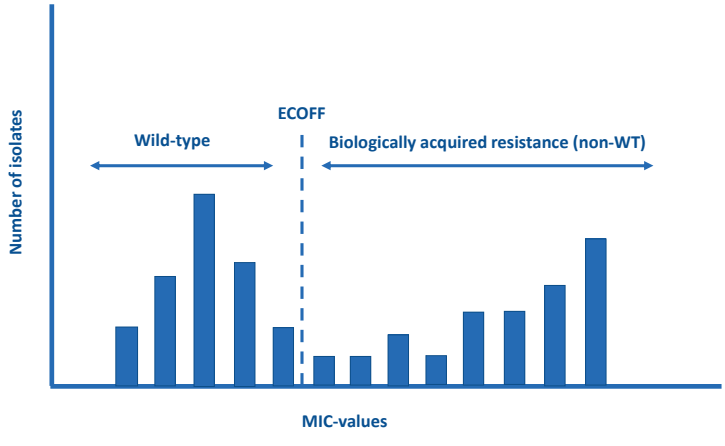

Figure 1 MIC-distribution where the wild-type (WT) population devoid of resistance mechanisms is shown. The upper limit of the WT is the epidemiologic cut-off value (ECOFF)

Most WT distributions cover 3-5 dilution steps and the ECOFF is the lowest possible clinical breakpoint since breakpoints always are set to avoid splitting the WT. The reason for this strategy is to minimize the risk of miscategorization of AST results due to assay variations of MIC-testing (64, 66, 67). In

many cases the species/agent combinations are simple and the ECOFF and the clinical S-breakpoint are the same (Figure 2).

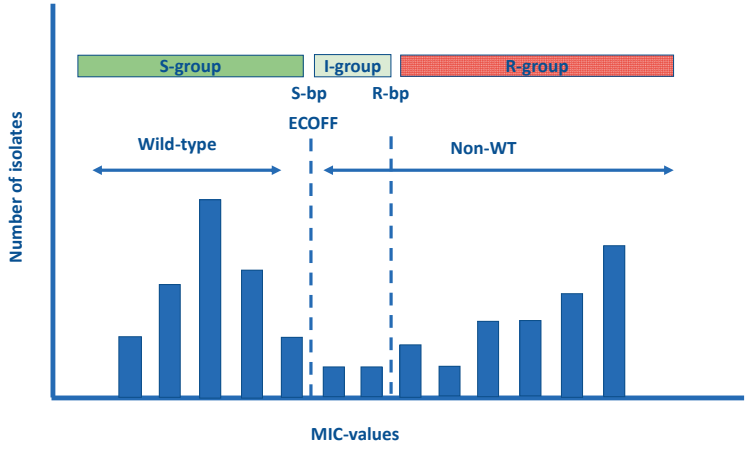

Figure 2 MIC-distribution when the ECOFF and the clinical S-breakpoint are identical

For some combinations, like *Enterobacterales* and meropenem, the situation is somewhat more complex (Figure 3).

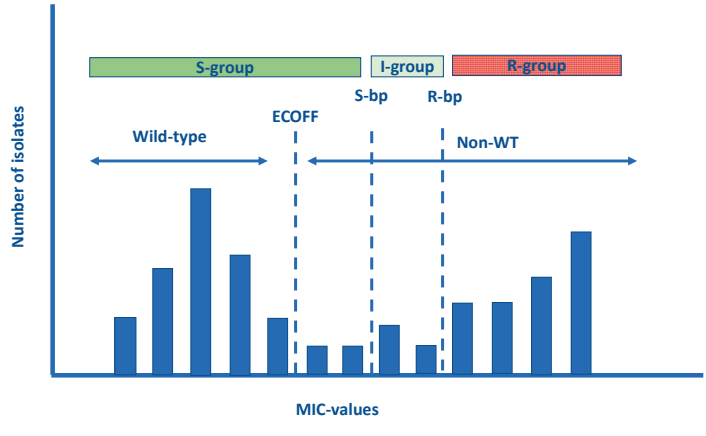

Figure 3 MIC-distribution when the clinical S-breakpoint is higher than the ECOFF

Broth microdilution (BMD) is considered the reference method for MIC-testing for most bacterial species by the International Organization for Standardisation (ISO) (27). For some slow-growing pathogens like the anaerobes and for some problematic antibiotics to test, like fosfomycin and mecillinam, the reference method is not BMD but agar dilution (62).

All other phenotypical methods including disk diffusion test, gradient test and semi-automated AST-systems are surrogate MIC-methods and must be carefully calibrated to the reference method as part of formal accreditation (67).
The EUCAST disk diffusion method was launched by EUCAST in 2009 and compared to other surrogate MIC-methods this method is well calibrated to MIC-results for BMD and the yearly updated breakpoint table includes both clinical breakpoints for MIC-testing as well as for EUCAST disk diffusion method. Each year EUCAST development laboratory (EDL) also performs calibration and validation of zone diameter breakpoints against MIC-values which is presented as a report on the website (64, 67, 68).

Reference-methods for MIC-testing

Broth dilution test including BMD

The macro-broth dilution method was one of the earliest AST-methods and the pictures of this method are often used to explain the MIC-concept. The test procedure includes a series of test tubes with broth and a rising 2-fold dilution of antibiotics. These tubes are inoculated with a standardized bacterial inoculum of the isolate you wish to test and following overnight incubation at 35°C the visible growth in the tubes is assessed. The tube with the lowest antibiotic concentration preventing growth represents the MIC-value of the isolate (69) (Figure 4).

Figure 4 Macro-broth dilution method

However, this method is very time consuming due to all the manual steps in preparing the antibiotic test tubes and has been replaced by the broth microdilution testing in trays, BMD (Figure 5). This test method is a development of the first macro broth dilution test and follows the same principle but instead of tubes the antibiotic series are in wells of a tray. The trays usually contain 96 wells that allow approximately 12 antibiotics to be tested in a range of doubling dilution steps. A standardized inoculum of the isolate you wish to

test is inoculated to the microtiter wells and incubated under standardized conditions for 16-24 hours depending on species. The well with the lowest antibiotic concentration preventing visible growth represents the MIC of the isolate/agent combination (69, 70). Nowadays these panels are commercially available from different manufacturers.

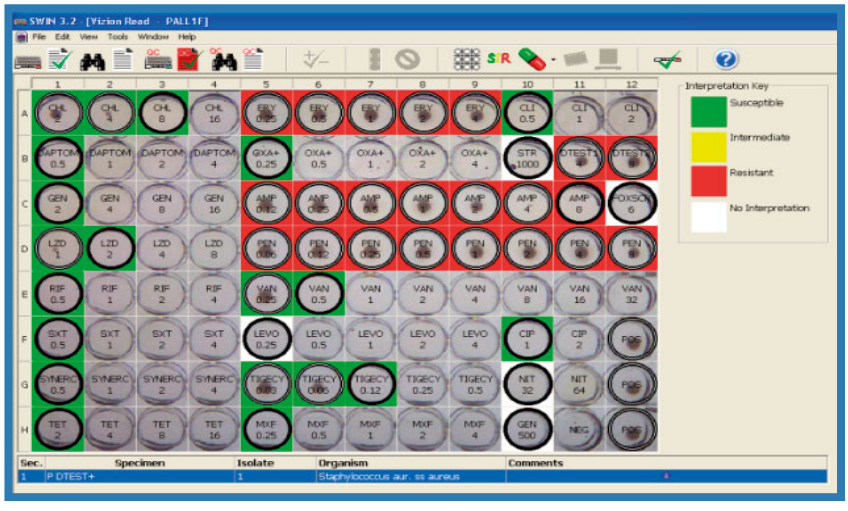

Figure 5 Reading of the broth microdilution method (BMD) in the SWIN-system

Agar dilution method

The agar dilution test is also one of the earliest AST- methods. Different 2-fold concentrations of the antibiotic agent you wish to test are added into several different Petri dishes containing agar medium. This means that each agar plate has a special antibiotic concentration and on every plate, several different clinical isolates, up to 32 including quality control strains, are inoculated under standardized conditions (71). When reading the results the lowest concentration of the antibiotic that will prevent macroscopically visible growth of the bacteria is considered the MIC of that isolate (Figure 6).

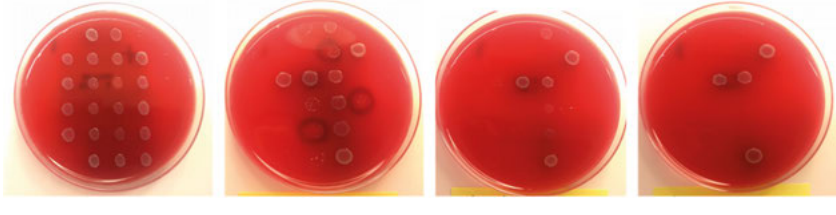

Figure 6 Agar dilution method.

Four plates with rising antibiotic concentrations of meropenem. From the left 1) Positive control without antibiotic. 2) With 2 mg/L. 3) With 4 mg/L and 4) with 8 mg/L

The advantage of the method is that you can test a large number of isolates at a relatively low cost but above all the method allows testing of fastidious pathogens like the anaerobe that do not grow well in broth media (70, 71). However, in a clinical routine laboratory, the disadvantages of this method are that the production and control of the agar plates are expensive and time consuming, especially when you only have a few clinical isolates to test making the use of this method often limited to reference laboratories.

Surrogate methods for MIC-testing

The EUCAST disk diffusion method

Disk diffusion is one of the oldest AST-methods and is also widely used in routine microbiology laboratories all over the world (72). However, it requires well educated and trained staff as well as quality control measures for providing accurate results but has many advantages compared to semi-automated systems (73). In the disk diffusion method you suspend bacterial colonies of the isolate you wish to test in a saline solution to a density of 0.5 McFarland. The suspension is inoculated on agar plates and antibiotic disks are applied within 15 minutes on the plate. After 16-20 hours of incubation, the inhibition zones are measured with a calliper or automated zone reader and the zone diameters are interpreted according to the EUCAST breakpoints (Figure 7) (72).

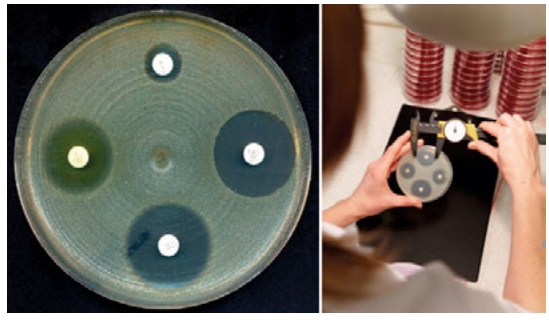

Figure 7 EUCAST disk diffusion method. Reading of zone diameters.

The greatest benefits are low cost for testing and is easy to implement since EUCAST provides free education material and support on the website (64, 72). The method is also very flexible and the antibiotics tested can easily be adapted fast for local needs. When EUCAST develops clinical breakpoints for new antimicrobials they also examine the best disk potency of the new agent parallel to clinical trials making the disk diffusion method commercially available within months after approval of new antibiotics (72, 73).

The EUCAST disk diffusion method is very standardised and provides precise rules for which agar medium to use, preparation of the bacteria inoculum and its turbidity, how to inoculate the agar plates, application of antimicrobial

disks, maximum time from inoculum to incubation, exact incubation temperature, atmosphere and time, examination of plates after incubation and also rules for measurement of inhibition zones and interpretation of results as well as rules for daily quality control (72). All steps of the procedure must be followed for the clinical breakpoints to be valid. In Sweden, all clinical laboratories perform AST using the EUCAST disk diffusion method as the standard method. All clinical isolates in this thesis were tested with the EUCAST disk diffusion method at the local clinical microbiology laboratories.

Basic concepts of PK/PD

The pharmacology of antimicrobial therapy depends on the pharmacokinetics (PK) and the pharmacodynamics (PD) of the drug and the relationship between them is described as the PK/PD-indices.

The PK describes how the body processes the drug in terms of how the concentration of a single dose changes over time depending on the four PK parameters: absorption, distribution, metabolism and excretion of the drug leading to a specific exposure of the drug (Figure 8).

The PD on the other hand describes how the drug exposure after a single dose impacts the body, or the dose-response relationship. In the case of treatment with a β-lactam agent in the ICU, this means the effect of the antimicrobial on the specific pathogen causing this infection depends on the MIC, or the susceptibility, of the pathogen (Figure 8),

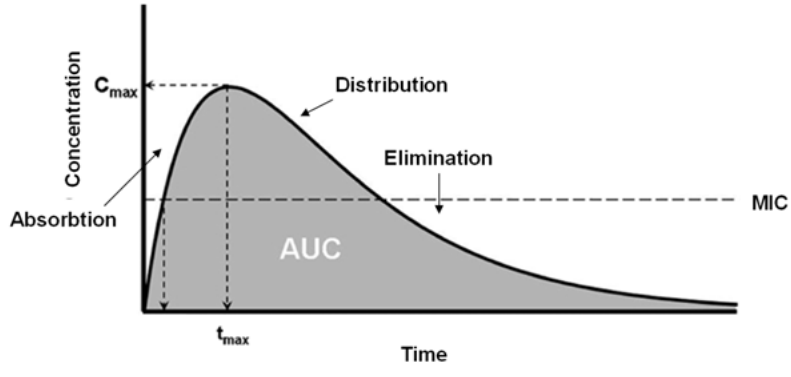

Figure 8 Basic concept of PK/PD showing:

a) Phases of PK: absorption, distribution, and elimination.

b) The PK/PD-parameters of the concentration/time curve used in PK/PD-indices: C_{max} (the maximal concentration from a single dose), the AUC (area under the curve) and the MIC (minimum inhibitory concentration of the pathogen)

c) In this example the plasma concentration remains above the MIC for around half of the dosing interval (=50%$T_{>MIC}$)

For antimicrobial agents, there are three different PK/PD-indices that describe the correlation between the exposure (PK), the MIC and the effective killing of the causative bacteria (PD), i.e. the treatment efficacy (Table 1) (74-76). The best PK/PD- index for the β-lactams is the percentage of time the antibiotic concentrations exceeds the MIC of the pathogen (74, 75)

Table 1. The three PK/PD-indices.

PK/PD-index correlating to bacterial killing/treatment efficacy	Explanation of PK/PD-index	Antimicrobial agent
$T_{>MIC}$	Percentage of time the concentration exceeds the MIC	Penicillins, cephalosporins, carbapenems, monobactams
$AUC_{0\text{-}24\ h}$/MIC	The area under the concentration curve divided by MIC during 24 hours.	Fluoroquinolones, vancomycin, tigecycline, aminoglycosides
C_{max}/MIC	The peak concentration divided by MIC	(Previously used for aminoglycosides)

Once the PK/PD-indices is known the magnitude of the index which is needed for antimicrobial efficacy can be established. This cut-off value, known as the pharmacodynamic target (PDT) or PK/PD-target is the magnitude of the index at which a desired level of predicted response is achieved. For new antimicrobials, the magnitude of the index is usually derived from a combination of animal studies, in vitro PD-studies on bacterial growth and clinical PK/PD-response studies. For the β-lactam class of antimicrobials, the PK/PD index is $T_{>MIC}$, and the magnitude of the index is the percentage of time that the agent's plasma concentration remains above the MIC.

The optimal PK/PD-target for β-lactam in critically ill patients is still unknown and has been a matter of discussion for many years but many experts suggest that 100%$fT_{>MIC}$ could be the best PK/PD-target for intermittent infusions to use in PK/PD-studies and in TDM situations (24) Some authors argue that a higher PK/PD-target of 50-100%$fT_{>3\text{-}5xMIC}$ could be beneficial to reduce the risk of antibiotic resistance developing during the ICU-treatment (8, 11, 77, 78). However, even if the β-lactams have a wide therapeutic window, neurotoxicity can occur with high exposure(41, 51), which must be considered if this PK/PD-target is used.

Important PK-parameters for the absorption phase of a specific antimicrobial agent include the peak plasma concentration (C_{max}) reached after a specific dose as well as the lowest (trough) concentration prior to the next dose (C_{min}). It is the free fraction of the total concentration of the antimicrobial

agents in plasma that is pharmacologically active and usually used in PK/PD-calculations. This is indicated by "*f*" in the PK/PD-indices.

The distribution phase describes what happens to the drug from the site of measurement, i.e. plasma, and its distribution to other parts of the body like the peripheral tissues. Each antimicrobial has its own volume of distribution (V_D) depending on the drug's chemical characteristics and this is a PK-parameter that quantifies the extent of this distribution. It is a theoretical volume the drug would have to occupy in the body and does not necessarily follow defined anatomical divides. However, each compartment is always considered homologous.

The phases of metabolism and excretion are often grouped and called the elimination phase. The most important PK-parameter of this phase is the drug clearance (CL), or the volume of the plasma that is cleared from the drug each time unit (L/h).

The PK-parameter half-life (T½) describes the time from C_{max} to a plasma reduction of 50%, and is dependent on the distribution and the elimination of the drug, i.e. on V_D and CL. The term "steady-state" is often used. This refers to a situation where there is a fairly dynamic equilibrium between the absorption and the elimination. For β-lactams, this state is considered to be reached for regular dosing within 3-5 times the agent's half-life (T½).

When developing new antimicrobials the PK/PD approach and mathematical models are used to describe the relationship between the kinetics and the dynamics to determine the optimal dosing regimens of the agent (76). The PK/PD approach is also used to help establishing clinical susceptibility breakpoints for bacteria/agent combinations by EUCAST (66). For already existing antimicrobial agents PK/PD analyses are also important to optimize dosing regimens to improve efficacy and to minimize toxic effects and also the emergence of resistance (76).

The variability of PD

The variability of MIC

Even when a reference method for MIC-testing is used in a laboratory with trained staff the precision of the BMD method is considered to be plus or minus one two-fold concentrations even under the best circumstances, due to assay variations of the method (28, 69). The variability of the MIC-measurement consist of both intra-laboratory and inter-laboratory components of differences in technical skills and degree of training among staff and to some extent also biological variations due to strain-to-strain differences within a species. The MIC is therefore not a strict MIC-value but rather an observable minimum concentration range and there is no such thing as a "true" MIC for a specific isolate (28). For many clinicians and often in TDM situations and

PK/PD-studies however, the variability of the MIC-parameter in PK/PD-calculation is not taken into account which may cause dosing adjustment errors when a single MIC-test result is used in the calculations. This can however be dealt with if we start to think about the MIC from a MIC-distribution point of view as pointed out by Mouton et al (28).

The MIC-distribution of a specific species/antimicrobial combination can be divided into two distribution groups each. The WT population, without any required resistance mechanisms, and the non-WT population with biologically acquired resistance mechanisms. In TDM situations a measured MIC-value within the WT should be handled by using the ECOFF (MIC_{ECOFF}) of the species/agent combination whereas a MIC within non-WT-population should be handled by adding a two 2-fold dilution step to the actual MIC measured (28) The use of the actual MIC-value (MIC_{ACTUAL}) within the WT-population is discouraged due to the method variation of MIC-testing (Figure 9) (28).

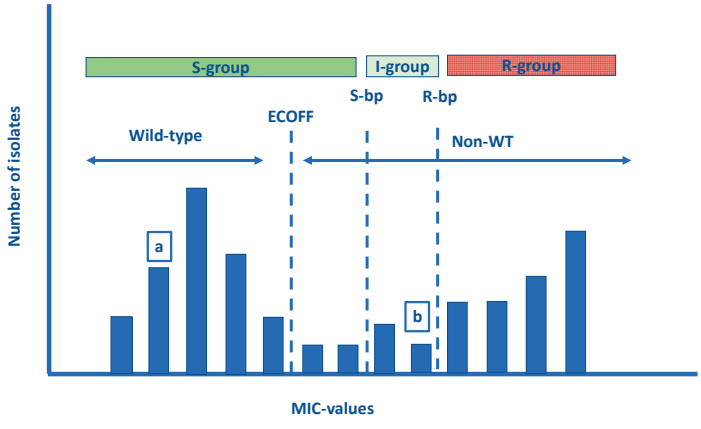

Figure 9 MIC-distribution with examples from Mouton et al. on what MIC-parameter to use for clinical therapeutic drug monitoring or PK/PD-studies depending on the actual MIC (MIC_{ACTUAL}) of the causative pathogen.

a) MIC_{ACTUAL} within the wild-type (WT) population: use the ECOFF (MIC_{ECOFF}).

b) MIC_{ACTUAL}) outside the WT population but still below the R-breakpoint: use MIC_{ACTUAL} + 2 dilution steps.

In many PK/PD-studies however, no causative pathogen is collected or tested with AST, instead a common approach is to use a tentative worst-case scenario MIC-value (MIC_{WCS}) which represents the highest MIC_{ECOFF} available for a pathogen still considered to be sensitive for the antimicrobial you study. From a clinical point of view, this represents the highest tentative MIC the initial empirical treatment should cover. For cefotaxime this MIC_{WCS} is the MIC_{ECOFF} for *S. aureus* of 4 mg/L (79). For piperacillin-tazobactam and meropenem, the MIC_{WCS} is the MIC_{ECOFF} for *P.aeruginosa* of 16 mg/L respectively 2 mg/L

(79). However, the relevance of this approach using MIC_{WCS} in target attainment studies is poorly investigated.

The variability of PK

Impact of critical illness on PK in the ICU

In critically ill ICU patients not only the naturally underlying infection alters the PK of a specific antimicrobial agent, but also the interventions associated with modern intensive care (18, 24, 80). The results in an increased risk that standard dosing regimens developed on healthy individuals may lead to either under- or overexposure depending on the class of antimicrobials in an ICU population.

Volume of distribution

As mentioned above V_D is an important PK-parameter influencing the absorption phase and the achieved plasma concentration of an antimicrobial agent. The β-lactams are hydrophilic agents with a low V_D which means that they are primarily distributed in the systemic circulation. However, in critically ill patients with sepsis or septic shock, the "capillary leak" syndrome causes fluid shifts from the intravascular compartment to the interstitial space resulting in an expanding V_D for hydrophilic antimicrobials (80, 81). Also, several medical interventions during the ICU care itself like aggressive fluid resuscitation, mechanical ventilation, extracorporeal circuits and total parental nutrition (TPN) have been reported to be associated with enlarged V_D for hydrophilic antimicrobials (82-85).

Since the β-lactams are hydrophilic, an enlarged V_D consequently leads to decreased concentrations in plasma in an ICU patient compared to a non-critically ill patient given the same dose.

Protein binding and hypoalbuminemia

Hypoalbuminemia in ICU patients is usually caused by extreme fluid extravasation, down-regulation of the hepatic synthesis or a combination of both. When a patient develops hypoalbuminemia the free fraction of the antimicrobials increases. For β-lactam antibiotics with a high protein-binding, like ceftriaxone, the higher free concentration gets more available for distribution and the V_D for these antimicrobials may be increased. Since β-lactams also are cleared renally, the increase in free concentration will also lead to a more rapid CL (80, 86). Cefotaxime and piperacillin-tazobactam are considered moderately protein-bound compounds whereas meropenem has a low protein-binding.

The altered V_D and CL may lead to low antibiotic concentrations, particularly at the end of the dosing interval in ICU patients treated with β-lactams. The risk is probably higher for moderate to highly protein-bound β-lactams(18, 87).

Changes in drug clearance

In critically ill patients with infections, renal function varies both within and between patients since they are at risk of both augmented renal clearance (ARC), and most commonly, acute kidney injury (AKI),

Many critically ill ICU patients with severe infections develop hyperdynamic cardiovascular responses as part of a systemic inflammatory response syndrome (SIRS) with an increased cardiac output leading to enhanced blood flow to major organs like the kidneys. The increased renal blood flow leads to increased glomerular filtration rates and/or tubular secretion leading to a higher CL of renally eliminated antimicrobials like the β-lactams. Also, the ICU treatment for hypotension itself, with large boluses of intravenous fluids and vasopressor infusions, increases the cardiac output and the glomerular filtration and causes an increased renal CL. In some cases all these mechanisms put together can lead to ARC, a state of hyperfunctioning kidneys, often defined as creatinine CL greater than 130 ml/min (80, 88-91). Although highly dependent on patient mix, the pooled prevalence of ARC in the ICU has been reported to be 39% in one meta-analysis (89). Risk factors for ARC include ICU populations with critical illness due to sepsis and septic shock, trauma or neurocritical care, especially if young age < 60 years without cardiovascular comorbidities and male sex (80, 92). ARC has been strongly associated with suboptimal β-lactam concentrations, and target attainment failures and is most often seen in the initial and most acute phase of severe infections (89-91, 93-96).

Decreased renal function due to AKI on the other hand, is associated with the risk of overexposure and toxicity of renally eliminated drugs like the β-lactams and also with increased risk of mortality and morbidity (18, 97-101). AKI can be seen in 60% of patients with sepsis or septic shock in the ICU (97), although the prevalence varies in different ICU cohorts.

In severe infection in critically ill patients' myocardial depression sometimes occurs leading to decreased organ perfusion and microcirculatory failure which may cause multi-organ dysfunction syndrome (102). This syndrome often includes both renal and hepatic dysfunction that alters the elimination of antimicrobials.

The use of renal replacement therapy (RRT) in the ICU has been shown to further alter the PK of β-lactams leading to variable CL and dosing requirements that can shift on a day-to-day basis(18, 80, 103)

Assessment of renal function

Correct renal function measures are crucial for several aspects of patient management in the ICU patient, including dosing to achieve adequate target organ levels of renally eliminated drugs like the β-lactams but also for predicting the risk of disease progression and determining the need for RRT(104).

The best indicator of global kidney function is the glomerular filtration rate (GFR). GFR cannot be measured directly, instead measured CL of inulin, iohexol or EDTA, all exogenous filtration markers, are used as reference methods for measured GFR (mGFR)(105). These methods are, however, not optimal to use in unstable ICU patients since they often are considered to be impractical, time-consuming and expensive (105, 106).

Instead estimated GFR (eGFR) from endogenous filtration markers like creatinine ($eGFR_{Creatinine}$) and cystatin C ($eGFR_{CystatinC}$) measured in serum are most commonly used in the ICUs. These eGFR methods based on creatinine and/or cystatin C have been reported to be good predictive biomarkers of risk for long-term cardiovascular death, mortality, and the need for acute RRT during ICU stay which is an advantage (107-111). However, both creatinine and cystatin C have limitations as eGFR markers of renal function, especially in critically ill patients without steady-state conditions, and can both under- and overestimate GFR in ICU patients (106, 112-115).

Creatinine

Serum levels of creatinine are influenced by gender, ethnicity, diet, muscle mass, fluid balance and physical activity since creatinine is produced in the muscle cells(116, 117). In ICU patients both muscle wasting and nutritional status are altered which will affect creatinine production.

The creatinine equations for eGFR are also based on patient cohorts with low severity of illness and without the alterations that can be seen in ICU patients(114).

AKI and RRT are other factors known to influence the creatinine level which makes this marker of renal function less reliable in ICU patients(106, 118)

Cystatin C

Serum levels of cystatin C are influenced by corticosteroids, thyroid hormones and possibly smoking, inflammation and obesity(106, 113, 118-121) . However, cystatin C is generated in all nucleated cells and thus is less affected by muscle mass and nutritional status compared to creatinine.

A systematic review of five ICU studies found that despite the improvements of eGFR based on cystatin C compared to creatinine, $eGFR_{CystatinC}$ both underestimates and overestimates renal function compared to mGFR in critically ill patients (113).

Strategies to improve antimicrobial exposure of β-lactams

Prolonged antibiotic infusion duration

β-lactams display time-dependent bactericidal activity depending on the time the free drug concentration remains above the MIC of the causative bacterial pathogen during the dosing interval (74). Prolonged infusion (PI) is thus a theoretical way to improve target attainment compared to short intermittent infusion, as it produces more even β-lactam concentrations maintained above the MIC for a longer period of time with the same daily dose (122). This is the rationale behind the increasing interest and use of prolonged infusion, above all continuous infusion in ICU patients in recent years.

Intravenous infusion <60 min is usually defined as short infusion (SI) whereas prolonged infusions are divided into extended infusion (EI) of 3-4 hours and continuous infusion (CI) of 24 h duration (9). A concentration-time illustration of the three different infusion strategies without bolus doses is depicted in Figure 10.

Recent consensus guidelines recommend PI given as EI or CI in critically ill patients (9). However, the impact on mortality compared to SI still remains unclear (123-125) and further studies that compare EI and CI are warranted.

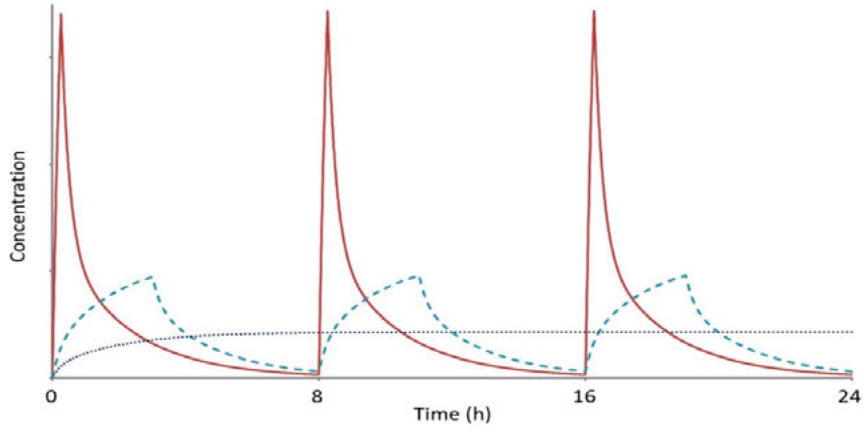

Figure 10 Illustration of concentration-time profiles for short (solid line), extended (dashed line) and continuous infusion (dotted line) for β-lactams following the same daily dose

TDM for dose individualisation

In situations where population-level dosing recommendations are insufficient to reach target attainment for a sufficient proportion of a specific patient cohort, methods for dose individualization including TDM, are important tools (126).

Traditionally, TDM on antimicrobials have focused on agents with a narrow therapeutic range and risk of nephrotoxicity levels like aminoglycosides and vancomycin, which have been well integrated into clinical routine practice for many years and are widely available. β-lactams, traditionally thought of as a group of antibiotics with low risk of toxicity, have not earlier been prioritised for TDM. (23). However, the rise of multi-drug resistant bacterial pathogens and the declining antimicrobial pipeline, in combination with increasing knowledge about the altered PK in ICU patients and the risk of insufficient antimicrobial exposure with standard doses as well as risk for toxicity with more aggressive doses/infusion strategies have resulted in a need to revise this approach (22, 25, 126, 127).

Since the β-lactams mostly used in Swedish ICU are hydrophilic with a low VD, predominately cleared by renal elimination and have a low (<30%) or moderate (30-70%) protein binding, the PK alterations caused by the infection and the ICU care itself in these critically ill patients is heterogeneous and unpredictable. Particularly in the earlier phase of critical illness, these PK alterations may lead to inadequate β-lactam concentrations which may affect treatment outcome (93-95). This is the rationale behind the possible role of TDM on β-lactam in ICUs and in a position paper from 2020 by among other European Society of Intensive Care Medicine (ESICM) and European Society of Clinical Microbiology and Infectious Diseases (ESCMID) TDM for β-lactams is recommended in the ICU (24).

However, there are many problems in adapting to this recommendation in clinical routine practice and it also comes with a cost. Among others, there is a lack of capacity in most clinical laboratories and slow turn-around times when the analysis is available. In Sweden today, TDM for β-lactams are only available in four of the university hospitals. There is also a need for the development of assays that measure the actual free concentration, not only the total concentration of the β-lactam, which today only is available in one of Sweden's laboratories.

There is also a need for PK/PD-studies on which particular groups of ICU patients could benefit from TDM and if TDM in a low resistance setting like Sweden would be meaningful. As the experts point out in the position paper there is still a lack of controlled studies focusing on comparing outcomes with and without TDM-guided antimicrobial dosing. However, in clinical TDM-situations not only the actual concentration of the antimicrobial in question is essential for dose adjustments. The understanding of the variability of the MIC measurement and the usage of appropriate MIC parameter is also essential.

It is important to remember that the antimicrobial dosing regimens to a great extent are based on PK/PD-studies on healthy young individuals volunteering for studies. Critically ill patients are rarely included in the different phases of drug development (18). This means that the dosing regimens for older antimicrobials like the β-lactams cefotaxime, piperacillin-tazobactam and meropenem might not be optimal for the critically ill ICU patients, where one or several of the four PK-phases described above can be altered compared to a healthy individual (18, 76).

Another approach to battle the reported underdosing in the ICU besides direct individual TDM is the use of statistical estimations on the possibility of achieving 100%$fT_{>MIC}$ for different β-lactam dosing strategies based on Monte Carlo mathematical simulations. This method can use TDM data with population PK-parameters of ICU populations and an appropriate MIC-parameter to investigate alternative dosing regimens' impact on target attainment with the goal of finding the optimal initial empirical dosing regimens of β-lactams in the ICU. For instance, Clinical pharmacology laboratories could make such simulations based on TDM data from their ICU patients. Typically, a Monte Carlo simulation will simulate around 10,000 individual plasma concentration profiles and based on this inflate the variation from smaller PK studies to what would be realistic on a population level.

Aims

The overall aim of this thesis was to investigate strategies for optimisation of β-lactam dosing in ICU patients during the early phase of infection.

The specific aims were:

- To investigate if antibiotic concentrations in plasma met the PK/PD targets during the first 72 hours of β-lactam treatment with standard dosing in an ICU cohort (paper I).
- To investigate the association between failure to reach the PK/PD target versus patient and treatment characteristics in the ICU cohort. The goal was to identify patients at risk for under- or overdosing where TDM could help guiding dose individualisation (paper I).
- To investigate how three different MIC-parameters influenced target attainment rates when the causative pathogen of the patient infection was considered. The overall goal was to investigate which MIC-parameter to use in future PK/PD-studies and clinical TDM practice (paper II).
- To predict the impact of three different β-lactam infusion strategies on PK/PD target attainment and potential risk for toxicity in an ICU cohort. By using short, extended and continuous infusion and two different MIC-levels representing two clinical scenarios i.e. community-acquired and hospital-acquired infections in the predictions. The goal was to give guidance on infusion type for antibiotic dose individualization in the ICU (paper III).
- To investigate if eGFR derived from gentamicin serum concentrations by a population PK model corresponds to eGFR derived from creatinine and cystatin C and to explore the association between the three methods of eGFR and RRT during ICU stay as well as short (30-day) and long-term mortality. The overall goal was to investigate the role of gentamicin as a potential exogenous marker for renal function in the ICU during the initial phase of infection (paper IV).

Materials and methods

Study design and patients

The ACCIS study (paper I-III)

The Antibiotic Concentrations in Critically ill ICU-patients in Sweden (ACCIS) study, was an observational prospective multi-centre study performed in the middle part of Sweden between December 2015 and July 2017 in seven ICUs in five counties.

The participating ICUs belonged both to general ICUs of the county hospitals of Gävle, Karlstad, Eskilstuna and Västerås as well as highly specialized ICUs such as the thoracic surgery ICU, the burn injury ICU and the general ICU of Uppsala University hospital (lead investigation site).

The aim of this quality assurance study was to investigate how the antibiotic concentrations of the ICU patients met the wanted PK/PD-target during the first three important days of antibiotic treatment with either cefotaxime, piperacillin-tazobactam, meropenem, gentamicin, tobramycin, amikacin, ciprofloxacin, or vancomycin. For the aminoglycoside group, only patients with continued treatment were eligible to be included, not one-dose regimens.

To follow reporting bias at all participating centres we conducted two point prevalence assessments on which antibiotics were used for the treatment of infection in the seven participating ICUs. The assessments were made on two different days two months apart at 9 am each day.

Inclusion and exclusion criteria

Consecutive ICU patients above 18 years of age with a suspected or confirmed infection where intravenous antibiotic treatment was initialized no longer than 24 hours prior to inclusion in the ACCIS study were eligible for inclusion.

Only patients that were given one of the antibiotics mentioned above could be included. Exclusion criteria were age below 18 years, known pregnancy, intermittent haemodialysis, limitations of care or prior inclusion in the ACCIS study. The patients were recruited 24 hours a day, every day of the week.

Patient data, cultures and sampling of antibiotic concentrations

Two blood samples were collected in serum tubes on each of the three study days for each patient as presented in the time-concentration illustration in Figure 11. One blood sample was taken between two doses (mid-dose) and one sample was taken as an end dose sample i.e., immediately before the next antibiotic dose was given (also called trough concentration).

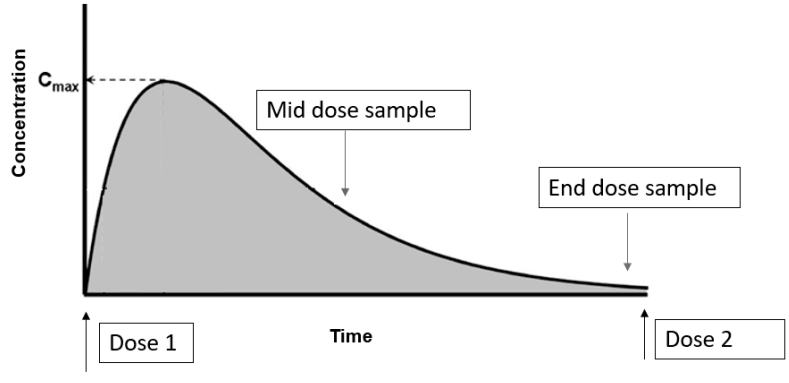

Figure 11 Time-concentration illustration of the sample times in relation to dosing

Demographic data at admission to the ICU were collected in a study database including age, gender, height, SAPS3 (Simplified Acute Physiology Score 3) and the presence of any underlying immunosuppression. Weight before ICU admission was also collected

Clinical data were registered in the database during the three days of the study for each patient including daily fluid balance, daily laboratory variables (albumin, haemoglobin, creatinine, cystatin C, prothrombin complex), time on renal replacement therapy (RRT), given doses of vasopressor treatment, given doses and time for antibiotic treatment, if blood cultures were taken before antibiotic initiation, concomitant medication and initial suspected infection type/diagnosis.

All patients were followed up 30 days after inclusion and mortality and final diagnosis of infection were collected from the medical records as well as ID consultants' assessments of the infection.

All patients were cultured and bacterial findings were handled according to local clinical routines at the local Clinical Microbiology laboratory. Each participating laboratory had a designated contact person in the ICU and followed a study protocol. The purpose was to optimize the collection of causative pathogens in the study.

The Gentamicin study (paper IV)

The Gentamicin study was an observational retrospective study performed in the general ICU of Uppsala University Hospital between January 2009 and December 2013. Patients above 18 years of age given at least one gentamicin dose during their ICU stay with a corresponding sample of gentamicin concentration taken according to local guidelines were included. Exclusion criteria were RRT before ICU stay or age below 18 years.

Demographic data, daily fluid balance, time on RRT, dose and administration time for gentamicin and as well as time for gentamicin sampling were collected from the medical records. Results of plasma creatinine, cystatin C and gentamicin concentrations following the first dose were collected from the laboratory information system of the Department of Clinical Chemistry and Pharmacology at Uppsala University Hospital. On the 8th of July 2020, a follow-up of the medical records was performed and the date of death if applicable was collected.

Ethical consideration

ACCIS study

The study protocol for the ACCIS study was approved by the regional ethics review board in Uppsala (registration number 2015/135). Informed consent was obtained from the patient or, if that initially was impossible, next of kin were consulted.

For each patient, the study continued for a maximum of three days and only resulted in 2 extra antibiotic concentration blood samples each day compared to standard care in the ICU. The attending physician and /or the ID consultant at the ICU decided the therapeutic strategy regarding the choice of antibiotic treatment and dosing. The study investigators had no influence on the patient's care.

All patient data was handled and entered into the electronic CRF by one or a maximum of two investigators at each study site. Only the responsible principal investigators had access to the whole database and the identification key. Finally, all results from the study have been presented at an aggregated level so no individual patients could be identified.

Gentamicin study

The study was approved by the regional ethics board in Uppsala (registration number 2016/157). Informed consent from patients was waived by the ethics board because of the retrospective study design and the distant time period. It was deemed not possible to obtain consent since some of the patients were deceased at the time of the study.

The value of the medical research obtained by the study was considered significant. The increased knowledge of gentamicin clearance (CL) as a possible exogenous biomarker of renal function could enable more correct dosing of other renally excreted drugs in the ICU.

For the included patients the study did not cause any extra intervention or sampling. All data for the study was collected from existing medical records and databases. All patient data was handled by two study investigators. Only the responsible principal investigator had access to the identification key.

Laboratory analysis

Analysis of antibiotic concentrations

ACCIS study (paper I-III)

Each antibiotic concentration sample was centrifuged for 7 min at 2400 g, transported to Nunc CryoTubes, stored locally at -70 to -80 degrees within 30 minutes of sampling and labelled to maintain integrity.

After the study ended all samples were transported on dry ice to Uppsala Biobank, Uppsala University Hospital and sorted before being sent for analysis as one batch for each beta-lactam antibiotic.

Total plasma concentrations of cefotaxime, piperacillin and meropenem were determined by LC-MS at the accredited Department of Clinical Pharmacology, Karolinska University Hospital Huddinge, Stockholm, Sweden. Tazobactam levels were not measured.

The bioanalytical method was validated according to the European Medicines Agency Guideline on bioanalytical method validation. The total coefficient of variation was 11.6% at the lower limit of quantification (LLOQ) and ≤6.0% in the quantification range for cefotaxime; 5.7% (LLOQ) and ≤6.6% for piperacillin, and 7.5% (LLOQ) and ≤6.5% for meropenem. Accuracy was within -6.8 to +4.4% for all analysts over the quantification range.

The quantification range was 0.50-50 µg/mL, 0.20-100 µg/mL and 0.20-50 µg/mL for cefotaxime, piperacillin and meropenem respectively.

Gentamicin study (paper IV)

Two different methods for analysing serum gentamicin concentrations were used during the study period at the accredited Department of Clinical Chemistry and Pharmacology at Uppsala University Hospital. Between 2009 and 2011 fluorescence polarization immune assay (F-PIA) on TDx Flex from Abbot was used. From September 2011 chemiluminescence microparticle immunoassay (CMIA) in Architect, Abbot was used instead. According to the validation of the new method performed by the accredited laboratory, the two methods produced comparable results. Since gentamicin has negligible protein binding no corrections of the total concentrations were needed.

Analysis of creatinine and cystatin C and estimation of eGFR (paper IV)

In paper IV measurements of plasma creatinine and cystatin C were performed at the accredited Department of Clinical Chemistry and Pharmacology at Uppsala University Hospital on Architect ci800 (Abbot laboratories, Abbot Park, Ill, USA) as part of the clinical routine for ICU patients.

Relative values of eGFR for creatinine and cystatin C were calculated using the LM-rev and CAPA-equations, respectively (128, 129).

The first measured gentamicin concentration for each patient was applied in a population PK model described by Hodiamont et al (130). The resulting estimated gentamicin CL (mL/min) was converted to a relative value (mL/min/1.73m$^{2)}$ by applying the equation for body surface area by du Bois (131).

Analysis of isolated bacterial pathogens (paper II)

All significant bacterial findings in cultures from each patient in the ACCIS study were handled according to clinical routine, and antimicrobial susceptibility testing was performed using EUCAST disk diffusion method at the local Clinical Microbiology laboratories.

All significant strains from two days before to three days after inclusion were collected and sent immediately to Clinical Microbiology of Uppsala University Hospital where the bacterial strains were stored at -70 degrees in a 12.5 % glycerol solution until the ACCIS study closed in 2017.

Bacterial findings considered being a sampling contamination or colonization strain, were not sent. Only pathogens deemed clinically significant by the local clinical microbiologist, preferably isolates from blood cultures or other sterile locations as well as primary pathogens from lower respiratory tract samples, intra-abdominal abscesses, infected wounds or urinary samples, were sent

After the ACCIS study closed in 2017 an additional independent assessment of the clinical relevance of the referred strains was made by the co-authors of paper II based on the type of culture and pathogen finding in relation to clinical diagnosis as well as the initial and final assessment made by the ID consultant at the time of ICU care according to medical records. The goal was to identify one clinically significant main pathogen for the target attainment analysis for each patient. In cases with more than one main pathogen, all strains were subjected to MIC testing.

MIC testing and interpretation for target attainment analysis

The ACCIS strain collection was sent for MIC-testing using reference methods at Sweden's two national reference laboratories for AST.

The aerobic strains were sent to EUCAST Development Laboratory (EDL) in Växjö Sweden for MIC-testing with BMD (27). The anaerobic strains were tested with the agar dilution MIC method at the Department of Clinical Microbiology Karolinska University Hospital, Stockholm, Sweden.

In cases where cultures had revealed more than one significant main pathogen, the pathogen with the highest MIC for the antibiotic in question was chosen for the target attainment analysis.

Considerations regarding PK/PD target attainment analysis (paper I-III)

PK/PD-targets

In paper I and II the PK/PD target for target attainment calculations of $100\%T_{>MIC}$ was chosen as proposed for short β-lactam infusion by ESCMID and ESICM TDM working group (24). We added $50\%T_{>4xMIC}$ as another PK/PD target in paper I and II for comparison with earlier studies as proposed by the DALI study group (11).

For the target attainment analysis in paper I and II, a simple clinical approach was used according to local clinical guidelines at the Department of Infectious Diseases at Uppsala University Hospital. For the $100\%T_{>MIC}$ target a plasma antibiotic concentration higher than the MIC of the bacteria at trough i.e., at the end of the dosing interval just before the next dose was considered as the achieved treatment target.

The corresponding target attainment for the target of $50\%T_{>4xMIC}$ was considered a concentration 4 times higher than the MIC of the causative bacterium at mid-dose i.e., at 50% of the dosing interval. Resulting in a simple yes or no if the target attainment was reached or not for each day of the study.

In paper III the PK/PD target of $100\%fT_{>MIC}$ was used to evaluate target attainment for the short and extended infusions in the predictions. For continuous infusion, the target fC_{ss}> 4xMIC was chosen as proposed by Hong et al (9).

MIC parameters

In total three different MIC parameters were used in the target attainment analysis in paper I-III. The definitions of the MIC parameters in Table 2 were developed for paper II from a position paper on how to use MIC in TDM calculations in clinical practice (28).

Table 2 Definitions of the MIC-parameters developed for this thesis

MIC-parameter	Definition	Usage in the PK/PD—calculations of paper II (n=patients)
$MIC_{ACTUAL+1}$	The actual MIC for the isolated pathogens adjusted by adding one 2-fold dilution MIC-step to account for technical variation of BMD	Used for all patients where a causative pathogen was isolated and MIC-testing with BMD was performed (n=72)
MIC_{ECOFF}	The epidemiological cut-off value (ECOFF); a value separating the WT population from the population with acquired resistance mechanisms	Used for all patients where a causative pathogen was isolated and BMD or disc diffusion according to EUCAST was performed (n=81)
MIC_{WCS}	The worst-case scenario of bacterial susceptibility, i.e., the MIC_{ECOFF} of the possible pathogen with the highest ECOFF for the antibiotic in question, reflecting the highest MIC the empiric treatment should cover.	MIC_{WCS} used for all patients in the study (n=138): Cefotaxime: MIC_{ECOFF} for *S. aureus* of 4 mg/L Piperacillin-tazobactam: MIC_{ECOFF} of *P. aeruginosa* of 16mg/L Meropenem: MIC_{ECOFF} of *P. aeruginosa* of 2 mg/L

In paper I the only used MIC-parameter was $MIC_{WCS,}$ reflecting the aim of the study.

In paper II all three MIC-parameters were used for comparison of the influence of the MIC-parameter on target attainment calculations.

In paper III MIC_{ECOFF} for primary and worst-case scenario (WCS) pathogens were used.

Protein binding

Since only total plasma concentration analysis were available at the time of the study corrections for protein binding had to be addressed.

In paper I and II no corrections were made to explore what the best possible outcome regarding target attainment could be.

In paper III free fraction was estimated from published values for protein binding for piperacillin (30%) and cefotaxime (30%) and used in the simulations when applicable (33, 132). No corrections were made for meropenem since it has negligible protein binding (132).

Considerations regarding the predictions in paper III

Infusion strategies and dosing

In the ACCIS study (paper I) all β-lactam antibiotics were given as SI with a duration of approximately 15 minutes with the exception of 79% (44/56) of the piperacillin-tazobactam treated patients, where an SI of 30 minutes was used according to local guidelines at the different ICUs. No initial higher loading doses were given in the original study.

In paper III target attainment predictions were made for three different infusion strategies; SI of 15 minutes, EI of 3 hours and CI over 24 h for each β-lactam. In this sub-study of the ACCIS study, the PK-data from the patients in each antibiotic group was used to predict target attainment at 24, 48 and 72 h of treatment when the same total daily dose of the β-lactam in question was given as SI, EI or CI for each patient. The dosing scenario for SI was based on the national Swedish dosing guidelines aligned with the suggested dosing from EUCAST (62, 133). The same total daily doses were kept for EI and CI but the infusion durations were prolonged in the predictions (Table 3).

Table 3 Dosing strategies, MIC scenarios and upper recommended/toxicity levels for SI, EI and CI applied in the predictions.

	Dosing scenarios	**Primary pathogen MIC_{ECOFF} (mg/L)**	**WCS pathogen MIC_{ECOFF} (mg/L)**	**Toxicity level C_{min} (mg/L)**	**Upper rec. level C_{min} (mg/L)**
Cefotaxime	2gx3 (SI) 2gx3 (EI) 6g/24h (CI)	0.25 (*E. coli*)	4 (*S. aureus*)	Missing	60
Piperacillin-tazobactam	4gx4 (SI) 4gx4 (EI) 16g/24h(CI)	8 (*E. coli*)	16 (*P. aeruginosa*)	361 (piperacillin)	NA
Meropenem	1gx3 (SI) 1gx3 (EI) 3g/24h (CI)	0.5 (*S. aureus*)	2 (*P. aeruginosa*)	44.5	NA

SI; short infusion, EI; extended infusion, CI; continuous infusion, NA; not applicable, WCS; worst-case scenario.

Toxicity levels

To evaluate the potential risk for toxicity for SI and EI in the predictions the toxicity C_{min} thresholds with a 50% risk of developing neuro- or nephrotoxicity identified for meropenem (44.5 mg/L) and piperacillin (361 mg/L) supported by European consensus guidelines were used (Table 3) (24).

There is no established toxicity threshold for cefotaxime, instead, the recommended upper level of trough concentration (C_{min}) of 60 mg/L suggested by the French consensus guideline was applied (8).

Regarding toxicity and CI, data are limited and consensus guidelines are missing, and as a consequence, the risk for toxicity for CI was not addressed in paper III.

Primary and worst-case scenarios MIC

In the predictions on target attainment in paper III, two MIC-scenarios were applied to reflect two different clinical scenarios based on EUCAST MIC-distributions and the ECOFFs of the most common human pathogens (Table 3).

A primary pathogen scenario (primary), mainly reflecting community-acquired infections, and a worst-case pathogen scenario (WCS) mainly reflecting hospital-acquired or opportunistic infections.

For cefotaxime, piperacillin-tazobactam and meropenem the "primary" corresponds to the MIC_{ECOFF} of *E. coli* respectively *S. aureus* (Figure 12). For piperacillin-tazobactam and meropenem the WCS is an infection with *P. aeruginosa* (Figure 12), a pathogen rarely causing community-acquired infections in immunocompetent patients, but is sometimes seen in hospital-acquired infections such as lower respiratory tract infections (LRTI).

For cefotaxime, the situation is more complex since the WCS-pathogen *S. aureus* at the same time is a primary pathogen in skin and soft tissue infections (SSTI) in community-acquired infections. Hence the WCS for cefotaxime in this study reflects both a worst-case scenario for hospital-acquired lower respiratory tract infection (LRTI) and a primary pathogen scenario in SSTI in all categories of patients.

The primary scenario for cefotaxime using *E. coli* reflects all other infections where *S. aureus* is an unlikely pathogen, like community-acquired urinary tract infections (UTI) and intra-abdominal infections (IAI).

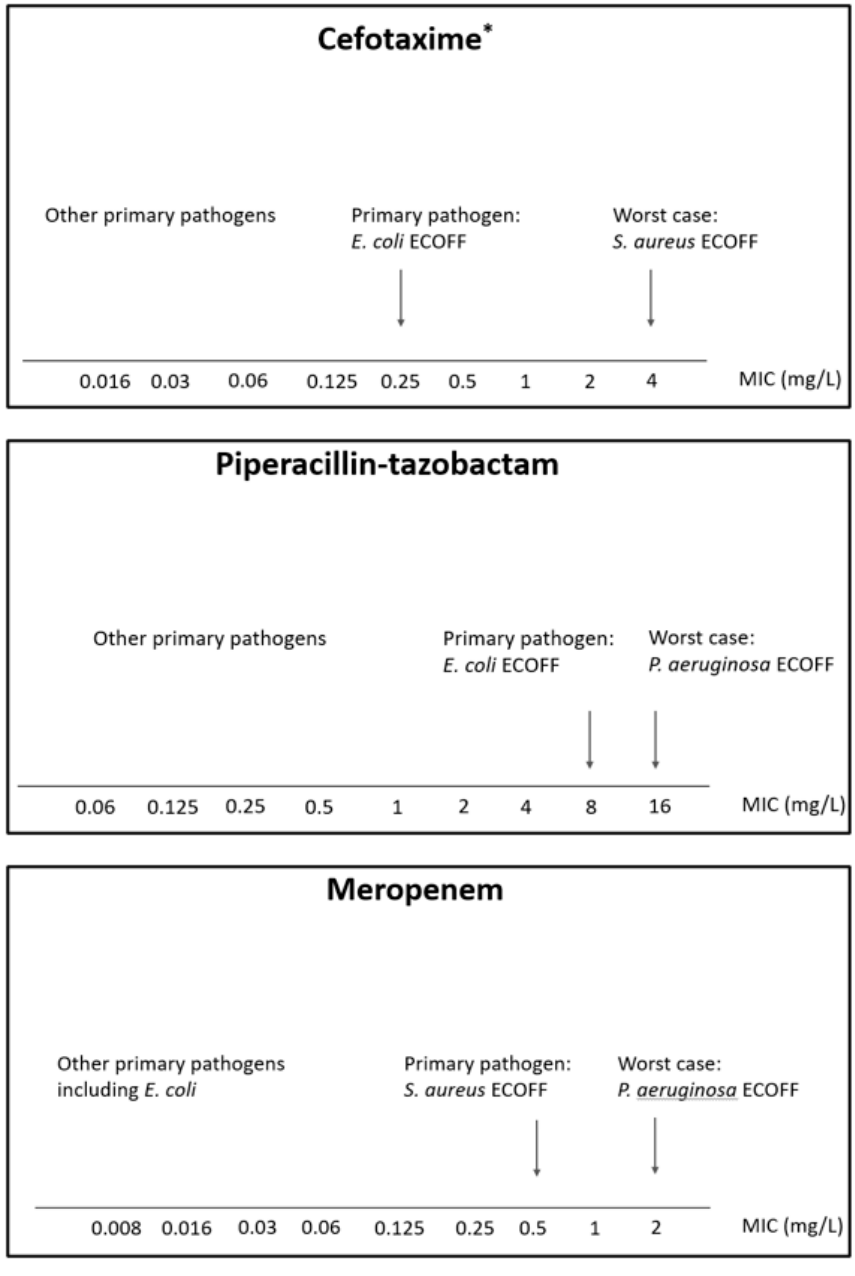

Figure 12 MIC_{ECOFF} distributions for worst-case scenario pathogens and primary pathogens for cefotaxime, piperacillin-tazobactam and meropenem.

*Cefotaxime has no clinically useful activity vs P. aeruginosa

Individual PK parameter estimation

In paper III and IV, Bayesian estimation was used together with the selected population PK models to obtain individual PK parameters.

In the process, the information of the model was weighted against information from actual measured concentrations in each patient resulting in revised PK parameter estimates for the individual.

In paper III the estimated individual PK parameters were used to predict C_{min} for SI and EI as well as fC_{ss} for CI at 24, 48 and 72 hours of treatment with each β-lactam. The $fT_{>MIC}$ was predicted the same way. In paper IV gentamicin clearance was estimated.

The published population PK models used in paper III and IV were selected based on population characteristics and dosing to resemble the ACCIS and Gentamicin cohorts as well as the model structure and included covariates. Predictions and Bayesian estimation in paper III and paper IV were performed using NONMEM version 7.5 (Icon Development Solutions, Hanover, MD, USA).

Statistical analysis

In all papers included in this thesis, descriptive statistics for demographic and clinical characteristics were presented as number of observations (%) for categorical variables and as median with interquartile range (IQR) for continuous variables, unless otherwise stated.

All statistical calculations were made using STATISTICA™ software version 14.1 (StatSoft Tulsa, OK, USA) in paper I-IV, unless otherwise stated. A $p<0.05$ was considered statistically significant.

Specific statistical analysis in paper I-III

Power calculation for the ACCIS study

To be able to show that 10% of the patients had plasma antibiotic concentration below the PK/PD target, and thus did not reach target attainment, at least 16 patients had to be included for each studied antibiotic for an alpha error of 0.05 and a beta error of 0.8. We aimed at including 150 patients, i.e. 10% more than suggested by the sample size calculation if all eight antibiotics should be included in the study, due to the risk of missing data, uneven distribution between groups etc.

Missing data

For some patients in the ACCIS study, not all concentration samples were taken according to the protocol regarding time intervals. Such sample results were excluded from the study. Both missing and excluded concentrations were

replaced by imputed values using the Multivariate Imputation by chained Equations (MICE, R software version 3.5.3) to make the data set complete for the analysis in paper I and II.

Concentrations below LLOQ were found only in the cefotaxime treated group (n=15) and those concentrations were replaced by LLOQ/2 (=0.25 mg/L) in the target attainment analysis in papers I-III (Beal 2001).

In paper III some patients had missing information regarding weight at ICU admission and missing data were imputed by using linear regression with documented weight before ICU admission (n=13). If no weight was available, the median weight in the ACCIS cohort before admission was applied (n=1).

Paper I and II

Target attainment results in paper I and II are presented as percentages reaching the target with a 95% Confidence Interval (CI).

In paper I Spearman Rank Order Correlations were used to assess associations between continuous variables. Univariate logistic regression was performed with the PK/PD-target $100\%T_{>MIC}$ (using MIC_{WCS}) to assess risk factors of not reaching treatment attainment and data presented in a forest plot.

Specific statistical analysis in paper IV

Pearson's correlation coefficients and coefficient of determination were used to assess correlations between $eGFR_{Gentamicin}$ and the two other eGFR methods. The limits of agreement and bias between $eGFR_{Gentamicin}$ and $eGFR_{Creatinine}$ as well as between $eGFR_{Gentamicin}$ and $eGFR_{CystatinC}$ were calculated and presented in Bland-Altman plots. To assess the association between the three eGFR methods and the risk of RRT during ICU stay and 30-day mortality univariate logistic regression was used, and receiver operating characteristic curves (ROC-curves) are presented. Cox proportional hazard regression was used to analyse the association between the three eGFR methods and overall mortality.

Missing data for weight (20 patients) and height (47 patients) were imputed with the median from all patients in the study.

Results

ACCIS study (paper I-III)

Patients and clinical characteristics

In total 161 patients were eligible of whom 144 could be included in the ACCIS-study. Fifty-five patients were treated with cefotaxime, 56 with piperacillin-tazobactam, 27 with meropenem and six patients treated with vancomycin. Flowchart illustrating the patients in the study is presented in Figure 13.

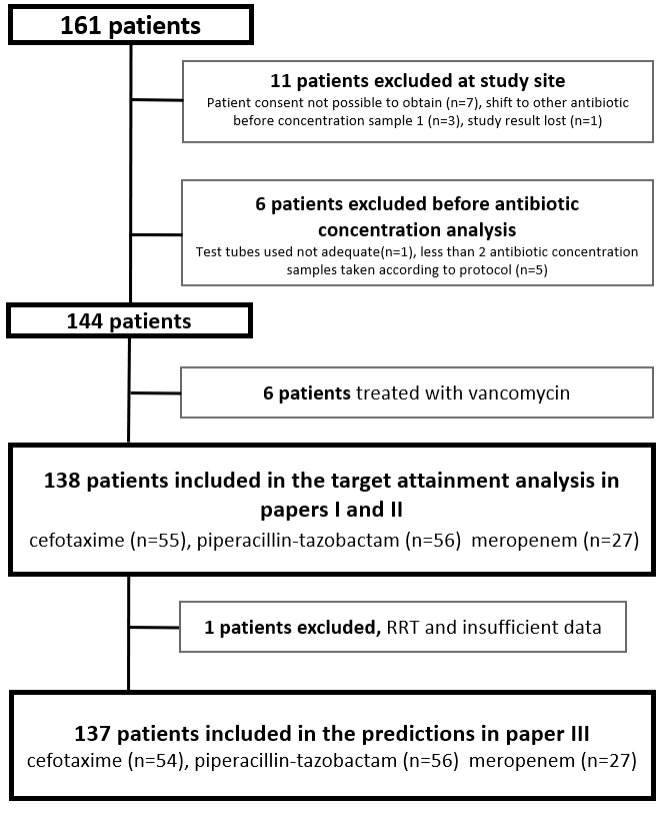

Figure 13 Flowchart illustrating the patients in the ACCIS study.

During the study period, only patients treated with cefotaxime, piperacillin-tazobactam or vancomycin were included in the study and no patients treated with ciprofloxacin, gentamicin, tobramycin or amikacin.

In the point prevalence assessment on two different days, 37% (15/41) respectively 56% (17/30) of the ICU patients received treatment for an infection. The proportion of patients receiving cefotaxime, meropenem, piperacillin-tazobactam or vancomycin was 73% (11/15) on day one and 71% (12/17) on day two.

The rest were treated with benzylpenicillin, cloxacillin, clindamycin or imipenem. No patient received treatment with an aminoglycoside or ciprofloxacin which supports the conclusion from the ACCIS study that fluoroquinolones and aminoglycosides (except for one-dose) are seldom used in ICU patients in Sweden in treatment for an acute infection.

In Table 4 demographics and clinical characteristics of the 144 patients in the ACCIS study are presented. Community-acquired infections (symptoms developed before hospitalization or within 48 h) were seen in 52% (75/144) of the patients. Of the rest of the patients, 48% (69/144) had a hospital-acquired infection (symptoms developed more than 48 h after hospital admission). In total 47 % (67/144) of the patients had a LRTI, 16 % (23/144) had an IAI, 16% (24/144) had a SSTI and 9 % (13/144) a UTI. The cefotaxime treated cohort had a higher proportion of patients with community-acquired infections, a lower SAPS3 at admission and a lower proportion on renal replacement therapy than the rest of the study population.

Table 4 Demographic and clinical characteristics of the included patients.

Characteristic	Cefotaxime (n=55)	Piperacillin-tazobactam (n=56)	Meropenem (n=27)	Vancomycin (n=6)	Total (n=144)
Age, year	64 (47-73)	68 (54-76)	67 (57-73)	70 (64-76)	67 (55-74)
Male gender	37 (67%)	34 (61%)	13 (48%)	5 (83%)	89 (62%)
Body weight, kg	92 (79-101)	80 (66-97)	83 (70-99)	87.7 (80-100)	85 (73-100)
SAPS3	55 (47-64)	63.5 (52-74.5)	62 (50-69)	78 (52-86)	59 (50-69)
Vasopressor treatment	37 (67%)	42 (75%)	23 (85%)	5 (83%)	107 (74%)
RRT[a]	4 (7%)	12 (21%)	9 (33%)	3 (59%)	28 (19%)
Immunosuppression[b]	1 (2%)	7 (13%)	6 (22%)	1 (16%)	15 (10%)
30-day mortality	4 (7%)	19 (18%)	9 (33%)	3 (50%)	26 (18%)
Hospital-acquired infection[c]	19 (35%)	32 (57%)	13 (48%)	5 (83%)	69 (48%)
Community-acquired infection[d]	36 (65%)	24 (42%)	14 (52%)	1 (17%)	75 (52%)
Focus of infection					
LRTI	30 (55%)	25 (45%)	10 (37%)	2 (33%)	67 (47%)
IAI	1 (2%)	16 (29%)	4 (15%)	2 (33%)	23 (16%)
SSTI	16 (29%)	2 (4%)	5 (19%)	1 (17%)	24 (17%)
UTI	4 (7%)	6 (11%)	3 (11%)	-	13 (9%)
Other	4 (7%)	7 (13%)	5 (19%)	1 (17%)	17 (27%)
Blood culture before start of antibiotic treatment	51 (92%)	52 (92%)	26 (96%)	5 (83%)	134 (93%)
Plasma creatinine concentration (µmol/L)	92 (68-155)	128 (79-187)	130 (71-190)	102 (69-299)	118 (71-185)
Plasma albumin (g/L)	23 (16-29)	26 (22-29)	24 (22-27)	21 (15-27)	24 (20-29)
Blood hemoglobin (g/L)	118 (99-131)	105 (93-121)	98 (90-108)	92 (88-98)	108 (95-124)
Daily dose (g/24 h)	4.0 (3.0-6.0)	12 (12.0-16.0)	3.0 (1.5-4.0)	3.0 (2.0-3.0)	NA
In day 1, 2 and 3 order	3.0 (3.0-6.0)	12 (12.0-12.0)	2.8 (1.5-3.0)	2.5 (2.0-3.0)	
	3.0 (3.0-6.0)	12 (12.0-12.0)	3.0 (1.5-3.0)	2.0 (1.0-3.0)	
Antibiotic Concentration (mg/L)	7.8 (4.0-15.0)	67.0 (38.0-99)	13.8 (5.2-18.8)	NA	NA
Mid dosing interval	8.5 (4.3-13.4)	64.1 (31.9-103)	12.7 (6.5-17.8)		
In day 1, 2 and 3 order	8.6 (4.1-15.5)	62.6 (36.4-112)	7.7 (4.8-14.5)		
Antibiotic Concentration (mg/L)	2.89 (1.25-6.85)	26.7 (7.5-57.7)	4.3 (1.8-9.5)	NA	NA
End dosing interval	2.45 (1.15-5.11)	30.0 (7.7-60.4	4.9 (2.4-8.1)		
In day 1, 2 and 3 order	2.22 (0.93-5.08)	19.5 (9.1-68.8)	2.6 (1.2-6.7)		

RRT[a]: Renal replacement therapy.

Immunosuppression[b]: Neutropenic fever, stem cell-/solid organ transplantation OR prednisolone treatment of more than 20 mg per day due to inflammatory rheumatic disease or haematological diseases at admission.

Hospital-acquired infection[c]: Symptoms developed more than 48 hours after admission to hospital.

Community-acquired infection[d]: Symptoms developed before hospitalisation or within 48 hours after admission.

Focus of infection: LRTI= lower respiratory tract infection. IAI: intra-abdominal infection. SSTI: soft skin and tissue infection. UTI: urinary tract infection.

Data are presented as median (IQR) or number (percentages)

The 138 patients treated with β-lactams were selected for the target attainment analysis part of the study since the six vancomycin-treated patients were considered too few to analyse further. Thus, the results below apply to the 138 β-lactam treated patients.

Antibiotic concentrations

Sixty-nine percent of the patients (95/138) were included in the study prior to dose one, 23% (32/138) on dose two, 6% (8/138) on dose three and 2% (3/138) prior to the fourth dose of the first day of antibiotic treatment in the ICU.

In total 715 antibiotic concentration samples were eligible for target attainment analysis for the β-lactam treated patients. Of the 138 patients, 55% (76/138) had six samples taken and another 23% (32/138) had five samples. In Table 4 the median antibiotic concentrations and the IQR for each β-lactam are given for both mid dosing interval and end dosing interval.

For cefotaxime, at least one out of the three concentration samples at the end of the interval taken per patient was below the lower level of detection (LLOD) of <0.5 mg/L in 15% (8/55) of the patients. Of these patients, 25 %

(2/8) had undetectable end-dosing levels all three days and the rest concentrations below 1.25 mg/L. In total 38% (21/55) of the cefotaxime treated patients had at least one end-dosing concentration below 1 mg/L.

None of the patients in paper I treated with meropenem or piperacillin-tazobactam achieved antibiotic concentrations at the end of the dosing interval (C_{min}) below LLOD.

Microbiology

A bacterial pathogen considered of clinical relevance by the clinicians was identified for 61 % of the patients (84/138). In forty percent of the patients with a clinically relevant pathogen identified, the finding was from a blood culture (34/84). Another 32% (27/84) of the patients had a positive culture from the lower respiratory tract, 12% from a wound culture and 10% had a positive culture from a deep abscess.

E. coli was the most common finding in 25 % (21/84) of the patients followed by *S. aureus* in 24 % (20/84) regardless of type of culture. Only one patient in the study had a positive culture with *P.aeruginosa* (Figure 14).

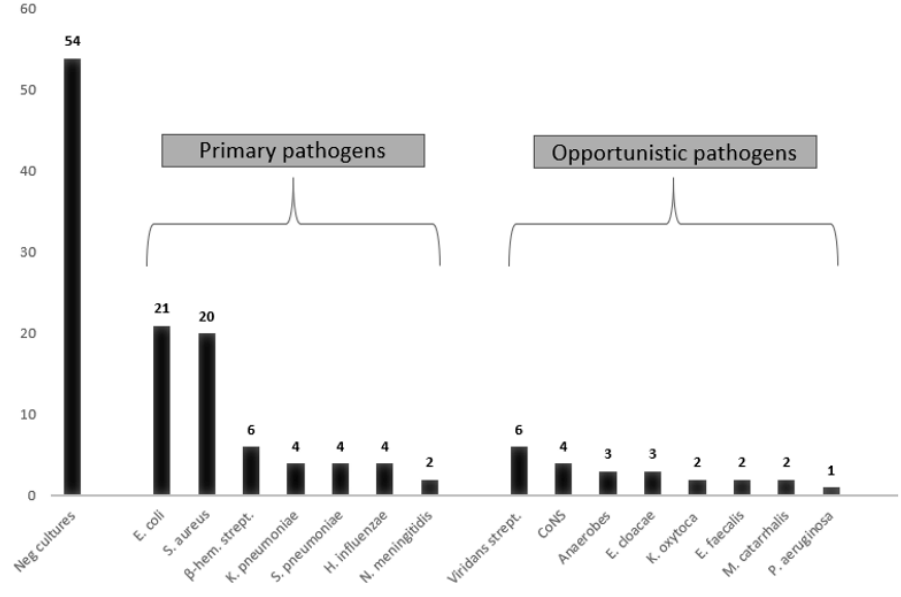

Figure 14 Culture findings of the ACCIS study divided into primary and opportunistic pathogens.

Three out of the 84 clinically significant isolates were resistant to the given β-lactam and thus excluded from the target attainment analysis in paper II. Nine of the 84 isolated bacterial pathogens were unfortunately discarded after sus-

ceptibility testing using the EUCAST disk diffusion method at the local laboratory and MIC-testing could not be performed. However, all nine isolates belonged to the WT population and a result for MIC_{ECOFF} was available.
In Figure 15 the flowchart for the 138 patients, isolated pathogens and available MIC parameters for the target attainment analysis in paper II is presented.

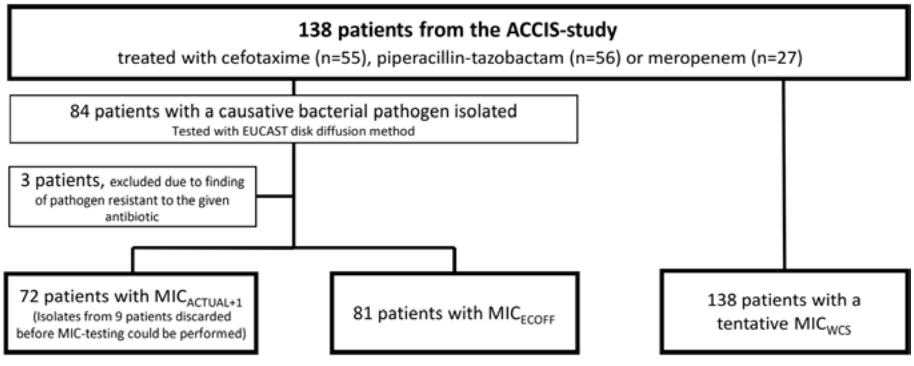

Figure 15 Flow chart illustrating the patients in the ACCIS study and the three MIC parameters used ($MIC_{ACTUAL+1}$, MIC_{ECOFF} and $MIC_{WCS)}$.

PK/PD target attainment for cefotaxime, piperacillin-tazobactam and meropenem (paper I)

Observed target attainment in the ACCIS cohort (paper I)

In the PK/PD target attainment calculations in paper I only the MIC parameter MIC_{WCS} was used. This reflects the highest tentative MIC the initial empirical treatment should cover for each studied β-lactam during the first important three days of antibiotic treatment in the ICU.

In the whole cohort, 45% (62/138) of the patients did not reach $100\%T_{>MIC}$ on Day 1 of treatment (Figure 16 A). When the PK/PD target of $50\%T_{>4xMIC}$ was used the target attainment was even lower (Figure 16 B). There was no trend towards better target attainment on Day 3 compared to Day 1 regardless of which PK/PD-targets that was used.

In the cefotaxime-treated group, 58 % (32/55) did not reach $100\%T_{>MIC}$ on Day 1. The corresponding results for piperacillin-tazobactam were 39% (22/56) and for meropenem 30% (8/27). The results were comparable for the following days of treatment (Figure 16 A).
The target attainment was lower when the PK/PD target $50\%T_{>4xMIC}$ was used for all antibiotics but in particular, in the cefotaxime treated patients where almost 80% did not meet the target (Figure 16 B).

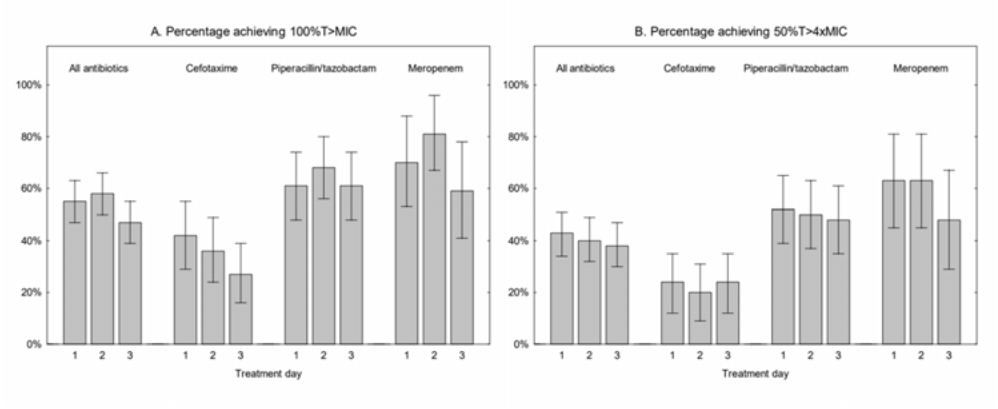

Figure 16 (A) Percentage with 95% confidence interval of the patients achieving the PK/PD-target 100%T>MIC_{WCS} on Day 1, 2 and 3 of the study.

Figure 16 (B) Percentage with 95% confidence interval of the patients achieving the PK/PD-target 50%T>4xMIC_{WCS} on Day 1,2 and 3 of the study.

Risk factors for low or high target attainment (paper I)

In paper I we present risk factors associated with a lower or higher risk of not meeting the PK/PD target 100% $T_{> MIC}$ in a forest plot (Figure 17). The MIC-parameter used in the calculations was MIC_{WCS}.

Increasing age, SAPS3 and plasma creatinine levels were associated with a lower risk of not reaching 100% $T_{>MIC}$. There was a statistically significant lower risk of target attainment failure if the patient had a UTI compared to an LRTI. Renal replacement therapy was also associated with a lower risk of not reaching the PK/PD-target. Comparing the antibiotic of choice, there was a three-fold higher risk of not reaching the target for patients treated with cefotaxime compared to meropenem. The total daily dose/kg did not significantly impact the target attainment.

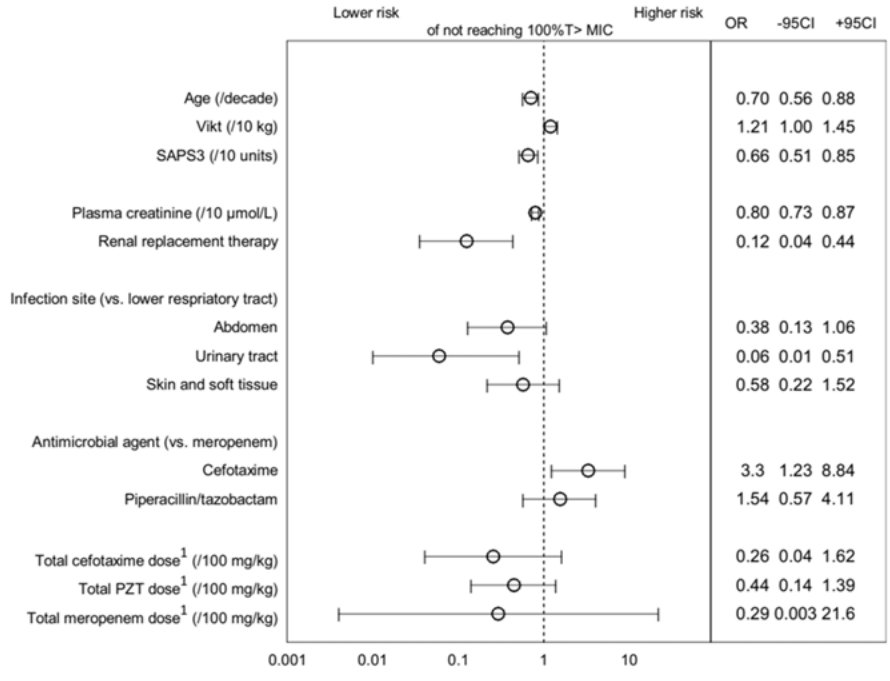

Figure 17 Forest plot illustrating predictive factors resulting in lower (to the left) or higher (to the right) risk for target failure of not reaching 100%T>MIC_{WCS}, including odds ratios (OR) with 95% confidence intervals from univariate logistic regression. [1] Daily doses of antibiotics per body weight.

Comparison of target attainment using different MIC parameters in the calculations (paper II)

In paper II we used the collected actual causative bacterial pathogens from the ACCIS patients and the three MIC-parameters MIC_{WCS}, $MICE_{COFF}$ and $MIC_{ACTUAL+1}$ for comparison of the impact of the MIC-parameter in target attainment calculations using two different PK/PD-targets, 100%$T_{>MIC}$ and 50%$T_{>4xMIC}$. The results for all patients are presented in Figure 18 and the results for each individual β-lactam-treated group are presented in Figure 19.

In the whole cohort, when comparing failure rates for the PK/PD-target of 100% $T_{>MIC}$ depending on MIC-parameter, 23% (19/81) using MIC_{ECOFF} and 45% (62/138) using MIC_{WCS} respectively, did not reach target attainment during Day 1 (Figure 18a).

For the cefotaxime treated patients on Day 1, 24% (10/42) of the patients using MIC_{ECOFF} and 58% (32/55) using MIC_{WCS} in the calculation did not reach the PK/PD target. The corresponding results for piperacillin-tazobactam were 31% (9/29) and 39% (22/56), respectively. For meropenem treated patients, no patient failed the target attainment when MIC_{ECOFF} was used compared to 30% (8/27) for MIC_{WCS} (Figure 19a-c).

The results on a cohort level for the other PK/PD-target, $50\%T_{>4x\ MIC}$, followed the same pattern as for $100\%T_{>MIC}$ with 25% (20/81) of the patients not reaching the target on day one when MIC_{ECOFF} was used (Figure 18a).

However, when using the MIC_{WCS} parameter instead in the PK/PD calculations the target failure differed for the two PK/PD-targets. For $50\%T_{>4xMIC}$ the target failure was 57% (79/138) for MIC_{ECOFF} compared with 23% (19/81) for MIC_{WCS} (Fig 18b). This pattern was seen for all antibiotics when using the MIC_{WCS} in the $50\%T_{>4xMIC}$ calculation compared to $100\%T_{>MIC}$ but in particular for cefotaxime-treated patients (Figure 19d-f)

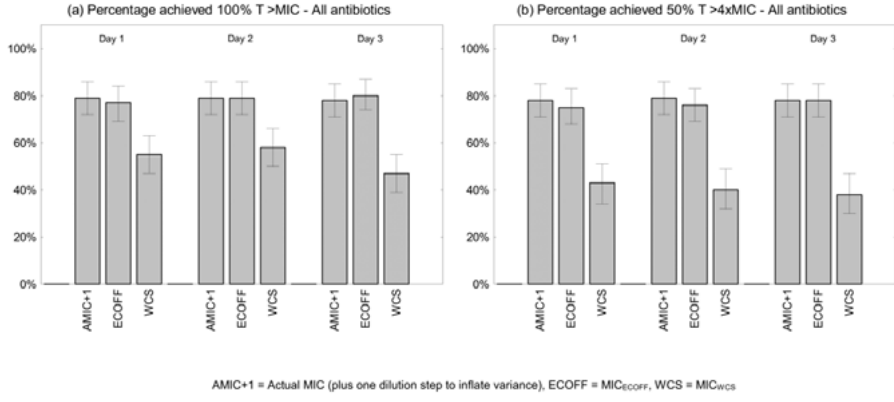

Figure 18 a Percentage with 95% CI of all patients achieving $100\%T_{>MIC}$ on Day 1, 2 and 3 depending on the MIC-parameter used in the calculation ($MIC_{ACTUAL\ +\ 1}$, MIC_{ECOFF} or MIC_{WCS})

Figure 18 b Percentage with 95% CI of all patients achieving $50\%T_{>4xMIC}$ on Day 1, 2 and 3 depending on MIC-parameter used in the calculation ($MIC_{ACTUAL\ +\ 1}$, MIC_{ECOFF} or MIC_{WCS})

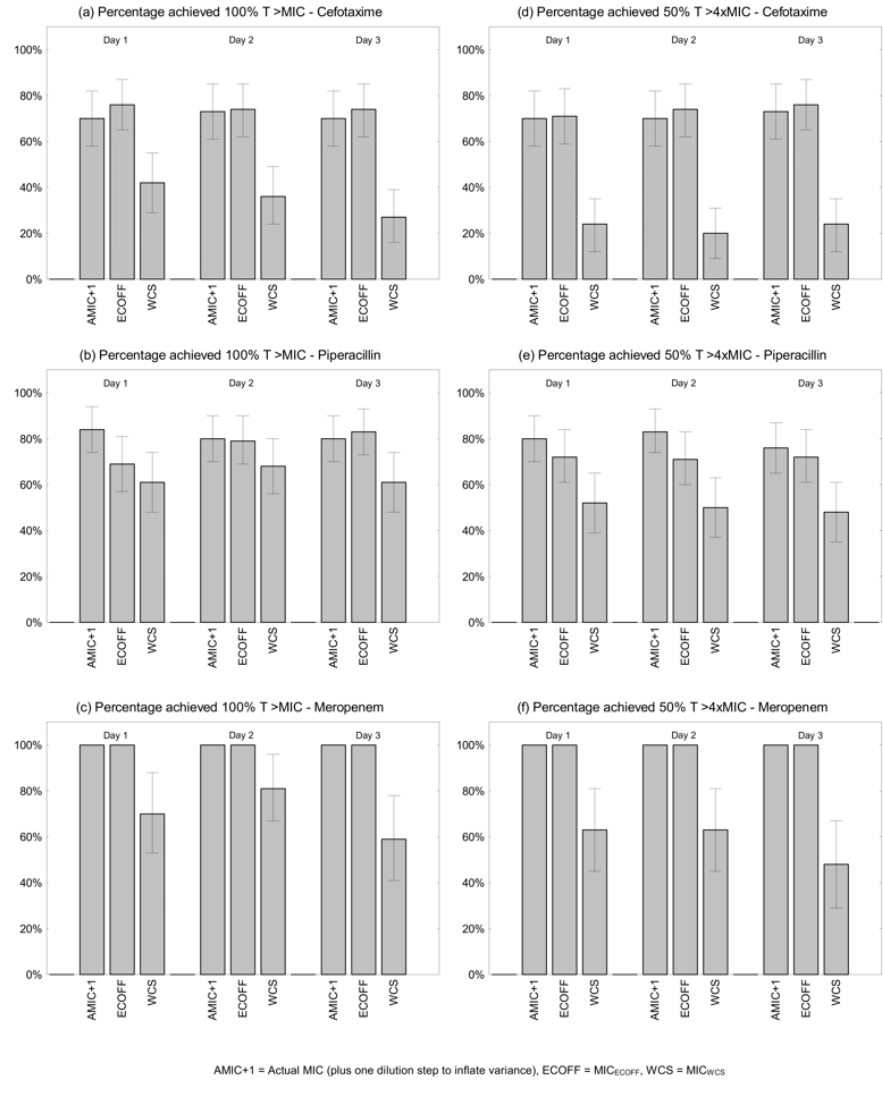

Figure 19 a-c Percentage with 95% CI of the (A) cefotaxime, (B) piperacillin respectively (C) meropenem treated patients achieving 100%T$_{>MIC}$ Day 1, 2 and 3 depending on the MIC-parameter used in the calculation (MIC$_{ACTUAL + 1}$, MIC$_{ECOFF}$ or MIC$_{WCS}$).

Figure 19 d-f Percentage with 95% CI of the (D) cefotaxime, (E) piperacillin respectively (F) meropenem treated patients achieving 50%T$_{>4xMIC}$ day 1, 2 and 3 depending on the MIC-parameter used in the calculation (MIC$_{ACTUAL + 1}$, MIC$_{ECOFF}$ or MIC$_{WCS}$).

Predicted impact of dosing strategies on target attainment (paper III)

In paper III 137 of the138 β-lactam treated patients of the ACCIS study were included in the predictions. One cefotaxime-treated patient was excluded because of insufficient data regarding RRT.

The predicted target attainments for Day 1 using different infusion regimens and two different MIC-levels representing different clinical scenarios are presented in Figure 20. Note that the PK/PD target for CI (fC_{ss}) is different from SI and EI (100% $fT_{>MIC}$).

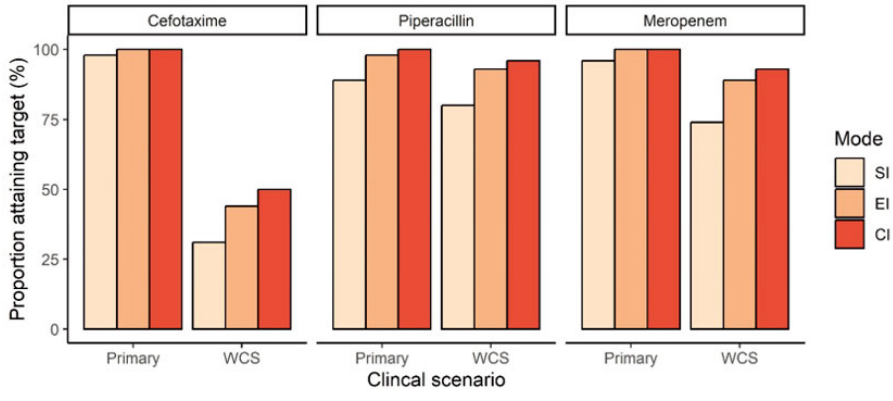

Figure 20 Proportion of individuals predicted to attain target 100% $fT_{>MIC}$ (SI and EI) or fC_{ss} >4xMIC (CI) for the primary pathogen scenario (primary) and a worst-case pathogen scenario (WCS), at antibiotic treatment Day 1, applying different antibiotic infusion modes on the cefotaxime (n=54), piperacillin-tazobactam (n=56) and meropenem (n=27) treated patients.

SI, short infusion; EI, extended infusion; CI, continuous infusion; fC_{ss}, free drug concentration at steady state

Overall, for the primary scenario, the target 100%$fT_{>MIC}$ for SI was reached in 94% (129/137) of all patients already during Day 1. In the treatment of WCS-pathogen with meropenem or piperacillin-tazobactam with either SI or EI, 78% (65/83) and 92% (76/83) of the patients reached the target. In the cefotaxime-treated group, the target attainment was lower, only 31% (17/54) reached the target during Day 1 using SI and 44% (24/54) when switching to EI.

In addition, Figure 21 presents the predicted $fT_{>MIC}$ for each individual and provides more insight regarding the difference between the infusion durations at treatment Days 1-3. For *S aureus*, the WCS-pathogen in relation to cefotaxime, the median $fT_{>MIC}$ increased during Day 1 when prolonging the infusion, from 75% (range 21-100%) for SI to 93% (42-100%) for EI and 99% (85-

100%) for CI. However, the consensus recommendation regarding the treatment target for CI is proposed to be fC_{ss} >4xMIC and not 100%$fT_{>MIC}$ (9).

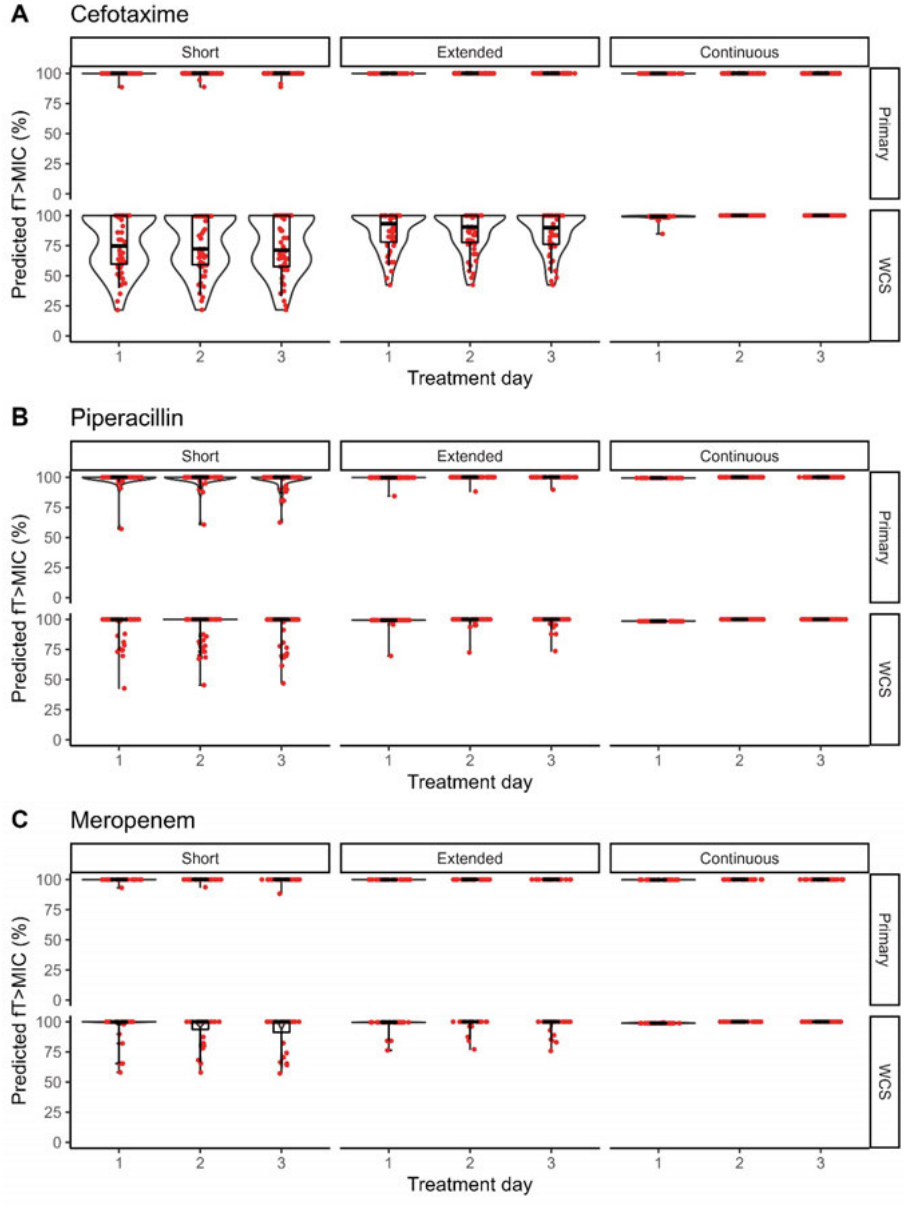

Figure 21 Violin plots of predicted $fT_{>MIC}$ (%) at treatment Day 1-3 for the individual patients treated with cefotaxime (A) (n=54), piperacillin-tazobactam (shown as piperacillin) (B) (n=56) or meropenem (C) (n=27), stratified for mode of infusion and MIC-scenario. The black line marks the median, the box marks IQR, and the whiskers mark the 5 and 95 percentiles of the data. Dots represent the individual predictions.
Primary: primary pathogen scenario; WCS: worst-case pathogen scenario.

The corresponding predictions of fC_{ss} after 24, 48 and 72 h of treatment in relation to 4xMIC in each clinical scenario are presented in Figure 22. Regardless of antibiotic group the target fC_{ss}>4xMIC was reached in 100% of the patients in the primary scenario all three days. In the WCS the target was reached in 50% (27/54) of the cefotaxime-treated patients on Day 1. The corresponding results for the piperacillin-tazobactam and meropenem-treated groups were 96% (54/56) and 93% (25/27) respectively.

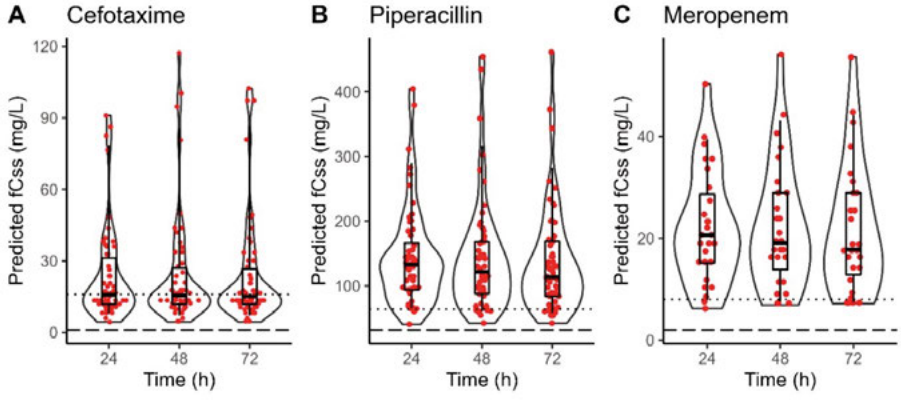

Figure 22 Violin plots of predicted fC_{ss} at 24, 48 and 72 h of treatment for the individual patients receiving cefotaxime (A) (n=54), piperacillin-tazobactam (B) (n=56) or meropenem (C) (n=27), following continuous infusion. The black line marks the median, the box marks IQR, and the whiskers mark the 5 and 95 percentiles of the data. Dots represent the individual predictions. The dashed lines represent 4xMIC for primary pathogens, and the dotted line marks 4xMIC for worst-case scenario pathogens.

fC_{ss}, free drug concentration at steady state

Observed and predicted toxicity in the ACCIS cohort (paper I and III)

None of the patients in paper I treated with meropenem or piperacillin-tazobactam achieved observed total antibiotic concentrations at the end of the dosing interval (C_{min}) above the threshold of toxicity suggested by European consensus guidelines of 44.5 and 361 mg/L, respectively (24).

However, two of the cefotaxime treated patients had a trough concentration (C_{min}) above 60 mg/L suggested as an upper recommended level by the French consensus guideline (8).

In paper III the predicted C_{min} after 24, 48 and 72 h of treatment with short (SI) or extended (EI) infusions in relation to toxic/upper recommended levels are presented in Figure 23. The predictions for both SI and EI were below the toxicity threshold in the meropenem-treated group. A minor increase in the

number of individuals predicted to have C_{min} above the threshold was seen when switching from SI (n=5) to EI (n=7) in the cefotaxime and piperacillin-tazobactam treated group.

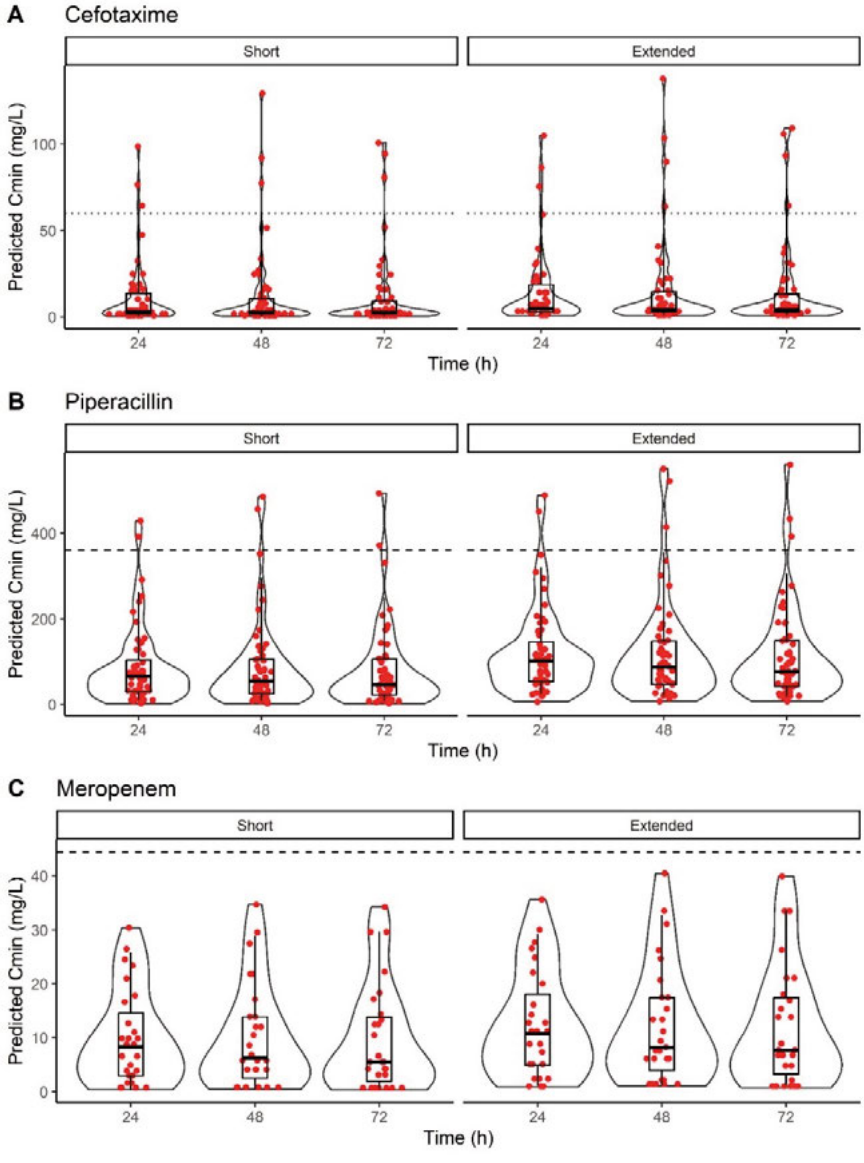

Figure 23 Violin plots of predicted C_{min} at 24, 48 and 72 h of treatment for the individual patients receiving cefotaxime (A) (n=54), piperacillin-tazobactam (B) (n=56), or meropenem (C) (n=27), stratified for the mode of infusion. The black line marks the median, the box marks IQR, and the whiskers mark the 5 and 95 percentiles of the data. Dots represent the individual predictions. The dashed line represents potentially toxic concentration levels, and the dotted line upper recommended level to avoid toxicity.

Gentamicin study (paper IV)

Patients and clinical characteristics

A total of 254 patients were included in the study. The demographic and clinical characteristics of the patients are presented in Table 5.

Overall the median age was 65.6 years (IQR 54.3-75.2) and 37% were females. The recommended dosing regimen in Uppsala during the major part of the study period was 4.5 mg/kg. The therapeutic range for 8-hour concentrations was 1.5-4 mg/L. The 30-day mortality was 19% (n=49) and the total mortality until the follow-up on the 8th of July 2020 was 63% (159/254).

Table 5 Demographic and clinical characteristics of the included patients

Characteristic	**All patients (n=254)**
Age, year	65.6 (54.3-74.2)
Male gender	159 (63%)
Body weight, kg	82.0 (72.0-95.0)
BSA, m^2	1.97 (1.82-2.10)
Gentamicin dose, mg/kg	3.2 (2.2-4.0)
Serum gentamicin concentration, mg/L (8 h)	3.1 (2.0-4.4)
$eGFR_{Creatinine}$, ml/min/1.73m^2 BSA	55 (33-83)
$eGFR_{CystatinC}$, ml/min/1.73m^2 BSA	45 (28-71)
$eGFR_{Gentamicin}$, ml/min/1.73m^2 BSA	43 (27-62)
Renal placement therapy during ICU care	28 (11%)
30-day mortality	49 (19%)
Days from gentamicin sampling to death	1475 (63-3129)

BSA, body surface area; eGFR, estimated glomerular filtration rate; ICU, intensive care unit. Data are presented as median (IQR) or number (percentages).

Correlation and agreement between $eGFR_{Gentamicin}$ and $eGFR_{Creatinine}$/$eGFR_{CystatinC}$

The correlation between $eGFR_{Gentamicin}$ and $eGFR_{Creatinine}$ as well as between $eGFR_{Gentamicin}$ and $eGFR_{CystatinC}$ are presented in Figure 24. The coefficient and coefficients of determination for both analyses were similar and indicated a positive linear relationship.

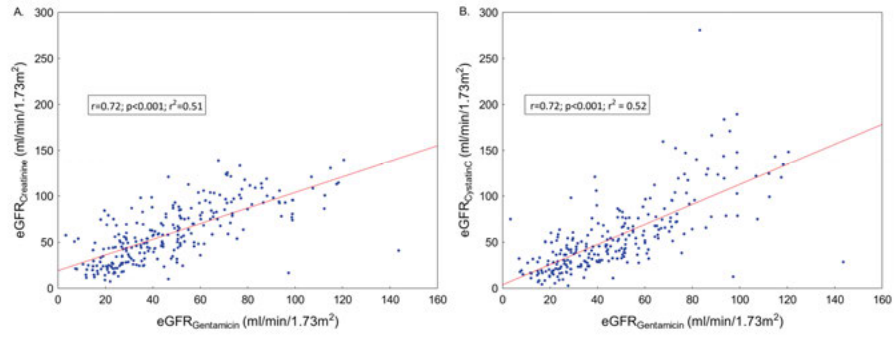

Figure 24 Scatter plot of the correlation between A) $eGFR_{Gentamicin}$ and $eGFR_{Creatinine}$ B) $eGFR_{Gentamicin}$ and $eGFR_{CystatinC}$.

eGFR; estimated glomerular filtration rate

The agreement between $eGFR_{Gentamicin}$ and $eGFR_{Creatinine}$ as well as between $eGFR_{Gentamicin}$ and $eGFR_{CystatinC}$ are presented in two Bland-Altman plots (Figure 25).

The Limits of Agreement (LoA) were 55 to -31 mg/ml/1.73 m^2 and 62 to -46 mg/ml/1.73 m^2, respectively. The bias was found to be 12 and 8 mg/ml/1.73 m^2, respectively.

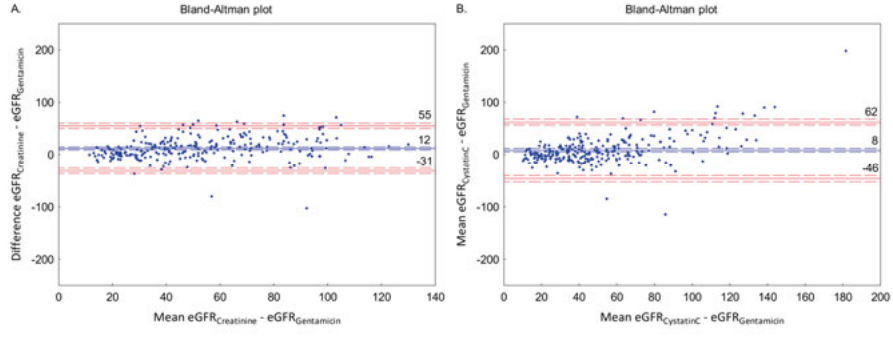

Figure 25 Bland-Altman plot of the agreement between A) $eGFR_{Gentamicin}$ and $eGFR_{Creatinine}$ B) $eGFR_{Gentamicin}$ and $eGFR_{CystatinC}$. The blue line marks the bias. Red lines mark the LoA. Dashed red respectively blue lines mark the 95% CI of the bias and the LoA.

eGFR; estimated glomerular filtration rate, LoA; limits of agreement

eGFR methods as biomarkers to predict RRT and mortality

The performance of the eGFR methods to predict RRT during ICU stay are presented as ROC-curves in Figure 26. The c-index, also called the ROC-AUC, was 0.80 (0.69-0.90) for $eGFR_{Gentamicin}$ compared to 0.75 (0.64-0.86) for $eGFR_{Creatinine}$, and 0.77 (0.66-0.88) for $eGFR_{CystatinC}$. The corresponding odds

ratios (OR) to predict RRT were 0.94 (0.92-0.97) for $eGFR_{Gentamicin}$, 0.96 (0.93-0.98) for $eGFR_{Creatinine}$ and 0.96 (0.93-0.98) for $eGFR_{CystatinC}$.

The performance of the eGFR methods to predict death within 30 days after the first Gentamicin dose in the ICU is presented in Figure 27. The c-index was 0.63 (0.54-0.72) for $eGFR_{Gentamicin}$ compared to 0.61 (0.52-0.70) for both $eGFR_{Creatinine}$ and $eGFR_{CystatinC}$. The OR to predict the risk of death was 0.98 (0.97-1.00) for $eGFR_{Gentamicin}$ and 0.99 (0.98-1.00) for both $eGFR_{Creatinine}$ and $eGFR_{CystatinC}$.

Hazard ratios (HR) were used to assess how the three eGFR methods performed to predict the risk of death over time during the follow-up period and were found to be 0.99 (0.98-0.99) for $eGFR_{Gentamicin}$, and 0.99 (0.99-1.00) for both $eGFR_{Creatinine}$ and $eGFR_{CystatinC}$.

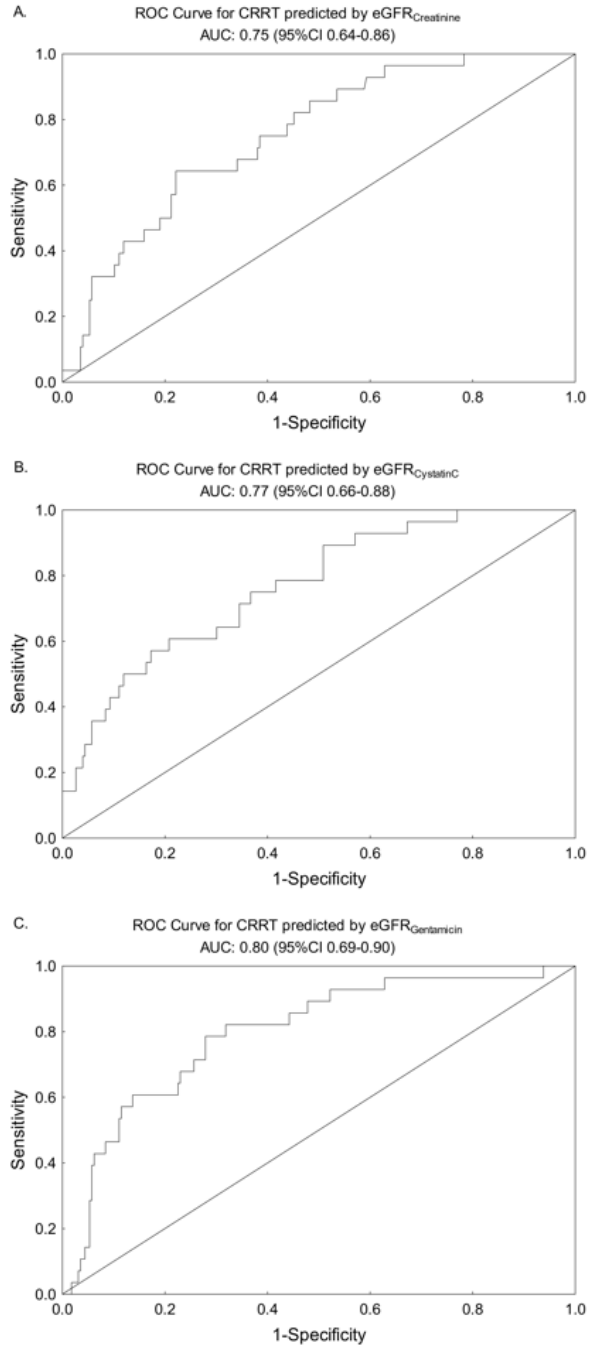

Figure 26 ROC-curve for RRT predicted by A) $eGFR_{Creatinine}$ B) $eGFR_{CystatinC}$ C) $eGFR_{Gentamicin}$

ROC; receiver operating characteristic, eGFR; estimated glomerular filtration rate

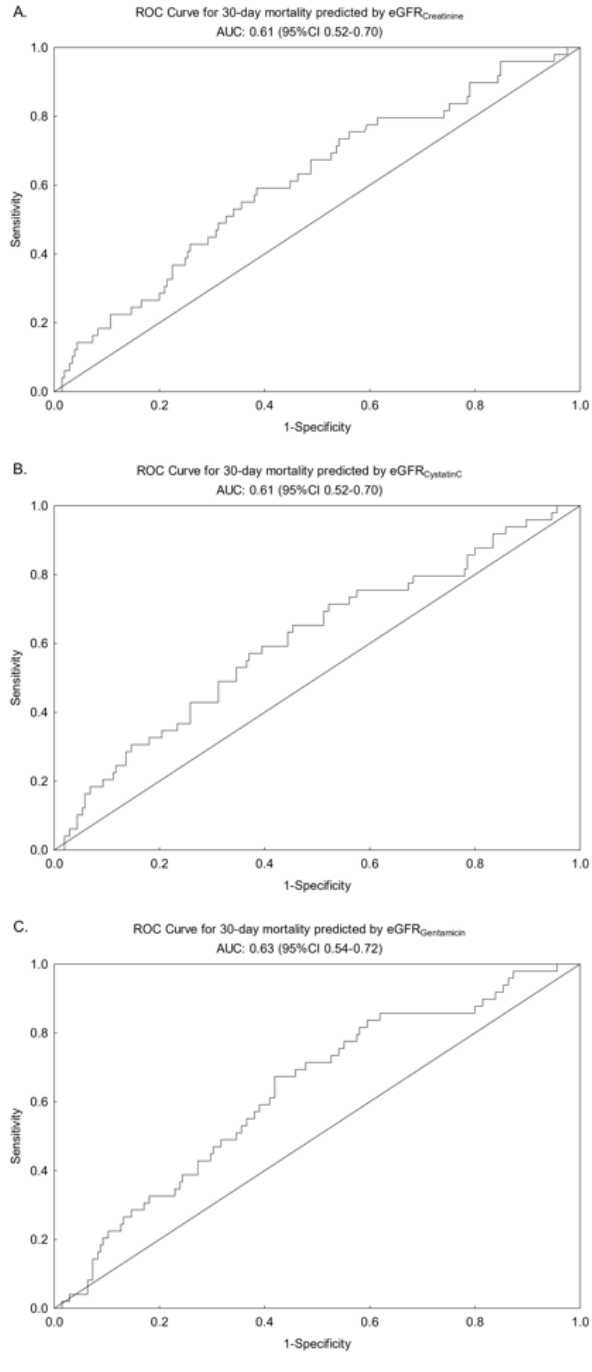

Figure 27 ROC-curve for 30-day mortality predicted by A) $eGFR_{Creatinine}$, B) $eGFR_{CystatinC}$, C) $eGFR_{Gentamicin}$

ROC; receiver operating characteristic, eGFR; estimated glomerular filtration rate

Discussion

In this thesis strategies for optimisation of antibiotic concentrations were investigated. Target attainment and β-lactam dosing in ICU patients during the three first important days of treatment with cefotaxime, piperacillin-tazobactam and meropenem were analysed. We explored the target attainment following standard intermittent infusions in an ICU cohort from the ACCIS study and patient factors influencing the risk of target attainment failure. Further, we investigated the impact of different MIC parameters on the calculations of target attainment as well as different PK/PD targets. Since we collected the actual causative bacteria of the patients' infections, the findings also have implications on how to use the MIC-parameter in both clinical TDM and in future PK/PD-studies on target attainment to make the results more reliable. We also performed predictions of short (SI), extended (EI), and continuous infusion (CI) based on the ACCIS cohorts PK data to further investigate the impact of different infusion strategies on target attainment of β-lactams and the risk for toxicity using two different clinical scenarios in the predictions and taking the causative pathogen into account.

Correct estimations of renal function in the ICU are crucial for adequate dosing of drugs with renal elimination like the β-lactams. Since standard eGFR methods based on creatine and cystatin C have limitations in an ICU setting we investigated if clearance of gentamicin, an aminoglycoside antibiotic often used in septic shock together with β-lactams, could be used as an exogenous marker for renal function in the early phase of infection. Since biomarkers of renal function are known to be strong predictors of mortality and risk for RRT in the ICU we also evaluated $eGFR_{Gentamicin}$ from this perspective compared to the other two commonly used eGFRs.

Target attainment of β-lactams

Target attainment rates will always depend on what PK/PD target you choose and what parameters you use in the PK/PD target calculation. Results from different studies can therefore be difficult to compare and studies of the impact of TDM or infusion strategies on mortality might be misleading. Many uncertainties still remain, including the optimal PK/PD-target in ICU patients even

if 100%$fT_{>MIC}$ for SI/ EI and fC_{ss}>4xMIC for CI are the targets mostly suggested in consensus documents (9, 24).

Impact of the PK/PD-target

In paper I we conclude that in the ACCIS ICU-cohort of 138 patients treated with cefotaxime, piperacillin-tazobactam or meropenem, 45% (62/138) of the patients did not reach the PK/PD-target of 100%$T_{>MIC}$ during the first important day of treatment when the MIC-parameter used was MIC_{WCS}. This is the MIC-parameter most PK/PD-studies have been using through the years instead of looking at the actual causative pathogen.

The result of target failure in our study of 45% is comparable with previous reports regarding β-lactam antibiotics in ICU patients of 37-65% of the patients not reaching the same PK/PD-target and using MIC_{WCS} in the calculations (11-14). In our study, the failure was even greater when using 50%$T_{>4xMIC}$ an alternative target sometimes suggested by some authors (11, 77).

The finding in paper I and other studies implicate that almost half of the ICU population treated with one of the three most used β-lactams in Swedish ICUs have inadequate dosing regimens to combat the WCS-pathogen the empirical treatments also are intended to cover during the first important day of treatment, when the bacterial load is the highest. However, in the ACCIS study, a bacterial pathogen of clinical relevance was found in 61% (84/138) of the patients and tested with reference method BMD to obtain their actual MIC values.

Impact of the MIC parameter and causative bacterial pathogen

In paper II we found that *S. aureus* and *E. coli* were the most common culture findings and together represented almost 50% of the cultured pathogens in the study. This finding is in line with previous Swedish studies (12, 134, 135). Overall, the bacterial findings and the resistance levels in paper II indicate that Swedish ICUs still can be considered low resistance settings. Only one patient in the study had a culture finding with *P. aeruginosa*, the worst case scenario pathogen for piperacillin-tazobactam and meropenem.

These findings question the traditional use of MIC_{WCS} in PK/PD-studies since the actual causative bacteria the β-lactams are supposed to kill seldom are collected and thereby are left out of the discussion regarding analysis of target attainment. To explore the impact of the MIC-parameter on target attainment calculations we performed further analyses in paper II on target attainment based on the culture findings and MIC results.

When using the PK/PD-target 100%$T_{>MIC}$ and three different MIC parameters in the calculations for all antibiotics in the study we found that the use of MIC_{WCS} overestimated the failure rate, 45% (62/138) compared to 23% (19/81) for MIC_{ECOFF} based on the actual causative bacterial pathogens. The

failure rate for MIC_{ECOFF} and $MIC_{ACTUAL+1}$ was comparable. This finding can be supported by the work of Woksepp were target failure was seen in 45% when MIC_{WCS} was used compared to 11% when actual MIC of the cultured pathogen was used (12). The overestimation in our study was most prominent in the cefotaxime-treated group where the corresponding target failure rates were 58% and 24% for MIC_{WCS} and MIC_{ECOFF}, respectively.

The impact of the used MIC-parameter was even more important when using the PK/PD-target of 50% $T_{>4xMIC}$ in the target attainment calculation. Target failure was then seen in 57% of the total cohort using the MIC_{WCS} in the calculations compared to 25% using MIC_{ECOFF}. This finding raises concern about the use 50% $T_{>4xMIC}$ in PK/PD-studies where no causative bacteria is collected or taken into consideration and MIC_{WCS} instead is used.

The data in paper II provides a unique description of the impact of the MIC-parameter used in target attainment calculations. The finding could be one of the reasons why it has been difficult to find evidence for the clinical impact /mortality of target attainment failures for β-lactams in ICU-studies, which use MIC_{WCS} and do not take the actual causative bacterial pathogen that causes the infection into account. Also, in studies on the impact of prolonged infusion strategies for β-lactams or TDM on mortality/morbidity, the absence of taking the actual causative bacterial pathogen and its MIC into account can be part of the explanation why there is still no clear evidence of the benefits.

Impact of patient risk factors

In paper I we report that younger age with increased renal clearance were associated with target attainment failure whereas a high SAPS3 and a high age were associated with target attainment.

Our results are in line with previous studies and further stress that TDM in young patients with augmented renal function in the ICU is important to consider early in the treatment (10, 12, 93). We also found that patients on RRT have a decreased risk of target attainment failure. However, in the RRT population piperacillin-tazobactam and meropenem were the most common β-lactams used which might have influenced this finding.

We did not see an association between the dosing regimen and target attainment, but the confidence intervals of antibiotic dosing were wide in the logistic regression model, probably due to low variation in dosing per body weight and small sizes for each antibiotic. For all three antibiotics, however, there was a trend towards not reaching target attainment for the lower doses. Additionally, the attending physician/ID specialist prescribing the antibiotic dosing may have adjusted the antibiotic dose to clinical factors influencing the pharmacokinetics which could have facilitated target attainment.

Impact of different infusion strategies

In paper I and II we found low target attainment, especially in the cefotaxime and piperacillin-tazobactam treated patients, when using SI during the first three days of treatment. This finding made us conclude the need for further studies on the optimal empirical initial dosing strategy for β-lactam treated patients in the ICU. Therefore, we decided to use the ACCIS cohort PK data to evaluate the impact of SI, EI and CI dosing strategies on target attainment and also the risk of toxicity further for cefotaxime, piperacillin-tazobactam and meropenem.

In the initial predictions of target attainment at 24, 48 and 72 h of treatment in paper III the PK/PD target of 100%$fT_{>MIC}$ was used together with two different MIC-levels, representing two clinical scenarios based on each β-lactams MIC_{WCS} and the MIC_{ECOFF} of the most difficult to treat primary pathogen when it comes to MIC values, i.e., the primary scenario and the worst case scenario. The same total daily dose of cefotaxime (6g/24 h), piperacillin-tazobactam (16g/24 h) or meropenem (3g/24h) was administered as SI (15 min), EI (3 h) and CI (24 h) in the predictions.

Overall, the target attainment for all antibiotics in the primary scenario was 94% (129/137) already with SI indicating that SI is sufficient in most community-acquired infections in the ICU in immunocompetent patients without risk factors for an infection caused by *P. aeruginosa* or *S. aureus.*

When the WCS pathogens were targeted in the predictions the numbers of patients above the efficacy target 100%$fT_{>MIC}$ increased as expected when switching from SI to EI and CI for all antibiotics. However, when applying the recommended target of fC_{ss}>4xMIC on CI the predicted target attainment was only slighter higher with CI than EI, for all antibiotics on each day indicating the approaches to be comparable. Other aspects, like economic or practical issues, may thus play a more important role in the selection of infusion strategy implemented in the ICU.

Another important aspect is that these predictions are made on plasma concentrations reflecting bacteriemia and not difficult to treat foci of infection like abscesses and bone. It is not well explored whether or not the top concentration in plasma also plays an important role in reaching adequate concentrations in the actual infection site making it difficult to suggest CI in these situations without further studies.

For cefotaxime, the predictions showed a high target attainment of 98% on Day 1 for the primary scenario indicating that infections caused by all cefotaxime susceptible bacterial pathogens except *S. aureus* could be treated with SI. In the case of *S. aureus*, which indeed can be both a primary and secondary pathogen, the median time of the dose above MIC increased from 75% (SI) to 93% (EI), and 99% (CI) but the predicted proportion of patients reaching target attainment remained low regardless of infusion strategy applied. In the predictions, 31% on SI compared to 44% on EI and 50% on CI (target fC_{ss})

reached target attainment suggesting that prolonging the infusion itself will not be sufficient. Minichmayr *et al.* found similar when investigating ceftaroline and treatment for *methicillin-susceptible S. aureus* and concluded that increasing the total daily dose was the most important factor to reach target attainment in isolates with MIC of 2-4 mg/L, not extending the duration of infusion(136). For cefotaxime treatment of *S. aureus,* a daily dose of 8 mg cefotaxime would be interesting to investigate further administered as SI, EI and CI.

The finding in the predictions indicates that 70% of the ICU patients on SI in Swedish ICUs fail to achieve target attainment when using cefotaxime on *S. aureus* infections which might not reflect the perceived clinical reality. The mortality in *S. aureus* bacteriemia (SAB) is reported to be 10-30% nowadays (137). In most cases, cefotaxime is switched to cloxacillin when the culture results come from the laboratory and the finding of target failure is thus mostly applicable to early *S. aureus* treatment. Cefotaxime also has an active metabolite, desacetylcefotaxime with reported antimicrobial activity 2-16 times lower than cefotaxime but the effect is species specific and the metabolite has almost no activity versus *S. aureus* (36). Thus, the activity of the metabolite cannot compensate for the low exposure to cefotaxime when treating *S. aureus* described above.

Another debatable issue is whether the PK/PD target of 100%$T_{>MIC}$ is unnecessarily high for treating *S. aureus* infections with cefotaxime. In the pre-antibiotic era, the mortality for SAB was reported to be around 80% which means that 20 % of the patients survived without antibiotic treatment(137). *S aureus* is a bacterial pathogen that has followed and colonized humans for possibly thousands of years (138), and perhaps the immune system is more adapted to this pathogen than for example opportunistic pathogens such as *P. aeruginosa* (139).

Toxicity and β-lactams

The toxicity thresholds for meropenem and piperacillin given as intermittent infusion used in paper I and III are, even if in line with European consensus recommendations(24), based on limited and uncertain data since they are based on one single study of 223 patients by Imani et al (44). It should also be noted that these thresholds represent the C_{min} where there is a 50% risk of developing neuro- or nephrotoxicity and not a strict toxicity level. No studies on toxicity levels studies for intermittent cefotaxime exist and the chosen upper recommended C_{min} level of 60 mg/L based on the French guidelines could only serve as an indication of possible toxicity even if the level can be supported by some case reports regarding neurotoxicity (8, 43, 46, 54).

In the ACCIS study, as reported in paper I, only two of the patients had observed end dose concentrations just above the upper recommended/toxicity

threshold. Both patients were treated with cefotaxime and had been given high initial doses in relation to the rapid development of decreased renal function.

In the predictions in paper III at 24, 48 and 72 h of treatment no meropenem treated patient had through concentrations above the suggested toxicity threshold regardless of infusion strategy.

When switching from SI to EI in the cefotaxime and piperacillin-tazobactam treated groups the total number of patients above the upper recommended/toxicity threshold increased slightly from 4.5% (5/110) for SI to 6.4% (7/110) for EI. The number of patients in the groups is small so no statistical conclusion can be made and the results interpreted with caution. However, the observed trend in the predictions is that there might be a slight increase in patients at risk for neuro- and/or nephrotoxicity when switching from SI to EI. Especially since the toxicity threshold for piperacillin recommended by European experts is based on levels where 50% of the patients have these side effects.

Regarding CI no consensus guidelines regarding toxicity levels exist and hence the risk of toxicity for CI was not evaluated in paper III. However, Quinton *et al.* reported that a concentration threshold of 157 mg/L for piperacillin given as continuous infusion with 96.7% specificity and 52.2% sensitivity predicted neurotoxicity in a cohort of 53 patients without prior neurological disorders. If applying the toxicity level of 157 mg/L, correlated for a protein binding of 30% to 110 mg/L, in the violin plots of predicted fC_{ss} for piperacillin it seems like the therapeutic range is narrow and TDM in these ICU patients could be of benefit to avoid toxicity.

Clinical implications on β-lactam dosing and TDM in the ICU

The findings of the ACCIS study and paper I-III have implications both on the treatment regimens with the three most used β-lactam antibiotics and the use of TDM in ICU patients in Sweden.

Infusion strategy and dosing

In a low-resistance setting like many Swedish ICUs, the results of the predictions in paper III together with the patient's immune status, medical history, type of infection and most likely causative bacterial pathogen can guide the clinician to which infusion type to use for the individual patient. Potentially, patients with community-acquired infections without immunosuppression or risk factors for a *P. aeruginosa* infection might still be treated with meropenem or piperacillin-tazobactam as SI without risk of underexposure.

In cases where a *P. aeruginosa* infection is more likely or even a confirmed causative bacterial pathogen, like in hospital-acquired LRTI or in immunocompromised patients in the ICU, it seems beneficial to give EI or CI, especially in severe infections where it is crucial to maintain antimicrobial exposure above the MIC.

For cefotaxime the results of the predictions suggest that in cases with possible *S. aureus* infection, like in SSTI or hospital-acquired LRTI, neither EI nor CI will help reach the selected target and a higher daily dose than 6g or switch to another β-lactam with better staphylococcal activity would be the action of choice. For patients receiving cefotaxime when *S. aureus* is an unlikely causative pathogen like in UTI, IAI and community-acquired LRTI the predictions in the study for the primary scenario indicate that all other cefotaxime susceptible pathogens could be treated with SI and still reach target attainment with a dose of 2g three times daily.

A recently published meta-analysis of randomized controlled trials found lower 90-day mortality in ICU patients receiving prolonged infusion of β-lactams (124), but since the individual groups of patients were not large enough it was not possible to assess whether all patients in the ICU could benefit from prolonged infusion or if this pertains mostly to certain groups of patients. To give all ICU patients CI could cause some patients to receive unnecessarily high antibiotic exposure and concentrations with a risk of developing neurotoxicity that can be very challenging to diagnose in sedated ICU patients.

Regarding toxicity and prolonged infusions of β-lactams, many things are still unknown and other exposure metrics than C_{min} (e.g., total AUC) as well as the impact of a β-lactam free interval need to be explored further. In Sweden, β-lactams are still regarded as very safe treatment options but in times of more aggressive β-lactam dosing with prolonged infusion, the risk of neurotoxicity in ICU patients might be underappreciated due to the diagnostic challenges in sedated patients.

When planning on implementing prolonged infusions of β-lactams in the local ICU, it could be prudent to develop a strategy for daily evaluations of neurotoxicity in these patients. Especially this could be important in ICUs where there is no access to daily TDM to guide the dosing.

Overall, when prolonged infusions are used in ICU patients, consideration of the suspected bacterial pathogen and site of infection is still highly important. The utility of CI or EI on infections such as deep abscesses, osteomyelitis and central nervous system infections needs to be investigated further. Potentially the top C_{max} could play a role in these more difficult to treat infections with penetration difficulties of β-lactams. There is also a difference in the anti-bacterial effect on dividing planktonic bacterial pathogens and bacteria in biofilms where the C_{max} might also play a role.

TDM

In Sweden, access to TDM for β-lactam antibiotics is very limited and can only be performed in four university hospitals which is a clear limitation for dose optimization and safe introduction of prolonged dosing strategies in ICU patients.

In paper I we report that 15% of the cefotaxime treated patients had at least one end sample below detectable concentration during the three days of the ACCIS study. If the concentration was low on day one it also continued for the following days. These findings suggest that TDM and end dose sampling already during the first day after β-lactam treatment initiation can help identify patients with a high risk of very low antibiotic concentrations and thus low antimicrobial exposure on the following days, especially in cefotaxime-treated patients. This finding is in contrast with current European recommendations that TDM can start first after 48 hours of treatment with β-lactams (24).

We conclude from the risk factor analysis in paper I, that patients of younger age, patients with signs of augmented renal clearance, patients with other focus of infection than UTI and especially if treated with cefotaxime, could benefit from early TDM already on day one in the ICU since they are at risk for insufficient antibiotic concentrations. With help from TDM, the β-lactam dosing can be more individualized with better target attainment. In times of more aggressive β-lactam treatment, the predictions of toxicity in this thesis indicate that TDM also needs to be introduced on a wider scale to prevent toxicity in patients given prolonged infusions, especially in elderly patients with reduced renal function and other neurological diseases where the risk of neurotoxicity is not negligible (51).

Even if TDM is implemented many other practical issues remain. One of them is which MIC value to use in the PK/PD calculations to compare your trough concentration against. The use of MIC_{ECOFF} in TDM situations where you have found a causative pathogen instead of $MIC_{ACTUAL+1}$ is recommended by Mouton et al (28). The findings in paper II support this conclusion since there is no difference in target attainment when you use either of these two MIC-parameters in our calculations.

Unfortunately, in clinical TDM situations, a causative pathogen is not always found in the cultures from the ICU. This can be solved by using MIC_{ECOFF} of the most likely causative bacterial pathogen in the calculations. You then have to take into consideration the focus of infection, the primary pathogens causing infections in that particular focus, the local epidemiological situation regarding common species causing infections in that specific ICU as well as the local level of resistance. The clinical microbiology laboratory could be an important resource for such discussions.

In many clinical TDM situations, the lack of a cultured causative pathogen has been solved by using the MIC_{WCS} of the β-lactam in question. The finding

in the ACCIS study that only one of the 138 patients had *P. aeruginosa* as the causative pathogen in cultures further stresses that in a Swedish ICU setting the use of MIC_{WCS} in clinical TDM situations is unsuitable.

The reason for this is that the MIC_{WCS} for both piperacillin-tazobactam and meropenem is the MIC_{ECOFF} of *P. aeruginosa*, corresponding to MIC of 16 mg/L for piperacillin-tazobactam and MIC of 2 mg/L for meropenem which are poorly adapted surrogate MIC-values to use in a low-resistance setting which many Swedish ICU still can be considered to be. From the findings in paper II, it would be more reasonable to use the MIC_{ECOFF} for *E. coli* with MIC 8 mg/L for piperacillin-tazobactam and MIC of 0.06 mg/L for meropenem since this was the most common culture findings.

However, in other countries as well as possibly in certain Swedish ICUs with an outbreak or future endemic situations with highly resistant *P. aeruginosa* infections, it could be adequate to consider *P. aeruginosa* MICs. Which MIC_{ECOFF} to choose in cases without culture findings depends on the local epidemiology but the worst-case scenario MIC should always be used with caution.

Another advantage of using MIC_{ECOFF} in clinical TDM situations as well as in PK/PD studies is that MIC-testing is not required if the strain has been categorised as S or I, with the EUCAST disk diffusion method. Due to the excellent calibration of the methods, it is possible to use the MIC_{ECOFF} of the pathogen/antimicrobial combination from the EUCAST website. This can be extra beneficial in low- and medium-income countries devoid of methods for MIC reference testing but also in Swedish clinical microbiology laboratories that have not yet implemented reference methodology for MIC-testing.

Implications for PK/PD-studies

Furthermore, the findings in paper II that the popular use of the MIC-parameter MIC_{WCS} in PK/PD-calculations leads to an overestimation of target failure have implications on future target attainment studies on ICU-patients and we suggest the use of MIC_{ECOFF} of the actual or suspected causative pathogen as a better option. As mentioned above this approach is a feasible way to take the causative bacterial pathogen and infection site of the patients into account in the studies without having to collect the strains or perform MIC-testing.

Strengths and limitations of the ACCIS study

To our knowledge, the ACCIS study is the first multi-centre study to study the PK/PD-target attainment during the first three critical days after the start of antibiotic treatment with cefotaxime, piperacillin-tazobactam or meropenem in ICU-patients treated for an infection. Contrary to previous studies we also

managed to collect the actual causative pathogen for the infections in 61% of the patients. In paper II we performed MIC-testing with the reference MIC-testing methods making our MIC-values in the target attainment analysis more reliable than in most previous studies. We also conducted a careful examination of the impact of using different MIC-parameters in the target attainment analysis which has not been carried out to this extent in earlier studies (11, 12).

We included patients from seven different ICUs in five regions and managed to get a good variation of patients with community- and hospital-acquired infections as well as a broad range of different infectious foci in the patients. In the study, both patients from small general ICUs in the county hospitals as well as from highly specified unit ICUs were included. Herein, the strength of the ACCIS study population is that it well represents a general Swedish ICU population which makes the results regarding target attainment representative and not just applicable for a narrow population.

However, there are also limitations of the ACCIS study regarding mainly the antibiotic concentration results. One very important limitation is that all measured antibiotic concentration levels are total antibiotic concentrations and not the free fraction in plasma. The classical approach for solving this problem in earlier studies has been to use previously published data on protein binding from healthy volunteers (74). That usually means that you calculate an assumed protein binding for cefotaxime of 30-40%, piperacillin of 30 % and meropenem of 2%. Some studies however have shown that these might not be appropriate figures to use in the ICU setting since data indicate that the protein levels and binding in ICU patients can be highly variable (87, 140). We therefore decided to follow the local instructions for TDM calculation in the ICU at the Uppsala University Hospital and leave protein binding out of our calculations to see what the best possible outcome regarding antibiotic concentrations and target attainment could be if the protein binding was zero for cefotaxime, piperacillin and meropenem in paper I and II.

In the prediction of paper III, we followed the classical approach where applicable and used the published values of protein binding for piperacillin (30%) and cefotaxime (30%) whereas meropenem concentrations were used without corrections due to negligible protein binding.

Another limitation is that the antibiotic concentrations from the first day were taken before steady-state was established which is usually not recommended since it will reduce the predictive value of a single TDM-value over the following days. However, in critically ill ICU patients with unstable PK, steady state might never be fully achieved. In the ACCIS study, low antibiotic concentrations for cefotaxime on Day 1 were reproducible on Day 2 and 3 indicating that early TDM after all can provide useful information in the ICU in order to find the patient at risk of target failure as soon as possible, at least

for cefotaxime-treated patients. In paper II the limitations regarding the antibiotic concentration just mentioned in paper I influenced all calculations equally regardless of MIC-parameter used and did not affect the comparison.

As in many other PK/PD-studies on ICU patients, we were only able to measure plasma antibiotic concentrations and thus calculate target attainment in the bloodstream and not at the site of infection where we can assume that the antibiotic concentrations and the target attainment were even lower.

Finally, the best PK/PD-target to use in calculations of ICU patients is still not known but we used $100\%T_{>MIC}$ and fC_{ss}>4xMIC as proposed by expert consensus guidelines (9, 24).

However, one PK/PD-target might not fit all ICU patients depending on immune status, focus of infection and causative bacterial pathogen meaning that for example, $100\%T_{>MIC}$ might be unnecessarily strict for immunocompetent patients which is important to have in mind when evaluating target attainment results.

Can gentamicin concentrations be used to estimate eGFR in ICU-patients?

In paper IV we investigated the possibility of using clearance derived from a population PK model based on gentamicin concentrations in an ICU cohort as an exogenous marker of renal function and compared to the endogenous eGFR markers derived from creatinine and cystatin C that are commonly used in Swedish ICUs.

When analysing the correlation between $eGFR_{Gentamicin}$ and $eGFR_{Creatinine}$ and $eGFR_{Gentamicin}$ $eGFR_{CystatinC}$, respectively we found a positive linear relationship for both analyses. In the analysis of agreement, we found low agreement despite low bias for both $eGFR_{Gentamicin}$ /$eGFR_{Creatinine}$ and $eGFR_{Gentamicin}$/$eGFR_{CystatinC}$, respectively.

However, since both creatinine and cystatin C are known to have limitations in ICU populations the findings cannot be used to rule out the usefulness of $eGFR_{Gentamicin}$ as a marker for renal function alone.

To investigate $eGFR_{Gentamicin}$ further, we also used univariate logistic regression, presented as ROC-curves, to assess the association between the three eGFR methods and the risk for RRT during ICU stay as well as 30-day mortality. The c-index for predicting RRT were similar for all three eGFR methods with a trend towards a higher c-index for $eGFR_{Gentamicin}$ of 0.80 (0.69-0.90) compared to 0.75 (0.64-0.86) and 0.77 (0.66-0.88) for $eGFR_{Creatinine}$ and $eGFR_{CystatinC}$, respectively. The c-index for predicting 30-day mortality had a similar trend. The risk of death over time calculated as hazard ratios was 0.99 for all three eGFR methods.

In conclusion, $eGFR_{Gentamicin}$ performs well in line with the other biomarkers of renal function known to be strong predictors of RRT and mortality which support exploring the use of estimated gentamicin clearance derived from 8 h concentrations as an exogenous marker of renal function in the ICU further.

Strengths, limitations and clinical implications of the Gentamicin study

The study has some strengths and limitations that merit mentioning. First of all, to our knowledge, this is the first study that uses $eGFR_{Gentamicin}$ derived from serum gentamicin concentrations using a population PK model-based approach to compare against $eGFR_{Creatinine}$ and $eGFR_{CystatinC}$, the standard estimates of eGFR in most countries. We also for the first time evaluated $eGFR_{Gentamicin}$ not only to other eGFR methods but also to clinical outcomes related to renal functions since eGFR methods are known to be strong predictors of mortality and need for RRT.

One important limitation of the study is that we only compared the estimated gentamicin clearance towards other biomarkers and not one of the reference methods for creatinine clearance like iohexol. Another objection might be that the use of gentamicin has declined in recent years in Sweden, and the usefulness of developing a new exogenous eGFR method could be questioned. But in patients with septic shock in the ICU, neonates and in other countries where gentamicin is used together with β-lactams as empirical treatment because of antimicrobial stewardship reasons, $eGFR_{Gentamicin}$ as a marker of renal function might be a promising option.

In recent years computerized population PK model-based methods have been developed to estimate PK parameters such as clearance and to predict individual dosing regimens, referred to as model-informed precision dosing (MIPD). This approach requires the development of a software program to be able to quickly convert the gentamicin concentration into eGFR but could be beneficial in the initial critical stage of infection to be able to better dose other renally eliminated drugs like the β-lactams since neither eGFR based on creatinine or cystatin C are well-functioning during that stage.

In this thesis, the findings of paper IV could be developed into a tool for optimisation of β-lactam concentrations and target attainment during the first day in critically ill ICU patients since the estimation of renal function is crucial for the dosing. An additional benefit is that $eGFR_{Gentamicin}$ also could be used to predict risk for mortality and RRT during ICU care.

Conclusions

- A high proportion of ICU patients treated with cefotaxime, piperacillin-tazobactam or meropenem did not reach the PK/PD-target of 100%$T_{>MIC}$ during the first three important days of treatment. TDM already on the first day of treatment could help identify patients who need higher individual dosing regimens. Younger age, signs of augmented renal clearance, treatment with cefotaxime, and treatment of other infections than UTI were identified as risk factors for target attainment failure where early TDM could be encouraged.
- The current use of the MIC-parameter MIC_{WCS} in PK/PD-calculations in studies of ICU patients was found to overestimate target attainment failures and its routine use should be questioned. MIC_{ECOFF} of the actual causative bacteria or in cases without such the MIC_{ECOFF} of the most likely pathogen is a better option than using MIC_{WCS}. These findings could be part of the explanation for failing to find a correlation between target attainment failure and mortality in PK/PD-studies on ICU patients.
- In predictions based on three different infusion strategies of β-lactams in a Swedish ICU cohort, target attainment rates for primary pathogen scenarios were high regardless of infusion type, indicating that SI is sufficient in most community-acquired infections except for infections with *S. aureus* treated with cefotaxime. In worst case scenario (WCS) pathogens, reflecting infections with *P. aeruginosa*, SI was insufficient and routine use of EI or CI could be beneficial for piperacillin-tazobactam and meropenem. However, in the WCS for cefotaxime reflecting *S. aureus* infections the target attainments were low regardless of infusion strategy indicating the need for a higher daily dose than 6 g daily or switching to another β-lactam.
- Well established thresholds to evaluate toxicity in an era of more aggressive β-lactam dosing strategies are largely lacking and future studies are needed. The use of the suggested PK/PD target of fC_{ss} >4MIC for CI might lead to unnecessarily high concentrations and risk of adverse side effects like neurotoxicity, especially in elderly patients with renal insufficiency and prior neurological events. TDM already on Day 1 can be an important tool to identify patients

at risk, as well as daily neurological evaluations in ICUs implementing CI and EI.
In ICU patients treated with gentamicin, the use of estimated gentamicin CL derived from measured 8 h concentrations using a population PK model, was found to be a potential exogenous marker of renal function in patients in an early phase of severe infection.

Future perspectives

Despite years of research on the optimisation of β-lactam treatment and efforts like the Surviving Sepsis Campaign, the mortality and morbidity caused by infections in ICUs remain high and knowledge gaps still persist. This thesis has tried to answer some of the questions, but much is left to explore.

One of many things that can facilitate the research on target attainment, toxicity levels and optimal dosing in ICU patients further is the recent appearance of analysis methods that can measure the free concentration of the β-lactam and not only the total concentrations. This will finally solve the problem with the uncertainty of the level of protein binding which is well known to be variable and difficult to predict in ICU patients and seldom match with the published values of protein binding from healthy volunteers. To repeat the ACCIS study with this tool available would be of great interest. However, in Sweden, this analysis is yet only available at one university hospital.

With the availability of measuring free β-lactam concentrations and the knowledge regarding which MIC parameter to use from this thesis, different PK/PD targets can be investigated with better precision than before, making them more clinically relevant. It would also be of interest to further explore if different bacterial pathogens and/or foci of infection require different PK/PD targets and in the long run different infusion strategies. Overall, improved research focusing also on the causative or suspected pathogen and the type of infection is warranted since no size fits all when it comes to β-lactam treatment of ICU patients. Great research has been made in the PK-field, but the PD-part of the PK/PD equation needs further exploring and cannot be reduced to MIC_{WCS} if we want the research to align with clinical practice. Also, further advances in measuring β-lactam concentrations at the site of infection are warranted.

When developing this thesis, one apparent knowledge gap is the limited data on β-lactam toxicity when using prolonged infusions in general and in particular CI. Also, for SI and EI the data behind the recommended toxicity thresholds are uncertain and limited. An implementation on a broad scale of EI/CI in ICUs is expected and possibly also further increases of recommended doses. At the same time, the research regarding risk for toxicity with more aggressive β-lactam treatment needs to be evaluated since the reports on nephro- and neurotoxicity for some β-lactams like cefepime and piperacillin-tazobactam are on the rise after the introduction of prolonged infusions and

higher doses. However, recommended toxicity levels for CI are missing and other PK metrics like AUC have not been explored. Future clinical studies on toxicity with prolonged infusion as well as PK/PD studies of the optimal drug exposure metrics are needed.

A more reliable assessment of renal function in ICU patients during the initial phase of infection is another important and warranted research area for many reasons, not the least for optimal dosing of β-lactams. Further exploring of the use of gentamicin clearance as an exogenous marker of renal function by comparing to iohexol in ICU patients would be an interesting first step.

Most ICUs in Sweden have limited access to TDM for β-lactams which is troublesome and makes individualised precision dosing very difficult to achieve. However, TDM is needed both to be able to detect patients at risk for underexposure but also to prevent toxic side effects like neurotoxicity when treatment strategies and dosing change. Otherwise, we are left in the dark and future studies in the field are also more difficult to perform outside of some of the university hospitals.

In a future perspective, with a further rise in MDR gram-negative bacterial pathogens with very limited treatment options causing clinical infections in our ICUs, we need to have the ability to give more individualised dosing when combining different antimicrobials - including the least resistant β-lactam for treatment. Rapid TDM results and extended knowledge as described above regarding PK/PD in relation to the causative bacteria and infection type will be crucial in these cases. Also, the new β-lactam/β-lactam inhibitor combinations are warranted to explore further in PK/PD studies and are important to implement rapidly in available TDM panels for the same reason.

Svensk sammanfattning

Intensivvård (IVA) är den dyraste och mest resurskrävande vårdformen på sjukhus och svåra infektioner utgör majoriteten av den akuta sjukdomsbördan hos patienterna. Tidig och korrekt antibiotikabehandling är avgörande för patienternas prognos. Adekvat behandling handlar dels om att ge korrekt antibiotika vid en viss infektion, dels att uppnå rätt koncentration av antibiotika i blodet för att avdöda orsakande bakterier. Hur antibiotika tas upp och utsöndras från kroppen kallas farmakokinetik (PK), medan sättet antibiotika dödar bakterier kallas farmakodynamik (PD). Koncentrationen av antibiotika som krävs för att döda bakterier kallas minst hämmande koncentration (MIC). När dessa tre är i balans säger man att man uppnår PK/PD-målen.

I denna avhandling har vi undersökt strategier för optimering av antibiotikakoncentrationer, måluppfyllelse och dosering under de tre första viktiga dagarna av behandling med tre av de mest använda antibiotika på IVA i Sverige, cefotaxim, piperacillin-tazobaktam och meropenem. Samtliga tre tillhör antibiotikaklassen som kallas β-laktamer.

Vi undersökte hur stor andel av IVA-patienterna i en svensk multicenterstudie som uppnådde de satta PK/PD-målen, dvs hade adekvata antibiotikakoncentrationer, efter korta standardinfusioner samt vilka patientfaktorer som påverkar risken för otillräcklig måluppfyllelse, dvs för låga antibiotikakoncentrationer i blodet.

Vidare undersökte vi effekten av att använda olika s.k. MIC-parametrar i beräkningarna av måluppfyllelse eftersom vi samtidigt samlade in de bakterierna som orsakat studiepatienternas infektioner.

Vi utvärderade hur förlängning av infusionstiden, dvs tiden som antibiotika tillförs blodet, påverkar måluppfyllelsen vid samma totaldos av respektive antibiotika vid kort, förlängd och kontinuerlig infusion. I matematiska simuleringar jämförde vi dessutom måluppfyllelsen mot två olika kliniska scenarier som representerar samhällsförvärvade respektive sjukhusförvärvade infektioner.

Korrekt uppskattning av njurfunktionen på intensivvårdspatienter är avgörande för adekvat dosering av antibiotika som utsöndras via njurarna. Eftersom standardmetoder för detta idag baseras på kroppsegna markörer med begränsningar i en intensivvårdssituation undersökte vi om gentamicin, ett antibiotikum som ofta används vid septisk chock tillsammans med β-laktamer,

kunde användas som en markör för njurfunktion i infektionernas initiala fas i en IVA-population.

Baserat på studierna i den här avhandlingen kan följande konklusioner dras:

I studie I fann vi att en hög andel intensivvårdspatienter som behandlades med cefotaxim, piperacillin-tazobaktam eller meropenem inte nådde de uppsatta PK/PD-målet under de första tre viktiga dagarna av behandlingen. TDM, terapeutisk läkemedelsövervakning där doseringen vägleds av upprepade koncentrationsmätningar av aktuellt antibiotika, redan på den första behandlingsdagen kan hjälpa till att identifiera patienter som behöver högre individuella doser eller andra infusionssätt. Yngre ålder, tecken på förstärkt njurfunktion, behandling med cefotaxim samt andra infektioner än urinvägsinfektioner identifierades som riskfaktorer för måluppfyllelse där tidig koncentrationsbestämning med TDM är önskvärt.

I studie II undersöktes MIC-parameterns roll och betydelse i PK/PD-beräkningar av måluppfyllelse vid behandling med β-laktamer. För varje antibiotika/bakterie-kombination finns en MIC-parameter som kallas $MIC_{ECOFF.}$ Det motsvarar det MIC-värde som avgränsar stammar av aktuell bakterie som har, respektive saknar mekanismer som kan orsaka att aktuellt antibiotika inte fungerar, s.k. antibiotikaresistens. I både PK/PD-studier av måluppfyllelsen på IVA och vid TDM i klinisk praxis har dock av tradition en annan MIC-parameter kallad MIC_{WCS} ofta använts, vilket reflekterar ett ”worst-case-scenario” motsvarande en infektion orsakad av den bakterien med högst tänkbara behandlingsbara MIC_{ECOFF} för antibiotikapreparatet i fråga. Vår studie visade att denna användning överskattar andelen patienter som inte når behandlingsmålen dvs underskattar den faktiska måluppfyllelsen med konsekvensen att man bl. a riskerar att ge onödigt höga doser β-laktamantibiotika med potentiell risk för toxicitet. Istället bör man använda MIC_{ECOFF} baserat på odlingsfynd eller på den bakterie man bedömer är den mest sannolika orsaken till infektionen. Detta är en mycket viktig slutsats för behandling med β-laktamer på IVA men även för design av framtida studier. Användningen av felaktigt för höga MIC-värde kan också vara en del av förklaringen till att man hittills misslyckats med att hitta en korrelation mellan låg måluppfyllelse och dödlighet i PK/PD-studier på IVA-patienter behandlade med β-laktamer.

I studie III utvärderade vi hur samma dos av en specifik β-laktam givet med tre olika infusionsstrategier påverkade måluppfyllelse. I utvärderingen var andelen patienter som nådde de uppsatta PK/PD-målen för det första kliniska scenariot, motsvarande MIC_{ECOFF} för de flesta bakterier som ger samhällsförvärvade infektioner, höga oavsett infusionstyp, vilket indikerar att korta standardinfusioner är tillräckligt för de flesta samhällsförvärvade infektioner med undantag för infektioner med *S. aureus* behandlad med cefotaxim. I det andra ”worst-case-scenariot”, som återspeglar sjukhusförvärvade infektioner med *P. aeruginosa*, var korta standardinfusioner otillräckliga och rutinmässig användning av förlängd eller kontinuerlig infusion fördelaktigt vid behandling

med piperacillin-tazobaktam och meropenem. I scenariot med cefotaxim och *S. aureus*-infektioner var måluppfyllelserna dock låga oavsett infusionsstrategi, vilket indikerar att högre daglig dos än 6 g dagligen eller byte till en annat antibiotikapreparat krävs för optimal behandling. Kontinuerlig infusion kan leda till onödigt höga antibiotikakoncentrationer, men tydliga gränsvärden för toxicitet saknas och framtida studier behövs. Med tanke på pågående diskussion om värdet av kontinuerliga infusioner ger denna studie, som beskriver vinster och risken med olika administrationsstrategier, central information. TDM redan på dag 1 på samt dagliga neurologiska utvärderingar av patienterna för att hitta tecken på neurotoxicitet (dvs biverkan som drabbar nervsystemet) på intensivvårdsavdelningar som implementerar förlängd infusionsstrategi är viktiga verktyg för att identifiera patienter i riskzonen för höga, potentiellt toxiska antibiotikakoncentrationer.

Hos intensivvårdspatienter som behandlats med gentamicin, visar vi i avhandlingen att användningen av uppmätta gentamicinkoncentrationer med hjälp av en PK-modell på populationsnivå för gentamicinutsöndring kan anses vara en lovande markör för njurfunktion hos IVA-patienter i en tidig fas av allvarlig infektion där dagens njurfunktionsmarkörer inte fungerar tillfredsställande. Fler studier behövs dock för att utvärdera detta

Ur ett svenskt perspektiv visar avhandlingen att den begränsade tillgången till TDM, dvs laboratorier som kan utföra koncentrationsbestämningar av β-laktamer, är bekymmersam för att uppnå optimerad och säker dosering av β-laktamer på IVA. I dagsläget är det endast fyra av landets universitetssjukhus som tillhandahåller analyserna och svarstiderna för IVA-avdelningar utanför dessa enheter är långa.

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References

1. Perner A, Gordon AC, De Backer D, Dimopoulos G, Russell JA, Lipman J, et al. Sepsis: frontiers in diagnosis, resuscitation and antibiotic therapy. Intensive Care Medicine. 2016;42(12):1958-69.
2. Kollef MH, Shorr AF, Bassetti M, Timsit JF, Micek ST, Michelson AP, et al. Timing of antibiotic therapy in the ICU. Crit Care. 2021;25(1):360.
3. Vincent JL, Sakr Y, Singer M, Martin-Loeches I, Machado FR, Marshall JC, et al. Prevalence and Outcomes of Infection Among Patients in Intensive Care Units in 2017. Jama. 2020;323(15):1478-87.
4. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. Jama. 2009;302(21):2323-9.
5. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Medicine. 2021;47(11):1181-247.
6. Seymour CW, Gesten F, Prescott HC, Friedrich ME, Iwashyna TJ, Phillips GS, et al. Time to Treatment and Mortality during Mandated Emergency Care for Sepsis. N Engl J Med. 2017;376(23):2235-44.
7. Liu VX, Fielding-Singh V, Greene JD, Baker JM, Iwashyna TJ, Bhattacharya J, et al. The Timing of Early Antibiotics and Hospital Mortality in Sepsis. Am J Respir Crit Care Med. 2017;196(7):856-63.
8. Guilhaumou R, Benaboud S, Bennis Y, Dahyot-Fizelier C, Dailly E, Gandia P, et al. Optimization of the treatment with beta-lactam antibiotics in critically ill patients-guidelines from the French Society of Pharmacology and Therapeutics (Société Française de Pharmacologie et Thérapeutique-SFPT) and the French Society of Anaesthesia and Intensive Care Medicine (Société Française d'Anesthésie et Réanimation-SFAR). Crit Care. 2019;23(1):104.
9. Hong LT, Downes KJ, FakhriRavari A, Abdul-Mutakabbir JC, Kuti JL, Jorgensen S, et al. International consensus recommendations for the use of prolonged-infusion beta-lactam antibiotics: Endorsed by the American College of Clinical Pharmacy, British Society for Antimicrobial Chemotherapy, Cystic Fibrosis Foundation, European Society of Clinical Microbiology and Infectious Diseases, Infectious Diseases Society of America, Society of Critical Care Medicine, and Society of Infectious Diseases Pharmacists: An executive summary. Pharmacotherapy. 2023;43(8):736-9.
10. Roberts JA, Abdul-Aziz MH, Lipman J, Mouton JW, Vinks AA, Felton TW, et al. Individualised antibiotic dosing for patients who are critically ill: challenges and potential solutions. Lancet Infect Dis. 2014;14(6):498-509.
11. Roberts JA, Paul SK, Akova M, Bassetti M, De Waele JJ, Dimopoulos G, et al. DALI: defining antibiotic levels in intensive care unit patients: are current beta-lactam antibiotic doses sufficient for critically ill patients? Clin Infect Dis. 2014;58(8):1072-83.

12. Woksepp H, Hallgren A, Borgstrom S, Kullberg F, Wimmerstedt A, Oscarsson A, et al. High target attainment for beta-lactam antibiotics in intensive care unit patients when actual minimum inhibitory concentrations are applied. Eur J Clin Microbiol Infect Dis. 2017;36(3):553-63.
13. Petersson J, Giske CG, Eliasson E. Standard dosing of piperacillin and meropenem fail to achieve adequate plasma concentrations in ICU patients. Acta Anaesthesiol Scand. 2016;60(10):1425-36.
14. Taccone FS, Laterre PF, Dugernier T, Spapen H, Delattre I, Wittebole X, et al. Insufficient beta-lactam concentrations in the early phase of severe sepsis and septic shock. Crit Care. 2010;14(4):R126.
15. Blot SI, Pea F, Lipman J. The effect of pathophysiology on pharmacokinetics in the critically ill patient--concepts appraised by the example of antimicrobial agents. Adv Drug Deliv Rev. 2014;77:3-11.
16. Zander J, Döbbeler G, Nagel D, Maier B, Scharf C, Huseyn-Zada M, et al. Piperacillin concentration in relation to therapeutic range in critically ill patients--a prospective observational study. Crit Care. 2016;20:79.
17. Sumi CD, Heffernan AJ, Lipman J, Roberts JA, Sime FB. What Antibiotic Exposures Are Required to Suppress the Emergence of Resistance for Gram-Negative Bacteria? A Systematic Review. Clin Pharmacokinet. 2019.
18. Roberts JA, Taccone FS, Lipman J. Understanding PK/PD. Intensive Care Med. 2016;42(11):1797-800.
19. Roger C. Understanding antimicrobial pharmacokinetics in critically ill patients to optimize antimicrobial therapy: A narrative review. J Intensive Med. 2024;4(3):287-98.
20. Whimbey E, Wong B, Kiehn TE, Armstrong D. Clinical correlations of serial quantitative blood cultures determined by lysis-centrifugation in patients with persistent septicemia. J Clin Microbiol. 1984;19(6):766-71.
21. Lipcsey M, Castegren M, Furebring M, Sjölin J. Should the Aminoglycoside β-Lactam Combination Be Abandoned in All Severely Ill Patients With Presumed Gram-Negative Infection? Clin Infect Dis. 2018;66(3):480-2.
22. Huttner A, Harbarth S, Hope WW, Lipman J, Roberts JA. Therapeutic drug monitoring of the β-lactam antibiotics: what is the evidence and which patients should we be using it for? J Antimicrob Chemother. 2015;70(12):3178-83.
23. Muller AE, Huttner B, Huttner A. Therapeutic Drug Monitoring of Beta-Lactams and Other Antibiotics in the Intensive Care Unit: Which Agents, Which Patients and Which Infections? Drugs. 2018;78(4):439-51.
24. Abdul-Aziz MH, Alffenaar JC, Bassetti M, Bracht H, Dimopoulos G, Marriott D, et al. Antimicrobial therapeutic drug monitoring in critically ill adult patients: a Position Paper(). Intensive Care Med. 2020;46(6):1127-53.
25. Abdul-Aziz MH, Brady K, Cotta MO, Roberts JA. Therapeutic Drug Monitoring of Antibiotics: Defining the Therapeutic Range. Ther Drug Monit. 2022;44(1):19-31.
26. Roelofsen EE, Abdulla A, Muller AE, Endeman H, Gommers D, Dijkstra A, et al. Dose optimization of cefotaxime as pre-emptive treatment in critically ill adult patients: A population pharmacokinetic study. Br J Clin Pharmacol. 2023;89(2):705-13.
27. ISO 20776-1:2019 Susceptibility testing of infectious agents and evaluation of antimicrobial susceptibility test device -Part 1, Broth microdilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.

28. Mouton JW, Muller AE, Canton R, Giske CG, Kahlmeter G, Turnidge J. MIC-based dose adjustment: facts and fables. J Antimicrob Chemother. 2018;73(3):564-8.
29. Bush K, Bradford PA. β-Lactams and β-Lactamase Inhibitors: An Overview. Cold Spring Harb Perspect Med. 2016;6(8).
30. Sjövall F, Edström M, Walther S, Hanberger H. A nationwide evaluation of antibiotics consumption in Swedish intensive care units. Infect Dis (Lond). 2022;54(10):713-21.
31. Georgopapadakou NH, Liu FY. Penicillin-binding proteins in bacteria. Antimicrob Agents Chemother. 1980;18(1):148-57.
32. Infektionsläkarföreningen. Vårdprogrammet för sepsis och septisk chock 2022 Available from: https://infektion.net/vardprogram/svar-sepsisseptisk-chock/.
33. Todd PA, Brogden RN. Cefotaxime. An update of its pharmacology and therapeutic use. Drugs. 1990;40(4):608-51.
34. Chin NX, Neu HC. Cefotaxime and desacetylcefotaxime: an example of advantageous antimicrobial metabolism. Diagn Microbiol Infect Dis. 1984;2(3 Suppl):21s-31s.
35. Jones RN. Cefotaxime and desacetylcefotaxime antimicrobial interactions. The clinical relevance of enhanced activity: a review. Diagn Microbiol Infect Dis. 1995;22(1-2):19-33.
36. Neu HC. Antibacterial activity of desacetylcefotaxime alone and in combination with cefotaxime. Rev Infect Dis. 1982;4 Suppl:S374-8.
37. Referensgruppen för Antibiotikafrågor (RAF), Antibiotikakompendium Available from: https://www.sls.se/raf/lakemedel/antibiotikakompendium/ Accessed on Feb 1 2023.
38. Perry CM, Markham A. Piperacillin/tazobactam: an updated review of its use in the treatment of bacterial infections. Drugs. 1999;57(5):805-43.
39. Nicolau DP. Carbapenems: a potent class of antibiotics. Expert Opin Pharmacother. 2008;9(1):23-37.
40. Mouton JW, van den Anker JN. Meropenem clinical pharmacokinetics. Clin Pharmacokinet. 1995;28(4):275-86.
41. Roger C, Louart B. Beta-Lactams Toxicity in the Intensive Care Unit: An Underestimated Collateral Damage? Microorganisms. 2021;9(7).
42. Legg A, Carmichael S, Chai MG, Roberts JA, Cotta MO. Beta-Lactam Dose Optimisation in the Intensive Care Unit: Targets, Therapeutic Drug Monitoring and Toxicity. Antibiotics (Basel). 2023;12(5).
43. Lacroix C, Kheloufi F, Montastruc F, Bennis Y, Pizzoglio V, Micallef J. Serious central nervous system side effects of cephalosporins: A national analysis of serious reports registered in the French Pharmacovigilance Database. J Neurol Sci. 2019;398:196-201.
44. Imani S, Buscher H, Marriott D, Gentili S, Sandaradura I. Too much of a good thing: a retrospective study of β-lactam concentration-toxicity relationships. J Antimicrob Chemother. 2017;72(10):2891-7.
45. Quinton MC, Bodeau S, Kontar L, Zerbib Y, Maizel J, Slama M, et al. Neurotoxic Concentration of Piperacillin during Continuous Infusion in Critically Ill Patients. Antimicrob Agents Chemother. 2017;61(9).
46. Zerbib Y, Gaulin C, Bodeau S, Batteux B, Lemaire-Hurtel AS, Maizel J, et al. Neurological burden and outcomes of excessive β-lactam serum concentrations of critically ill septic patients: a prospective cohort study. J Antimicrob Chemother. 2023;78(11):2691-5.

47. Beumier M, Casu GS, Hites M, Wolff F, Cotton F, Vincent JL, et al. Elevated β-lactam concentrations associated with neurological deterioration in ICU septic patients. Minerva Anestesiol. 2015;81(5):497-506.
48. Grill MF, Maganti R. Cephalosporin-induced neurotoxicity: clinical manifestations, potential pathogenic mechanisms, and the role of electroencephalographic monitoring. Ann Pharmacother. 2008;42(12):1843-50.
49. Doerr BI, Glomot R, Kief H, Kramer M, Sakaguchi T. Toxicology of cefotaxime in comparison to other cephalosporins. J Antimicrob Chemother. 1980;6 Suppl A:79-82.
50. Doerr BI, Glomot R, Kief H, Kramer M, Sakaguchi T. Cefotaxime toxicity studies: a review of preclinical studies and some clinical reports. Rev Infect Dis. 1982;4 Suppl:S354-9.
51. Grill MF, Maganti RK. Neurotoxic effects associated with antibiotic use: management considerations. Br J Clin Pharmacol. 2011;72(3):381-93.
52. Deshayes S, Coquerel A, Verdon R. Neurological Adverse Effects Attributable to β-Lactam Antibiotics: A Literature Review. Drug Saf. 2017;40(12):1171-98.
53. De Sarro A, De Sarro GB, Ascioti C, Nisticó G. Epileptogenic activity of some beta-lactam derivatives: structure-activity relationship. Neuropharmacology. 1989;28(4):359-65.
54. Pascual J, Liaño F, Ortuño J. Cefotaxime-induced encephalopathy in an uremic patient. Nephron. 1990;54(1):92.
55. Chow KM, Hui AC, Szeto CC. Neurotoxicity induced by beta-lactam antibiotics: from bench to bedside. Eur J Clin Microbiol Infect Dis. 2005;24(10):649-53.
56. Lagacé-Wiens P, Rubinstein E. Adverse reactions to β-lactam antimicrobials. Expert Opin Drug Saf. 2012;11(3):381-99.
57. Olaison L, Alestig K. A prospective study of neutropenia induced by high doses of beta-lactam antibiotics. J Antimicrob Chemother. 1990;25(3):449-53.
58. Jansson AK, Enblad P, Sjölin J. Efficacy and safety of cefotaxime in combination with metronidazole for empirical treatment of brain abscess in clinical practice: a retrospective study of 66 consecutive cases. Eur J Clin Microbiol Infect Dis. 2004;23(1):7-14.
59. Edson RS, Terrell CL. The aminoglycosides. Mayo Clin Proc. 1999;74(5):519-28.
60. Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: An Overview. Cold Spring Harb Perspect Med. 2016;6(6).
61. Chen C, Chen Y, Wu P, Chen B. Update on new medicinal applications of gentamicin: evidence-based review. J Formos Med Assoc. 2014;113(2):72-82.
62. EUCAST (European Committee on Antimicrobial Susceptibility Testing). EUCAST clinical breakpoint table, version 14.0, year 2024 Available from: https://www.eucast.org/clinical_breakpoints.
63. Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW, Osterlund A, et al. European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J Antimicrob Chemother. 2003;52(2):145-8.
64. Giske CG, Turnidge J, Cantón R, Kahlmeter G. Update from the European Committee on Antimicrobial Susceptibility Testing (EUCAST). J Clin Microbiol. 2022;60(3):e0027621.

65. EUCAST (European Committee on Antimicrobial Susceptibility Testing). Setting breakpoints for new antimicrobial agents, EUCAST SOP 1.4, 2021. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/EUCAST_SOPs/2021/EUCAST_SOP_1.4_Setting_breakpoints_new_agents_20211202.pdf.
66. Mouton JW, Brown DF, Apfalter P, Cantón R, Giske CG, Ivanova M, et al. The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach. Clin Microbiol Infect. 2012;18(3):E37-45.
67. Kahlmeter G. The 2014 Garrod Lecture: EUCAST - are we heading towards international agreement? J Antimicrob Chemother. 2015;70(9):2427-39.
68. EUCAST (European Committee on Antimicrobial Susceptibility Testing). Calibration of zone diameter breakpoints against MIC values. 2024. Available from: https://www.eucast.org/ast_of_bacteria/calibration_and_validation.
69. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin Infect Dis. 2009;49(11):1749-55.
70. Kowalska-Krochmal B, Dudek-Wicher R. The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. Pathogens. 2021;10(2).
71. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: general principles and contemporary practices. Clin Infect Dis. 1998;26(4):973-80.
72. Matuschek E, Brown DF, Kahlmeter G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clin Microbiol Infect. 2014;20(4):O255-66.
73. Humphries RM, Kircher S, Ferrell A, Krause KM, Malherbe R, Hsiung A, et al. The Continued Value of Disk Diffusion for Assessing Antimicrobial Susceptibility in Clinical Laboratories: Report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. J Clin Microbiol. 2018;56(8).
74. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis. 1998;26(1):1-10; quiz 1-2.
75. Mouton JW, Dudley MN, Cars O, Derendorf H, Drusano GL. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. J Antimicrob Chemother. 2005;55(5):601-7.
76. Rodríguez-Gascón A, Solinís M, Isla A. The Role of PK/PD Analysis in the Development and Evaluation of Antimicrobials. Pharmaceutics. 2021;13(6).
77. Goessens WH, Mouton JW, ten Kate MT, Bijl AJ, Ott A, Bakker-Woudenberg IA. Role of ceftazidime dose regimen on the selection of resistant Enterobacter cloacae in the intestinal flora of rats treated for an experimental pulmonary infection. J Antimicrob Chemother. 2007;59(3):507-16.
78. Tam VH, McKinnon PS, Akins RL, Rybak MJ, Drusano GL. Pharmacodynamics of cefepime in patients with Gram-negative infections. J Antimicrob Chemother. 2002;50(3):425-8.
79. EUCAST (European Committee on Antimicrobial Susceptibility Testing). MIC distributions. Antimicrobial wild type distributions. Available from: https://mic.eucast.org/search/.
80. Abdul-Aziz MH. IDSAP PK/PD in special populations. 2020. p. 7-30.
81. Gonçalves-Pereira J, Póvoa P. Antibiotics in critically ill patients: a systematic review of the pharmacokinetics of β-lactams. Crit Care. 2011;15(5):R206.

82. Ocampos-Martinez E, Penaccini L, Scolletta S, Abdelhadii A, Devigili A, Cianferoni S, et al. Determinants of early inadequate vancomycin concentrations during continuous infusion in septic patients. Int J Antimicrob Agents. 2012;39(4):332-7.
83. Conil JM, Georges B, Fourcade O, Seguin T, Lavit M, Samii K, et al. Assessment of renal function in clinical practice at the bedside of burn patients. Br J Clin Pharmacol. 2007;63(5):583-94.
84. Adnan S, Li JX, Wallis SC, Rudd M, Jarrett P, Paterson DL, et al. Pharmacokinetics of meropenem and piperacillin in critically ill patients with indwelling surgical drains. Int J Antimicrob Agents. 2013;42(1):90-3.
85. Ronchera-Oms CL, Tormo C, Ordovás JP, Abad J, Jiménez NV. Expanded gentamicin volume of distribution in critically ill adult patients receiving total parenteral nutrition. J Clin Pharm Ther. 1995;20(5):253-8.
86. Ulldemolins M, Roberts JA, Rello J, Paterson DL, Lipman J. The effects of hypoalbuminaemia on optimizing antibacterial dosing in critically ill patients. Clin Pharmacokinet. 2011;50(2):99-110.
87. Roberts JA, Pea F, Lipman J. The clinical relevance of plasma protein binding changes. Clin Pharmacokinet. 2013;52(1):1-8.
88. Cook AM, Hatton-Kolpek J. Augmented Renal Clearance. Pharmacotherapy. 2019;39(3):346-54.
89. Hefny F, Sambhi S, Morris C, Kung JY, Stuart A, Mahmoud SH. Drug Dosing in Critically Ill Adult Patients with Augmented Renal Clearance. Eur J Drug Metab Pharmacokinet. 2022;47(5):607-20.
90. Egea A, Dupuis C, de Montmollin E, Wicky PH, Patrier J, Jaquet P, et al. Augmented renal clearance in the ICU: estimation, incidence, risk factors and consequences-a retrospective observational study. Ann Intensive Care. 2022;12(1):88.
91. Mikami R, Hayakawa M, Imai S, Sugawara M, Takekuma Y. Onset timing and duration of augmented renal clearance in a mixed intensive care unit. J Intensive Care. 2023;11(1):13.
92. Hefny F, Stuart A, Kung JY, Mahmoud SH. Prevalence and Risk Factors of Augmented Renal Clearance: A Systematic Review and Meta-Analysis. Pharmaceutics. 2022;14(2).
93. Huttner A, Von Dach E, Renzoni A, Huttner BD, Affaticati M, Pagani L, et al. Augmented renal clearance, low β-lactam concentrations and clinical outcomes in the critically ill: an observational prospective cohort study. Int J Antimicrob Agents. 2015;45(4):385-92.
94. Carrie C, Bentejac M, Cottenceau V, Masson F, Petit L, Cochard JF, et al. Association between augmented renal clearance and clinical failure of antibiotic treatment in brain-injured patients with ventilator-acquired pneumonia: A preliminary study. Anaesth Crit Care Pain Med. 2018;37(1):35-41.
95. Udy AA, Varghese JM, Altukroni M, Briscoe S, McWhinney BC, Ungerer JP, et al. Subtherapeutic initial β-lactam concentrations in select critically ill patients: association between augmented renal clearance and low trough drug concentrations. Chest. 2012;142(1):30-9.
96. Udy AA, Roberts JA, Boots RJ, Paterson DL, Lipman J. Augmented renal clearance: implications for antibacterial dosing in the critically ill. Clin Pharmacokinet. 2010;49(1):1-16.

97. Bagshaw SM, Lapinsky S, Dial S, Arabi Y, Dodek P, Wood G, et al. Acute kidney injury in septic shock: clinical outcomes and impact of duration of hypotension prior to initiation of antimicrobial therapy. Intensive Care Med. 2009;35(5):871-81.
98. Bouchard J, Acharya A, Cerda J, Maccariello ER, Madarasu RC, Tolwani AJ, et al. A Prospective International Multicenter Study of AKI in the Intensive Care Unit. Clin J Am Soc Nephrol. 2015;10(8):1324-31.
99. Zarbock A, Koyner JL, Gomez H, Pickkers P, Forni L. Sepsis-associated acute kidney injury-treatment standard. Nephrol Dial Transplant. 2023;39(1):26-35.
100. Hughes S, Heard KL, Mughal N, Moore LSP. Optimization of antimicrobial dosing in patients with acute kidney injury: a single-centre observational study. JAC Antimicrob Resist. 2022;4(4):dlac080.
101. Blot S, Lipman J, Roberts DM, Roberts JA. The influence of acute kidney injury on antimicrobial dosing in critically ill patients: are dose reductions always necessary? Diagn Microbiol Infect Dis. 2014;79(1):77-84.
102. Hites M, Dell'Anna AM, Scolletta S, Taccone FS. The challenges of multiple organ dysfunction syndrome and extra-corporeal circuits for drug delivery in critically ill patients. Adv Drug Deliv Rev. 2014;77:12-21.
103. Veiga RP, Paiva JA. Pharmacokinetics-pharmacodynamics issues relevant for the clinical use of beta-lactam antibiotics in critically ill patients. Crit Care. 2018;22(1):233.
104. Porrini E, Ruggenenti P, Luis-Lima S, Carrara F, Jiménez A, de Vries APJ, et al. Estimated GFR: time for a critical appraisal. Nat Rev Nephrol. 2019;15(3):177-90.
105. Soveri I, Berg UB, Björk J, Elinder CG, Grubb A, Mejare I, et al. Measuring GFR: a systematic review. Am J Kidney Dis. 2014;64(3):411-24.
106. Levey AS, Coresh J, Tighiouart H, Greene T, Inker LA. Measured and estimated glomerular filtration rate: current status and future directions. Nat Rev Nephrol. 2020;16(1):51-64.
107. Peralta CA, Shlipak MG, Judd S, Cushman M, McClellan W, Zakai NA, et al. Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. Jama. 2011;305(15):1545-52.
108. Sitbon A, Darmon M, Geri G, Jaubert P, Lamouche-Wilquin P, Monet C, et al. Accuracy of clinicians' ability to predict the need for renal replacement therapy: a prospective multicenter study. Ann Intensive Care. 2022;12(1):95.
109. Helmersson-Karlqvist J, Lipcsey M, Ärnlöv J, Bell M, Ravn B, Dardashti A, et al. Addition of cystatin C predicts cardiovascular death better than creatinine in intensive care. Heart. 2022;108(4):279-84.
110. Shlipak MG, Matsushita K, Ärnlöv J, Inker LA, Katz R, Polkinghorne KR, et al. Cystatin C versus creatinine in determining risk based on kidney function. N Engl J Med. 2013;369(10):932-43.
111. Ni L, Lü J, Hou LB, Yan JT, Fan Q, Hui R, et al. Cystatin C, associated with hemorrhagic and ischemic stroke, is a strong predictor of the risk of cardiovascular events and death in Chinese. Stroke. 2007;38(12):3287-8.
112. Sunder S, Jayaraman R, Mahapatra HS, Sathi S, Ramanan V, Kanchi P, et al. Estimation of renal function in the intensive care unit: the covert concepts brought to light. J Intensive Care. 2014;2(1):31.
113. Adingwupu OM, Barbosa ER, Palevsky PM, Vassalotti JA, Levey AS, Inker LA. Cystatin C as a GFR Estimation Marker in Acute and Chronic Illness: A Systematic Review. Kidney Med. 2023;5(12):100727.

114. Roy R, MacDonald J, Dark P, Kalra PA, Green D. The estimation of glomerular filtration in acute and critical illness: Challenges and opportunities. Clin Biochem. 2023;118:110608.
115. Molitoris BA. Measuring glomerular filtration rate in the intensive care unit: no substitutes please. Crit Care. 2013;17(5):181.
116. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem. 1992;38(10):1933-53.
117. Levey AS, Perrone RD, Madias NE. Serum creatinine and renal function. Annu Rev Med. 1988;39:465-90.
118. Inker LA, Titan S. Measurement and Estimation of GFR for Use in Clinical Practice: Core Curriculum 2021. Am J Kidney Dis. 2021;78(5):736-49.
119. Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, et al. Factors other than glomerular filtration rate affect serum cystatin C levels. Kidney Int. 2009;75(6):652-60.
120. McMahon GM, Waikar SS. Biomarkers in nephrology: Core Curriculum 2013. Am J Kidney Dis. 2013;62(1):165-78.
121. Foster MC, Levey AS, Inker LA, Shafi T, Fan L, Gudnason V, et al. Non-GFR Determinants of Low-Molecular-Weight Serum Protein Filtration Markers in the Elderly: AGES-Kidney and MESA-Kidney. Am J Kidney Dis. 2017;70(3):406-14.
122. Abdul-Aziz MH, Portunato F, Roberts JA. Prolonged infusion of beta-lactam antibiotics for Gram-negative infections: rationale and evidence base. Curr Opin Infect Dis. 2020;33(6):501-10.
123. Dulhunty JM, Brett SJ, De Waele JJ, Rajbhandari D, Billot L, Cotta MO, et al. Continuous vs Intermittent β-Lactam Antibiotic Infusions in Critically Ill Patients With Sepsis: The BLING III Randomized Clinical Trial. Jama. 2024;332(8):629-37.
124. Abdul-Aziz MH, Hammond NE, Brett SJ, Cotta MO, De Waele JJ, Devaux A, et al. Prolonged vs Intermittent Infusions of beta-Lactam Antibiotics in Adults With Sepsis or Septic Shock: A Systematic Review and Meta-Analysis. JAMA. 2024;332(8):638-48.
125. Zhao Y, Zang B, Wang Q. Prolonged versus intermittent β-lactam infusion in sepsis: a systematic review and meta-analysis of randomized controlled trials. Ann Intensive Care. 2024;14(1):30.
126. Pai Mangalore R, Peel TN, Udy AA, Peleg AY. The clinical application of beta-lactam antibiotic therapeutic drug monitoring in the critical care setting. J Antimicrob Chemother. 2023;78(10):2395-405.
127. Dhaese S, Van Vooren S, Boelens J, De Waele J. Therapeutic drug monitoring of β-lactam antibiotics in the ICU. Expert Rev Anti Infect Ther. 2020;18(11):1155-64.
128. Björk J, Grubb A, Sterner G, Nyman U. Revised equations for estimating glomerular filtration rate based on the Lund-Malmö Study cohort. Scand J Clin Lab Invest. 2011;71(3):232-9.
129. Grubb A, Horio M, Hansson LO, Björk J, Nyman U, Flodin M, et al. Generation of a new cystatin C-based estimating equation for glomerular filtration rate by use of 7 assays standardized to the international calibrator. Clin Chem. 2014;60(7):974-86.
130. Hodiamont CJ, Janssen JM, de Jong MD, Mathôt RA, Juffermans NP, van Hest RM. Therapeutic Drug Monitoring of Gentamicin Peak Concentrations in Critically Ill Patients. Ther Drug Monit. 2017;39(5):522-30.

131. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. 1916. Nutrition. 1989;5(5):303-11; discussion 12-3.
132. Wong G, Briscoe S, Adnan S, McWhinney B, Ungerer J, Lipman J, et al. Protein binding of β-lactam antibiotics in critically ill patients: can we successfully predict unbound concentrations? Antimicrob Agents Chemother. 2013;57(12):6165-70.
133. Referensgruppen för Antibiotikafrågor (RAF), Doseringstabell vuxna 2024 [Available from: https://www.sls.se/raf/kunskapsunderlag/doseringtabell/.
134. Holmbom M, Giske CG, Fredrikson M, Östholm Balkhed Å, Claesson C, Nilsson LE, et al. 14-Year Survey in a Swedish County Reveals a Pronounced Increase in Bloodstream Infections (BSI). Comorbidity - An Independent Risk Factor for Both BSI and Mortality. PLoS One. 2016;11(11):e0166527.
135. Mellhammar L, Kahn F, Whitlow C, Kander T, Christensson B, Linder A. Bacteremic sepsis leads to higher mortality when adjusting for confounders with propensity score matching. Sci Rep. 2021;11(1):6972.
136. Minichmayr IK, Wicha SG, Matzneller P, Kloft C, Zeitlinger M. Impact of Key Components of Intensified Ceftaroline Dosing on Pharmacokinetic/Pharmacodynamic Target Attainment. Clin Pharmacokinet. 2024;63(1):121-31.
137. van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB. Predictors of mortality in Staphylococcus aureus Bacteremia. Clin Microbiol Rev. 2012;25(2):362-86.
138. Liesenborghs L, Verhamme P, Vanassche T. Staphylococcus aureus, master manipulator of the human hemostatic system. J Thromb Haemost. 2018;16(3):441-54.
139. Karauzum H, Datta SK. Adaptive Immunity Against Staphylococcus aureus. Curr Top Microbiol Immunol. 2017;409:419-39.
140. Hayashi Y, Roberts JA, Paterson DL, Lipman J. Pharmacokinetic evaluation of piperacillin-tazobactam. Expert Opin Drug Metab Toxicol. 2010;6(8):1017-31.

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