Patient tailored antibiotic therapy in critically ill patients

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Summary

Infection is a common problem for critically ill patients. About half of the intensive care patients are considered to have an infection. Despite the advances made in modern medicine, the mortality and morbidity due to infection in critically ill remains unacceptably high.

Timely initiation of antibiotic therapy with an appropriate spectrum for the likely pathogen after source control has been shown to have a significant impact on outcome, and have therefore been widely promoted. However, almost no information is available about the effect of appropriate dosing.

The antibiotic dosing regimen, administered to the infected critically ill patient, is determined by pharmacokinetic studies in healthy volunteers with normal physiology. Research has shown that the key pharmacokinetic determinants are markedly different in critically ill patients. Moreover, infections in critically ill patients are often caused by microorganisms with decreased susceptibility compared to other clinical settings, which further renders optimal dosing more difficult. The overall result is that a standard dose of antibiotic leads to very variable concentrations in critically ill patients and that a significant proportion of patients may not reach optimal concentrations associated with maximal effect.

Considering this, individually tailored antibiotic therapy may be a useful strategy to maximize antibiotic efficacy while minimizing toxicity. The aim of this work was to investigate the pharmacokinetic variability of β-lactam antibiotics in critically ill patients and investigate methods to improve dosing in this patient population.

As first part of this thesis, a fast and accurate chromatographic method was developed for quantification of the most commonly used β-lactam antibiotics in our hospital, and we also explored the pre-analytical stability of these compounds. Multiple pharmacokinetic studies were undertaken and we demonstrated high pharmacokinetic variability both between patients, as well as within the same patient over time. We also demonstrated that a high creatinine clearance is a risk factor for subtherapeutic drug concentrations after standard dosing, even when extended infusions are used. For cefepime during continuous renal replacement therapy, we investigated the influence of dialysis settings on cefepime

concentrations. In a simulation study, we found that the probability to achieve therapeutic exposure was lower for narrower spectrum antibiotics using conventional dosing compared to the broad-spectrum antibiotics for a selection of microorganisms for which de-escalation can be undertaken. A more practical part of this research consisted of stability experiments of meropenem, amoxicillin and amoxicillin/clavulanic acid for their use as a continuous infusion. Last but not least, a randomized controlled trial was undertaken investigating the potential of dose adaptations based on daily therapeutic drug monitoring of meropenem and piperacillin/tazobactam in critically ill patients with normal kidney function.

As an overall conclusion, this project has contributed to the knowledge on the altered pharmacokinetics in critically ill patients, and has investigated some strategies to improve dosing, however there are still many questions that need to be answered before we can truly move to patient-tailored antibiotic therapy, such as the relationship between plasma concentrations and concentrations in the infected tissue, as well as the relation between antibiotic concentrations and outcome.

Samenvatting

Infecties zijn een ernstig probleem bij kritiek zieke patiënten. Ongeveer de helft van de patiënten op intensieve zorgen hebben af te rekenen met een infectie. Ondanks de vooruitgang in de moderne geneeskunde blijft de mortaliteit en morbiditeit te wijten aan infecties in deze patiënten zeer hoog.

Het tijdig toedienen van antibiotica met een geschikt spectrum vormt hierbij de hoeksteen van de behandeling. Het belang hiervan is aangetoond in verschillende studies. Er is echter heel weinig evidentie omtrent het belang van geschikte dosering, en het bereiken van adequate concentraties.

Welke dosis van het antibioticum moet toegediend worden aan de kritiek zieke patiënt met een infectie is bepaald aan de hand van farmacokinetische studies uitgevoerd in gezonde vrijwilligers. Onderzoek heeft echter aangetoond dat de belangrijkste farmacokinetische determinanten in intensieve zorg patiënten sterk verschillend zijn ten opzichte van gezonde vrijwilligers. Bovendien zijn infecties in deze patiënten vaak veroorzaakt door minder gevoelige microorganismen, in vergelijking met andere klinische settings, wat de antibioticadosering nog meer bemoeilijkt. Hierdoor leidt één standaarddosering van antibiotica tot erg variabele concentraties in kritiek zieke patiënten, waarbij een significant deel van de patiënten suboptimale concentraties bereikt.

Mogelijks kan individueel aangepaste antibioticatherapie een goede strategie zijn om dosering te optimaliseren met maximale efficaciteit en minimale toxiciteit. Het doel van dit werk was om de farmacokinetische variabiliteit van β-lactam antibiotica in kritiek zieke patiënten beter in kaart te brengen, alsook om methoden te onderzoeken die kunnen leiden tot betere dosering in deze patiëntengroep.

Als eerste onderdeel van deze thesis werd een snelle en accurate chromatografische methode ontwikkeld en gevalideerd voor de kwantificatie van de meest gebruikte β-lactam antibiotica in dit ziekenhuis, en ook de pre-analytische stabiliteit werd onderzocht. Meerdere farmacokinetische studies werden uitgevoerd, waarbij we een grote farmacokinetische variabiliteit aantoonden, zowel tussen verschillende patiënten, als binnen eenzelfde patiënt. We toonden ook aan dat een hoge creatinineklaring een risicofactor is

voor subtherapeutische concentraties na standaarddosering, zelfs wanneer de antibiotica toegediend werden over een langere tijdsperiode (extended infusie). Voor cefepime tijdens continue niervervangende therapie onderzochten we de invloed van dialysesettings op cefepimeconcentraties. In een simulatiestudie toonden we aan dat de kans om therapeutische concentraties te bereiken voor smalspectrum antibiotica in conventionele dosering lager is dan voor breedspectrum antibiotica in conventionele dosering, voor een aantal typische pathogenen waarvoor de-escalatie kan ondernomen worden.

Een meer praktisch deel van dit onderzoek behandelde de stabiliteit van meropenem, amoxicilline en amoxicilline/clavulaanzuur voor hun gebruik als continu infuus. Als laatste rapporteren we de resultaten van een gerandomiseerde gecontroleerde studie over het effect van dosisadaptaties gebaseerd op dagelijkse therapeutische drug monitoring van meropenem en piperacilline in kritiek zieke patiënten met normale nierfunctie.

Om te concluderen kunnen we stellen dat dit project bijgedragen heeft tot de kennis over de veranderde farmacokinetiek van hydrofiele antibiotica in kritiek zieke patiënten. We hebben ook strategieën onderzocht die dosering kunnen verbeteren. Vooraleer we echter volledig kunnen overgaan tot antibiotica therapie op maat van de patiënt moeten een aantal cruciale vragen beantwoord worden, zoals onder meer de relatie tussen plasmaconcentraties en concentraties ter hoogte van het geïnfecteerde weefsel, alsook de relatie tussen concentraties en de klinische uitkomst van de antibioticatherapie.

Chapter One : Introduction

1. Infections in the ICU

1.1. Epidemiology

Infection is an extremely important problem in critical care medicine. In a recent point prevalence study, more than half of the patients were assumed to have an infection [1]. The mortality rate of these infected patients was found to be more than two times higher than the mortality rate of the non-infected patients, with infection being an independent predictor of mortality [1]. It is estimated that annually 135 000 patients are dying from sepsis in the European Union [1]. It is the leading cause of mortality in non-coronary intensive care unit (ICU) patients with up to 30% of patients dying within one month of diagnosis [1, 2] and mortality rates exceeding 50% for septic shock [3]. Despite the advances in modern medicine and intensive care, the incidence of sepsis is still increasing [4, 5].

Various factors may contribute to this increased risk of infection and the associated poor outcomes. First of all, compared to the general hospital population, ICU patients have a more frail physical condition. They have more comorbidities and more severe physiologic derangements and are therefore less fit to fight an infection [6]. Secondly, the large use of indwelling catheters among critically ill patients serves as a port of entry of microorganisms into the body. Last but not least, multi-drug resistant microorganisms such as methicillin-resistant *Staphylococcus aureus* and multi drug resistant gram negatives such as extended spectrum beta lactamases producing Enterobacteriaceae, *Acinetobacter baumannii* and carbapenem-resistant *Pseudomonas aeruginosa* are more frequently isolated in the ICU compared to the general wards [7, 8]. The emerging resistance to broad spectrum antibiotics among gram-negative microorganisms is particularly worrisome since treatment options are very limited, and sometimes no effective antimicrobial agent is available at all [9].

The most common nosocomial infections in critically ill patients are device related and therefore the most common sites of infection are the lungs, urinary tract and blood stream [7].

1.2. Treatment principles

Sepsis is a major cause of mortality and morbidity. In order to maximize outcome, sepsis must be timely recognized and treated.

Adequate antibiotic therapy after source control is the cornerstone of treatment. The mantra for antibiotics in severe sepsis is 'hit hard, early and appropriately', so when optimizing antibiotic therapy after source control, one should take three issues into account: spectrum, timing and dosing [10].

1.2.1. Source control

Source control is critical for therapeutic success as antimicrobial therapy and other interventions may fail if the source of infection is not properly controlled. It is defined as those measures that can be used to control the focus of an infection [11]. The principles of source control are [11]:

1) Drainage of abscesses containing infected fluids
2) Removal of contaminated devices or foreign bodies which serve as a reservoir of microorganisms (for example, a colonized intravascular catheter or infected prosthetic heart valve) whenever feasible
3) Debridement of infected or necrotic tissue
4) Correction of anatomic derangements which result in ongoing microbial contamination (for example perforation of the colon)

1.2.2. Spectrum

It is of utmost importance that the spectrum of the chosen antibiotic covers the causative pathogen ("adequate" antibiotic therapy). The importance of initial antimicrobial choice on mortality in patients with sepsis has been repeatedly shown by separate investigations [12-15]. Harbarth and colleagues found the 28-day mortality to be 24% for patients in the adequately treated group versus 39% (82/211) for patients receiving inappropriate initial antimicrobial therapy in a large cohort of patients with microbiologically confirmed severe sepsis of multiple origin [14]. It was found to be an independent predictor of mortality with an adjusted odds ratio of 1.8. This was also confirmed by Ibrahim et al who assessed the influence of inadequate antimicrobial treatment on outcome in critically ill

patients with blood stream infections. They found the hospital mortality to be twice as high in patients who received inadequate antimicrobial treatment compared to those who received adequate therapy [13]. Multivariate analysis of these data has identified inadequate treatment as the most important risk factor of hospital mortality, with an adjusted odds ratio of 6.9 (95 % confidence interval 5.1-9.2). This has also been confirmed in peritonitis, where inadequate initial antibiotic therapy was an independent predictor of mortality with an adjusted odds ratio of 1.6. These findings have led to the widespread use of broad-spectrum antibiotic therapy for the empirical treatment of infections, when the causative microorganism is still unknown. The situation is less clear when studying ventilator-associated pneumonia where some researchers have found an excess mortality caused by inappropriate initial therapy estimated to be 21.4%, while others have found an attributable mortality as low as 1 % [12, 16]. Although it seems quite clear from studies that the initial spectrum has a significant effect on mortality, it is apparently dependent on many variables such as the type of infection, and the underlying health status of the patient. Moreover, these studies may overestimate the effect of adequate antibiotic therapy, as it is not achievable in all cases, since empiric antibiotic therapy is chosen based on local epidemiology, but in rare cases the patient may be infected with a resistant pathogen.

The unadjusted mortality rates for the patient groups receiving appropriate and inappropriate initial antibiotic therapy are graphically shown in figure 1.
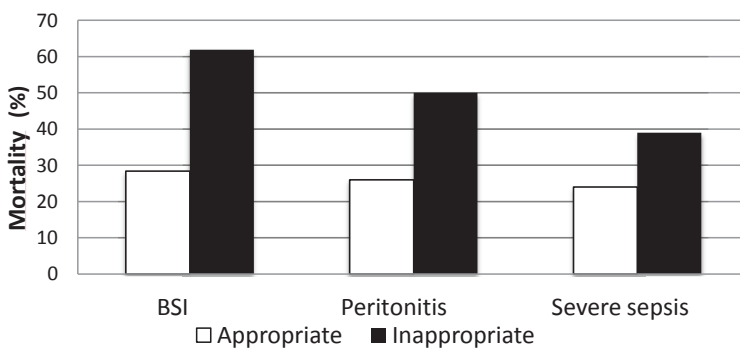
Fig. 1 Influence of adequate vs. inadequate antibiotic spectrum on mortality in different types of infections: blood stream infections (BSI)[13], peritonitis [15] and severe sepsis of mixed origin [14] (figure from J. De Waele, copied with permission)

1.2.3. Timing

Sepsis is a serious medical condition with a high mortality rate if left untreated. However, a major challenge with complicated infections is early recognition [17]. As such, severe sepsis is often not immediately recognized upon admission to the emergency department. As a consequence, these patients may develop shock and multi-organ failure. These patients are then admitted to the ICU in a moribund state, and may be beyond salvage [18]. Many factors contribute to this, such as age, general health status, how long it took to reach the emergency department/intensive care unit and how long the infection has already been present [19].

Based on anecdotal evidence, similar to inadequate spectrum, delay in the initiation of appropriate antibiotic therapy is also considered as an important risk factor for mortality.

Kumar and colleagues retrospectively analyzed the impact of antibiotic timing on hospital mortality in septic shock patients and found a strong correlation between delay in effective therapy and mortality. They found a decrease in survival by 7.6% for each hourly delay over the next 6 hours after the onset of recurrent or persistent hypotension [20]. Another study by Gaieski and colleagues in patients with severe sepsis also examined the effects of time from triage to antibiotic administration on mortality. They did not find a correlation between delay in antibiotic therapy and survival, but they did find that patients who received appropriate antibiotic therapy within 1 h after triage had a significantly better chance of survival compared to patients that were delayed (odds ratio = 0.3 p<0.03) [21]. Puskarich et al performed a retrospective analysis of data collected for a multicenter randomized controlled trial on early sepsis resuscitation in the emergency department and did not find an influence of delay of antibiotic therapy on mortality up to 6 h after triage or after the onset of shock. However, patients who were receiving antibiotics before the recognition of shock had a lower mortality compared to patients that were given antibiotics after recognition of shock (odds ratio = 2.35) [22]. Ferrer et al performed a retrospective analysis from the Surviving Sepsis Campaign database (17 990 patients with severe sepsis and septic shock) and found an significant increase in mortality associated with the hours of delay in antibiotic administration following recognition of severe sepsis (significant after 1 hour of delay) [23]. However, it is important to realize that the impact of the timing of

antibiotic therapy can never be separated from the severity of the disease before the antibiotic was administered, which is not taken into account by these retrospective studies.

Based on this available (suboptimal) evidence, timely administration and appropriateness of the spectrum of antibiotic therapy have been massively promoted in sepsis guidelines such as the Surviving Sepsis Campaign, which recommend to begin intravenous antibiotic as early as possible and always within the first hour of recognizing severe sepsis and septic shock, often called the "golden hour" [24]. Although these guidelines have been shown to reduce mortality from severe sepsis and septic shock [25], it must be mentioned that too early use of antibiotics can lead to excessive and unnecessary use of broad spectrum antibiotics, which may promote the emergence of resistance.

A Cochrane review looking at the evidence on timing of administration of antibiotics was published in 2012 [19]. The authors concluded that they were unable to make a recommendation on the early or late use of broad spectrum antibiotics in adult patients with severe sepsis in the ED pre-ICU admission [19]. To collect better evidence on the optimal timing of antibiotic delivery, randomized controlled trials would be needed. However, this may not be feasible, as it may be ethically wrong to randomize these patients to a seemingly inferior treatment arm. As performing randomized controlled trials would not be ethical, large and highly qualitative retrospective datasets incorporating many confounding factors may also improve our knowledge on this issue.

1.2.4. Dosing

Last but not least, when antibiotics are administered, the dose should be based on knowledge about the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of the drug, in order to maximize the effect and minimize concentration-related toxicity. A good example of the importance of knowledge on PK/PD is how dosing of aminoglycosides has changed over time. Traditionally, aminoglycosides were dosed twice or three times daily. However, when it became clear that aminoglycosides exert concentration-dependent killing and have an important post-antibiotic effect, it makes more sense to dose them once-daily, which gives rise to a higher peak concentration (more efficient killing) and lower trough concentrations (less nephrotoxicity). More than 30 randomized controlled trials have been performed comparing once versus multiple daily dosing, and all meta-analyses of these trials

favored once daily dosing [26-34]. Once daily dosing for aminoglycosides is now widely accepted as standard of care therapy.

2. β-lactam antibiotics

More than 70 years after their introduction, β-lactam antibiotics remain the mainstay of treatment for many bacterial infections. They are given alone or in combination with β-lactamase inhibitors. Advantages of these drugs are their broad spectrum of activity and their minimal intrinsic toxicity. Although toxicity occurring from these antibiotics is rare, it is associated with high concentrations [35].

2.1. Physicochemical properties

As revealed by the name, all β-lactam antibiotics share a common β-lactam group, which is a cyclic amide. Another common feature is the carboxylate or sulfonate.

β-lactam antibiotics are classified into different groups depending on the chemical structure: penicillins, cephalosporins, monobactams and carbapenems. Penicillins all share the common penam group, which is a fusion of the β-lactam and thiazolidine ring. Cephalosporins all contain the cephem group, consisting of the β-lactam and dihydrothiazine ring. Monobactams only have one ring. Carbapenems are different from penicillins because these antibiotics have an unsaturated bond and a carbon atom replacing sulphur at position 1 of the thiazolidine ring, which makes them highly resistant to most prevalent β-lactamases, which are enzymes that hydrolyze the β-lactam ring, and so deactivate the antibiotic [36].

A schematic overview of the four major β-lactam antibiotic classes is given in figure 2.
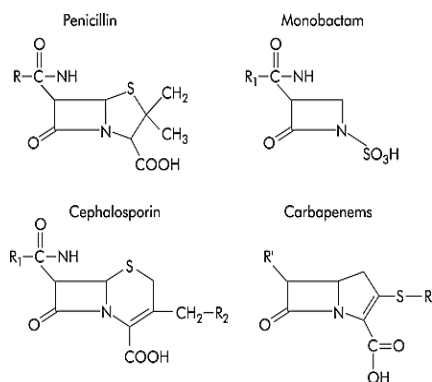
Fig. 2 Common core structures of four major β-lactam antibiotics. Penicillins, cephalosporins and carbapenems have a bicyclic core. Monobactams have a single central cyclic structure (copied from [37] with permission)

2.2. Mechanism of action

The main targets of the β-lactam antibiotics are the penicillin-binding proteins. These proteins play an important role in the peptidoglycan cell wall synthesis through cross-linking strands of peptidoglycan into a polymer surrounding the bacterial cell. β-lactam antibiotics resemble the natural enzyme substrate and form a stable bond between the antibiotic and the enzyme. The hydrolysis of this acylated protein occurs only very slowly, therefore the formation of this bond between antibiotic and penicillin-binding proteins results in enzyme inactivation and an impaired synthesis of the bacterial cell wall [38].

2.3. Pharmacodynamics

For antibiotics, pharmacodynamics (PD) describe the relationship between the antibiotic concentration and the ability to kill or inhibit the growth of bacterial pathogens. These exposure-response relationships have been studied primarily using *in vitro* experiments, as well as in animals. Most commonly used are the neutropenic thigh model and pneumonia model in mice. In brief, neutropenia is induced in these mice by administering cyclophosphamide, after which they are infected by injection of bacteria. Antibiotic treatment is usually started 2 hours after infection and serial plasma concentrations are obtained, which are used to calculate exposure. The total bacterial count from the infected tissue is determined after a fixed time interval. Different dosing regimens result in ranges of exposure, from which the relationship between exposure (time above the minimum inhibitory concentration (MIC), concentration/MIC, area under the curve/MIC) and response (number of colony forming units, death , emergence of resistance, …) can be derived.

Antibiotics can be classified according to their PD characteristics as concentration-dependent, time-dependent or both, as shown in figure 3.
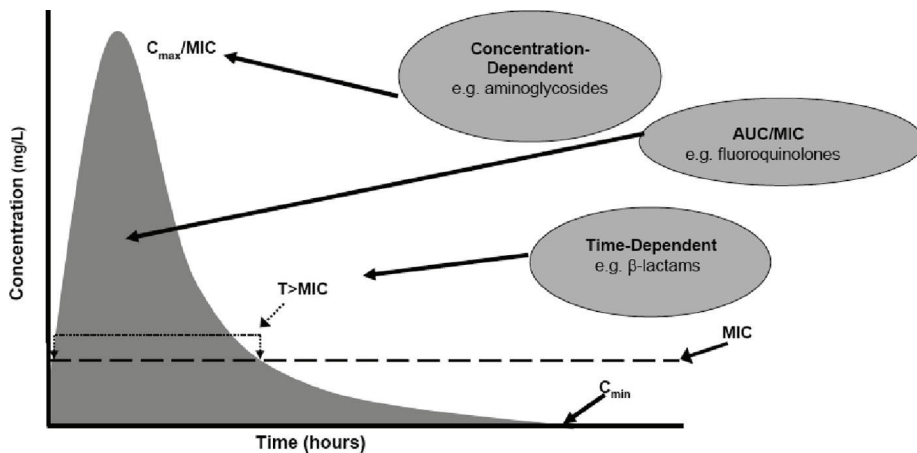
Fig. 3 Pharmacokinetic and pharmacodynamic parameters of antibiotics on a concentration vs. time curve. T>MIC—The time for which a drug's plasma concentration remains above the minimum inhibitory concentration (MIC) for a dosing period; Cmax/MIC, the ratio of the maximum plasma antibiotic concentration (Cmax) to MIC; AUC/MIC, the ratio of the area under the concentration time curve during a 24-hour time period (AUC0 −24) to MIC (copied from [39] with permission)

β-lactam antibiotics exhibit a time-dependent killing pattern, meaning that the percentage of the dosing interval for which the unbound concentration exceeds the minimal inhibitory concentration ($fT_{>MIC}$) of the microorganism is considered the best descriptor of efficacy [40]. *In vitro* and animal models have suggested that 30 to 70% $fT_{>MIC}$ (depending on the antibiotic) is necessary to treat infections [41]. However, a number of studies have suggested that higher targets may be needed to maximize the effect in humans. The available studies that have investigated toe relationship between achievement of PK/PD targets and outcome in patients with an infection are summarized in table 1.

Table 1 : Summary of studies investigating relationship between PK/PD targets and outcome in humans

Ref	Type of infection	Antibiotic	N	PK/PD target	Outcome	Odd's ratio (95 % confidence interval)	Limitations
[42]	Pneumonia	Meropenem	101	100% $fT_{>5\times MIC}$	Clinical success	3.6 (1.1–13.1)	• Calculated concentrations using covariate • Some MIC's determined using agar disk diffusion
[42]	Pneumonia	Meropenem	101	100% $fT_{>5\times MIC}$	Bacteriological eradication	4.4 (1.3-15.8)	• Calculated concentrations using covariates Some MIC's determined using agar disk diffusion
[43]	Sepsis with bacteremia, pneumonia or complicated UTI	Ceftazidime	76	100% $fT_{>MIC}$	Clinical success	2.5 (NA)	• Calculated concentrations using covariates method used to determine MIC not mentioned
[43]	Sepsis with bacteremia, pneumonia or complicated UTI	Ceftazidime	76	100% $fT_{>MIC}$	Bacteriological eradication	2.2 (NA)	• Calculated concentrations using covariates method used to determine MIC not mentioned
[44]	Gram negative pneumonia	Cefazidime	154	54% $fT_{>MIC}$	Bacterial eradication	9.22 (NA)	• Most patients had calculated concentrations using covariates • All patients were co-treated with linezolid

Ref	Type of infection	Antibiotic	N	PK/PD target	outcome	Odd's ratio (95 % confidence interval)	Limitations
[45]	Gram negative pneumonia	Ceftazidime and cefepime	73	53% $fT_{>MIC}$	Bacterial eradication	10.3 (1.1 -92.3)	• Calculated concentrations using covariates method used to determine MIC not mentioned
[46]	Gram negative pneumonia	Cefepime	33	100% $fT_{>2.1x\ MIC}$	Clinical success	9 (1.5 -50)	• Calculated concentrations using covariates MIC's determination using an automated analyser
[47]	Pneumonia, skin infection, bacteremia	Cefepime	56	60% $fT_{>MIC}$	Clinical success	8.1 (1.2-55.6)	• Calculated concentrations using covariates
[48]	Pneumonia	Ceftobiprole	159	51% $fT_{>MIC}$	Clinical success	2.7 (NA)	• Most patients had calculated concentrations using covariates
[49]	Mixed	8 Different β-lactams	248	50% $fT_{>MIC}$	Clinical succes	3.2 (2.1-11.1)	• Pathogens were only grown in 73% of patients, • MIC was only available in 34% of these patients

Ref : reference; NA : not available ; UTI : urinary tract infection, % $fT_{>MIC}$: the percentage of the dosing interval for which the concentration exceeds the minmum inhibitory concentration

3. Susceptibility of the microorganism

3.1. Importance

Only a few years after the mass production of penicillin in 1943, hospitals already experienced problems with resistant *Staphylococcus aureus*, which was thought to be uniformly susceptible. The importance of testing the bacterial culture for susceptibility, and treating the patient only with these antibiotics that were active in vitro, became increasingly recognized in later years [50].

In order to select the appropriate antibiotic for empirical therapy, it is important to have a good understanding of the trends in pathogen incidence and antimicrobial resistance, which can differ significantly between countries (northern versus southern Europe), between hospitals and even between departments (general hospital settings versus ICU).

Knowing the susceptibility is crucial, as the microorganism must be sensitive to the chosen antibiotics, both in the empirical phase, as well as after identification, in order to maximize the chances of patient survival. In order to choose the right antibiotic for the empirical phase, it is important to have an accurate idea about the local ecology and susceptibility of microorganisms. Surveillance studies provide important information on this subject and have shown that the incidence of antimicrobial-resistant pathogens is increasing [51, 52]. On a more local level, hospital antibiograms provide the percentage of samples for a given organism together with their antibiotic sensitivity. This is used to select the appropriate empirical therapy, to assist in determining if coverage for multidrug resistant organisms in the empiric therapy are necessary and to monitor resistance rates, such as the incidence of methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE), extended spectrum beta-lactamase (ESBL) producing bacteria and carbapenemase-producing Enterobacteriaceae (CPE) [53].

After identification of the causative organism, antibiotic therapy may be adapted to the susceptibility profile, preferably switching to narrower-spectrum antibiotics to decrease selective pressure and therefore to reduce the development of resistance, a process which is called de-escalation [54]. It is generally considered safe as most studies could not find a negative effect on outcome [55, 56], and some studies even showed improved outcomes [57, 58], although the reason for this is not quite clear. As these studies were all non-

interventional, it is possible that de-escalation was only performed in patients who were improving and therefore selection bias may be responsible for this effect.

De-escalation to the most appropriate single therapy as soon as the susceptibility profile is known is recommended in the 2013 Surviving Sepsis Campaign guideline, be it with poor qualities of evidence and strengths of recommendations [59]. Therefore, it is often incorporated in antibiotic stewardship programs in critically ill patients [60, 61], which has been defined as "the optimal selection, dosage, and duration of antimicrobial treatment that results in the best clinical outcome for the treatment or prevention of infection, with minimal toxicity to the patient and minimal impact on subsequent resistance"[62]. It is estimated that de-escalation is applied in 13-46 % of the patients (depending on the definition used and the context) [58, 63-66] although in clinical practice there seem to be a number of obstacles to use it widely [67].

The generally accepted principle that de-escalation is safe has been challenged by the results of a recent randomized controlled multicenter trial, which did not suffer from the above mentioned selection bias as all patients in whom de-escalation was possible were included in the study. Leone et al found that de-escalation to narrow spectrum antibiotics did not reduce patient ICU length of stay and was associated with an increased number of antibiotic days for patients who had been de-escalated. The authors also reported that superinfections were more frequent in patients who were de-escalated, with about half of the superinfections being caused by the same pathogens as the initial infection [68]. It is therefore questionable whether de-escalation is actually safe in terms of preserving outcome while reducing broad-spectrum antibiotic use [67].

3.2. Susceptibility testing

The minimum inhibitory concentration (MIC) is considered to be the gold standard for determination of the susceptibility of organisms to antimicrobials. It is defined as the lowest antibiotic concentration that inhibits visual bacterial growth.

The reference technique to determine the MIC values is by using broth microdilution (ISO 20776-1:2006) [69]. Basically, a standardized bacterial inoculum is applied to a standardized broth containing serial twofold dilutions of antibiotic. The cups are left to incubate at standardized conditions. The MIC is determined after overnight incubation as the antibiotic

concentration for which there is no visible growth. MIC determination can be performed manually, automatically or semi-automatically. It is generally accepted that the test result is reproducible within ± 1 well in a dilution series.

While broth dilution used to be associated with a high workload, impracticalities such as manual preparation of antibiotic solutions, possibilities of errors and the large amount of reagents and space required, this has now been miniaturized by using small microdilution trays which can be purchased commercially [70]. After incubation, MICs can be determined using automated devices for inspection of each of the panel wells for growth. The disadvantage of this technique is the inflexibility of antibiotics available in standard commercial panels [70]. A suitable alternative is the antimicrobial gradient method, where a strip containing the dried antibiotic in serial dilutions, is placed on the surface of a culture plate inoculated with the bacterial suspension. The antibiotic gradient on the strip diffuses from the strip to the matrix, creating the same gradient in the culture medium. After overnight incubation, a symmetrical inhibition ellipse centered along the strip is seen. The MIC value is determined as the value on the scale where the lower part of the ellipse intersects the test strip. This approach is shown to have a high concordance with broth dilution techniques and offers flexibility by being able to test only the drugs of choice. However it is quite expensive [71-74]. Automated systems to determine susceptibility (such as Vitek2® from Biomerieux, and BD Phoenix® from Becton Dickinson) are also available but may not be as accurate compared to the gold standard technique. Moreover they only test a very limited range of dilutions. Failure of these systems to detect resistance has been reported by several studies [75-78].

Another very commonly used method to determine the susceptibility is based on the zone distribution of the disk diffusion test. In this test, antibiotic disks impregnated with antibiotics are placed on a culture plate swabbed with bacteria, which is left to incubate at a defined temperature and environment [79]. If the antibiotic inhibits bacterial growth, there will be a zone of inhibition where bacterial growth is not visible. Based on the diameter, bacteria are classified as sensitive, intermediary resistant or resistant, which will be further discussed in section 3.3.

3.3.   Clinical breakpoints

There are two important factors that determine the antimicrobial efficacy of the drug, which are the in vitro susceptibility of the microorganism (the MIC), and the exposure of the drug to the bacterium in vivo (PK), as shown in figure 4.

```
┌──────────────┐   ┌──────────────┐      ┌──────────────┐
│Susceptibility of│   │ Exposure to the│ ◄─── │Dosing regimen │
│ the bug (MIC)│   │  drug (PK)   │      │              │
└──────────────┘   └──────────────┘      └──────────────┘
         │                  │
         ▼                  ▼
      ┌──────────────────────────┐
      │ Antimicrobial efficacy of the│
      │  drug (microbiological cure) │
      └──────────────────────────┘
                   │
                   ▼
            ┌──────────────┐
            │ Effect on host│
            │ (clinical cure)│
            └──────────────┘
```
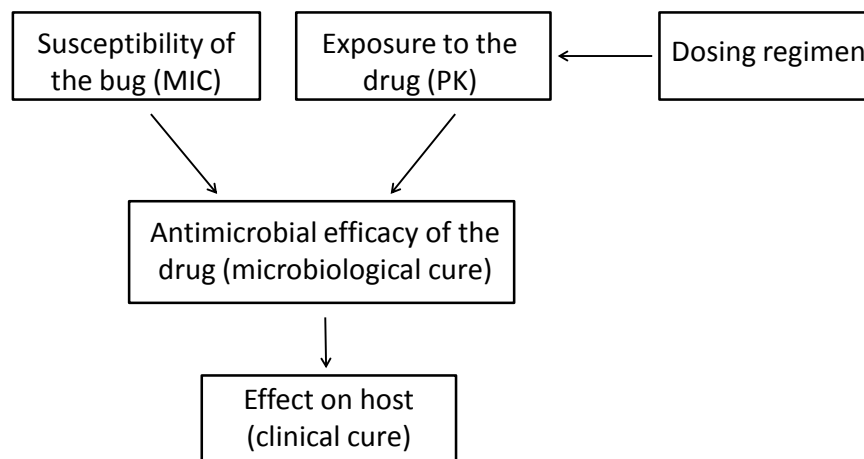
Fig. 4 Relationship between the susceptibility of a microorganism, exposure of an antimicrobial drug and antimicrobial drug effects (adapted from [80] with permission)

Based on the MIC (microbroth dilution or gradient diffusion test) or based on the diameter of the inhibition zone (in the case of disk diffusion), a microorganism is classified as sensitive (S), intermediary resistant (I) or resistant (R). A sensitive bacterial strain is defined as a strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic success. Intermediary resistant strains are defined as bacterial strains inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with uncertain therapeutic effect. Resistant bacterial strains are strains inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic failure [81].

This clinical breakpoint is derived by performing dosing simulations using the most common doses and taking into account the pharmacokinetic variability between patients. The clinical sensitive breakpoint is then defined as the MIC that will result in attainment of the PK/PD target for 99 % of the patients [82]. As an example, the results of the dosing simulations for meropenem which are used to determine the clinical breakpoint according to the European Society on Antimicrobial Susceptibility testing (EUCAST) for this antibiotic are shown in figure 5. Administering a dose of 1 g every 8 hours results in achievement of the

PK/PD target of 40 % $fT>_{MIC}$ for 99% of the patients if the MIC ≤ 2 mg/L. Therefore the MIC of 2 mg/L is the clinical breakpoint. Unfortunately, the key PK parameters used to perform these dosing simulations are based on PK from healthy volunteers, which is different from PK in critically ill patient, and does not take into account the vast variability seen in critically ill patients, which will be discussed in section 4.
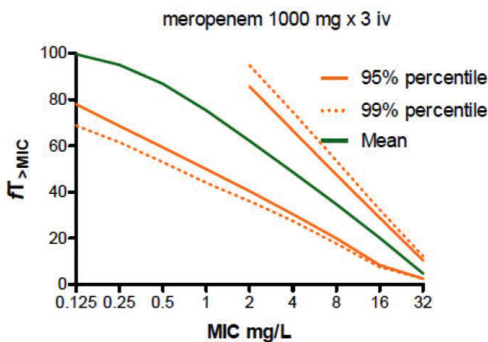
Fig. 5 Percentage time exceeding the minimum inhibitory concentration ($fT>_{MIC}$) for meropenem 1000 mg x 3 times daily. The following pharmacokinetic parameters were used : volume of distribution 20.8 L (coefficient of variation (CV) 13%), elimination half-life 1.04 h (CV 19%), fraction unbound 91%, infusion time 0.5 h (Rationale document EUCAST [83])

4. Pharmacokinetic alterations of hydrophilic antibiotics during critical illness

Pharmacokinetics (PK) is the study of the movement of drugs into, through and out of the body, and describes the concentration of the drug versus the time.

Antibiotic dosing regimens are usually determined in healthy adults with normal physiology. Moreover, clinical breakpoints are also determined using PK data from non-critically ill patients. However, patients in intensive care units are distinctly different from those in general wards and from healthy volunteers. A recent multinational pharmacokinetic point prevalence study in critically ill patients has shown that β-lactam antibiotic concentrations vary greatly between ill patients and that the plasma concentration halfway through the dosing interval did not exceed the MIC (assuming a worst case scenario) in 16 % of the patients, who were therefore considered underdosed [84]. Both volume of distribution (V_d) and clearance are the key PK determinants. Unfortunately, many pathophysiological changes occur in critical illness that may have a significant impact on

these PK determinants [39, 85, 86]. Figure 6 summarizes the possible PK alterations in critically ill patients.
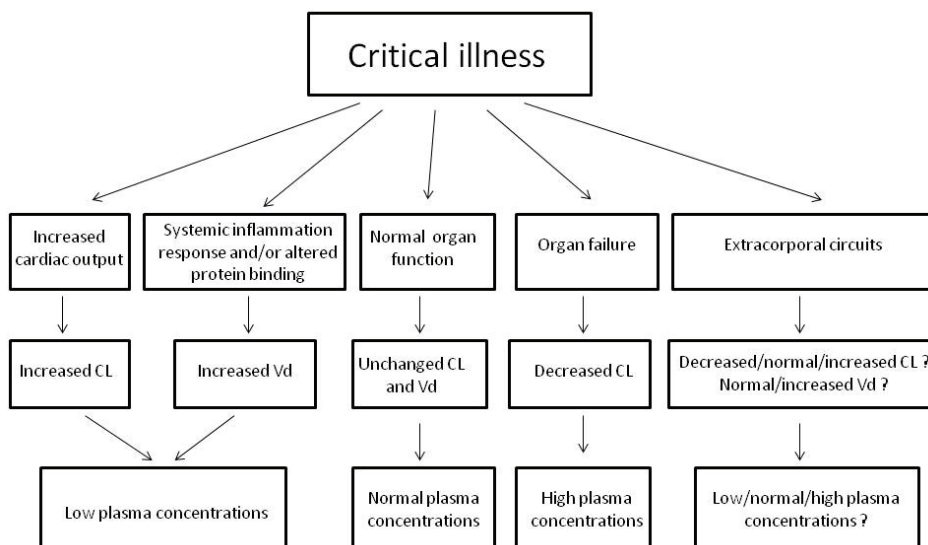
Fig. 6 Pharmacokinetic alterations in critically ill patients. CL : clearance, Vd : volume of distribution (adapted from [39] with permission)

4.1. Changes in volume of distribution

The (apparent) volume of distribution (V_d) is defined as the theoretical volume in which the drug would need to be distributed in order to produce the blood concentration. It is a constant factor that relates the plasma concentration (C_p) to the dose (equation 1) [87].

Dose = C_p x V_d (1)

β-lactam antibiotics are hydrophilic drugs and predominantly distribute into the intravascular and interstitial fluid. Therefore, these drugs have a small V_d, usually consistent with the volume of extracellular body water (approximately 0.1-0.6 L/kg), which results in high plasma concentrations [88].

Critically ill patients often have a larger V_d for hydrophilic drugs compared to healthy adults, mainly because of a systemic inflammatory response syndrome (SIRS) [39]. This response is part of the innate immune response, characterized by physiological and

laboratory alterations which are due to an infectious or a non-infectious cause, such as trauma, pancreatitis, burn injuries, hematological derangements and surgery [6]. Sepsis is defined as SIRS with an infectious cause. This inflammation response triggers a capillary leak and fluid extravasation into the interstitial space (also called third spacing) and results in hypotension. In order to maintain blood pressure, large amounts of fluids are often administered, which also distribute into the interstitial fluid, thereby substantially increasing the interstitial volume. This rise in interstitial volume leads to a large increase of volume of distribution of hydrophilic antibiotics, resulting in lower initial drug concentrations [85]. A large volume of distribution in critically ill patients has been demonstrated for β-lactam antibiotics, aminoglycosides and vancomycin [89-96]. The volume of distribution of some β-lactam antibiotics compared to healthy volunteers is shown in figure 7.
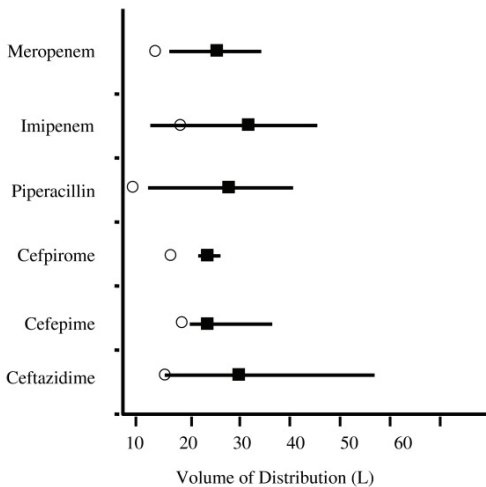
Fig. 7 Heterogeneity of volume of distribution (V_d) of β-lactam antibiotics in critically ill patients. Open circles: volume of distribution in healthy volunteers; filled squares: weighted means of volume of distribution in critically ill patients; straight lines: ranges of the means of volume of distribution in the studies (copied from [97] with permission).

Figure 8 illustrates the effect of increased V_d on concentrations in plasma and tissue after a single dose.

Healthy volunteer / General ward patient
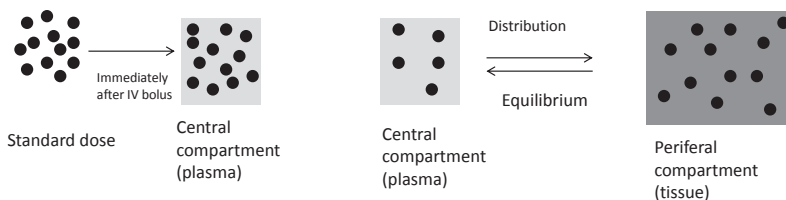
Critically Ill patient

Systemic inflammation response syndrome
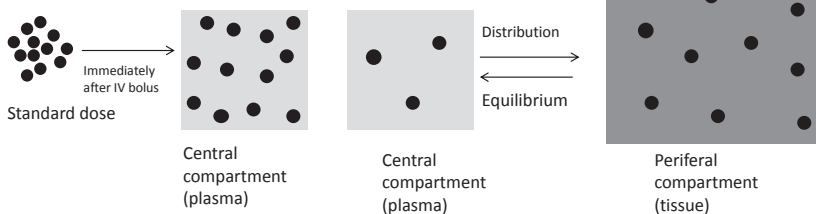
Fig. 8 Concentrations in plasma (central compartment) and tissues (peripheral compartment) in healthy volunteers/general ward patients and critically ill patients after standard dosing

4.2. Changes in clearance

Clearance is defined as the volume of plasma cleared of drug per unit of time. The main route of elimination for most β-lactam antibiotics is renal excretion, and therefore the concentrations will be highly affected by changes in renal function. In critically ill patients, both a decreased as well as an increased clearance can occur.

4.2.1. Decreased clearance

Acute kidney injury (AKI) is generally defined as the sudden and sustained loss of kidney function which results in the inability to excrete nitrogenous waste and xenobiotics and in the dysregulation of extracellular volume and electrolytes [98].

The lack of a standard definition for AKI has resulted in a large variation in the reported incidence [99]. In 2004, the Acute Dialysis Quality Initiative has published a consensus definition on AKI: the risk, injury, failure, loss, and end-stage renal disease classification (RIFLE). The classification is based on a relative increase in serum creatinine and on urine output [98]. In the case of a severe infection and associated sepsis, AKI is a

common sequel. The incidence of AKI (RIFLE category injury or failure) in sepsis has been reported to be around 40% [100, 101]. AKI will lead to a reduction in β-lactam antibiotic clearance [102, 103], and hence a significant increase in plasma concentrations. Although not very common, toxicity from β-lactam antibiotics may occur and is associated with high concentrations [35].

4.2.2. Extracorporeal clearance

In the case of severe AKI, initiation of renal replacement therapy may be needed. This may consist of continuous renal replacement therapy (CRRT), intermittent hemodialysis or a hybrid form, such as sustained low-efficiency dialysis. Solute removal occurs by convection and/or diffusion. Many factors may influence the antibiotic clearance from the circuit such as the filter material, blood flow rate, ultrafiltration rate, dialysate rate, location of fluid replacement, interruptions because of filter clotting or for therapeutic interventions [104]. The modality and dosage of the renal replacement therapy are unstandardized and individualized to the patient needs and therefore antibiotic clearance varies tremendously with method and settings [105].

These extracorporeal circuits may further complicate PK, and have not been properly investigated because these studies are not recommended in existing US Food and Drug Administration (FDA) guidance documents or required for new drug approval [106]. Observational studies have shown a wide variability in antibiotic concentrations during CRRT, with many patients having low concentrations early in therapy, and accumulation occurring in the next days [107-109].

To date, there are relatively little clinical data on the removal of specific drugs by CRRT. Moreover, it is unclear how the specific dialysis settings influence drug concentrations. Current recommendations on antibiotic dosing during CRRT are based on studies that included a limited number of patients who received different types of CRRT and therefore larger and better designed studies are necessary.

4.2.3. Augmented renal clearance

The exact opposite phenomenon of acute kidney injury is also frequently encountered in critically ill patients and has been coined "augmented renal clearance" (ARC). This phenomenon describes an increased renal elimination of solutes, such as metabolites,

toxins, waste products and pharmaceuticals and has been defined as a creatinine clearance of 130 mL/min/1.73m² or higher [110].

This hyperdynamic circulation, characterized by a high cardiac output and low systemic vascular resistance is a result of the SIRS which triggers cytokine release. How this influences renal function is still being studied. It is assumed that renal blood flow is correlated with cardiac output, which has been investigated by Udy et al. These researchers have shown a weak correlation (R= 0.346) between the cardiac index using pulse contour analysis and creatinine clearance in septic patients [111]. In order to normalize cardiac function, fluid resuscitation and vasopressors are often prescribed. These events all result in an enhanced blood flow to the major organs, including the kidneys which enhances glomerular filtration rate (GFR). Altered tubular function and recruitment of renal reserve, are also thought to contribute to this phenomenon [112]. This increases drug delivery to the glomerulus and subsequent elimination of renally cleared antibiotics, which in turn may possibly lead to therapeutic failure and selection of drug resistant strains [113, 114]. The potential physiologic mechanisms are graphically shown in figure 9, however it must be noted that these mechanisms are still being investigated. In order to further elucidate the pathophysiology, Udy et al have investigated the changes in GFR and renal tubular function in 20 critically ill patients at risk of augmented renal clearance (ARC), using exogenous marker compounds. Sinistrin was used to measure GFR, p-aminohippuric acid for assessment of tubular anion secretion, pindolol for cationic secetion and fluconazole for tubular reabsorption. They found that GFR was markedly elevated. Tubular anion secretion and tubular reabsorption were also elevated, was also elevated. Net tubular cationic secretion was impaired..

Although ARC has been discovered a long time ago (the first report on ARC dates from 1978), it is being increasingly described in critically ill patients [116]. ARC typically occurs in younger male patients with trauma, sepsis, burns, malignant disease or pancreatitis [111, 117].
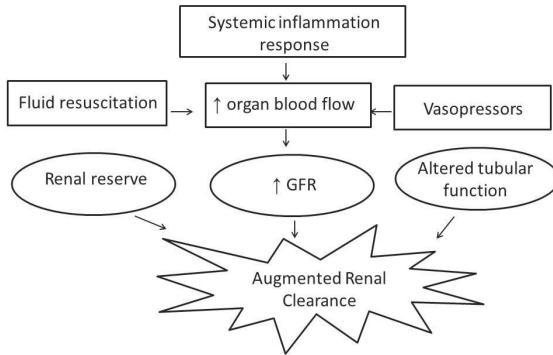
Fig. 9 Mechanisms driving augmented renal clearance; GFR: glomerular filtration rate (adapted from [115] with permission)

Udy et al. also found that 65% of all ICU patients without evidence of absolute renal impairment had evidence of ARC on at least one occasion during the first seven study days, and of those patients manifesting ARC, 74% did so on at least 50% of the study period (7 days) [118]. De Waele et al. reported that at least one episode of ARC was observed in 55.8 % of the patients, with an incidence of 36.6 episodes/100 patient days. Moreover, 60.9 % of the patients manifested ARC on more than 50 % of their ICU days[119]. These data suggest that ARC is likely to be common. Moreover, Udy et al. have also demonstrated that up to 82% of the patients with ARC had subtherapeutic β-lactam plasma concentrations after standard dosing [120].

Not only is ARC very common as shown above, research has also shown that ARC is associated with worse outcomes: Claus et al studied 128 patients in a mixed cohort of surgical and medical ICU patients receiving antimicrobial therapy and found 27.3% of the patients with ARC had therapeutic failure (defined as an impaired clinical response and the need for alternate antimicrobial therapy by two investigators blind for creatinine clearance), versus 12.9 % in the patients without ARC (p=0.04) [117].

4.3. Changes in protein binding

Many drugs, including β-lactam antibiotics bind to proteins. The degree of protein binding greatly affects PK. The most important binding proteins are albumin and α_1-acid glycoprotein. This binding between drug and protein must be seen as a reversible equilibrium, which is dependent on both the concentration of drug and protein. Figure 10

graphically represents a two-compartment model for a drug, including protein binding. The bound fraction acts as a drug-reservoir in the vascular compartment.
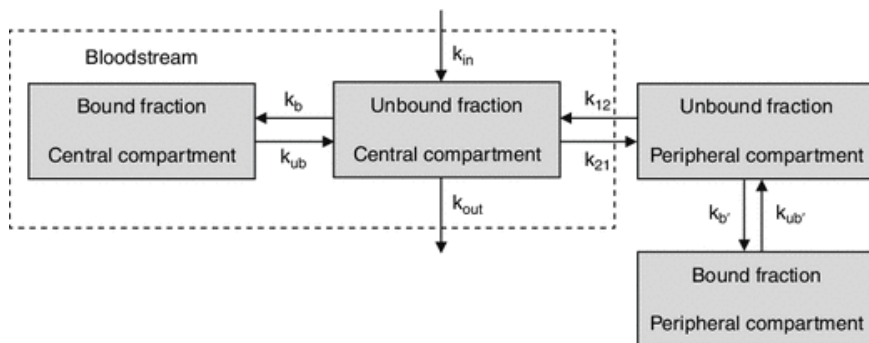
Fig. 10 The equilibrium between unbound, bound and distributed drug in the body in a two-compartment model. The bloodstream is the central compartment and the peripheral compartment represents the extravascular tissues where the drug distributes from the central compartment. k_{in} corresponds to the absorption constant (in oral administration) or the infusion rate (in intravenous infusion), k_{12} corresponds to the constant that describes the movement of drug from the central compartment (1) to the peripheral compartment (2). k_{21} describes the movement from the peripheral compartment(s) back to the central compartment. k_b and k_{ub} describe the equilibrium between bound and unbound drug, respectively, and albumin in the bloodstream. k_b and k_{ub} will depend on the binding affinity. $k_{b'}$ and $k_{ub'}$ describe the equilibrium between bound and unbound drug and albumin in the peripheral compartment where binding can occur to extravasated albumin or to cell membranes (including intracellular distribution). The albumin binding equilibrium will displace depending on the plasma albumin concentration and the plasma drug concentration. k_{out} corresponds to the elimination constant from the central compartment (copied from [121], with permission)

Protein binding affects PK in two ways. First, only the unbound antibiotic is able to penetrate into the extravascular space and distribute into the infected tissue to exert its pharmacological effect. Protein binding is therefore an important determinant of the extent of tissue distribution and the volume of distribution. Secondly, only the free drug can be filtered by the glomerulus, and therefore, protein binding also affects drug clearance.

Hypoalbuminaemia, defined as serum albumin levels < 25 g/L by the SAFE (Saline versus Albumin Fluid Evaluation) study, is very common in critically ill patients (incidence around 40-50%), mainly caused by loss through the capillaries mediated by the inflammation response [122]. A lower albumin concentration gives rise to a decreased binding to albumin. Therefore, free concentrations in plasma will initially increase. However, this increased unbound fraction is also available for distribution and elimination, which leads to a higher volume of distribution and a faster elimination from the body and thus leading to low concentrations later in the dosing interval, which is unfavorable for time-dependent antibiotics [123].

The importance of changes in protein binding is often not clinically relevant, since an increase in free fraction does not always result in an increased free concentration. A change in protein binding, causing a higher free fraction may initially result in higher concentrations, but as this concentration is available both for distribution and elimination, these processes which will decrease the free concentration [124].

Because of the dynamic equilibrium between higher unbound fraction and increased distribution and elimination, the influence of hypoalbuminaemia is probably most relevant for highly bound compounds, where small changes in protein binding may result in a large increase of unbound fraction. An decreased protein binding from 50 to 40 % results in only 20 % increase in free concentration while a decreased protein binding from 99 to 98 % results in a 100 % increase in free concentration, which may lead to toxic effects if the drug has a narrow therapeutic-toxic window. In the case of highly renally cleared drugs such as β-lactam antibiotics, this increased free concentration will be distributed and cleared, giving rise to low trough concentrations, which may impact the effect of these drugs. It has been shown for highly bound β-lactam antibiotics, such as ertapenem that conventional dosing of 1 g once daily, as determined in studies on healthy volunteers with normal serum albumin concentration, leads to suboptimal PK exposure in critically ill patients: the volume of distribution was found to be 4 times larger than the volume of distribution in healthy volunteers, and clearance 2 times higher [125-127]. Similar observations have been made for other highly protein bound antibacterials such as ceftriaxone, teicoplanin, cefazolin and flucloxacillin [128-132].

4.4. Impaired tissue penetration

In order to be effective, antibiotics need to penetrate from the plasma into the site of infection. Another factor complicating dosing in critically ill patients is the fact that severe infection may cause vascular dysfunctions, which can impair the penetration into the infected tissue. Although not much is known on this subject, there is some literature suggesting impaired tissue penetration and subsequent subtherapeutic tissue concentrations for many antibiotics in critically ill patients with a severe infection [133-136].

5. Dose – optimization

Considering that the PK of β-lactam antibiotics are significantly altered in critically ill patients and that administering standard doses that are based on pharmacokinetic data from healthy volunteers may lead to both under-as well as overdosing, individually tailored antibiotic therapy may be a useful strategy to improve efficacy and prevent toxicity. At this moment, PK/PD modeling, and antibiotic therapeutic drug monitoring (TDM) are being explored to achieve this, together with prolonged infusion strategies, which form the 3 components of β-lactam antibiotic dose optimization.

5.1. Prolonged infusion time

Prolonged infusion encompasses both terms extended (defined as a discontinuous infusion of 2 hours or more) and continuous infusion.

There is compelling evidence that administration of β-lactam antibiotics by prolonged infusion increases the time for which the concentration exceeds the MIC of the pathogen (both in blood as well as in interstitial fluid), and therefore results in a better PK profile in critically ill patients than intermittent infusion when using the same dose, particularly for bacteria with high MIC values. This is graphically illustrated for piperacillin in figure 11 [133, 137-140].
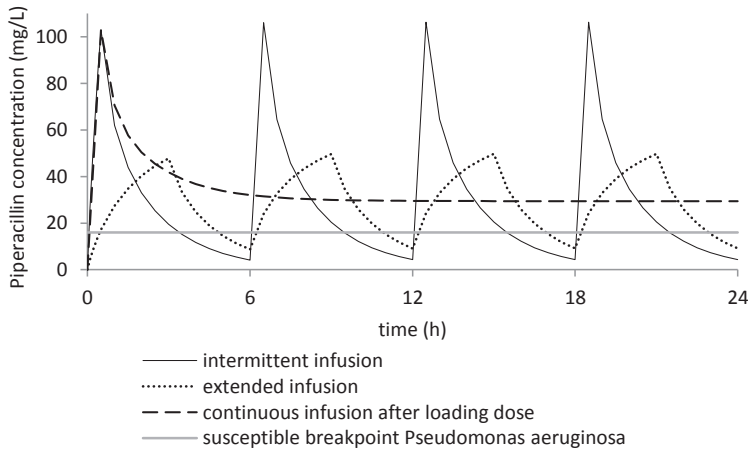
Fig. 11 Simulated concentration time profiles for a patient with fixed pharmacokinetic parameters only changing the duration of infusion (30 minute infusion, 3 hour infusion and continuous infusion), showing that prolonging infusion time increases the time above the minimal inhibitory concentration for less susceptible organisms.

Whether or not this results in improved outcome is still up for debate as comparative clinical studies between intermittent and prolonged infusion did not demonstrate significant differences in patient outcome. Abdul-Aziz et al explored the methodological flaws and inconsistencies of the published clinical studies, such as heterogeneous patient populations, patients with a low level of illness severity, inconsistent dosing regimens, pathogens with high susceptibilities and insufficient sample sizes. They concluded that continuous infusion of β-lactam antibiotics will not be beneficial to all patients but only to a specific subset, namely the critically ill with severe infections [141]. Currently, a 420 patient phase II study has been completed which should shed light on the benefit of bolus vs. continuous infusion. A proof-of-concept randomized controlled trial from this research group did already show that in this patient population, continuous infusion resulted in higher clinical cure rates [142].

Therefore, administering antibiotics as a continuous infusion is increasingly used in intensive care units (ICU) around the world [49]. However, this way of administration brings about some practical issues such as the need for a loading dose to ensure rapid achievement of therapeutic plasma concentrations, which otherwise may take several hours. Drug

stability and compatibility should also be taken into account, as some β-lactam antibiotics are not stable for 24 hours at room temperature. Most critically ill patients receive multiple drugs simultaneously, and often drug-drug compatibilities are not investigated, which requires the need for a dedicated line for continuous infusion [143].

5.2 Patient tailored antibiotic therapy

Even with the use of prolonged infusion techniques, the large PK variability between patients will continue to exist, and blind dosing may still lead to under-or overdosing in some patients. Because of this variability, individualized dosing, adapted to the physiology of the patient and the susceptibility of the causative pathogen may be better suited to ensure maximum efficiency and minimal toxicity.

5.2.1. Population pharmacokinetics

Population PK is the study of the variability in drug concentrations between individuals of a specific target population. It includes both the extent, as well as the sources and the correlates of this variability [144]. The major difference between population PK and traditional PK studies is that in traditional PK studies, the mean plasma-concentration time profile is the main point of interest. However, the focus of population PK is to provide estimates of the mean PK parameters (called population-typical values) together with the components of variability (called population variability values) [145].

Non Linear Mixed Effects Modeling is widely used as an estimation method of population PK. Population parameters are composed of so called fixed-effects and random-effect parameters, which are estimated simultaneously. Fixed effects include the population typical values, which define the average value for a PK parameter in the population, and the relationship between measureable patient characteristics (called covariates, for example creatinine clearance) and PK parameters. Random-effects parameters are the population-variability values, which quantify variation. The model is defined by a structural model, a random effect model and a covariate model [146].

5.2.1.1. Building blocks of a population pharmacokinetic model

5.2.1.1.1. Structural model

The structural model defines the structure of the PK process. It is the PK model best describing the concentration data without covariates. It is part of the fixed-effects part of the population PK model. Most commonly used are one, two or three compartmental models [145].

5.2.1.1.2. Random effect model

The random effect model describes the variability of the PK parameters. The random effects can be subdivided into 3 categories: between subject variability, residual unexplained variability and between occasion variability [145].

Between subject variability measures the unexplained random differences between subjects (also called the inter-individual variability). It quantifies the deviation from the individual PK parameter to the population PK parameter. It is mostly described by the coefficient of variation [145].

Residual unexplained variability measures the remaining unexplained variability when all other sources of variability have been taken into account. It includes errors in the analytical assay, in the drug administration, in the sample timing, etc. It can be described by an additive, a proportional, a combined additive-proportional or an exponential error model [145].

Between occasion variability (also called inter occasion variability) is a measure of unexplained random differences within the same individual on different occasions and is similarly to between subject variation mostly described by the coefficient of variation [147].

5.2.1.1.3. Covariate model

A covariate is any measurable variable specific to an individual that may affect the drug PK and therefore explain part of the between subject variation. The goal of a covariate model is to reduce the between subject variation. Most important covariates are weight, renal function and age. The covariate model describes the effect of covariates on the PK parameters of the structural model. It is also part of the fixed-effects part of the population pharmacokinetic model. The decision to include a covariate in the final model should be based on statistical significance, biological plausibility and clinical significance [146].

 If the PK parameters can be precisely predicted based on the covariates, individualization of drug therapy becomes possible.

5.2.1.2 Monte Carlo simulations

After a well-evaluated and robust pharmacokinetic model including covariates has been developed in a population pharmacokinetic analysis of patient data, Monte Carlo simulations can be performed to make predictions about the future. A set of pharmacokinetic parameter values is generated for each simulated subject by random sampling within the predefined parameter distribution for each simulation. A concentration-time profile can be generated for each simulated subject, which can then be used to evaluate the likely result of different therapeutic approaches. Examples can be: the effect of achieving therapeutic targets of different dosing strategies, or the effect on the development of resistance, drug toxicity and so on [148]. This technique is very valuable, as this allows researchers to ask many "what if" questions without having to perform a new clinical trial, which gives the possibility to maximize knowledge in absence of large studies [148].

It must be noted that the appropriateness and robustness of the PK model is crucial. A model based on a very limited sample size will probably not be able to describe all PK variability likely to be encountered in the critically ill. However, Monte Carlo Simulations based on these small studies can still be instructive of the results of new dosing strategies, although the results should not be considered definitive [148]. Moreover, it is important to realize that Monte Carlo Simulations use a random sampling within a pre-defined distribution, which is often large and therefore the results will not be correct for each individual patient.

5.2.2. Therapeutic drug monitoring

If there are no population PK studies available, or if the random effects are considered to be too high to allow for dose individualization, then a more individualized approach using therapeutic drug monitoring (TDM) is likely to be a strategy that is better suited to optimize dosing [105].

TDM requires direct measurement of antibiotic concentrations with timely reporting to the clinicians, who then interpret the result and decide if dosing alterations are necessary

by comparing the concentration to a therapeutic target, or by the use of sophisticated software which estimates the antibiotic exposure [105].

Components subjected to TDM are generally drugs with a narrow therapeutic index and with large intersubject PK variability, that cannot be easily be titrated to effect and which have a clear concentration-effect relationship.

For aminoglycosides it has also been shown that individualized antibiotic therapy, with dosing adapted to specific pharmacokinetic targets using therapeutic drug monitoring (TDM) positively affects outcome [149-151]. An impressive study conducted in 1999 in the Netherlands found mortality to be lower in patients admitted with an infection treated with aminoglycosides subjected to active TDM compared to patients admitted with an infection treated with aminoglycosides subjected to nonguided TDM [149]. All available studies found TDM to be cost effective and associated with lower nephrotoxicity [150, 151].

For vancomycin, the benefit of TDM remains somewhat controversial. There are conflicting reports whether or not vancomycin-associated nephrotoxicity is concentration dependent. However, a recent meta-analysis on the benefits of TDM of vancomycin suggested that TDM significantly improves clinical efficacy while reducing the likelihood of developing nephrotoxicity [152].

TDM has not widely been investigated for β-lactam antibiotics, and has traditionally been considered unnecessary because of their wide therapeutic index [153]. Currently there is almost no information about the effect of TDM on outcome for patients treated with β-lactam antibiotics. To date, there is only one study that investigated the influence of feedback dose alterations of different drugs (aminoglycosides, fluoroquinolones and β-lactam antibiotics) and found that adjustment of dose was independently associated with the probability of obtaining a positive clinical outcome in a multivariate analysis (p< 0.0002) [154].

Unfortunately, TDM of β-lactam antibiotics is currently challenging with long turnaround times, expensive equipment, logistical problems related to the instability of the samples and the need for well-trained personnel. A detailed review on the available assays for TDM of β-lactam antibiotics can be found in chapter 4.

Chapter Two : Research Objectives

Despite the clinical experience with β-lactam antibiotics and high clinical cure rates for non-critically ill patients, outcomes in infected critically ill patients are still poor despite apparently appropriate and timely antibiotic therapy. In recent years, very few new antibiotics have become available, and the same is to be expected in the next years. With increasing rates of antimicrobial resistance, a rational use of antibiotics has been advocated. Optimized use of antibiotics to improve outcome and reduce antibiotic resistance is therefore the next challenge.

In the heterogeneous population of an intensive care setting, correct antibiotic dosing is problematic because of highly variable and unpredictable pharmacokinetic changes in critically ill patients. The decreased susceptibility is an additional factor that makes dosing even more problematic. Considering this wide variability of antibiotic concentrations in critically ill patients, individually tailored antibiotic therapy may be a useful strategy to improve dosing.

The aim of this thesis is to provide valuable information that can be used as a basis for patient tailored antibiotic therapy. For this, antibiotic concentrations need to be accurately measured in plasma. The first aim of this PhD is therefore to develop and validate an analytical method to accurately determine the plasma concentration of the most commonly used β-lactam antibiotics in Ghent University Hospital. As these antibiotics are considered to be quite unstable, we will also explore the pre-analytical stability of these antibiotics in plasma and whole blood in order to investigate whether labor-intensive measures taken to prevent degradation are really warranted.

The second aim of this research is to gain more insight in the pharmacokinetics of these antibiotics, and their determinants. Therefore, we will conduct a number of pharmacokinetic studies in critically ill patients. In an era of emerging resistance and few new available antibiotics, it is necessary to use all remaining armamentarium optimally. Critical illness has an effect on the pharmacokinetics of both broad-as well as smaller spectrum antibiotics, and therefore research to optimize dosing should be focused on both broad-as well as smaller spectrum antibiotics.

We will investigate the population pharmacokinetics of amoxicillin/clavulanic acid and cefuroxime in critically ill patients without acute kidney injury. We will also investigate the population pharmacokinetics of cefepime in septic shock patients during continuous renal replacement therapy. We will investigate the pharmacokinetics of meropenem and piperacillin administered as an extended infusion and compare it to bolus infusion. Another study will focus on the pharmacokinetic variability within the same patient over an entire course of treatment of piperacillin. We will also examine the adequacy of dosing of β-lactam antibiotics when de-escalating from empirical broad spectrum antibiotics to more targeted narrow spectrum antibiotics by performing dosing simulations using previously published pharmacokinetic studies.

The third and last aim of this thesis is to perform a number of studies focused on optimizing antibiotic therapy in clinical practice. Firstly, we will investigate the stability of drug infusions containing meropenem or amoxicillin/clavulanic acid reconstituted in physiologic saline, to evaluate the potential to be administered as a continuous infusion. Secondly, we will investigate the influence of creatinine clearance on pharmacokinetic/pharmacodynamic target attainment. Finally, we will analyze the effect of a dose-adaptation strategy based on daily therapeutic drug monitoring on target attainment of meropenem and piperacillin/tazobactam by performing a randomized controlled trial.

Chapter Three : Personal research

My contribution to the work was based on the following papers :

1. Carlier M, Stove V, Roberts JA, Van De Velde E, De Waele JJ, Verstraete AG. Quantification of seven β-lactam antibiotics and two β-lactamase inhibitors in human plasma using a validated UPLC-MS/MS method. *Int J Antimicrob Agents.* 40(5):416-422 (2012).

2. Carlier M, Carrette S, Roberts JA, Stove V, Verstraete AG, Hoste E, Depuydt P, Decruyenaere J, Lipman J, Wallis SC, De Waele JJ. Meropenem and piperacillin/tazobactam prescribing in critically ill patients: does augmented renal clearance affect pharmacokinetic/pharmacodynamic target attainment when extended infusions are used? *Crit Care.* 17(3):R84 (2013).

3. Carlier M, Noë M, De Waele JJ, Stove V, Verstraete AG, Lipman J, Roberts JA. Population pharmacokinetics and dosing simulations of amoxicillin/clavulanic acid in critically ill patients. *J Antimicrob Chemother.* 68: 2600-8 *(*2013).

4. De Waele JJ, Carrette S, Carlier M, Stove V, Boelens J, Claeys G, Leroux-Roels I, Hoste E, Depuydt P, Decruyenaere J, Verstraete AG. Therapeutic drug monitoring-based dose optimisation of piperacillin and meropenem: a randomized controlled trial. *Intensive Care Med*. 40(3):380-7 (2014).

5. Carlier M, Carrette S, Stove V, Verstraete AG, De Waele JJ. Does consistent piperacillin dosing result in consistent therapeutic concentrations in critically ill patients? A longitudinal study over an entire antibiotic course. *Int J Antimicrob Agents.* 43 (5):470-473 (2014).

6. De Waele JJ, Carlier M, Hoste E, Depuydt P, Decruyenaere J, Wallis S, Lipman J, Roberts J. Extended versus bolus infusion of meropenem and piperacillin: a pharmacokinetic analysis. *Minerv. Anestesiol.* 80(12):1302-9 (2014).

7. Carlier M, Noë M, Roberts JA, Stove V, Verstraete AG, Lipman J, De Waele JJ. Population pharmacokinetics and dosing simulations of cefuroxime in critically ill

patients – non-standard dosing approaches are required to achieve therapeutic exposures. *J Antimicrob Chemother.* 69(10):2797-803 (2014).

8. Carlier M, Stove V, Verstraete AG, De Waele JJ. Stability of generic brands of meropenem reconstituted in isotonic saline. *Minerv Anestesiol.* 81(3):283-7 (2015).

9. Carlier M, Stove V, De Waele JJ, Verstraete AG. Ultrafast quantification of β-lactam antibiotics in human plasma using UPLC-MS/MS. *J Chrom B.* 978-979:89-94 (2015).

10. Carlier M, De Waele JJ, Verstraete AG, Stove V. Exploration of the pre-analytical stability of β-Lactam antibiotics in plasma and blood – implications for therapeutic drug monitoring and pharmacokinetic studies. *Clin Chem Lab Med*. Epub ahead of print doi: 10.1515/cclm-2014-0833 (Published ahead of print November 2014).

11. Carlier M, Roberts JA, Stove V, Verstraete AG, Lipman J, De Waele JJ. Lack of PK/PD target attainment in de-escalated antibiotic therapy in critically ill patients: Less is not always more. *Antimicrob Agents Chemother* (accepted).

12. Carlier M, Taccone FS, Beumier M, Seyler L, Cotton F, Jacobs F, Roberts JA. Population pharmacokinetics of cefepime during continuous renal replacement therapy in septic shock. *Int J Antimicrob Agents* (accepted).

13. Carlier M, Verstraete AG, De Waele JJ, Stove V: Stability of amoxicillin and amoxicillin/clavulanic reconstituted in isotonic saline. *J Chemother* (accepted).

14. Carlier M, Stove V, Wallis SC, De Waele JJ, Verstraete AG, Lipman J, Roberts JA. Assays for therapeutic drug monitoring of β-lactam antibiotics: a structured review. *Int J Antimicrob Agents* (submitted).

Chapter Four : Analytical Methods

Performing pharmacokinetic studies requires accurate measurement of drugs in patient samples. This chapter summarizes the analytical work that has been performed as part of this thesis.

This first part of this chapter is a review of all currently available methods that can be used for TDM-purposes. An important part of this PhD consisted of developing a reliable method to quantify the most commonly used β-lactam antibiotics in Ghent University Hospital, which is described in section 2. For this research, 2 analytical methods were developed. The first described method is able to quantify 6 β-lactam antibiotics amoxicillin, ampicillin, piperacillin, ceftazidime, cefazolin, meropenem and 2 β-lactamase inhibitors, clavulanic acid and tazobactam. Although this method proved to be very accurate and reliable, it was associated with a high workload because of the intensive sample clean-up procedure. Moreover, the chosen mobile phases were different from the mobile phases used for other routine analyses performed on the same machine. Therefore, we developed a new method with minimal sample preparation using the standard mobile phases that were also used for other methods. The last part of this chapter summarizes the pre-analytical research, as little is known on this subject for β-lactam antibiotics. As they are generally considered to be quite unstable, labor intensive measures are currently often used, which makes routine therapeutic monitoring of these drugs even more challenging. This pre-analytical research investigated whether these measures are indeed warranted.

1. **Assays for therapeutic drug monitoring of β-lactam antibiotics: a structured review**

Authors : Mieke Carlier, Veronique Stove, Steven C. Wallis, Jan J. De Waele, Alain G. Verstraete, Jeffrey Lipman, Jason A. Roberts

Abstract

In some patient groups - including critically patients - the pharmacokinetics of β-lactam antibiotics may be profoundly disturbed due to pathophysiological changes in distribution and elimination. Therapeutic drug monitoring (TDM) is a strategy that may be helpful to optimize dosing. The aim of this review was to review and analyze the published literature of the methods used for β-lactam quantification in TDM programs. Sixteen reports described methods for simultaneous determination of 3 or more β-lactam antibiotics in plasma/serum. Measurement of these antibiotics, due to low frequency of usage relative to some other tests, is generally limited to in-house chromatographic methods coupled to ultraviolet or mass spectrometric detection. Although many published methods state they are fit for TDM, they are inconvenient because of intensive sample preparation and/or long run times. Ideally, methods used for routine TDM should have a short turn around time (fast run-time and fast sample preparation), a low limit of quantification and a sufficiently high upper limit of quantification. The published assays included a median of 6 analytes (interquartile range (IQR) 4-10), with meropenem and piperacillin as most frequently measured β-lactam antibiotics. The median runtime was 8 minutes (IQR 5.9 - 21.3). There are also a growing number of methods measuring free concentrations. An assay that measures antibiotics without any sample preparation would be the next step toward real-time monitoring, however no such method is currently available.

1.1 Introduction

Infection is a severe problem in many areas of medicine. Sepsis alone is the leading cause of mortality in non-cardiac intensive care units with up to 30% of patients dying within one month of diagnosis [2]. Timely and appropriate antibiotic therapy after source control is considered to be the mainstay of treatment [24]. Achieving adequate antibiotic exposure is equally important, however, because of pathophysiological changes in the pharmacokinetics of the drugs, optimal dosing remains very difficult [39, 49].

β-lactam antibiotics are the most commonly used antibiotics because of their broad spectrum of activity and wide therapeutic index. They exhibit time-dependent pharmacodynamics, meaning that the duration that the free antibiotic concentration exceeds the minimal inhibitory concentration (MIC) of the pathogen ($fT_{>MIC}$) determines the bactericidal effect. Subtherapeutic concentrations using standard dosing have been reported in many patients groups, in particular, critically ill patients, [128, 140, 155-161] which in turn may result in clinical failure as well as development of antibiotic resistance. Toxicity of β-lactam antibiotics is less common, but severe when it occurs, with seizures from high concentrations being reported previously [35, 162-165].

A more individualized approach using therapeutic drug monitoring (TDM) with dosing adapted to the altered pharmacokinetics of the individual patient is likely to be a strategy that can help optimize dosing [105]. TDM is mostly used for drugs with a narrow therapeutic index (such as aminoglycosides and glycopeptides) to maximize efficacy and minimize toxicity, although only aminoglycosides have supportive published outcome data [150]. TDM of β-lactam antibiotics is a relatively new technique, and although to date, there is no evidence that this leads to improved clinical outcomes, it is increasing in popularity as a means to optimize dosing in difficult patient populations, mostly for reasons of efficacy [49, 166].

However, unlike TDM of aminoglycosides and glycopeptides, no commercial assays such as immunoassays are available for routine monitoring of β-lactam antibiotics. A comparison of TDM of β-lactam antibiotics to aminoglycosides and glycopeptides is shown in figure 1.

The aim of this review was to identify and analyze the published literature of the methods used for β-lactam quantification during TDM programs. In this review we describe

and compare the available methods to determine β-lactam antibiotics in plasma/serum and in other matrices, both for total and free concentrations.
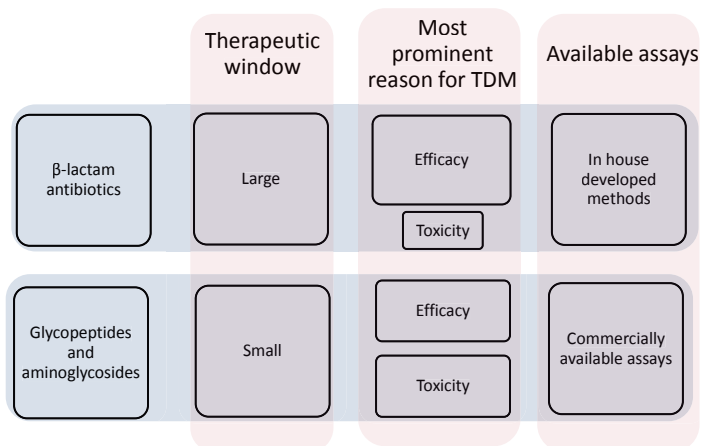
Fig. 1 : Comparison of therapeutic drug monitoring of β-lactam antibiotics to aminoglycosides and glycopeptides

1.2 Search strategy

1.2.1   Search terms

Data for the present review were identified using a literature search of Pubmed from 1951 to January 2015, as well as references from within relevant papers and the extensive files of the authors. The search terms included: (((Beta-lactam OR penicillin OR cephalosporin OR carbapenem OR monobactam)) AND ("quantification" OR "determination" OR "assay" OR "chromatographic" OR "immunoassay")) AND ("dosage" OR "patients" OR "therapeutic drug monitoring" OR "TDM" OR "clinical samples").

1.2.2. Principles for the preferred method

Simultaneous analysis of 3 or more β-lactam antibiotics in serum/plasma was considered as an inclusion criterion for a method to be included in this review (1 for alternative biological fluids, e.g. peritoneal fluid). The characteristics extracted from the included assays were runtime, precision and accuracy of the method, the calibration range, the lower limit of quantification (LLOQ) as well as the upper limit of quantification (ULOQ). We also listed

whether selectivity was tested, in case photometric detection was used, and whether the matrix effect was tested, in the case of mass-spectrometric detection.

1.3 Results

A total of 588 articles were reviewed for qualitative synthesis, of which 476 titles did not describe assay methods for quantification of β-lactam antibiotics, but described general microbiological research, veterinary research, or pharmaceutical research. Seventeen reports described methods for simultaneous determination of 3 or more β-lactam antibiotics in plasma/serum: 15 chromatographic methods [167-181], and 2 non-chromatographic methods [182, 183]. Forty-two methods were found determining one or two β-lactam antibiotics, 11 of which determined at least one β-lactam antibiotic in other body fluids such as cerebrospinal fluid, ultrafiltrate or used an alternative sampling strategy such as dried blood spots [184-195]. Eleven papers reporting immunoassays were also found [196-207].

1.3.1. Methods to measure β-lactam antibiotic concentrations in plasma or serum

1.3.1.1. Chromatographic assays

Fourteen of the 15 chromatographic methods used reversed phase separation, coupled to ultraviolet (UV) (n = 9/15) or to mass spectrometric (MS) detection (n = 6/15). There seemed to be no difference whether plasma or serum was used. The characteristics of these methods are summarized in table 1.

Three methods did not report data on specificity, and one method only reported the results of one blank sample [171, 172, 175, 177]. Most of the manuscripts did not report on stability testing, although this is of major importance for β-lactam antibiotics, which in some cases are relatively unstable [208].

1.3.1.1.1.   Analytes

The most frequently measured β-lactam antibiotic was meropenem, which was included in 14 of 15 methods, followed by piperacillin (n = 11). The amount of analytes per run ranged between 3 and 12, with a median of 6 antibiotics (interquartile range (IQR) 4-10).

Table 1 : Characteristics of the evaluated chromatographic methods for determination of β-lactam antibiotics in plasma

Ref	No of β-lactam antibiotics	Analytes	Column type	Detection	Total /free	Sample preparation	Run time (min)	LLOQ (mg/L)	ULOQ (mg/L)
[178]	10	ampicillin benzylpenicillin cefazolin ceftriaxone cephalotin dicloxacillin ertapenem flucloxacillin meropenem piperacillin	C18	UV at 210 nm for ampicillin, piperacillin, benzylpenicillin, flucloxacillin and dicloxacillin UV at 260 nm for ceftriaxone, cefazolin and cephalotin UV at 304 nm for meropenem and ertapenem	Free	Ultrafiltration and stabilization	7	0.1	50
[169]	7	amoxicillin ampicillin cefazolin ceftazidime cefuroxime meropenem piperacillin	C18	MS/MS	Total	Protein denaturation followed by back extraction of acetonitrile	5.5	0.5	100
[181]	5	Amoxicillin ceftazidime cefuroxime meropenem piperacillin	C18	MS/MS	Total	Protein denaturation followed by dilution	2.5	1 (amoxicillin and piperacillin) 0.5 (ceftazidime, meropenem, cefuroxime)	150 for piperacillin, 100 for amoxicillin, cefuroxime and ceftazidime, 75 for meropenem

Ref	No of β-lactam antibiotics	Analytes	Column type	Detection	Total /free	Sample preparation	Run time (min)	LLOQ (mg/L)	ULOQ (mg/L)
[176]	8	amoxicillin ampicillin cefepime ceftazidime ceftriaxone imipenem meropenem piperacillin	C18	MS/MS	Total	Protein denaturation followed by dilution	6	0.5	5
[170]	11	amoxicillin ampicillin cefadroxil cefazolin cefepime ceftazidime cefuroxime flucloxacillin phenoxymethyl penicillin piperacillin meropenem	C18	MS/MS	Total	Solid phase extraction followed by dilution	4	0.05 for phenoxymethylpenicillin and cefepime 0.1 for meropenem 0.2 for cefuroxime and ceftazidime 0.25 for cefazolin 0.4 for amoxicillin 0.5 for flucloxacillin 0.6 for ampicillin and piperacillin 0.8 for cefadroxil	6 for phenoxymethylpenicillin and cefepime 9 for meropenem 20 for cefuroxime and ceftazidime 28 for cefazolin 50 for amoxicillin 58 for flucloxacillin 70 for ampicillin and piperacillin 91 for cefadroxil
[179]	4	doripenem ertapenem imipenem meropenem	5F-phenyl	UV at 295 nm	Total for doripenem, meropenem and imipenem Free for ertapenem	Stabilization of the plasma sample, followed by protein denaturation and evaporation of the supernatant and reconstitution in buffer. Ultrafiltration for ertapenem	7	0.5	100

Ref	No of β-lactam antibiotics	Analytes	Column type	Detection	Total /free	Sample preparation	Runtime (min)	LLOQ (mg/L)	ULOQ (mg/L)
[171]	5	cefepime ceftazidime cefuroxime meropenem piperacillin	C8	256 nm for cefepime and ceftazidime 270 nm for cefuroxime 300 nm for meropenem 220 for piperacillin	Total	Solid phase extraction followed by evaporation and reconstitution in buffer	30	0.5 for ceftazidime, piperacillin and meropenem 1 for cefepime and cefuroxime	50
[173]	3	ertapenem imipenem meropenem	C8	UV at 298 nm	Total	Stabilization of the plasma sample, protein denaturation, evaporation of the supernatant and reconstitution in buffer	25	0.5	80
[175]	8	ampicillin cefazolin cefepime cefmetazole cefotaxime doripenem meropenem piperacillin	C18	MS/MS	Total	Solid phase extraction followed by evaporation and reconstitution in buffer	13	0.005 for piperacillin 0.01 for cefepime 0.05 for cefmetazole and ampicillin 0.1 for cefazolin, cefotaxime and meropenem 0.5 for doripenem	50
[177]	3	Cefotaxime cefradine cefuroxime	C18	UV at 260 nm	Total	Solid phase extraction followed by evaporation to 2 mL	5	0.5 for cefuroxime 1 for cefotaxime 5 for cefradine	15 for cefuroxime 20 for cefotaxime 20 for cefradine

Ref	No of β-lactam antibiotics	Analytes	Column type	Detection	Total /free	Sample preparation	Run time (min)	LLOQ (mg/L)	ULOQ (mg/L)
[174]	12	amoxicillin cefepime cefotaxime ceftazidime ceftriaxone cloxacillin imipenem meropenem oxacillin penicillin G piperacillin ticarcillin	C18	UV at 210 nm for cloxacillin, oxacillin, penicillin G, piperacillin, ticarcillin UV at 230 nm for amoxicillin, cefepime, ceftazidime, cefotaxime and ceftriaxone UV at 298 nm for imipenem and meropenem	Total	Protein denaturation using acetonitrile and dilution of the supernatant	20	2 for amoxicillin, cefepime, cefotaxime, ceftazidime, meropenem and imipenem 5 for ceftriaxone, cloxacillin, oxacillin, penicillin G, piperacillin and ticarcillin	250
[168]	12	ampicillin benzylpenicillin cefazolin ceftazidime ceftriaxone cephalotin dicloxacillin ertapenem flucloxacillin meropenem piperacillin ticarcillin	C18	UV at 210 nm for ampicillin, piperacillin, benzylpenicillin flucloxacillin and dicloxacillin 260 nm for ceftriaxone, cefazolin and cephalotin 304 nm for meropenem and ertapenem	Total	Protein denaturation using acetonitrile and back extraction of the acetonitrile	25	5 for all analytes except for piperacillin and ticarcillin (10 mg/L)	250

Ref	No of β-lactam antibiotics	Analytes	Column type	Detection	Total /free	Sample preparation	Run time (min)	LLOQ (mg/L)	ULOQ (mg/L)
[172]	5	biapenem doripenem imipenem meropenem panipenem	C18	UV at 300 nm	Free	Dilution in mobile phase followed by ultrafiltration	9	0.04	25
[167]	6	aztreonam cefepime ceftazidime cefuroxime meropenem piperacillin	C18	UV at 240 nm for aztreonam and piperacillin UV at 260 nm for cefepime, ceftazidime, cefuroxime UV at 300 nm for meropenem	Total	Precipitation with methanol followed by evaporation of the supernatant and reconstitution in buffer	17	2	200
[180]	7	benzylpenicillin cefazolin ceftazidime ertapenem flucloxacillin meropenem piperacillin	C18	MS/MS	Total	Protein denaturation using acetonitrile with 0.1 % formic acid followed by dilution in water with 0.1 % formic acid	7	0.1 for all analytes except flucloxacillin (0.25)	50 for meropenem, ertapenem and ceftazidime 25 for benzylpenicillin, piperacillin and flucloxacillin

Ref : Reference ; LLOQ : lower limit of quantification ; ULOQ : upper limit of quantification

1.3.1.1.2. Calibration range

The ideal assay should have an LLOQ that is lower than the minimal inhibitory concentration (MIC) of the likely causative pathogen. The LLOQ of most methods were indeed around MIC values for most pathogens (≤0.5 mg/L). Some methods reported a very high LLOQ such as 2 or 5 mg/L for meropenem (higher than the MIC breakpoint for the least susceptible pathogens such as *Pseudomonas aeruginosa*), 5 mg/L for flucloxacillin, or 10 mg/L for piperacillin [167, 168, 174]. Using a method with a high LLOQ may result in many trough samples measured as undetectable concentrations potentially leading to unnecessary dose adjustments for infections caused by lower MIC pathogens.

As both very high and low concentration values can be expected when performing TDM in ICU patients where such a wide range of organ functions is possible, and turn around time is strongly delayed if samples have to be re-analyzed after dilution, it is important that the ULOQ is sufficiently high. An ULOQ of 100 mg/L or higher should be preferred, especially for piperacillin, for which the most commonly used daily dosage is 12-16 g, resulting in high concentrations in a many patients. However, 6 out of 10 methods that quantified piperacillin reported an ULOQ of < 100 mg/L [170, 171, 175, 176, 178, 180]. One method reported the highest calibrator to be as low as 5 mg/L, which would not be considered convenient for routine TDM [176].

1.3.1.1.3. Runtime

The median runtime per sample was 8 minutes (IQR 5.9 - 21.3). However for routine TDM, shorter runtimes are desirable, as a batch with multiple calibrators and quality control samples can require a high number of samples to be analyzed consecutively.

1.3.1.1.4. Sample preparation

When developing an assay to determine the total concentration in plasma, a range of sample preparation procedures can be used, depending on the way the sample is pre-treated (protein precipitation using organic solvents or using solid phase extraction) and, depending on optional evaporation, used to concentrate the sample. Four methods used protein denaturation and subsequent dilution of the supernatant [174, 176, 180, 181], only one used solid phase extraction and subsequent dilution [170], 2 methods used protein denaturation and back-extraction of acetonitrile [168, 169], 2 methods used protein

denaturation, evaporation of the supernatant and reconstitution of the residue [173, 179], and three methods used solid phase extraction, evaporation of the supernatant and reconstitution of the residue [171, 175, 177]. Four assays were found that determined free concentrations, all of which used ultrafiltration [167, 172, 178, 179].

1.3.1.2. Dried blood spots

Dried blood spots are a form of biosampling where a drop of blood is collected as a spot on a filter paper. It is most commonly used for the screening of metabolic disorders in neonates, but is increasingly being used for TDM purposes as well, because of advantages including low volume of blood sampling, more convenient transportation, storage without special treatment and enhanced analyte stability, which make it very attractive for TDM and/or pharmacokinetic studies [209].

We found one method which measures ertapenem from dried blood spots for TDM in neonates, in whom sampling of larger volumes is not practical and 2 which measured piperacillin/tazobactam [184, 195, 210]. However, it must be noted that using this sampling strategy, it is not possible to determine free concentrations, which may be a problem for highly protein bound drugs like ertapenem (ca. 90% protein bound). Moreover, the drying process after sampling takes at least 2 h, and dried blood spots may suffer from issues affecting reliability of results including variations in the blood volume spotted, blood spot homogeneity, and haematocrit concentrations. Variability in haematocrit (the volume percentage of red blood cells in blood) is a widely discussed challenge, which has an analytical and a physiological aspect [209]. When a fixed-diameter punch is taken from these spots, punches with a high haematocrit will contain a higher blood volume, which results in overestimation [209]. Compounds that do not enter erythrocytes will display low blood to plasma ratios (the ratio between the concentration of a compound measured in blood and the concentration measured in plasma). Hence, the presence of erythrocytes may be seen as a dilution of the plasma fraction of whole blood [209], and therefore bridging studies in which both dried blood spots and plasma samples are collected in order to evaluate the correlation between the concentrations are needed. A bridging study has been performed for piperacillin and tazobactam, and found concentrations in dried blood spots to be on average 62 and 52 % lower compared to plasma, suggesting that piperacillin and tazobactam do not partition into red blood cells. A large range in the dried blood spots to plasma ratios

was observed [210]. Moreover, one of the biggest potential advantages of dried blood spots, namely stability has been shown to be insufficient to allow for transportation on room temperature [195]. These findings may limit its use for TDM purposes.

1.3.1.3. Other methods to determine β-lactam antibiotics in blood

1.3.1.3.1 Thermal biosensing

Thermal biosensing detects the heat generated by enzymatic reactions, in this case, the reaction of penicillinase with the β-lactam antibiotic [182]. This technique was used by Chen and colleagues to determine concentrations of penicillin G, penicillin V and ampicillin in whole blood and serum without any sample preparation.

Avoidance of sample preparation with this method reduces the turnaround time (time to which results are available) drastically and could even allow for point of care testing. However, there are issues that need to be resolved first. Chen et al. determined penicillin V, G and ampicillin, which are all susceptible to penicillinase, which is a requirement for the detection principle of this method. Firstly, it is unclear whether this system would be able to monitor meropenem, which is one of the most widely prescribed β-lactam antibiotics, developed to be greatly resistant to β-lactamases. Secondly, other frequently used β-lactam antibiotics are co-formulated with a β-lactamase inhibitor such as amoxicillin/clavulanic acid, piperacillin/tazobactam and ampicillin/sulbactam. Finally, similar to the dried blood spot analysis, bridging studies are needed to correlate the concentrations in whole blood to plasma concentrations.

1.3.1.3.2. Spectrofluorimetric determination

Some β-lactam antibiotics produce fluorescent degradation products. Therefore, the amount of light emitted by the degradation product after acid or alkaline degradation correlates with the initial concentration of the β-lactam antibiotic. Omar and colleagues developed a method to quantify 7 cephalosporins, which used spectrofluorimetric determination based on alkaline degradation. This method is inexpensive as long as a luminescence spectrometer is available. However, a fairly complicated sample pretreatment is needed with strict pH control and therefore it is not readily applicable for routine TDM [183].

1.3.1.3.3.   Immunoassays

An immunoassay measures the concentration of an analyte in a solution using an antibody. Such immunoassays are commercially available for aminoglycosides and glycopeptides and are widely used for TDM of these compounds. Currently, no immunoassays are available for quantification of β-lactam antibiotics in human plasma. However, there are multiple assays available for trace analysis of antibiotics in milk and other food sources, with very fast analysis times [196-207]. Most of these immunoassays only give qualitative results, but some also give quantitative results, however in a concentration range which is far too low for TDM purposes, as these tests are designed to quantify in the µg/L range (which is necessary to detect antibiotic residues in these dietary products) while therapeutic values for these antibiotics in human plasma are 100-1000 times higher.

The advantage of using an immunoassay over the previous described chromatographic methods is that the equipment needed to perform an immunoassay is available in all clinical laboratories and should be easy to use. However, immunoassays can be troubled by interferences and cross-reactivity from similar compounds. Taking into account that patients may be switched from one antibiotic to another, the presence of the previous antibiotic may be problematic if the immunoassay is not sufficiently specific. Moreover, many different immunoassays, each with specific calibrators and controls, should be available in order to quantify all the available β-lactam antibiotics

1.3.1.3.4.   Biosensors

Accurate quantification of small molecules using biosensors is emerging and seems very promising. Important applications of biosensing include glucose measurements in diabetic patients, or detecting bacterial DNA using micro-arrays. A biosensor is made out of 3 parts, firstly a biological sensor, such as an enzyme or a cell, secondly a transducer, which transduces the signal to the third part, the physicochemical detector, which for example has an increased fluorescence intensity when the ligand is added.   Biosensors have been designed for some β-lactams yet they have not been properly validated and compared to a reference method [211, 212].

1.3.1.4. Free drug concentrations

Drugs are bound to serum proteins to varying degrees. The bound fraction is in equilibrium with the free (unbound) fraction. There is increasing interest in measuring this free concentration of antibiotics, given that the free concentration is responsible for bacterial killing as well as toxicity.

The two most relevant drug-binding serum proteins are albumin and α_1-acid glycoprotein. It is often assumed that measuring free concentrations is only advisable for highly bound drugs ($\geq$ 95 % protein binding). Although this may be the case for healthy volunteers and general ward patients, many special patient groups (such as the critically ill, burn patients, undernourished patients or patients with nephrotic syndrome) frequently suffer from hypoalbuminaemia, which may significantly alter protein binding and therefore, basic pharmacokinetic parameters of the free antibiotic such as volume of distribution and clearance [123, 213].

Ultrafiltration is a simple method for measuring protein binding: the plasma sample is transferred to the upper chamber of a two-piece container separated by a molecular weight cutoff filter and when centrifuged separates the free fraction of drug into the lower chamber. However, ultrafiltration may be susceptible to non specific drug adsorption to the container [214] and to variations in the experimental conditions such as pH, temperature and centrifugal force, which has been reported for vancomycin, but also for the β-lactam antibiotics cefazolin and ertapenem [215-217]. Kratzer and colleagues report a free fraction for ertapenem of about 12.5 % at 4°C, but 20% when centrifuged at 37°C.[216] Briscoe and colleagues were the first to report on a method to determine free concentrations of a range of β-lactam antibiotics using ultrafiltration.[178] Connor and colleagues report free concentrations of piperacillin when centrifuged at 4°C, which may not be the ideal temperature to accurately measure free concentrations [185].

Importantly, there are few comparative data between ultrafiltration and the technique that is considered the gold standard, equilibrium dialysis [218]. In equilibrium dialysis, two chambers are separated by a semipermeable membrane. These chambers are filled with serum/plasma and a buffer, respectively. This method needs a long time for equilibrium to be reached, which may pose a problem for some β-lactam antibiotics that are relatively unstable.

Wong and colleagues have compared the measured free concentration (using ultrafiltration) to the free concentration predicted from published protein binding values for seven β-lactam antibiotics using blood samples obtained from critically ill patients. Significant differences between measured and predicted free drug concentrations were found only for highly protein-bound β-lactam antibiotics, such as flucloxacillin (bias of 56.8% overprediction) and ceftriaxone (bias of 83.3% overprediction). No correlation between free and bound concentrations was found for these antibiotics, therefore direct measurement is considered essential for these drugs. For low to moderately protein bound antibiotics (such as piperacillin and meropenem), free concentrations appear to be predictable from the total concentrations [219].

1.3.2 *Measuring β-lactam antibiotics in alternative matrices*

Measuring β-lactam concentrations in other biological fluids may be beneficial, as these fluids may be more closely related to the site of infection. Only methods determining β-lactam antibiotics in cerebrospinal fluid and ultrafiltrate were found in our review with no assays published for TDM in other fluids, such as ascites fluid or epithelial lining fluid.

1.3.2.1   *Cerebrospinal fluid*

Nine articles were found reporting methods that determine β-lactam antibiotics in cerebrospinal fluid. Two methods were found for meropenem [186, 187], 3 for cefepime [188-190], 2 for ceftazidime [191, 192], 1 for ceftriaxone [193] and 1 for cefotaxime [194]. Four out of 9 methods used protein precipitation with acetonitrile as a sample preparation [188, 192-194], 4 did not use any sample preparation [187, 189-191] and one used ultrafiltration [186]. The detection method was LC–UV in 5 cases [186, 188, 192-194] and micellar electrokinetic capillary chromatography in 3 cases [187, 189, 191]. Micellar electrokinetic chromatography has the advantage of eliminating the need for sample preparation, which is advantageous to improve turn-around-time. However, the migration time is quite long (10 minutes) and conditioning between runs is necessary [189-191].

1.3.2.2.   *Renal replacement therapy ultrafiltrate*

Connor et al. investigated the relationship between free plasma concentration of piperacillin and tazobactam and the concentration in dialysate in patients treated with continuous venovenous dialysis in 50 samples from 19 patients and concluded that dialysate

drug concentrations accurately predicted free plasma free drug concentrations ($R^2 = 0.91$ for piperacillin and 0.92 for tazobactam) [185]. However, the most evident deviation from unity was found in the lower-concentration piperacillin data, where dialysate concentrations underestimated plasma concentrations by as much as 50%. Therefore, it is unsure whether ultrafiltrate may replace plasma as a way to measure free concentrations although targeting higher concentrations with this technique may overcome this potential inadequacy.

1.4   General comments on setting up a method for TDM purposes

As routine TDM requires frequent runs (preferably once daily), the consequences of an extensive sample preparation and long runtimes for the laboratory personnel and on equipment occupation are important. Therefore, when developing a method, minimizing sample preparation is desirable, while still retaining sufficient assay sensitivity. The ideal method should be able to measure both low (around MIC values of most commonly causative pathogens), as well as high analyte concentrations without dilution. Moreover, turn around time should be kept to a minimum. As with all bioanalytical methods, the method should be thoroughly validated before it is used in clinical routine. Validation should include assessment of precision and accuracy, linearity, stability, interferences and matrix effect (in cases where mass spectrometry is used as the mechanism of detection). If one of the analytes is a β-lactam which is co-administered with a β-lactamase inhibitor (such as piperacillin/tazobactam or amoxicillin/clavulanic acid), it should be made sure that the β-lactamase inhibitor does not interfere. It is unclear whether or not the β-lactamase inhibitor should also be quantified during TDM, as there is currently no pre-defined target for the β-lactamase inhibitors and it is the βlactam antibiotic that is responsible for the antibacterial effect.

The developed assay should include all commonly used antibiotics, both small as well as broad spectrum agents, as only applying TDM for the broad spectrum antibiotics such as piperacillin and meropenem might lead to unnecessary switch from smaller spectrum antibiotics to these broad spectrum drugs while the reason for therapeutic failure might have been the altered pharmacokinetics of the smaller spectrum antibiotics, and a dose increase might have been enough to ensure optimal efficacy and therefore TDM of smaller spectrum drugs may potentially even spare the broad spectrum and more potent antibiotics.

If highly protein bound antibiotics are to be measured, free concentrations are preferred over total concentrations.

Pre-analytical stability must be guaranteed, as β-lactam antibiotics are generally considered to be quite unstable. However, this limited stability should not confound monitoring as these compounds are still stable for an adequate amount of time. The hospital staff must be aware that stability is limited, and therefore samples should be sent to the lab as soon as the sample is taken, and the isolated plasma should be immediately frozen in the lab.

1.5 Future directions

The best of all cases would be a bedside sample collection device that requires only a small volume of whole blood but immobilizes the proteinaceous and cellular component of the specimen to isolate the free fraction, thus yielding a sample requiring minimal preparation and providing maximal pharmacokinetic information; unfortunately, no such device is currently available.

Presently, TDM of β-lactam antibiotics requires the use of relatively expensive chromatographic techniques. As not all hospitals have this equipment available, easy transportation of the samples to a reference laboratory without the need for costly measures to prevent degradation would be most convenient. Therefore, more research into the utility of dried blood spots would be useful. Recently, two other promising sampling devices have been described. The first is the volumetric absorptive microsampler, which consists of a polymeric tip and is designed to absorb a fixed volume of blood independent of haematocrit [220]. The second device is a paper collection disk bearing plasma from a fingertip drop of blood that can be air dried in fifteen minutes and transported to the lab in a mailing envelope [221].

The cost of personnel to run chromatographic assays is also likely to be higher per sample than for the immunoassays used to measure aminoglycosides and glycopeptides. Therefore, another logical step would be to develop an easy to use assay that does not require expensive equipment or highly trained personnel. Immunoassays are available for the analysis of β-lactam antibiotics in food products, so technically it should be possible, as milk is an equally complex matrix as plasma. Therefore, more research in this field is also

recommended. Liquid chromatography coupled to tandem mass spectrometric detection is a very powerful tool, and has many advantages, such as enhanced specificity and the ability to simultaneously measure multiple analytes in highly complex biological matrices, but the adoption for clinical use has been limited because of instrument cost, expertise, training, quality assurance and standardization [222]. However progress is now being made in these areas with new instruments that allow analysis to be performed by general medical analysts, and the development of more user-friendly workstations with simplified sample preparation procedures. Finally, the commercial availability of reagent kits eliminates method development [222].

Last but not least, measuring free concentrations is an area that is in constant development. More research is needed about how to rapidly determine free concentrations to provide more accurate data.

1.6   Conclusion

Several methods have been developed and validated for TDM of β-lactam antibiotics. As quantification of these antibiotics is presently limited to in-house methods, most of the published methods use chromatographic separation coupled to UV or MS detection. There is currently no immunoassay available for TDM of β-lactam antibiotics.

Although many publications state the method under study is fit for TDM, some may not be considered highly practical because of intensive sample preparation and/or long assay run times. In order to develop a method for routine TDM, rapid sample preparation, short turn around time, low limit of quantification and sufficiently high upper limit of quantification are vital. The antibiotics that require monitoring are dependent on the hospital usage. Indeed, most of the published assays monitored meropenem and piperacillin because they are widely used at many institutions.

There is a growing interest in measuring concentrations in other matrices, such as cerebrospinal fluid, as well as measuring free concentrations. Ideally, a sample would be taken at bedside using a collection device that requires only a small volume of blood and can isolate the free fraction, which would yield a sample that requires minimal sample preparation and would be able to provide maximal pharmacokinetic information, unfortunately, no such device is currently available.

2. Developed analytical methods

2.1. *Quantification of seven β-lactam antibiotics and two β-lactamase inhibitors in human plasma using a validated UPLC-MS/MS method*

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Abstract

There is an increasing interest in monitoring plasma concentrations of β-lactam antibiotics. The objective of this work was to develop and validate a rapid ultra-performance liquid chromatographic method with tandem mass spectrometric detection (UPLC-MS/MS) for simultaneous quantification of amoxicillin, ampicillin, cefuroxime, cefazolin, ceftazidime, meropenem, piperacillin, clavulanic acid and tazobactam. Sample clean-up included protein precipitation with acetonitrile and back-extraction of acetonitrile with dichloromethane. Six deuterated β-lactam antibiotics were used as internal standards. Chromatographic separation was performed on a Waters Acquity UPLC system using a BEH C_{18} column (1.7 μm, 100 x 2.1 mm) applying a binary gradient elution of water and acetonitrile both containing 0.1 % formic acid. The total runtime was 5.5 minutes. The developed method was fully validated in terms of precision, accuracy, linearity, matrix effect and recovery. The assay has now been successfully used to determine concentrations of amoxicillin/clavulanic acid, cefuroxime and meropenem in plasma samples from intensive care patients.

2.1.1. Introduction

Infections are an extremely important problem in critical care medicine. Sepsis alone is the leading cause of mortality in non-cardiac intensive care units [1]. Adequate antibiotic therapy is one of the mainstays in the treatment, with the emphasis on timely administration and appropriateness of the spectrum [24]. The β-lactam antibiotics are central to the treatment of sepsis and life-threatening infections in the intensive care units. They are generally considered as time-dependent antibiotics, which means they exert optimal bactericidal effect when drug concentrations at the site of infection are maintained above the minimum inhibitory concentration (MIC) [40].

Although timely administration and appropriateness of spectrum are important, antibiotic dosing is also highly likely to affect clinical efficacy [39]. Recent data demonstrate that the concentration of antibiotics in plasma and at the site of infection is highly variable and that standard doses of antibiotics lead to underdosing in a considerable number of patients [39, 93]. This is most commonly caused by pharmacokinetic changes in volume of distribution and increased clearance compared to non-critically ill patients and may result in decreased efficacy. On the other hand, overdosing is also possible, leading to toxicity without increased efficacy [39]. This wide spectrum of pharmacokinetics makes empiric dosing choices highly challenging and likely to result in sub-optimal antibiotic exposures or toxicity.

Based on these considerations, monitoring antibiotic concentrations and subsequent dose-adaptation might offer a solution to maximize efficacy and minimize toxicity, especially in patients with considerable pharmacokinetic variability, such as intensive care patients [223, 224].

As current research is primarily focused on the study of broad-spectrum antibiotics, the pharmacokinetic study of narrower-spectrum β-lactams has received little attention. Patient-tailored antibiotic therapy may allow reliable use of the full spectrum of antibiotics available, including narrow-spectrum antibiotics that are only rarely used in most intensive care units. If these antibiotics can be adequately monitored, physicians may be more confident to prescribe them in severe infections. With this in mind, we believe a method capable of the simultaneous quantification of both narrow- and broad-spectrum antibiotics is required. At

this time, the concentration-effect relationship for the β-lactamase inhibitors is poorly described. We decided to incorporate these compounds in this method because if this assay were to be used for therapeutic drug monitoring, patients could perhaps reach toxic levels of these compounds when increasing the penicillin compound in co-formulations. Whilst HPLC methodology for individual analytes and some combinations exists in the literature, no published method is available that simultaneously quantifies the nine drugs we are interested in.

The objective of this report is to describe a newly developed UPLC-MS/MS-analysis for determination of drugs most frequently used in Ghent University hospital : three penicillins (amoxicillin, ampicillin and piperacillin), one carbapenem (meropenem), three cephalosporins (cefuroxime, ceftazidime and cefazolin) and two β-lactamase inhibitors (clavulanic acid and tazobactam).

2.1.2. Materials and methods

Chemicals and reagents

High purity powder of piperacillin, amoxicillin, ampicillin, ceftazidime, cefuroxime, tazobactam, clavulanic acid and bovine serum were purchased from Sigma-Aldrich (Bornem, Belgium), meropenem and cefazolin from Molekula (München, Germany). D_5-piperacillin and D_6- ceftazidime were bought from Alsachim (Strassbourg, France). D_4-amoxicillin, D_5-ampicillin, D_6-meropenem and D_3-cefuroxime were obtained from Toronto Research Chemicals (Ontario, Canada). Sodium dihydrogen orthophosphate and potassium orthophosphoric acid were analytical grade, acetonitrile was LC-MS grade, dichloromethane was HPLC grade. Pure water (CLSI type I), provided by a purification system (Elga LabWater, Analis, Namur, Belgium), was used throughout the study. Blank plasma was obtained from healthy volunteers.

Instruments

The UPLC-MS/MS system consisted of a Waters Acquity UPLC instrument coupled to a TQD triple-quadrupole mass spectrometer (Waters Corp., Milford, MA). Separations were performed on an Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm) with a 1.7 µm particle size equipped with a 0.2 µm precolumn filter unit and a guard column (Waters Corp.,

Milford, MA). Analytes were measured in the multiple reaction monitoring (MRM) mode. The flow rate was set at 0.4 mL/min. The column and autosampler tray temperature were set at 50°C and 4 °C respectively. Ten µL of the extract was injected into the column. The MS/MS instrument was operated with a capillary voltage of 1.00 kV, a source temperature of 140°C and desolvation gas (nitrogen) at 400°C with a flow of 800 L/h. The interchannel delay was 20 ms. Parent and daughter ions, cone voltage and collision energy were optimized by automatic infusion of 1 mg/L in a mixture of 50/50 water/acetonitrile containing 0.1% formic acid, except for clavulanic acid and tazobactam, for which a higher (10 mg/L) concentration was needed. Most analytes were measured in ESI$^+$ mode, only clavulanic acid, tazobactam and cefuroxime gave better signal in ESI$^-$ mode. Dwell times were adjusted individually to obtain an optimal amount of data points to describe the peak. For quantification of amoxicillin, meropenem, ceftazidime, ampicillin, cefuroxime and piperacillin, their deuterated analogue was used as internal standard. D$_4$-amoxicillin was used as internal standard for clavulanic acid and tazobactam, D$_5$-ampicillin was used for cefazolin. For each compound two transitions were followed. One transition was used for quantification (the quantifier), the other transition was monitored for identification (the qualifier). The proportion of these two peak areas is a fixed value which is used for identification. This should not deviate by more than 20 % from the fixed value. The optimized MRM, ratio of the peak areas for both transitions, cone voltage, collision energy and dwell time are listed in table 1. Because of the large number of mass transitions being followed, a separate channel was created for each compound. Breaking up the section into different channels which are all being measured for a specific amount of time allows more scans per analyte. The different channels and the time during which they are being measured is shown in figure 1. Data were acquired using Masslynx 4.1 software and processed using Quanlynx 4.1 software (Waters Corp., Milford, MA).

Chromatographic conditions

The mobile phase consisted of a mixture of solution A (0.1 % formic acid in water) and solution B (0.1 % formic acid in acetonitrile) with an initial composition of 5% solution B. The mobile phase composition changed linearly from 5 % B at 0.5 min to 55 % at 4.0 min, after which the column was rinsed with 95 % B for 0.5 min and re-equilibrated to starting conditions for one minute.

Table 1. Acquisition parameters used in the UPLC-MS/MS method on the Waters TQD detector

Analyte	Parent ion (m/z)	Daughter ions (m/z)	CV (V)	CE (eV)	Dwell time (s)	ESI mode
Clavulanic acid	198.03	108.00	20	11	0.050	-
		136.00		7	0.050	
Amoxicillin	366.16	113.94	20	20	0.050	+
		349.00		10	0.050	
D_4-amoxicillin	370.22	113.90	20	19	0.050	+
Tazobactam	299.11	138.00	22	19	0.050	-
		254.90		7		
Meropenem	384.18	141.03	20	15	0.021	+
		340.13		11	0.021	
D_6-meropenem	390.22	147.00	34	18	0.021	+
Ceftazidime	547.22	167.00	28	30	0.020	+
		468.10		13	0.020	
D_6 -ceftazidime	553.28	167.00	28	23	0.020	+
Ampicillin	350.16	106.00	26	12	0.020	+
		160.00		21	0.020	
D_5 -ampicillin	355.22	111.00	26	18	0.020	+
Cefazolin	455.16	155.90	20	16	0.030	+
Cefuroxime	423.09	207.00	28	15	0.030	-
		318.00		7	0.030	
D_3 -cefuroxime	426.09	210.00	20	20	0.030	-
Piperacillin	518.26	143.00	25	15	0.030	+
		359.09		10	0.030	
D_5 -piperacillin	523.28	148.10	24	20	0.030	+

CV, cone voltage; CE, collision energy ; ESI, electron spray ionisation
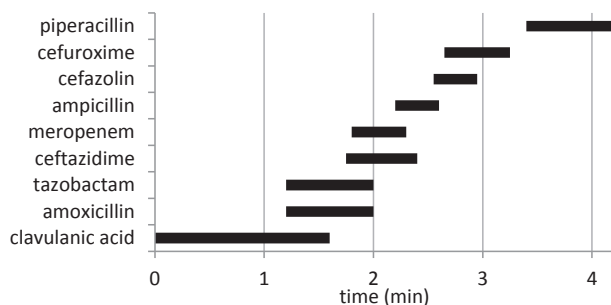
Fig. 1 Use of separate channels for all compounds measured during a specific amount of time increases the amount of scans per analyte

Preparation of standards and quality controls

Calibrators were prepared in 0.066 M aqueous phosphate buffer pH 6, as described by Reyns [225]. Briefly, a stock solution was prepared by weighing 10 mg of each compound was and dissolving these compounds altogether in 100.0 ml phosphate buffer. Aliquots were stored in microfuge tubes at -80°C. The calibrators were prepared freshly from the stock solution with the same buffer.

Quality controls were also prepared in aqueous phosphate buffer. The highest quality control (QCH) was prepared by weighing 8 mg of each compound and dissolving these compounds altogether in 100.0 ml phosphate buffer. Aliquots were stored in microfuge tubes at -80°C. The medium and low concentration quality controls were prepared by diluting QCH with phosphate buffer and were also stored at -80°C.

Stock solutions of the deuterated internal standards were prepared by diluting 1 mg of each internal standard in 10 ml of the appropriate solvent (methanol or water according to the certificate of analysis) and were stored at -80°C. A working solution in methanol was made by adding equal amounts of the stock solution in methanol, and similarly a working solution in water was prepared. These working solutions were also stored at -80°C. Before analysis, these two working solutions were added together and diluted with water to obtain a concentration of 11 mg/L of each internal standard.

Sample preparation

To 20 µl calibrator or quality control in phosphate buffer, 15 µL internal standard and 20 µL of bovine serum was added in a microfuge tube. Similarly, to 20 µL of sample, 15 µL of internal standard and 20 µL of phosphate buffer was added. The tubes were pulse-centrifuged to collect the mixture at the bottom of the tube and were then vortexed using a vortex-mixer for 3 minutes at maximum intensity (1400 rounds per minute) at 4°C. 100 µL of acetonitrile was added to precipitate proteins and the microfuge tube vortex-mixed for 3 minutes at 1400 rounds per minute at 4°C. Precipitated proteins were separated by centrifugation for 5 minutes at 16 000 g. Hundred µl of the supernatant was transferred into another microfuge tube containing 100 µL of water which was automatically vortex-mixed for 3 minutes at 1400 rounds per minute after which 100 µL of dichloromethane was added. The microfuge tube was vortex-mixed for 3 minutes at 1400 rpm to partition the acetonitrile and lipid-soluble plasma components into the dichloromethane organic phase, and centrifuged at 16 000 g for 2 minutes to separate the layers. An aliquot (100 µL) of the upper aqueous phase was transferred to an autosampler vial for chromatographic analysis, and 10 µL was injected into the chromatographic system.

Method validation

Specificity and selectivity

Selectivity was demonstrated by analyzing plasma samples from ten different healthy volunteers and from 25 intensive care patients not receiving any of the studied compounds. The absence of interference of the internal standards was checked by analyzing zero samples, which are blank bovine serum samples spiked with the internal standards. Similarly, blank samples of bovine serum each spiked with the compounds at their highest expectable concentration (without the internal standard) were used to check for absence of interference of the analyte with the internal standard.

Matrix effect and extraction recovery

Matrix effect was assessed as described by Matuszewski et al [226]. This involved determination of the peak areas of the analytes in three different sets of samples: one prepared in blank matrix spiked before extraction (set A), one prepared in blank matrix extract and spiked after extraction (set B), and one in neat mobile phase (set C). The matrix effect was then calculated as a percentage of the response of set B in relation to set C samples. The extraction recovery was determined by the response of set B in relation to set A. These experiments were performed using five different sources of blank matrix from healthy human volunteers at two concentrations.

Linearity

The choice of an appropriate calibration model is necessary for reliable quantification. For this experiment, six calibration samples at nine concentration levels were analyzed. The data were tested for homoscedasticity. When the assumption of homoscedasticity was not met, which was the case for all analytes, a weighting factor was applied. In order to find the appropriate weighting factor and calibration model, the sum of the relative errors for different weighting factors and regression models was calculated. The method that gave the smallest sum of the relative errors was chosen the most appropriate calibration model [227].

Accuracy and precision

Accuracy is used to describe the systematic error in the sense of bias, precision is the closeness of agreement between a series of measurements [228]. Accuracy and precision were determined from the analysis of quality control samples at three concentration levels analyzed in duplicate during eight days. The recommended value for precision and accuracy is ± 15 %, except for the lower limit of quantification (LLOQ), for which a limit of ± 20 % is acceptable[229]. Accuracy was calculated as the percent difference between the mean of the analyzed quality control sample and the true value. The lower limit of quantification (LLOQ) was also determined from the analysis of spiked samples at a low concentration (0.5 mg/L, 0.75 mg/L and 1 mg/L). The total precision was calculated using one-way ANOVA with the varied factor (day) as grouping variable-as described by Peters [230].

To assess whether bovine serum can be used to correct the matrix of the quality controls which are prepared in phosphate buffer the following experiment was conducted : thirty QC samples were assessed using bovine serum to correct the matrix, and thirty QC samples were assessed in the same run using human plasma from five healthy volunteers to correct the matrix. These two groups were compared using a paired t-test.

Application to biological samples

The present method was successfully applied to construct time-concentration profiles of amoxicillin/clavulanic acid, cefuroxime and meropenem in plasma samples obtained from intensive care patients. This study was carried out according to the principles of the Declaration of Helsinki and was approved by the local Ethics Committee. Written informed consent according to local practice was obtained for every patient. Amoxicillin/clavulanic acid and cefuroxime were administered as an intermittent infusion over 30 minutes, meropenem as an extended infusion over 180 minutes. Blood samples were collected into heparinized plasma sampling tubes at different time intervals. These samples were centrifuged promptly after sampling, sent to the lab on ice and frozen at -80°C until analysis

2.1.3. Results

Chromatography

The total run time was 5.5 minutes. Fig. 2 shows representative MRM chromatograms for all analytes obtained from human serum spiked with the seven β-lactam antibiotics and two β-lactamase inhibitors.

Specificity and selectivity

Nor the blank plasma from healthy volunteers or intensive care patients who did not receive any of the studied compounds, nor the zero samples were associated with any detectable interference. The blank samples of bovine serum that were spiked with the compounds at their highest expected concentrations also showed no interference of the analyte with the internal standard or with other (co-eluting) compounds.

Recovery and matrix effect

Mean absolute recovery (AR) for all compounds (determined on 5 sources of different blank plasma from healthy volunteers spiked at two concentrations) ranged from 60 to 72 %, with a maximum relative standard deviation (RSD) less than 15 %. For all compounds, ion enhancement was observed. The mean matrix effect ranged from 105 % to 135 % with a maximum RSD of less than 15 %. Table 2 summarizes the data for absolute recovery and matrix effects for the nine compounds.

Linearity

All compounds could be quantified between 0.5 mg/L and 100 mg/L, except for piperacillin, which was quantified between 1.5 mg/L and 100 mg/L. Because of the wide calibration range, a weighting factor was found to be necessary.

Amoxicillin and ceftazidime gave best results using linear regression and a weighting factor $1/x^2$. For meropenem, cefazolin, cefuroxime, clavulanic acid and tazobactam, quadratic regression and weighting factor $1/x$ provided optimal results. The best calibration model for piperacillin was found to be quadratic using weighting factor $1/x^2$.

Table 2
Absolute recovery (AR) and matrix effect (ME) for the analyzed compounds in order of retention time

Compound	AR (%)	RSD (%)	ME (%)	RSD (%)
clavulanic acid	68.1	14.6	117.0	9.8
amoxicillin	61.2	11.3	131.4	11.3
tazobactam	71.5	9.2	108.9	7.8
ceftazidime	67.3	8.5	128.5	12.2
meropenem	59.9	13.1	125.0	5.6
ampicillin	65.9	6.0	114.8	7.9
cefazolin	66.8	10.6	115.7	6.9
cefuroxime	69.4	9.1	104.1	12.2
piperacillin	67.2	9.7	136.2	14.7

AR, absolute recovery ; RSD relative standard deviation ; ME, matrix effect
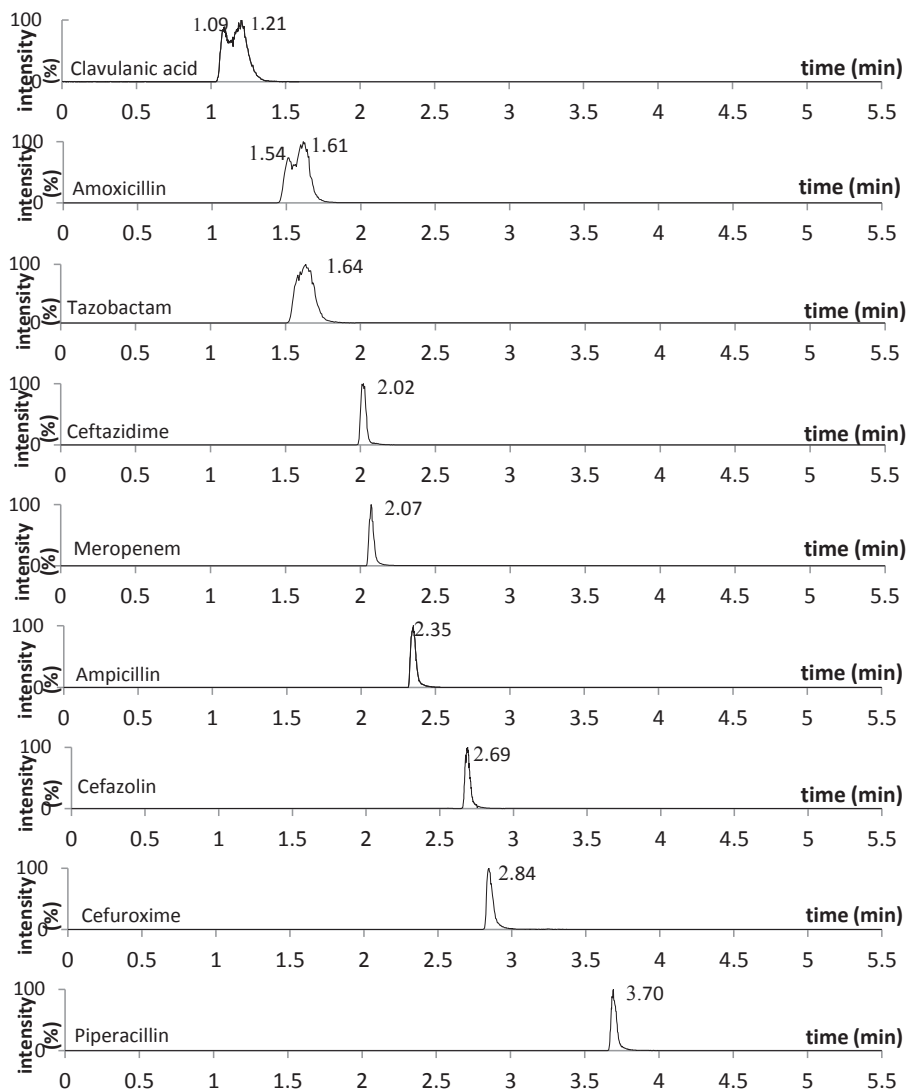
Fig. 2 MRM chromatograms and retention times for a mixture of the compounds at a concentration of 10 mg/mL spiked to blank plasma.

Accuracy, precision and limit of quantification

Data on total precision are summarized in Table 3. The LLOQ of each compound was 0.5 mg/L, except for piperacillin (1.5 mg/L). The total precision ranged from 10 to 20 % at LLOQ-

level, and from 3 to 15 % at higher levels. Mean accuracy values ranged from 89.9 to 101.5 %.

There was no difference between the QC samples corrected with bovine serum and the QC samples corrected with human plasma (p > 0.05 for all nine compounds at 3 different concentrations).

Application to biological samples

The results are shown in figures 3 to 6. Fig. 3 shows plasma concentrations of meropenem from a patient with acute kidney injury. As β-lactam antibiotics are principally eliminated by the kidneys, this explains the high concentration reached in this patient. Figure 4, 5 and 6 clearly state the pharmacokinetic variability in intensive care patients.
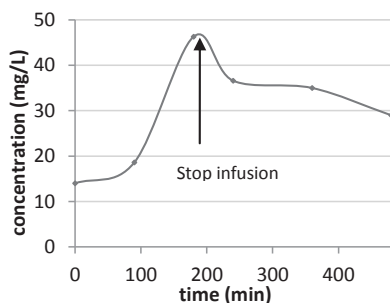
Fig. 3 Time-concentration profile of 1 g meropenem administered as an extended infusion over a period of 240 minutes (n=1)
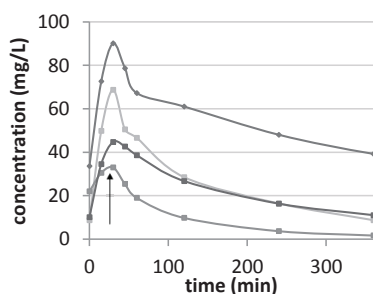
Fig. 4 Time-concentration profile of 1 g amoxicillin administered as an intermittent infusion over a period of 30 minutes , co-administered with 0.2 g clavulanic acid (n=4).
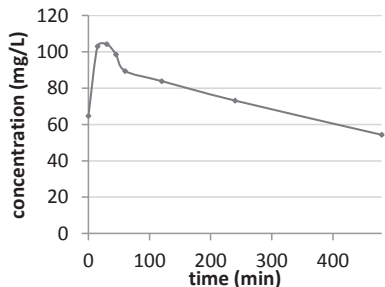
Fig. 5 Time-concentration profile of 1 g cefuroxime administered as an intermittent infusion over a period of 30 minutes (n=1).
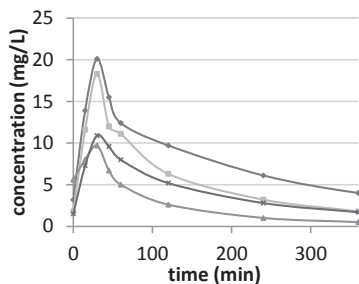
Fig. 6 Time-concentration profile of 0.2 g clavulanic acid administered as an intermittent infusion over a period of 30 minutes, co-administered with 1 g amoxicillin (n=4).

Table 3

Total precision and accuracy.

	LLOQ (mg/L)			QCL (mg/L)			QCM (mg/L)			QCH (mg/L)		
	mean ± SD	RSD (%)	Accuracy (%)	mean ± SD	RSD (%)	accuracy (%)	mean ± SD	RSD (%)	accuracy (%)	mean ± SD	RSD (%)	accuracy (%)
Clavulanic acid	0.51 ± 0.05	12.3	99.5	1.44 ± 0.13	7.4	94.6	13.2 ± 0.46	3.0	86.8	45.0 ± 3.3	7.3	100.1
Amoxicillin	0.50 ± 0.05	10.7	100.0	1.49 ± 0.16	10.5	99.2	15.3 ± 1.1	7.0	101.5	80.6 ± 7.2	9.0	100.5
Tazobactam	0.47 ± 0.08	15.9	93.8	1.48 ± 0.20	13.9	98.3	14.7 ± 1.1	7.5	97.7	47.4 ± 3.5	7.8	94.7
Ceftazidime	0.44 ± 0.09	19.2	92.4	1.31 ± 0.16	11.6	91.1	13.6 ± 1.2	8.5	94.5	70.5 ± 5.0	6.5	91.7
Meropenem	0.53 ± 0.07	14.8	99.0	1.59 ± 0.24	15.0	94.2	15.7 ± 1.5	10.4	95.6	79.7 ± 8.2	10.3	99.7
Ampicillin	0.50 ± 0.07	13.7	98.0	1.38 ± 0.17	11.1	89.9	14.8 ± 0.87	5.7	96.2	77.8 ± 5.2	7.2	95.0
Cefazolin	0.46 ± 0.05	10.6	98.4	1.34 ± 0.11	8.4	95.5	13.9 ± 0.81	6.0	99.7	72.7 ± 4.4	6.3	97.7
Cefuroxime	0.49 ± 0.05	12.5	97.5	1.48 ± 0.13	9.6	98.7	15.0 ± 1.2	7.9	99.8	77.9 ± 5.6	7.0	97.7
Piperacillin	1.59 ± 0.19	12.8	98.5	1.59 ± 0.19	12.8	98.5	15.1 ± 0.48	3.1	96.0	79.1 ± 3.5	4.1	94.3

LLOQ, lower limit of quantification; QCL, quality control low level; QCM, quality control medium level; QCH, quality control high level

2.1.4. Discussion

Monitoring the concentrations of β-lactam antibiotics in plasma might be a useful tool for optimizing the treatment of patients, especially those with altered pharmacokinetics, such as critically ill patients. To enable maximal use of the full antibiotic spectrum, monitoring of narrow-spectrum antibiotics is likely to be valuable.

In this study, we validated an UPLC-MS/MS method for the simultaneous quantification of seven β-lactam antibiotics and two β-lactamase inhibitors in human plasma. We used gradient elution, which gave optimal separation within minimal time limits. Because of their hydrophilic behavior, clavulanic acid, amoxicillin and tazobactam show minimal retention on conventional reversed-phase C_{18} columns. The peak shape of these compounds could be improved by starting at a very low percentage of solution B and steeply increasing this percentage. However, this approach was not further used as all nine compounds eluted at the same time (data not shown). Other gradient curve types or other percentages did not solve this problem. No problems have been encountered during validation due to the biphasic peak shape of clavulanic acid, amoxicillin and tazobactam. Full separation of the nine compounds was not possible: amoxicillin and tazobactam both elute at the same time, the same applies for meropenem and ceftazidime. As tested in the selectivity experiments, this did not cause any problems because of the high selectivity of the detector. The method has been shown to be accurate and precise, as acceptance criteria were met for all compounds (accuracy between 85-115 %, precision within 15 %).

The major advantage of our method is its speed of analysis (5.5 minutes per sample), which is considerably faster than other methods for multiple analytes previously reported [168, 171, 174, 175, 179, 231-234]. This allows high sample throughput and enables fast reporting of the results if this method were to be used for therapeutic drug monitoring. Moreover, our sensitivity was significantly better than reported by other methods [168, 171, 174]. Our limit of quantification was 0.5 mg/L for all components, except for piperacillin (1.5 mg/L). Sufficient sensitivity could be useful, as for many bacteria the MIC is than 1 mg/L [235]. An additional advantage of our method is its low sample volume as it requires only 20 µL plasma. We have also confirmed the clinical suitability of this method and have applied it to the analysis of plasma samples obtained from patients. A potential drawback of this assay

is the fact that it only measures total antibiotic concentrations, although the unbound concentration is pharmacologically active. Determining the unbound concentration can be done using ultracentrifugation for patients where this information is deemed essential.

2.2 *Ultrafast quantification of β-lactam antibiotics in human plasma using UPLC-MS/MS*

Authors : Mieke Carlier, Veronique Stove, Jan J. De Waele, Alain G. Verstraete

Abstract

There is an increasing interest in monitoring plasma concentrations of β-lactam antibiotics. The objective of this work was to develop and validate a fast ultra-performance liquid chromatographic method with tandem mass spectrometric detection (UPLC-MS/MS) for simultaneous quantification of amoxicillin, cefuroxime, ceftazidime, meropenem and piperacillin with minimal turn around time. Sample clean-up included protein precipitation with acetonitrile containing 5 deuterated internal standards, and subsequent dilution of the supernatant with water after centrifugation. Runtime was only 2.5 minutes. Chromatographic separation was performed on a Waters Acquity UPLC system using a BEH C_{18} column (1.7 μm, 100 x 2.1 mm) applying a binary gradient elution of water and methanol both containing 0.1 % formic acid and 2 mmol/L ammonium acetate on a Water TQD instrument in MRM mode. All compounds were detected in electrospray positive ion mode and could be quantified between 1 and 100 mg/L for amoxicillin and cefuroxime, between 0.5 and 80 mg/L for meropenem and ceftazidime, and between 1 and 150 mg/L for piperacillin. The method was validated in terms of precision, accuracy, linearity, matrix effect and recovery and has been compared to a previously published UPLC-MS/MS method.

2.2.1. Introduction

There is a growing interest in monitoring plasma concentrations of β-lactam antibiotics, as concentrations are variable in critically ill patients with both very low and high concentrations occurring [236]. Low concentrations are most commonly caused by changes in volume of distribution and increased clearance compared to non-critically ill patients which may result in decreased efficacy [39]. On the other hand, overdosing is also possible in the presence of organ dysfunction such as acute kidney injury, leading to toxicity without increased efficacy [39]. Because of the wide spectrum of pathophysiological changes in critically ill patients, antibiotic concentrations are very difficult to predict. Furthermore, recent data suggest that there is a relationship between serum concentrations of β-lactams and clinical outcomes in the critically ill [49]. Based on these considerations, therapeutic drug monitoring (TDM) of antibiotic concentrations and subsequent dose-adaptations might offer a solution to maximize efficacy and minimize toxicity.

TDM of β-lactam antibiotics requires an assay method with a short turn around time in order to allow a quick dose adaptation. However, unlike TDM of aminoglycosides and glycopeptides, for which immunoassays have been developed and are commercially available, quantification of β-lactam antibiotics are generally limited to in-house developed methods using liquid chromatography coupled to ultraviolet detection or liquid chromatography coupled to mass spectrometric detection. Most methods use extensive sample clean up and have fairly long run times [168, 169, 171, 173, 237]. Although some of them state they are fit for daily TDM, the extensive sample preparation and fairly long runtime do not make them an ideal method, unless an instrument is dedicated to only this assay.

The objective of this report is to describe a newly developed ultra performance liquid chromatographic tandem mass spectrometric (UPLC-MS/MS) analysis which has been optimized to ensure minimal turn around time for the determination of 5 frequently used β-lactam antibiotics: two penicillins (amoxicillin and piperacillin), one carbapenem (meropenem), two cephalosporins (cefuroxime and ceftazidime).

2.2.2   Materials and methods

Chemicals and reagents

High purity powder of piperacillin, amoxicillin, ceftazidime, cefuroxime, meropenem and bovine serum were purchased from Sigma-Aldrich (Bornem, Belgium). The deuterated standards D_5-piperacillin, D_4-amoxicillin, D_6-meropenem, D_5-ceftazidime and D_3-cefuroxime were obtained from Toronto Research Chemicals (Ontario, Canada). Pure water (Clinical Laboratory Reagent Water standard), provided by a purification system (Elga LabWater, Analis, Namur, Belgium), was used throughout the study. Blank plasma was obtained from healthy volunteers.

Instruments

The UPLC-MS/MS system consisted of a Waters Acquity UPLC instrument coupled to a TQD triple-quadrupole mass spectrometer (Waters Corp., Milford, MA). Separations were performed on an Acquity UPLC BEH C_{18} column (100 mm x 2.1 mm) with a 1.7 µm particle size equipped with a 0.2 µm precolumn filter unit and a guard column (Waters Corp., Milford, MA). Analytes were measured in the multiple reaction monitoring (MRM) mode. The flow rate was set at 0.4 mL/min. The column and autosampler tray temperature were set at 50°C and 4 °C respectively. Forty µL of the extract was injected into the column. The MS/MS instrument was operated with a capillary voltage of 1.00 kV, a source temperature of 140°C and desolvation gas (nitrogen) at 400°C with a flow of 800 L/h. Parent and daughter ions, cone voltage and collision energy were optimized by infusion of 1 mg/L in a mixture of 50/50 water/methanol containing 0.1% formic acid and 2 mM ammonium acetate. All analytes were measured in the electrospray positive (ESI$^+$) mode. For each of the antibiotics, a deuterated analogue was used as internal standard. The optimized MRM, cone voltage, collision energy and dwell time are listed in table 1. Data were acquired using Masslynx 4.1 software and processed using Quanlynx 4.1 software (Waters Corp., Milford, MA).

Table 1. Acquisition parameters used in the UPLC-MS/MS method on the Waters TQD detector

Analyte	Parent ion (m/z)	Daughter ion (m/z)	CV (V)	CE (eV)	Dwell time (s)
Amoxicillin	366.1	114.0	18	20	0.150
D_4-amoxicillin	370.2	114.0	16	20	0.150
Meropenem	384.2	141.2	26	15	0.045
D_6-meropenem	390.2	147.0	26	15	0.045
Ceftazidime	547.1	468.0	20	12	0.045
D_5-ceftazidime	552.0	468.0	24	12	0.045
Cefuroxime	442.2	364.1	20	10	0.045
D_3-cefuroxime	445.1	367.1	18	9	0.045
Piperacillin	518.2	143.1	32	17	0.045
D_5-piperacillin	523.3	148.1	32	17	0.045

CV, cone voltage; CE, collision energy

Chromatographic conditions

The mobile phase consisted of a mixture of solution A (0.1 % formic acid and 2 mM ammonium acetate in water) and solution B (0.1 % formic acid and 2mM ammonium acetate in methanol) with an initial composition of 2% solution B. The mobile phase composition changed from 2 % B at 0.4 min to 98 % at 0.5 min, after which the column was rinsed with 98 % B for one minute and re-equilibrated to starting conditions for another minute. Total runtime was 2.5 minutes.

Preparation of standards and quality controls

Calibrators were prepared in bovine serum. A stock solution was prepared by weighing 10-20 mg of each compound and dissolving these compounds altogether in 10.0 mL water, which was then diluted 10 times in bovine serum to prepare the highest calibrator. The other calibrators were prepared by diluting the highest calibrator with bovine serum. Aliquots were stored in microfuge tubes at -80°C.

Quality controls were also prepared in bovine serum. A stock solution was prepared by weighing 8-18 mg of each compound and dissolving these compounds altogether in 10.0 mL water, which was then diluted 10 times in bovine serum to prepare the highest quality control. Aliquots were stored in microfuge tubes at -80°C. The medium and low concentration quality controls were prepared by diluting QCH with bovine serum and were also stored at -80°C.

Stock solutions of the deuterated internal standards were prepared by diluting 1 mg of each internal standard in 10 ml of the appropriate solvent (methanol or water according to the certificate of analysis). Aliquots were stored at -80°C. A working solution was prepared freshly for each analysis by adding 35 µL of each stock solution to 1325 µL acetonitrile.

Sample preparation

To 15 µL calibrator, quality control or patient sample, 100 µL internal standard working solution was added in a microfuge tube. The tubes were vortexed using a vortex-mixer (Eppendorf, Hamburg, Germany) for 3 minutes at maximum intensity (1400 rounds per minute). Precipitated proteins were separated by centrifugation for 5 minutes at 16 000 g. Hundred µL of the supernatant was transferred into an autosampler vial which contained 400 µL of water, which were then vortexed using a vortex-mixer for 3 minutes at maximum intensity, after which they were ready for chromatographic analysis. Forty µL was injected into the chromatographic system.

Method validation

Specificity and selectivity

Selectivity was demonstrated by analyzing plasma samples from ten different healthy volunteers and from 10 intensive care patients not receiving any of the studied compounds. The absence of interference of the internal standards was checked by analyzing zero samples, which are blank bovine serum samples spiked with the internal standards. Similarly, blank samples of bovine serum each spiked with the compounds at their highest expectable concentration (without the internal standard) were used to check for absence of interference of the analyte with the internal standard.

Matrix effect and extraction recovery

Matrix effect was assessed as described by Matuszewski et al [226]. This involved determination of the peak areas of the analytes in three different sets of samples: one prepared in blank matrix spiked before extraction (set A), one prepared in blank matrix extract and spiked after extraction (set B), and one in neat mobile phase (set C). The matrix effect was then calculated as a percentage of the response of set B in relation to set C samples. The extraction recovery was determined by the response of set B in relation to set A. These experiments were performed using four different sources of blank matrix from healthy human volunteers, and 1 source from bovine serum (analyzed four times) at a low (between 1.5 and 4 mg/L depending on the compound) and high (between 80 and 150 mg/L depending on the compound) concentration.

Linearity

For this experiment, six calibration samples at six concentration levels were analyzed. In order to find the appropriate weighting factor and calibration model, the sum of the relative errors for different weighting factors and regression models was calculated. The method that gave the smallest sum of the relative errors was chosen the most appropriate calibration model [227].

Accuracy and precision

Accuracy and precision were determined from the analysis of quality control samples at 4 concentration levels (lower limit of quantification (LLOQ), low quality control (QC), medium QC and high QC). Within-run accuracy and precision were determined by analysis of 5 replicates in a single run for each concentration level. For assessment of the between-run accuracy and precision, 1 measurement of 4 samples at different concentration levels was performed over 8 different days. In order to pass validation, the mean concentration should be within 15 % of the nominal value (except for the LLOQ where 20 % is tolerated) for both within- as well as between run accuracy [229]. The coefficient of variation (CV) for both within- as well as between-run precision should not exceed 15 % (except for the LLOQ where 20 % is tolerated) [229].

Carryover

Carryover was assessed according to EMA guidelines by injection of a blank sample after the highest standard. The peak area of the analyte in the blank sample must be less

than 20% of the peak area of the LLOQ of the analyte and less than 5% of the peak of the internal standard [229]. This experiment was performed in triplicate.

Stability

Stability of the internal standard working solution

Stability of the internal standard working solution was assessed by comparing the response of the calibrators injected directly after sample preparation and the response of a new calibrator set extracted with an internal standard working solution which had been stored for 15 h at 4°C. The internal standard working solution was considered stable if there was no significant difference using the related samples Wilcoxon signed rank test for each of the antibiotics.

Autosampler stability of the extracts

The stability of plasma-extracts was evaluated by repeated injection after 15 h in the autosampler at 4°C using blank plasma from 5 different volunteers spiked at 3 concentrations (low, medium, high). The extracts were considered stable if the confidence interval for the mean degradation did not include -10%.

Freeze thaw stability

The influence of freeze thaw cycles was investigated by analyzing plasma, taken from 5 different volunteers, and spiked at 3 concentrations (low, medium, high) after 0, 1, 2 or 3 freeze thaw cycles. One freeze thaw cycle consisted of thawing the 80 µL sample 15 min at room temperature, after which it was transferred to -80°C for one hour. The concentrations were compared for each concentration level using an analysis of variance (ANOVA) test, a p value ≤0.05 was considered significant. The samples with a p value >0.05 where considered stable. For the samples with a p value ≤ 0.05 , the percentage degradation was calculated. These samples were still considered stable if the confidence interval for the mean degradation did not include -10%.

Method comparison with previously published method

Patient samples that have previously been collected for were re-analyzed using this new method and using our previously published method, which has a more extensive clean-up and different chromatographic conditions [169]. We compared the results using Bland and

Altman plots. For study samples, the difference between the two values obtained should be within 20% of the mean for at least 67% of the repeats, as specified by the European Medicine Agency (EMA) [229] .

2.2.3 Results

Chromatography

The total run time was 2.5 minutes. Fig. 1 shows representative MRM chromatograms for all analytes obtained from human plasma spiked with the 5 β-lactam antibiotics at LLOQ. We decided to measure all compounds in the ESI$^+$ mode. Because cefuroxime normally requires ESI$^-$ mode, we measured the ammonium adduct for cefuroxime in positive mode (M + 18). Because of its hydrophilicity, amoxicillin showed almost no retention on the column and eluted after only 0.8 minutes. Retention could be improved by injecting a smaller volume of sample onto the column (10 µL instead of 40 µL), however, this resulted in a decreased signal to noise ratio. As we used a deuterated internal standard, we were able to compensate for this effect.

Validation

Specificity and selectivity

No detectable interferences were found when analyzing the blank plasma from healthy volunteers or intensive care patients who did not receive any of the studied compounds, and the zero samples. The blank samples of bovine serum that were spiked with the compounds at their highest expected concentrations also showed no interference of the analyte with the internal standard or with other compounds.

Recovery and matrix effect

Mean absolute recovery (AR) for all compounds ranged from 67 to 100 %, with a maximum relative standard deviation (RSD) less than 11 %. For meropenem, ion enhancement was observed, while for amoxicillin, there was ion suppression. Table 2 summarizes the data for recovery and matrix effects, both absolute and compensated by the internal standards and shows that the internal standard compensates very efficiently for the ion suppression for amoxicillin and the ion enhancement of meropenem.
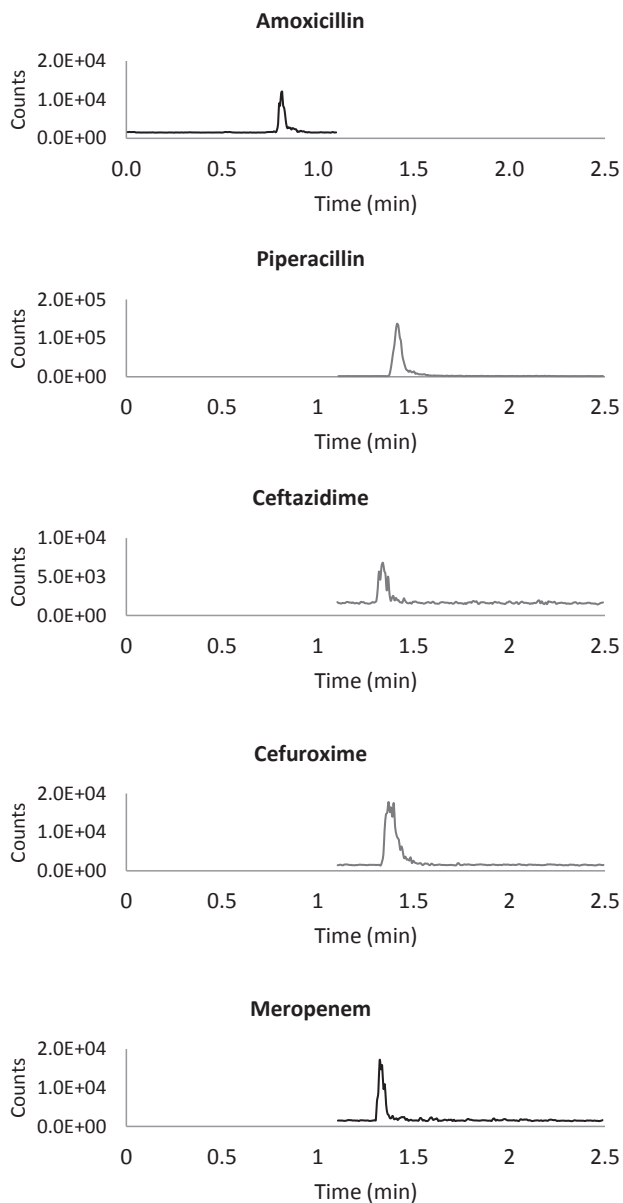
Fig. 1 Multiple reaction monitoring chromatograms for a mixture of compounds at LLOQ spiked in blank plasma (0.5-1 mg/L depending on the compound)

Linearity

A linear regression curve using weighting factor 1/x provided best results for meropenem, piperacillin and amoxicillin, a quadratic curve using weighting factor 1/x for cefuroxime, and a quadratic curve using weighting factor $1/x^2$ for ceftazidime.

Accuracy, precision and limit of quantification

Data on total precision are summarized in Table 3. The LLOQ of each compound was between 0.52 and 1.1 mg/L. For the different analytes, the between-run imprecision ranged from 4.8 to 16.7 % at LLOQ-level, and from 1.6 to 10.8 % at higher levels. Within-run imprecision ranged from 3.7 to 11.4 % at LLOQ level, and from 1.0 to 4.8 % at higher levels. Within-run accuracy values ranged from 92.6 to 107.6 %. Between-run accuracy ranged from 95.8 to 109.2 %.

Carryover

No peak was found in the blank sample for all tested antibiotics, except for piperacillin. The peak found in the blank sample was only 10 % of the peak of the LLOQ of piperacillin. No peak was found for the internal standards. Therefore all antibiotics passed the acceptance criterion.

Stability

Stability of the internal standard working solution

For each of the five tested antibiotics, there was no statistically significant difference (p= 0.916 for piperacillin, p=0.5 for meropenem, p=0.173 for cefuroxime, p=0.6 for ceftazidime, p=0.345 for amoxicillin) between the responses for the 6 calibrators using a freshly prepared internal standard working solution or using a working solution that has been stored at 4°C for 15 h.

Autosampler stability of the extracts

The confidence interval of the mean degradation did not encompass -10 % for all analytes.

<u>Freeze/thaw stability</u>

For meropenem, ceftazidime and amoxicillin, the ANOVA test showed no significant difference between the concentrations of the samples at each of the tested levels. For piperacillin, the ANOVA test revealed a difference for the low and high level control sample, and for cefuroxime for the medium control sample. However, the confidence interval for the mean degradation did not encompass -10%. Therefore, all analytes are considered stable during 3 freeze/thaw cycles.

Method comparison

The Bland and Altman plots are shown in figure 2. The mean difference ranged between -2.1 and + 3.7 %. For meropenem, piperacillin cefuroxime and ceftazidime, none of the samples differed by more than 20 % of the mean. For amoxicillin, 2 out of 34 samples differed by more than 20 % of the mean.

Table 2. Matrix-effect and recovery

Compound		Low concentration		High concentration	
		Matrix effect (%) ± CV	Recovery (%) ± CV	Matrix effect (%) ± CV	Recovery (%) ± CV
Amoxicillin					
Absolute	Human plasma	48 ± 6	93 ± 11	55 ± 6	85 ± 6
	Bovine serum	47 ± 9	85 ± 5	50 ± 3	100 ± 6
Compensated	Human plasma	95 ± 8	95 ± 15	99 ± 2	99 ± 2
	Bovine serum	100 ± 5	91 ± 10	97 ± 2	98 ± 3
Ceftazidime					
Absolute	Human plasma	83 ± 27	83 ± 11	81 ± 20	75 ± 4
	Bovine serum	102 ± 21	67 ± 7	85 ± 8	86 ± 3
Compensated	Human plasma	91 ± 8	103 ± 13	100 ± 5	88 ± 5
	Bovine serum	102 ± 2	89 ± 9	101 ± 2	104 ± 2
Cefuroxime					
Absolute	Human plasma	98 ± 6	87 ± 6	86 ± 9	86 ± 8
	Bovine serum	107 ± 9	88 ± 8	93 ± 3	98 ± 3
Compensated	Human plasma	102 ± 5	105 ± 4	100 ± 3	97 ± 9
	Bovine serum	102 ± 9	111 ± 6	100 ± 2	110 ± 5
Meropenem					
Absolute	Human plasma	165 ± 16	81 ± 7	139 ± 16	78 ± 4
	Bovine serum	192 ± 13	73 ± 4	145 ± 7	81 ± 3
Compensated	Human plasma	100 ± 10	107 ± 12	99 ± 3	103 ± 3
	Bovine serum	103 ± 4	106 ± 5	100 ± 6	110 ± 5
Piperacillin					
Absolute	Human plasma	107 ± 11	91 ± 3	93 ± 7	85 ± 5
	Bovine serum	107 ± 7	85 ± 2	97 ± 2	99 ± 2
Compensated	Human plasma	106 ± 14	105 ± 4	99 ± 3	97 ± 6
	Bovine serum	100 ± 9	100 ± 1	99 ± 2	112 ± 3

CV: coefficient of variation

Table 3. Imprecision and accuracy

Compound	Nominal concentration(mg/L)	Within run accuracy (%) (n=5 in a single run)	Between run accuracy (%) (n=8 on 8 different days)	Within run imprecision (% CV) (n=5 in a single run)	Between run imprecision (% CV) (n=8 on 8 different days)
Amoxicillin	1.05 - 3.31 - 53.57 - 125.3	107.6-98.1-96.3-93.1	102.8-109.2-106.3-97.2	7.0-4.8-1.4-1.8	16.7-10.8-9.8-8.3
Ceftazidime	0.62 - 1.85 - 36.5 - 88.7	98.9-101.7-94.0-94.1	98.6-106.9-105.6-95.8	11.3-2.9-4.6-3.3	11.9-7.7-6.6-6.6
Cefuroxime	0.81 - 2.55 - 49.4 - 116.1	98.6-98.7-96.0-94.7	102.7-106.3-107.0-100.2	7.1-3.3-2.9-4.4	4.8-4.2-3.3-1.6
Meropenem	0.52 - 1.54 - 30.6 - 75.4	100.9-99.0-97.8-97.2	105.6-103.3-101.1-101.8	11.4-2.1-3.1-4.6	9.1-6.0-2.8-4.7
Piperacillin	1.09 - 3.15 - 63.0 - 156.8	98.9-95.7-93.4-92.6	99.5-100.8-100.3-100.0	3.7-1.6-2.2-1.0	8.1-3.6-2.9-2.6

CV : coefficient of variation
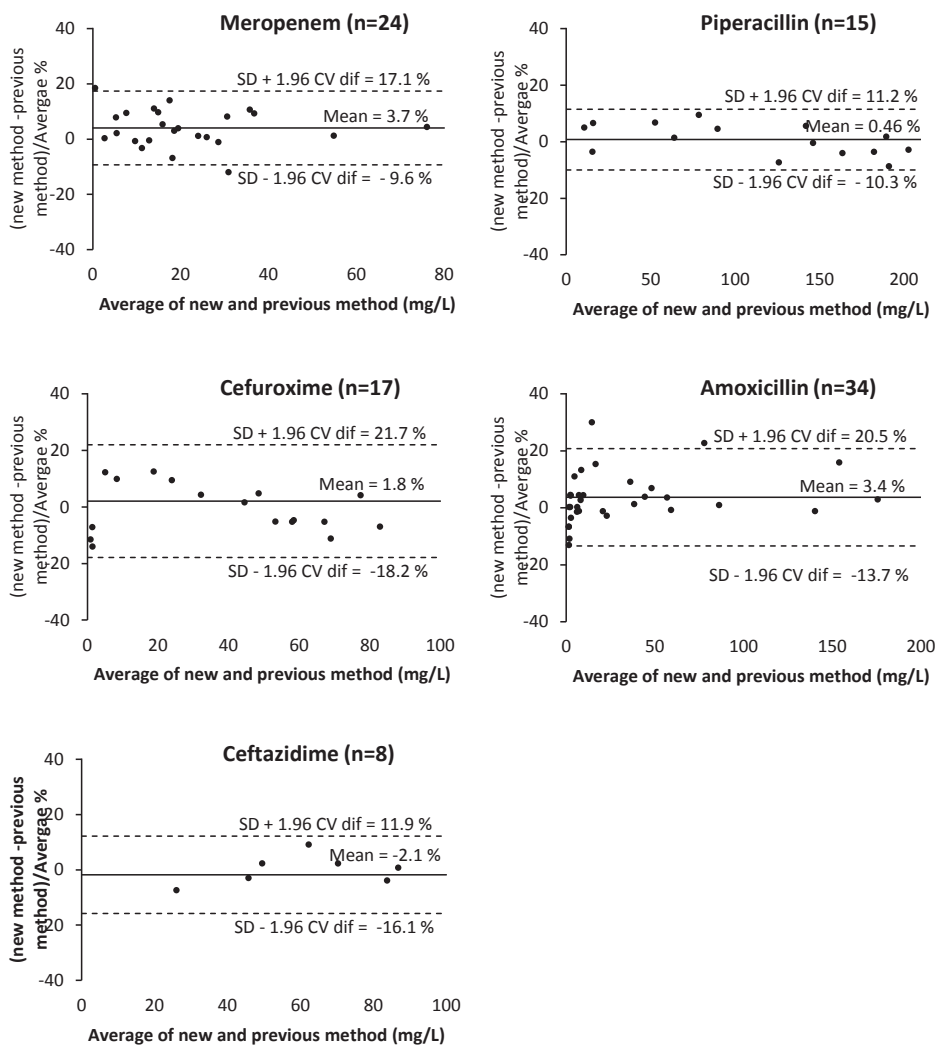
Fig 2. Method comparison: Bland and Altman plots

Black line: mean difference (%) , dashed lines : SD ± 1.96 CV dif : standard deviation of the average difference ± 1.96 times the coefficient of variation (CV) of the average difference.

2.2.4. Discussion

In this study, we validated a UPLC-MS/MS method for the simultaneous quantification of 5 frequently used β-lactam antibiotics. Bovine serum was used as a matrix for the calibrator and control solutions as this can be easily purchased and it has an accompanying certificate

of analysis, guaranteeing a constant composition and the absence of infectious agents. As bovine serum is not identical to human plasma, it is of utmost importance to investigate the matrix effect of both human plasma and bovine serum, used for preparation of the calibrators and control samples. The internal standard working solution is stable for up to 15 h when stored at 4°C, and the extracts are stable for at least 15 h in the autosampler at 4°C. The pre-analytical stability of the plasma samples has been investigated and reported separately. We found that amoxicillin, piperacillin and meropenem are stable for at least 4 h at room temperature followed by 4 h of storage at 4°C [169].

To ensure minimal turn around time, we used minimal sample preparation including protein precipitation and subsequent dilution. We did not focus on full chromatographic separation of the compounds, because the subsequent mass spectrometric detection offers plenty of selectivity and specificity. The method has been shown to be accurate and precise. Only few publications are describing a method for quantification of β-lactam antibiotics using deuterated standards [169, 170]. We assume this is because of the cost of deuterated analogues. However, because of our small sample volume, we only add 285 ng of deuterated standard to each sample. Consequently, one mg of deuterated standard is enough for analyzing 3500 samples. It is clear that the cost of this deuterated internal standard is only a mere fraction of the total cost, mostly consisting of the depreciation of the UPLC-MS/MS instrument and the personnel costs. Moreover, using a deuterated internal standard has significant advantages, such as ideal compensation for undesired effects.

The major advantage of our method is its speed, as it includes minimal sample preparation, and a chromatographic runtime of only 2.5 minutes per sample, which is considerably faster than all other methods for multiple analytes previously reported. This allows high sample throughput and enables fast reporting of the results. Sample preparation of a run consisting of 6 calibrators, 3 internal quality control samples and 10 patient plasma samples would take up maximum of 40 minutes sample preparation (the initial centrifugation of the blood samples not taken into account) and less than 50 minutes chromatographic runtime. Therefore, the workload associated with this daily TDM of β-lactam antibiotics is low. Using this method, TDM of β-lactams can be easily combined with TDM of other drugs, such as immunosuppressive drugs, on the same instrument, which in our case also use the same chromatographic solvents. Hence, delays due to through priming and equilibration of solvents are minimized. Another advantage of our method is that minimal sample volume is needed (15

µL plasma), which is relevant in critically ill patients and in neonates. A potential drawback of this assay is the fact that it only measures total antibiotic concentrations, although the unbound concentration is pharmacologically active. However, research has shown that for low to moderately protein bound drugs, the free concentration can be estimated from the total concentration [219].

2.2.5. Conclusion

In this study, we validated an ultrafast UPLC-MS/MS method for the simultaneous quantification of 5 frequently used β-lactam antibiotics. To ensure minimal turn around time, we used a minimal sample preparation including protein precipitation and subsequent dilution. We have performed a method comparison and have shown comparable results to our previous published assay, which confirms confirmed the clinical suitability of this method

3.   **Exploration of the pre-analytical stability of β-lactam antibiotics in plasma and blood: implications for therapeutic drug monitoring and pharmacokinetic studies**

Authors : Mieke Carlier, Jan J. De Waele , Alain G. Verstraete, Veronique Stove

Severe infection and sepsis are the most common causes of morbidity and mortality in non-cardiac intensive care units worldwide [1]. Research has shown that initiating appropriate and timely antibiotic therapy is crucial for survival [24]. However, reaching adequate antibiotic concentrations may also be important, as recent data suggested a correlation between serum concentrations of β-lactam antibiotics and clinical outcomes in the critically ill [49]. However, because of pathophysiological changes and treatment interventions, dose optimization in these patients remains difficult for the treating physician [39]. There is a growing interest in therapeutic drug monitoring (TDM) of plasma concentrations of β-lactam antibiotics, as this may maximize efficacy and minimize toxicity [166, 236]. However, little is known about the pre-analytical stability of these antibiotics, which are generally considered to be very unstable. Therefore, labor intensive measures are currently used such as stabilization of carbapenems using non-nucleophilic buffers, transportation of the blood sample on ice and immediate centrifugation and subsequent storage of the plasma at -80°C, which makes routine therapeutic monitoring of these drugs more challenging [107, 174, 238, 239].

The objective of this study was to evaluate the pre-analytical stability of three commonly used β-lactam antibiotics, both in whole blood and in plasma. This study was conducted at the intensive care unit of Ghent University Hospital, Belgium between February and April 2014. The trial was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Ghent University Hospital (registration number 2012/229). Patients were invited to participate but, as no patient data were used and no extra blood was taken, the need to obtain written informed consent was waived.

To evaluate the pre-analytical stability of these antibiotics, two blood tubes were drawn at the same time from patients treated with amoxicillin (n=8, range 4 to 45 mg/L), meropenem (n=7, range 9 to 36 mg/L) or piperacillin (n=10, range 34 to 263 mg/L): one Li-

heparinized tube with (Venosafe VF-052SAHL, tube A) and one without (Venosafe VF-052SHL, tube B) a gel separator (Terumo Europe, Leuven, Belgium). One mL of whole blood from tube B was removed to an Eppendorf cup and stored at 4°C (postponed centrifugation condition, tube C). Tube A and the remaining part of tube B were both centrifuged (8 min, 1885 g, room temperature (RT)). Tube A (plasma in contact with the gel separator) was first stored at RT for 4 h and was then placed at 4°C for further storage (mimicking the worst case scenario in our laboratory). Tube B (plasma in contact with the blood cells) was stored at 4°C immediately after centrifugation. The tubes on the bench at room temperature were not protected from light. An aliquot of plasma was taken from tube A and B. For tube C, an aliquot of whole blood was transferred to a new cup which was centrifuged. The resulting plasma was transferred to a new cup. Collection of aliquots occurred at serial time points up to 72 h (0 h, 4 h, 6 h, 8 h, 24 h, 48 h and 72 h) and were stored at -80°C until analysis. The aliquots were analyzed in duplicate using an adapted and optimized version of an in house developed ultra high performance liquid chromatography tandem mass spectrometric method [169]. In brief, 15 µL plasma was precipitated with 100 µL acetonitrile containing the internal standard (a deuterated analogue of each of the antibiotics) at a concentration of 1.5 mg/L, which was then vortexed and centrifuged. Hundred µL of the supernatant was diluted in 400 µL of water and 40 µL was injected onto the chromatographic column. Imprecision was < 10% at all concentrations. The influence of one freeze thaw cycle was investigated during validation and no significant degradation occurred.

The drug was considered stable if the mean recovery was ≥ 95 % of the reference condition. The aliquot immediately sampled after centrifugation of tube B was considered the reference condition. In 5% of the samples, the analysis could not be performed because of too small sample volume.

For the different storage conditions and tested β-lactam antibiotics, mean recovery and mean percentage degradation (+ standard deviation) are shown in Figure 1 and Table 1, respectively. Meropenem was stable for 8 h in whole blood or plasma in contact with cells at 4°C, while amoxicillin and piperacillin were stable for 48 h under this condition. The tube containing a gel separator stored for 4 h at RT followed by storage at 4°C was stable up to 8 h for amoxicillin, but only 6 h for meropenem and piperacillin. We first assumed that the limited stability of piperacillin might be caused by adsorption of piperacillin to the gel

barrier. However, our initial experiment was not appropriate to test this, as the storage conditions during the first 4 hours were different (RT for samples with separator gel and 4°C for plasma without gel barrier) and storage at a higher temperature could possibly also explain the higher instability of piperacillin in tube A. Therefore, we carried out an additional gel-adsorption experiment and compared 6 piperacillin plasma concentrations which were sampled with and without gel barrier and were stored at identical conditions (4°C). The recovery was calculated as the ratio of the piperacillin concentration in the sample to the reference (concentration of piperacillin immediately sampled in the tube without gel separator). This recovery was compared for each time point using the related samples Wilcoxon signed rank test and the difference in recovery between the tube with and without gel separator reached statistical significance after 48 h (p=0.046) and after 72 h (p=0.028). The difference in recovery between gel and no gel was around 10% after 48 and 72 h. As the percentage recovery for the gel tube in the first experiment was much lower than the second after 24, 48 and 72 h, this is due to the period stored at room temperature.

These experiments were performed using Venosafe heparin tubes from Terumo®, and the results are therefore only applicable on these tubes. Although only a limited number of samples was used in our experiment, we believe they give already a good estimation on the stability of the different compounds tested.

In conclusion, this study shows that the pre-analytical stability of these selected β-lactam antibiotics is relatively good and is dependent on the compound. Meropenem is slightly less stable than amoxicillin and piperacillin. Labor-intensive measures, now often taken to prevent degradation, such as transportation on ice, immediate centrifugation and stabilization of meropenem using non-nucleophilic buffers may be unwarranted. This can considerably simplify storage and transportation to the laboratory and therefore facilitate the implementation of TDM in clinical practice. Tubes not containing a gel separator are preferred, as there seems to be some adsorption of piperacillin to the gel barrier if the plasma is in contact with the gel for more than 24 hours.
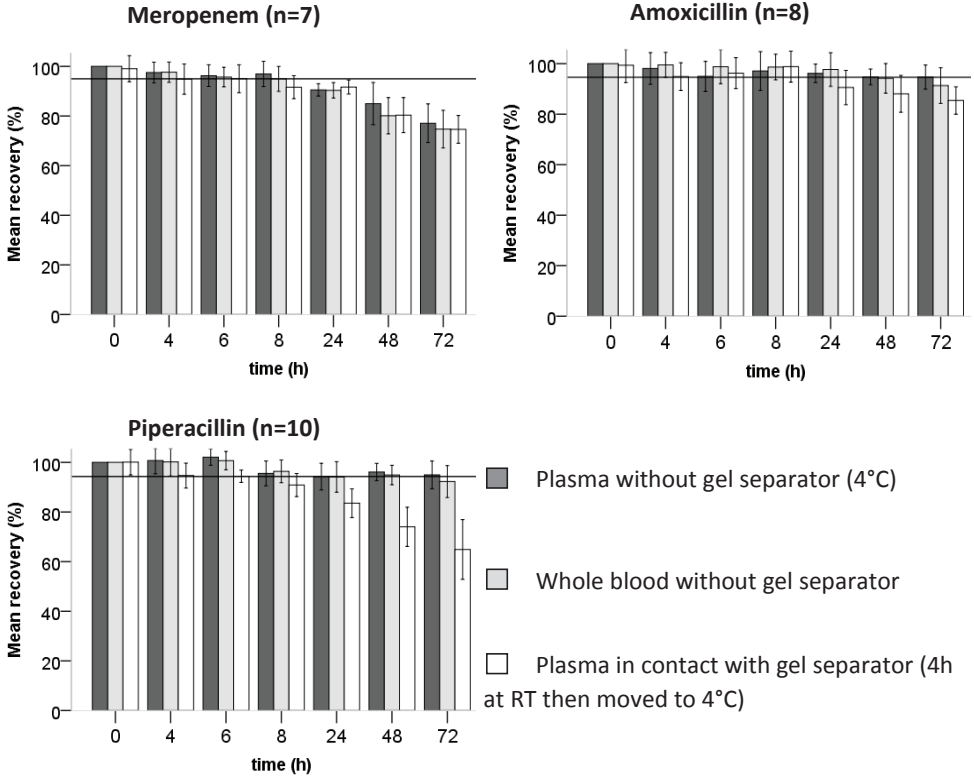
Figure 1: Mean recovery in function of time and storage conditions for meropenem, amoxicillin and piperacillin. Error bars : ± 1 standard deviation. Black line : limit of stability (95% recovery). RT : room temperature

Table 1 : Mean recovery (%) ± standard deviation (%)

	Condition A (mean recovery %)					
	4 h	6h	8h	24h	48h	72 h
Amoxicillin (n=8)	95 ±5	96 ±6	97±8	91±7	87±7	85±5
Meropenem (n=7)	96±7	95±6	93±7	93±4	81±6	75±7
Piperacillin (n=10)	95±4	95±4	91±5	84±6	74±9	65±13
	Condition B (mean recovery %)					
	4 h	6h	8h	24h	48h	72 h
Amoxicillin (n=8)	98±6	95±6	97±5	96±4	95±3	94±4
Meropenem (n=7)	98±4	96±4	97±7	89±3	83±7	76±7
Piperacillin (n=10)	101±5	102±3	96±5	95±5	96±3	94±6
	Condition C (mean recovery %)					
	4 h	6 h	8h	24h	48h	72 h
Amoxicillin (n=8)	100±5	99±7	100±5	98±7	95±6	92±7
Meropenem (n=7)	97±4	96±4	95±7	90±3	79±6	73±6
Piperacillin (n=10)	100±6	101±4	96±5	95±5	95±4	92±6

Condition A : plasma in contact with gel separator, stored for 4 h (not protected from light) at room temperature after which it was placed at 4°C (protected from light) ; Condition B : plasma without gel separator in contact with cells stored at 4°C (protected from light) ; Condition C : postponed centrifugation : whole blood stored at 4°C (protected from light)

Chapter Five : Pharmacokinetic Studies

This chapter describes 6 pharmacokinetic studies performed as part of this PhD thesis. Section 1 and 2 describe the population pharmacokinetics of amoxicillin/clavulanic acid and cefuroxime in critically ill patients without renal failure. Data were collected in Ghent University Hospital. The samples were analyzed using the first developed method described in chapter 4. Analysis of the data was performed as part of an international research stay at the Burns, Trauma, and Critical Care Research Centre in Brisbane, Australia. Section 3 describes the population pharmacokinetic analysis of cefepime during continuous renal replacement therapy using data collected at the Erasme Hospital in Brussels. A pharmacokinetic analysis of meropenem and piperacillin administered as an extended infusion in critically ill patients, and a comparison with bolus infusion is described in section 4. Patients treated with meropenem and piperacillin administered as an extended infusion were sampled in Ghent University Hospital. The pharmacokinetic data were compared with pharmacokinetic data from patients administered meropenem and piperacillin as a bolus infusion in the Royal Brisbane and Women's hospital, Brisbane, Australia. The plasma samples were analysed at the Burns, Trauma and Critical Research Centre in Brisbane, Australia. The fifth section describes the variability in piperacillin concentrations within the same patient over an entire course of therapy using data that were collected as part of the TDM study (described in chapter 6). The last section of this chapter describes a simulation study investigating the pharmacokinetic target attainment of both broad spectrum and narrower spectrum β-lactam antibiotics for a selection of microorganisms in which de-escalation may occur.

1. **Population pharmacokinetics and dosing simulations of amoxicillin/clavulanic acid in critically ill patients**

Authors : Mieke Carlier, Michaël Noë, Jan.J. De Waele, Veronique Stove, Alain G. Verstraete, Jeffrey Lipman, Jason A. Roberts

Abstract

Objective: The objective of this study was to investigate the population pharmacokinetics and pharmacodynamics of amoxicillin and clavulanic acid in critically ill patients.

Methods: In this observational pharmacokinetic study, multiple blood samples were taken over one dosing interval of intravenous amoxicillin/clavulanic acid (1000/200 mg). Blood samples were analysed using a validated ultra high performance liquid chromatography tandem mass spectrometry technique. Population pharmacokinetic analysis and dosing simulations were performed using non-linear mixed effects modeling.

Results: One hundred and four blood samples were collected from 13 patients. For both amoxicillin and clavulanic acid, a two-compartment model with between subject variability on both clearance and the volume of distribution of the central compartment described the data adequately. For both compounds, 24 h urinary creatinine clearance was supported as a descriptor of drug clearance. The mean clearance of amoxicillin was 10.0 L/h and mean volume of distribution was 27.4 L. For clavulanic acid mean clearance was 6.8 L/h and mean volume of distribution was 19.2 L. Dosing simulations for amoxicillin supported use of standard dosing regimens (30-minute infusion of 1g 4 times daily or 2g 3 times daily) for most patients when using a target MIC of 8 mg/L and a pharmacodynamic target of 50%fT$_{>MIC}$, except for those with creatinine clearance > 190 mL/min. Dosing simulations for clavulanic acid showed little accumulation when high doses were administered to patients with high creatinine clearance.

Conclusions: Although vast pharmacokinetic variability exists for both amoxicillin and clavulanic acid in ICU patients, current dosing regimens are appropriate for most patients, except for those with very high creatinine clearance.

1.1. Introduction

Infection is an important problem in critical care medicine. In a recent point prevalence study, 71 percent of over 13000 patients admitted to intensive care units (ICU) around the world received antibiotic therapy [1]. Sepsis alone is the leading cause of mortality in non-cardiac intensive care ICU's with up to 30% of patients dying within one month of diagnosis [2]. Currently, timely and appropriate antibiotic therapy after source control is considered to be the mainstay in treatment [24]. However, it is important that adequate concentrations are achieved [236].

Amoxicillin is a semisynthetic penicillin which has been in clinical use for decades. It is commonly administered with the beta-lactamase inhibitor clavulanic acid to broaden its antibacterial spectrum of activity. In ICU, amoxicillin/clavulanic acid is used for community acquired infections caused by both gram positive, and gram negative organisms inclusive of anaerobes [240]. Specific indications include community acquired pneumonia, intra-abdominal, skin and soft tissue infections.

β-lactam antibiotics exhibit a time-dependent killing pattern, meaning that the percentage time above the minimal inhibitory concentration ($fT_{>MIC}$) of the micro-organism is considered the best determinant of efficacy of these antibiotics. For penicillins, 50% $fT_{>MIC}$ is considered the minimum pharmacodynamic target for maximal bacterial killing [40]. However, research in critically ill patients shows that higher PK/PD targets such as 100% $fT_{>MIC}$ or even 100% $fT_{>4xMIC}$ may be associated with better outcomes [42, 43].

Numerous studies have already investigated the population PK of broad-spectrum antibiotics in critically ill patients [148, 157-161, 238, 241, 242], all of which highlight the different PK of these drugs in comparison with healthy volunteers and highlight the need of individual dosing of these antibiotics in critically ill patients. However, if research is only focused on these broad spectrum antibiotics, this may encourage physicians to favor using these antibiotics, even when more targeted therapies could be just as effective, only because these broad spectrum antibiotics have been investigated in this special patient population. This is why data on more targeted therapies are equally relevant [128].

Although amoxicillin/clavulanic acid is commonly used in critically ill patients, there are little data to guide dosing of amoxicillin and clavulanic acid in this specific patient population. Therefore the aim of this study was to evaluate the population pharmacokinetics

of amoxicillin and clavulanic acid in ICU patients and investigate if PK/PD targets are achieved with current dosing strategies, as well as investigate the potential of alternative dosing regimens and strategies.

1.2. Methods

Patients

This prospective, open-label PK study was conducted at the ICU of the Ghent University Hospital, Belgium between March and July 2012. The trial was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of the Ghent University Hospital (registration number 2012/078) and was registered with the European Union Drug Regulating Authorities Clinical Trials (EudraCT), registration number 2011-006107-35. Written informed consent was obtained from all patients or a legally authorized representative before enrolment. Patients were enrolled in the study if they were admitted to the ICU and were prescribed amoxicillin/clavulanic acid. The exclusion criteria included: <18 years of age, a hematocrit of <21% , absence of an arterial catheter or need for renal replacement therapy.

Drug administration

Amoxicillin/clavulanic acid (Augmentin®, GlaxoSmithKline, Genval, Belgium), was infused intravenously over 30 minutes using a syringe pump. Amoxicillin/clavulanic acid (1000 mg/200mg) was dosed 4 times daily for patients with normal renal function, and 3 times daily for patients with renal impairment.

Study procedures

Blood samples for assay were obtained at assumed PK steady state (at least 24 hours of therapy) through a separate arterial catheter. Blood samples were collected just before the start of infusion (time 0), and after 0.25, 0.5, 0.75, 1, 2, 4, 6 or 8 hours (depending on dose interval) in lithium-heparinized collection tubes (Venosafe, Terumo, Leuven, Belgium). The blood samples were centrifuged for 10 min at 3000 g (ALC Centrifugette 4206, Analys, Gent, Belgium) immediately after sample collection and then frozen on dry ice and finally stored at -80°C (within one hour after sample collection) for maximum 4 weeks until assay.

In order to determine 24 hour creatinine clearance, the patient's urine was collected, starting at the time of initiation of the antibiotic infusion. The plasma sample at time 0 was also used to determine the concentration of creatinine in blood. Additional data were obtained from the medical record and included participant demographics, clinical details, measures of illness severity, microbiological results, and laboratory investigations.

Analytical methods

The plasma samples were analysed at the toxicology laboratory of the Department of Laboratory Medicine at Ghent University Hospital. The plasma concentrations of amoxicillin and clavulanic acid were determined by validated ultra high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). The details of this method have been previously described elsewhere [169]. In brief, sample preparation included protein precipitation with acetonitrile and back-extraction of acetonitrile with dichloromethane. Amoxicillin-d_4 was used as an internal standard. Chromatographic separation was performed on a Waters Acquity UPLC system using a BEH C_{18} column (1.7 µm, 100 x 2.1 mm) applying a binary gradient elution of water and acetonitrile both containing 0.1 % formic acid. The total runtime was 5.5 minutes. The lower limit of quantification was 0.5 mg/L and imprecision was < 15 % at all levels. Observed concentrations for amoxicillin were corrected for protein binding (17 %) [240].

Creatinine was measured in both plasma and urine using the rate blanked, compensated and uncompensated Jaffe technique, respectively (Modular P and Cobas 6000, Roche Diagnostics GmbH, Mannheim, Germany). The creatinine clearance was calculated as follows : 24 hour creatinine clearance = V_u x U_{cr}/(1440 x S_{cr}), where V_u is the urinary volume (mL), U_{cr} the urinary creatinine concentration (µmol/L) and S_{cr} the serum creatinine concentration (µmol/L).

Pharmacokinetic analysis

The concentration-time data were analysed using non-Linear mixed-effects modeling (NONMEM version 6.1, Globomax LLC, Hanover, USA). A Digital Fortran compiler was used and the runs were executed using Wings for NONMEM (http://wfn.sourceforge.net). The first-order conditional estimation (FOCE) method with interaction was used throughout the model building.

For the population PK analysis, the plasma amoxicillin concentrations were fitted to one-, two-, or three-compartment linear models using subroutines from the NONMEM library.

Between subject variability (BSV)

BSV was evaluated using an exponential variability model. Various models for residual unexplained variability (RUV) were also tested.

Model diagnostics

Visual inspection of diagnostic scatter plots and the NONMEM objective function value (OFV) were used to evaluate goodness of fit. Statistical comparison of nested models was undertaken in the NONMEM program using log-likelihood ratios, which are assumed to be chi square distributed. On the basis of a $\chi 2$ test of the difference in OFV, a decrease in the OFV of 3.84 units ($p < 0.05$) for one degree of freedom was considered statistically significant. Decreases in BSV of one of the parameters of at least 10% were also accepted for inclusion of a more complicated model.

Covariate screening

Covariate model building was performed in a stepwise fashion with forward inclusion and backward deletion based upon the aforementioned model selection criteria. Creatinine clearance, age, sex, weight, Acute Physiology and Chronic Health Evaluation (APACHE) II score and Sequential Organ Failure Assessment (SOFA) score were evaluated as covariates.

Bootstrap

A nonparametric bootstrap method (n= 1000) was used to study the uncertainty of the pharmacokinetic parameter estimates in the final model. From the bootstrap empirical posterior distribution, we have been able to obtain the 95 % confidence interval (2.5 to 97.5 % percentile) for the parameters, as described previously [243].

Dosing simulations

As creatinine clearance was the only covariate retained in the final model, only the effects of different creatinine clearances were simulated. The creatinine clearances simulated were 10 ml/min, 30 mL/min, 50 mL/min, 100 mL/min, 130 mL/min, 150 mL/min and 190 mL/min. The simulated dosages for amoxicillin are summarised in table 1.

Table 1 : simulated dosages for amoxicillin

intermittent	extended	Continuous
No loading dose	No loading dose	Loading dose : 1 g over 0.5 h
Infusion time 0.5 h	Infusion time = half of dosing interval	Constant infusion over 24 hours
0.5 g q4h	0.5 g q4h	
0.5 g q6h	0.5 g q6h	
0.5 g q8h	0.5 g q8h	
1 g q4h	1 g q4h	6 g q24h
1 g q6h	1 g q6h	4 g q24h
1 g q8h	1 g q8h	3 g q24h
2 g q6h	2 g q6h	8 g q24h
2 g q8h	2 g q8h	
	3 g q6h	12g q24h

q4h : every 4 h, q6h : every 6 h, q8h : every 8 h, q24h : every 24 h

The ability of each dosing regimen to achieve predefined pharmacodynamic targets (50 % fT$_{> MIC}$) was then assessed. The target MIC was the highest MIC for which the antibiotic is used according to EUCAST breakpoints, which is 8 mg/L, the EUCAST breakpoint for *Enterobacter spp.*, *Klebsiella spp., Citrobacter spp* and *Escherichia coli.*

Performing dosing simulations for clavulanic acid to evaluate efficacy was not undertaken as the pharmacodynamic target is not clear. Therefore, simulations for clavulanic acid could only be undertaken to investigate whether accumulation of clavulanic acid occurs if the dose or frequency of amoxicillin/clavulanic acid administration is increased. Dosing simulations were performed for creatinine clearances of 30, 50, 130 and 190 mL/min for both a low and high dose of amoxicillin/clavulanic acid (table 2).

Validation of the model

The model for amoxicillin was validated using data from 14 independent patients enrolled as part of another pharmacokinetic study [84]. Two concentrations were available per patient. Validation was performed by comparing the observed versus predicted concentrations using a coefficient of determination (R^2).

Table 2 : Tested doses for dosing simulations to determine potential accumulation of clavulanic acid

CrCl (mL/min)	High dose amoxicillin/clavulanic acid (mg)	Low dose amoxicillin/clavulanic acid (mg)
30	2000/400 q8h	500/100 q8h
50	2000/400 q6h	500/100 q6h
130	2000/400 q4h	500/100 q4h
190	2000/400 q4h	500/100 q4h

CrCl : creatinine clearance , q4h : every 4 h, q6h : every 6 h, q8h : every 8 h, q24h : every 24 h

1.3. Results

Patient characteristics

A total of 104 blood samples and 13 creatinine clearances were analysed from 13 patients enrolled in this study. Demographic and general clinical characteristics are shown in table 3. The most frequent reason for the antibiotic therapy was a pulmonary infection.

Table 3 : patient characteristics. Values are displayed as median (interquartile range)

Patient characteristic	Value
Age (years)	62 (58-72)
Weight (kg)	75 (70-79)
BMI	24 (21-25)
Sex (% M-F)	85% - 15%
Apache II score on ICU admission	25 (18-29)
SOFA score on ICU admission	9 (5-12)
SOFA score following dose administration	6 (4-12)
Creatinine clearance (mL/min)	102 (50-157)

BMI : body mass index, Apache : Acute Physiology and Chronic Health Evaluation; SOFA: Sequential Organ Failure

Pharmacokinetic analysis

For both compounds, the best base model consisted of a two-compartment linear model with zero order input with exponential residual unknown variability for amoxicillin, and combined additive-exponential residual unknown variability for clavulanic acid. Between-subject variability was included for both clearance and for volume of distribution of the central compartment for both compounds.

The typical value of clearance (TVCL) was calculated as a function of creatinine clearance, normalized to the population's median creatinine clearance, 102 mL/min (equation 1 for amoxicillin, equation 2 for clavulanic acid) , where θ_{1a} is the typical value of amoxicillin

clearance (TVCL$_a$) in the population and θ_{1c} is the typical value of clavulanic acid clearance (TVCL$_c$) in the population.

TVCL$_a$= θ_{1a}*(CrCl/102)   (equation 1)

TVCL$_c$= θ_{1c}*(CrCl/102)   (equation 2)

The addition of creatinine clearance as a covariate greatly improved model fit, for both compounds. None of the other covariates statistically significantly improved the model, and therefore, they could not be included.

Figure 1 displays the goodness-of-fit plots for the final model for both compounds. Of the 104 samples included in the analysis, only 5 samples had a concentration greater than 2 standard deviations outside that predicted by the model for amoxicillin, and only 2 samples had a concentration greater than 2 standard deviations outside that predicted by the model for clavulanic acid, which we considered acceptable given the level of sickness severity and likely pharmacokinetic heterogeneity of the patient cohort. All other visual predictive checks were acceptable and confirmed the goodness of fit of the model. The plots in Fig. 1 show that the final PK model describes the measured concentrations adequately. All subsequent dosing simulations were then based on this model.

The values of the parameters for the final models are given in Table 4 and include the 95% confidence intervals for the parameters computed from all bootstrap runs.

(a)   (b)

99
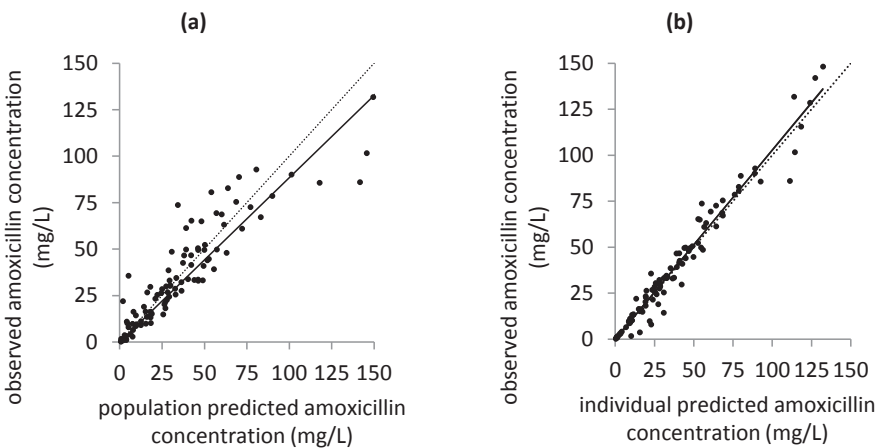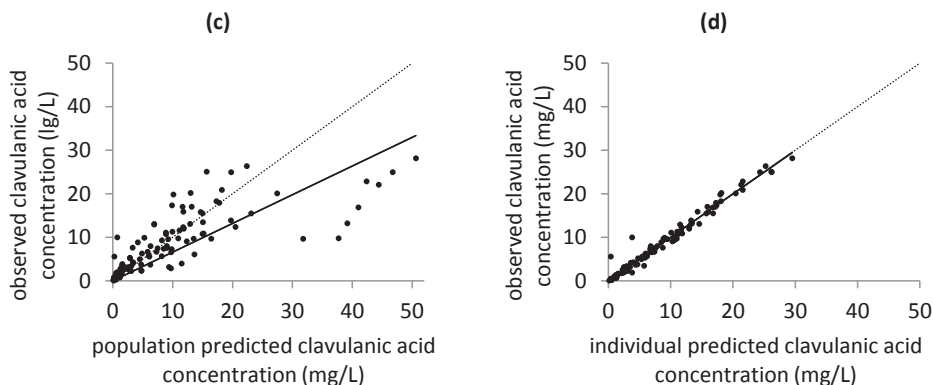
Fig 1. Diagnostic plots for the final population pharmacokinetic covariate model. (a) population predicted amoxicillin concentrations versus observed amoxicillin concentrations (R^2 0.87). (b) individual predicted amoxicillin concentrations versus observed amoxicillin concentrations (R^2 0.96). (c) population predicted clavulanic acid concentrations versus observed clavulanic acid concentrations (R^2 0.44). (d) individual predicted clavulanic acid concentrations versus observed clavulanic acid concentrations (R^2 0.98). The nonlinear regression line of fit is shown by the solid black line, and the line of xy is the gray dotted line.

Table 4 : Bootstrap parameter estimates of the final covariate model

Parameter	Amoxicillin				Clavulanic acid			
	Model	Bootstrap			Model	Bootstrap		
	Mean	Mean	95 % confidence interval		Mean	Mean	95 % confidence interval	
			2.5 %	97.5 %			2.5 %	97.5 %
Fixed effects								
Cl (L/h)	10.0	10.3	8.6	12.6	6.8	9.4	6.3	12.9
Vc (L)	13.7	13.5	10.2	17.7	7.6	8.1	6.8	9.9
Vp (L)	13.7	14.1	11.7	27.7	11.6	14.7	8.4	63.1
Q (L/h)	15.6	15.7	12.2	19.6	10.4	10.0	8.6	11.5
Random effects								
BSV (% CV)								
Cl (L/h)		39.9	25.3	53.6		57.8	31.1	85.5
Vc (L)		38.7	4.0	67.1		34.7	22.8	44.8
Random error								
RUV (% CV)		22.0	10.1	32.9				
RUV (SD, mg/L)						1.2	0.7	1.7

Cl = Clearance, Vc = Volume of distribution of the central compartment ; Vp = Volume of distribution of the peripheral compartment; Q = Intercompartmental clearance; BSV = between subject variability; RUV = residual unexplained variability; CV = coefficient of variation, SD = standard deviation

Dosing simulations

The results of the dosing simulations for amoxicillin are summarised in Table 5, which shows whether the target of 50 % or 100 % $fT_{>MIC}$ will be achieved for different values for creatinine clearance and different dosing strategies.

The standard dose of 1 g q6h or 2 g q8h amoxicillin results in adequate exposure for both low and normal creatinine clearances. However, dependent on the chosen target, standard dosing will not suffice for patients with high creatinine clearance infected with a micro organism with a high MIC_{90} (8mg/L). Patients with a creatinine clearance of 190 mL/min do not even achieve 50% $f_{>TMIC}$, which is considered the minimal PK target needed for bacterial killing, if standard dosing regimens are used.

The results of the dosing simulations for clavulanic acid are shown in figure 2 a-d, which shows the concentrations of amoxicillin and clavulanic acid over a 7 day course for both a low and high dose for different values of creatinine clearance. These figures show that little accumulation of clavulanic acid occurs if higher doses of amoxicillin/clavulanic acid are administered to the patients with normal-high creatinine clearance.

Validation

Similar to the characteristics of the patients used to build the model, the main indication for antibiotic therapy was also treatment of a pulmonary infection. Median creatinine clearance was 97.5 (IQR 44-125) mL/min, which was comparable to the creatinine clearance of the patients in the present study, for whom the median creatinine clearance was 102 (IQR 50-157) mL/min (p= 0.685).

The results of the external validation are graphically shown in Figure 3. The coefficient of determination was 0.75 and was found to be statistically significant (p<0.0001).
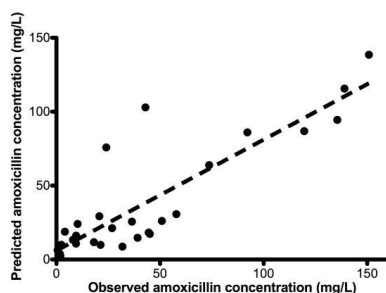
Fig. 3 – Observed amoxicillin concentrations versus predicted concentrations for 28 samples from 14 independent patients
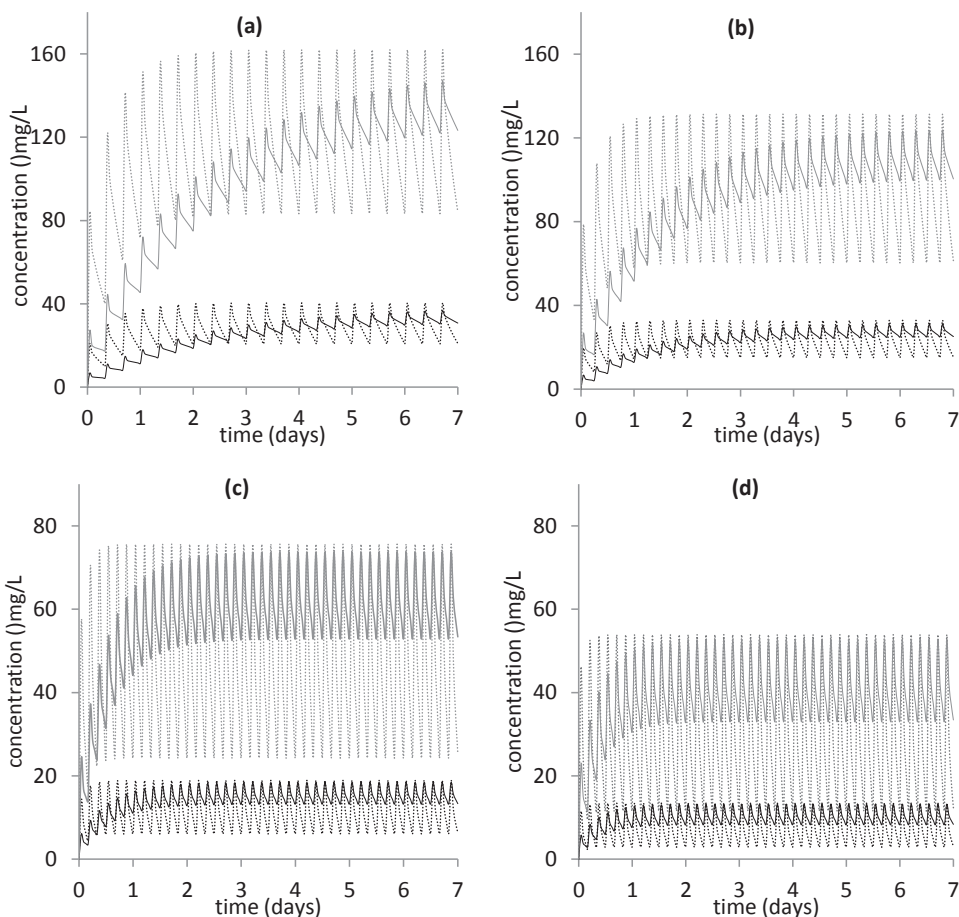
Fig. 2. Dosing simulations for amoxicillin and clavulanic acid in high and low dose for different creatinine clearances. (**a**) Concentration vs time for a patient with creatinine clearance 30 mL/min ; Low dose : 500mg/100mg amoxicillin/clavulanic acid q8h ; High dose : 2000 mg/400 mg amoxicillin/clavulanic acid q8h. (**b**) Concentration vs time for a patient with creatinine clearance 50 mL/min ; Low dose : 500mg/100mg amoxicillin/clavulanic acid q6h ; High dose : 2000 mg/400 mg amoxicillin/clavulanic acid q6h. (**c**) Concentration vs time for a patient with creatinine clearance 130 mL/min ; Low dose : 500mg/100mg amoxicillin/clavulanic acid q4h ; High dose : 2000 mg/400 mg amoxicillin/clavulanic acid q4h. (**d**) Concentration vs time for a patient with creatinine clearance 190 mL/min ; Low dose : 500mg/100mg amoxicillin/clavulanic acid q4h ; High dose : 2000 mg/400 mg amoxicillin/clavulanic acid q4h. Legend : Amoxicillin low dose : black dotted line; Clavulanic acid low dose : black solid line; Amoxicillin high dose : grey dotted line; Clavulanic acid high dose : grey solid line

1.4. Discussion

Although amoxicillin/clavulanic acid is frequently used to treat severe infections in critically ill patients, this is the first paper to investigate its population pharmacokinetics in ICU patients. We found that both amoxicillin and clavulanic acid clearance were proportional to creatinine clearance, with important variability between patients for antibiotic clearance. Current dosing schemes are adequate for patients without increased creatinine clearances when minimal PK/PD targets are used.

Table 5: The effect of creatinine clearances and different dosing strategies on the probability of target attainment for amoxicillin (50% $fT_{> MIC}$ and 100% $fT_{> MIC}$)

Creatinine clearance 30 mL/min					
MIC Dose	<4 mg/l		8 mg/l		16 mg/l	
	$50\%fT_{>MIC}$	$100\%fT_{>MIC}$	$50\%fT_{>MIC}$	$100\%fT_{>MIC}$	$50\%fT_{>MIC}$	$100\%fT_{>MIC}$
II 0.5 g q6h	+	+	+	+	+	-
II 0.5g q8h	+	+	+	+	+	-
II 1 g q8h	+	+	+	+	+	+
II 1 g q6h	+	+	+	+	+	+
Creatinine clearance 50 mL/min						
MIC Dose	<4 mg/l		8 mg/l		16 mg/l	
	$50\%fT_{>MIC}$	$100\%fT_{>MIC}$	$50\%fT_{>MIC}$	$100\%fT_{>MIC}$	$50\%fT_{>MIC}$	$100\%fT_{>MIC}$
II 0.5g q6h	+	+	+	+	+	-
II 1g q8h	+	+	+	+	+	-
EI 1g q8h	+	+	+	+	+	+
II 1g q6h	+	+	+	+	+	+
CI 4g q24h	+	+	+	+	+	+
Creatinine clearance 130 mL/min						
MIC Dose	<4 mg/l		8 mg/l		16 mg/l	
	$50\%fT_{>MIC}$	$100\%fT_{>MIC}$	$50\%fT_{>MIC}$	$100\%fT_{>MIC}$	$50\%fT_{>MIC}$	$100\%fT_{>MIC}$
II 1g q8h	+	-	-	-	-	-
II 1g q6h	+	+	+	-	-	-
EI 1g q6h	+	+	+	-	+	-
CI 4g q24h	+	+	+	+	-	-
II 1g q4h	+	+	+	+	+	-
CI 6g q24h	+	+	+	+	+	+

Creatinine clearance 190 mL/min						
MIC / Dose	<4 mg/l		8 mg/l		16 mg/l	
	50%fT$_{>MIC}$	100%fT$_{>MIC}$	50%fT$_{>MIC}$	100%fT$_{>MIC}$	50%fT$_{>MIC}$	100%fT$_{>MIC}$
II 1gq6h	+	-	-	-	-	-
EI 1g q6h	+	+	+	-	-	-
CI 4g q24h	+	+	+	-	-	-
II 1g q4h	+	+	+	-	-	-
CI 6g q24h	+	+	+	+	+	-
EI 2g q6h	+	+	+	-	+	-
CI 8g q24h	+	+	+	+	+	+
EI 3g q6h	+	+	+	+	+	-

II : intermittent infusion, EI : extended infusion, CI : continuous infusion, + : target attained, - : target not attained

Clearance appears to be an important factor in the variability described. This pharmacokinetic variability is typical for ICU patients and has been shown for other beta-lactams as well. The available pharmacokinetic studies in healthy volunteers show for amoxicillin a mean clearance of about 12.5 L/h with a CV (coefficient of variation) around 20 % [244-246]. This is in contrast to our findings of a mean clearance of 10.0 L/h with a CV of more than 80 % in the 13 patients included in this study, which highlights the importance of studying this specific patient population. The values observed for volume of distribution (IQR 24.7-30.7 L) seem to be comparable with those found in literature for healthy volunteers [244-246]. As there are no population pharmacokinetic studies published for clavulanic acid, it was not possible to compare our results to the results previously described in the literature.

The EUCAST breakpoint for amoxicillin for common respiratory pathogens such as staphylococcus aureus (2 mg/L) and streptococcus pneumonia (0.064 mg/L) is low. However, this breakpoint MIC is far higher for other species, such as *Enterobacter spp., Escherichia coli* and *Klebsiella spp.* , for which the breakpoint MIC is 8 mg/L, which are potential considerations with community acquired intra-abdominal infection[235].By performing dosing simulations for amoxicillin and investigating the probability of target attainment we have demonstrated that intermittent infusion of amoxicillin 1 g q6h or 2 g q8h will ensure plasma free concentrations exceeding this breakpoint MIC for at least 50% fT$_{>MIC}$ -which is considered the minimum PK/PD target to achieve bacterial killing- for patients with low and normal kidney function. However, using the same dosing strategy, patients with very high

creatinine clearances (190 mL/min) will not reach this target. In order to achieve sufficient exposure, these patients need more frequent antibiotic administration (1g 6 times daily) or need to be treated with alternate dosing strategies. This means that standard dosing should lead to sufficient PK/PD exposure when treating an infection caused by an organism with a low MIC (in the case of a respiratory tract infection), but may fail to achieve sufficient PK/PD exposure when treating an infection caused by an organism with a high MIC (in the case of an intra-abdominal infection), when the creatinine clearance is > 190 mL/min. It is important to state that estimations of GFR such as the cockroft gault equation and the modified diet in case of renal disease (MDRD) are not reliable in ICU patients, and 8 or 24 hour urinary creatinine clearance should be preferred in these patients [247-249].

Moreover, research in critically ill patients shows that higher PK/PD targets may be associated with better outcomes [42, 43]. If one aims to achieve these higher targets such as 100% $T_{>MIC}$ or even 100% $T_{>4xMIC}$ more frequent dosing or administration by prolonged infusion is necessary for patients with normal to high renal function. Amoxicillin is stable for up to 24 h for a concentration range from 20-40 g/L [250]. However, stability of clavulanic acid when used as a prolonged infusion is unknown. In addition, more frequent dosing or alternate dosing strategies could also be a way to treat more resistant microorganisms, which would otherwise be classified as not sensitive to this antibiotic, which may be very valuable in this era of increasing resistance. The advantage of using extended or continuous infusion on PK/PD target attainment has already been shown for other β-lactams as well [133, 140, 251].

As for clavulanic acid, dosing simulations were only performed to evaluate accumulation, since the pharmacodynamic target for efficacy is unknown. We have shown that there is little accumulation of clavulanic acid in patients with high creatinine clearance treated for 7 days with a high dose amoxicillin/clavulanic acid.

This paper has a number of limitations. First, we have not investigated free concentrations or concentrations at the site of infection. Instead, we have measured total drug concentrations with correction for protein binding based on literature. This is an oversimplification, but our (unpublished) data show that this approach is acceptable for low protein bound drugs such as amoxicillin (17% protein binding), although it is not accurate for more highly protein bound drugs. Moreover, we have only included 13 patients in this study,

which may not be sufficient to describe the variability present in ICU patients. However, this small study still provides important guidance for dosing this drug in the ICU given that the data is presently not available [148].

1.5. *Conclusion*

We found great variability in antibiotic clearance, which is not found in healthy volunteers, which points out the importance of individual dosing in ICU patients. We have shown that current dosing regimens of 1000/200 mg 4 times daily or 2000/400 mg 3 times daily for patients with low to normal creatinine clearance lead to sufficient pharmacokinetic exposure. However, patients with very high creatinine clearance need more frequent dosing or alternate dosing strategies to achieve the minimal PD target of 50 % $T_{> MIC}$ (8mg/L), with little accumulation of clavulanic acid. To achieve higher targets such as 100% $fT_{>MIC}$ in patients with high creatinine clearance, administration of higher doses as a prolonged infusion is necessary.

2. **Population pharmacokinetics and dosing simulations of cefuroxime in critically ill patients – non-standard dosing approaches are required to achieve therapeutic exposures**

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Abstract:

Objective: The objective of this study was to investigate the population pharmacokinetics of cefuroxime in critically ill patients.

Methods: In this observational pharmacokinetic study, multiple blood samples were taken over one dosing interval of intravenous cefuroxime. Blood samples were analysed using a validated ultra high performance liquid chromatography tandem mass spectrometry technique. Population pharmacokinetic analysis and dosing simulations were performed using non-linear mixed effects modeling.

Results: One hundred and sixty blood samples were collected from 20 patients. Creatinine clearance ranged between 10 and 304 mL/min. A two-compartment model with between-subject variability on clearance, volume of distribution of the central compartment and volume of distribution of the peripheral compartment described the data adequately. Twenty-four hour urinary creatinine clearance was supported as a descriptor of drug clearance. The population model for clearance was $Cl = \theta_1 x \frac{CrCl}{100}$, where θ_1 is the typical of cefuroxime clearance in the population, which is 9.0 L/h. The mean volume of distribution was 22.5 L. Dosing simulations showed failure to achieve the pharmacokinetic/pharmacodynamic (PK/PD) target of 65%fT$_{>MIC}$ for an MIC of 8 mg/L with standard dosing regimens for patients with creatinine clearance ≥ 50 mL/min.

Conclusions: Administration of standard doses by intermittent bolus is likely to result in underdosing for many critically ill patients. Continuous infusion of higher than normal doses after a loading dose is more likely to achieve pharmacokinetic/pharmacodynamic targets. However, even continuous infusion of high doses (up to 9 g per day) does not guarantee adequate levels for all patients with a creatinine clearance of 300 mL/min or higher if the MIC is 8 mg/L.

2.1.   Introduction

Cefuroxime is a second-generation cephalosporin that has been in clinical use for over two decades.

Like other β-lactam antibiotics, cefuroxime is a time-dependent antibiotic, which means antibacterial activity is related to the time for which the unbound concentration is maintained above the MIC during a dosing interval ($fT_{>MIC}$). The $fT_{>MIC}$ required for optimal bactericidal activity for cefuroxime has been reported to be somewhere between 40 and 70 % from *in vitro* animal models.[252] Although this may be adequate for minor infections, for treatment of serious infection in critically ill patients, higher pharmacokinetic/pharmacodynamic (PK/PD) targets such as 100% $fT_{>MIC}$ or even 100% $fT_{>4xMIC}$ have been associated with better outcomes, both clinical and microbiological [42, 43].

Research has shown that the pharmacokinetics of hydrophilic antibiotics in critically ill patients may differ from healthy volunteers and from non-critically ill patients. Subtherapeutic concentrations using standard dosing have been reported for many antibiotics [128, 140, 155-161]. This shows that pharmacokinetic data from healthy volunteers cannot just be extrapolated to critically ill patients and that population pharmacokinetic studies are needed to define robust drug doses for this specific patient population.

To date, there are little data to guide dosing of cefuroxime in critically ill patients, which may preclude the use of cefuroxime in this setting. Although cefuroxime is not commonly used as empirical therapy in critically ill patients, because it has a relatively narrow spectrum and does not cover most nosocomial pathogens. However, it may have a role in de-escalation when the pathogens are found to be susceptible to the drug. Therefore knowledge about the pharmacokinetics in the critically ill is important to use the drug appropriately.

Therefore the aim of this study was to evaluate the population pharmacokinetics of cefuroxime in critically ill patients and investigate if PK/PD targets are achieved with current dosing strategies, as well as investigate the potential of alternative dosing regimens and strategies.

2.2 Methods

Patients

This prospective, open-label pharmacokinetic study was conducted at the ICU of Ghent University Hospital, Belgium between March 2012 and January 2014. The trial was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Ghent University Hospital (registration number 2012/078) and was registered with the European Union Drug Regulating Authorities Clinical Trials (EudraCT), registration number 2011-006107-35. Written informed consent was obtained from all patients or a legally authorised representative before enrolment. Patients were enrolled in the study if they were admitted to the ICU and were prescribed cefuroxime. The exclusion criteria included: <18 years of age, a haematocrit of <21% , absence of an arterial catheter or need for renal replacement therapy.

Drug administration

Cefuroxime (Zinacef®, GlaxoSmithKline, Genval, Belgium), was infused intravenously over 30 minutes using a syringe pump. The dose was 1500 mg every 8 hours for all patients except for those with renal impairment (defined as a creatinine clearance < 20 mL/min), for whom the dose was reduced to 750 mg every 8 hours.

Study procedures

Blood samples for assay were obtained after at least 24 hours of therapy through a separate arterial catheter. Blood samples were collected just before the start of infusion (time 0), and after 0.25, 0.5, 0.75, 1, 2, 4, and 8 hours in lithium-heparinised collection tubes (Venosafe, Terumo, Leuven, Belgium). The blood samples were centrifuged for 10 min at 3000 g (ALC Centrifugette 4206, Analis, Gent, Belgium) immediately after sample collection and then frozen on dry ice and finally stored at -80°C (within one hour after sample collection) for maximum 4 weeks until assay.

In order to determine 24-hour creatinine clearance, the patient's urine was collected, starting at the time of initiation of the antibiotic infusion. The plasma sample at time 0 was also used to determine the concentration of creatinine in blood. Additional data were obtained from the medical record and included participant demographics, clinical details, measures of illness severity, microbiological results, and laboratory investigations.

Analytical methods

The plasma concentrations of cefuroxime were determined by a validated ultra high performance liquid chromatography method coupled to tandem mass spectrometry (UPLC-MS/MS). The details of this method have been previously described elsewhere [169]. Observed concentrations for cefuroxime were corrected for protein binding (33 %) [240, 253].

Creatinine was measured in both plasma and urine using the rate blanked, compensated and uncompensated Jaffe technique, respectively (Modular P and Cobas 8000, Roche Diagnostics GmbH, Mannheim, Germany).

Pharmacokinetic analysis

The concentration-time data were analysed using non-linear mixed-effects modeling (NONMEM version 7.3, Globomax LLC, Hanover, USA). A Digital Fortran compiler was used and the runs were executed using Wings for NONMEM (http://wfn.sourceforge.net). The first-order conditional estimation (FOCE) method with interaction was used throughout the model building.

Model development

For the population pharmacokinetic analysis, the plasma cefuroxime concentrations were fitted to one-, two-, or three-compartment linear models using subroutines from the NONMEM library. BSV was evaluated using an exponential variability model. Various models for residual unexplained variability (RUV) were also tested.

Model diagnostics

Visual inspection of diagnostic scatter plots and the NONMEM objective function value (OFV) were used to evaluate goodness of fit. Statistical comparison of nested models was undertaken in the NONMEM program on the basis of a $\chi 2$ test of the difference in OFV. A decrease in the OFV of 3.84 units ($p < 0.05$) was considered statistically significant for 1 degree of freedom. Decreases in BSV of one of the parameters of at least 10% were also accepted for inclusion of a more complicated model.

Covariate screening

Covariate model building was performed in a stepwise fashion with forward inclusion based upon the aforementioned model selection criteria for those clinical parameters which showed significant correlation with one of the pharmacokinetic parameters. Creatinine

clearance, serum albumin concentration, age, sex, weight, Acute Physiology and Chronic Health Evaluation (APACHE) II score and Sequential Organ Failure Assessment (SOFA) score were evaluated as covariates.

Bootstrap

A nonparametric bootstrap method (n= 1000) using NONMEM was used to study the uncertainty of the pharmacokinetic parameter estimates in the final model. From the bootstrap empirical posterior distribution, we obtained the 95 % confidence interval (2.5 to 97.5 % percentile) for the parameters, as described previously [243].

Dosing simulations

Different dosing regimens were simulated using Monte Carlo simulations. The creatinine clearances simulated were 50, 100, 200 and 300 mL/min. Five hundreds subjects were simulated per dosing strategy and per creatinine clearance. The simulated dosages are summarised in table 1. Each Monte Carlo Simulation generated concentration time profiles for 500 subjects per dosing regimen using the parameters from the final covariate model. From this data, the fT>$_{MIC}$ was calculated for each simulated subject using linear interpolation. The PTA was obtained by counting the subjects who achieved 65 % fT$_{>MIC.}$ The target MIC's were 0.5, 1, 2, 4, 8 and 16 mg/L.

Table 1: Simulated dosages

Intermittent	Extended	Continuous
No loading dose	No loading dose	Loading dose: 750 mg over 0.5 h
Infusion time 0.5 h	Infusion time = half of dosing interval	Constant infusion over 24 hours
1.5 g q8h	1.5 g q8h	4.5 g q24h
	1.5 g q6h	6.0 g q24h
		7.5 g q24h
		9.0 g q24h

q8h : every 8 hours ; q6h : every 6 hours, q24h : over 24 hours

2.3. Results

Patient characteristics

A total of 160 blood samples and 20 creatinine clearances were analysed from 20 patients enrolled in this study. Demographic and general clinical characteristics from the patients used for model building are shown in table 2. Eighteen patients received antibiotic

therapy for treatment of a pulmonary infection, and 2 for prevention of a pulmonary infection after aspiration. Twelve causative microorganisms were cultured from nine patients which are described in table 3.

Table 2: Patient characteristics. Values are displayed as median (range)

Patient characteristic	
Age (years)	69 (26-85)
Weight (kg)	80 (65-100)
Number of doses between start of therapy and start of study	3 (3-5)
BMI	28 (22.6-35)
Sex (% M-F)	73% /- 27%
Apache II score on ICU admission	19 (13-32)
SOFA score on ICU admission	9 (3-13)
SOFA score on day of sampling	7 (1-12)
Creatinine clearance (mL/min)	57 (10-304)
Albumin concentration (g/L)	28.5 (17-42)

BMI = Body Mass Index; M = male; F = female; ICU = intensive care unit; APACHE = Acute Physiology and Chronic Health Evaluation; SOFA = Sequential Organ Failure Assessment

Table 3 : Isolated micro-organisms and their susceptibility

Micro-organism	Number of positive cultures	Breakpoint MIC[*]
Escherichia coli	4/12	8
Staphylococcus aureus	2/12	4
Haemophilus influenza	1/12	2
Klebsiella oxytoca	1/12	8
Raoultella ornithinolytica	1/12	ND
Proteus mirabilis	1/12	ND
Streptococcus pneumoniae	1/12	1
Morganella morganii	1/12	ND

[*]As described by EUCAST ;[235] ND = not determined

Pharmacokinetic analysis

The best base model consisted of a two-compartment linear model with zero order input (ADVAN3 TRANS4 subroutine) with combined additive-proportional residual unknown variability. Between-subject variability was supported on clearance, for volume of distribution of the central compartment and for volume of distribution of the peripheral compartment.

The only covariate that statistically improved the base model was creatinine clearance, normalized to the population's mean creatinine clearance, 100 mL/min, which decreased the objective function value by 37.9 points and decreased BSV on clearance from

0.94 to 0.29. All other covariates showed no correlation with any of the PK parameters and were therefore not further investigated. The final model is represented as: TVCL= $\theta_1 * (CrCl/100)$

The typical value of clearance (TVCL) was calculated as a function of creatinine clearance, normalized to the population's mean creatinine clearance, 100 mL/min where θ_1 is the typical value of cefuroxime clearance in the population.

Figure 1 displays the goodness-of-fit plots for the final covariate model. The fit of the model was acceptable in terms of visual or statistical biases for the prediction. The plots in figure 1 show that the final PK model describes the measured concentrations adequately. All subsequent dosing simulations were then based on this model.

The values of the parameters for the final models are given in table 4 and include the 95% confidence intervals for the parameters computed from all bootstrap runs.

Dosing simulations

The probability of target attainment for different dosing regimens and different creatinine clearances are shown in figure 2.

The standard dose of 1.5 g cefuroxime 3 times daily results in inadequate target attainment for patients with a creatinine clearance ≥ 50 mL/min. This standard dose leads to a 87 % probability of target attainment for patients with a creatinine clearance of 50 mL/min and MIC 8 mg/L.
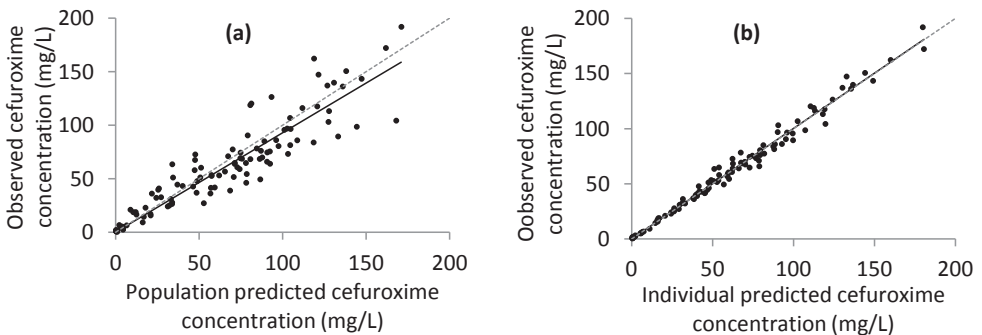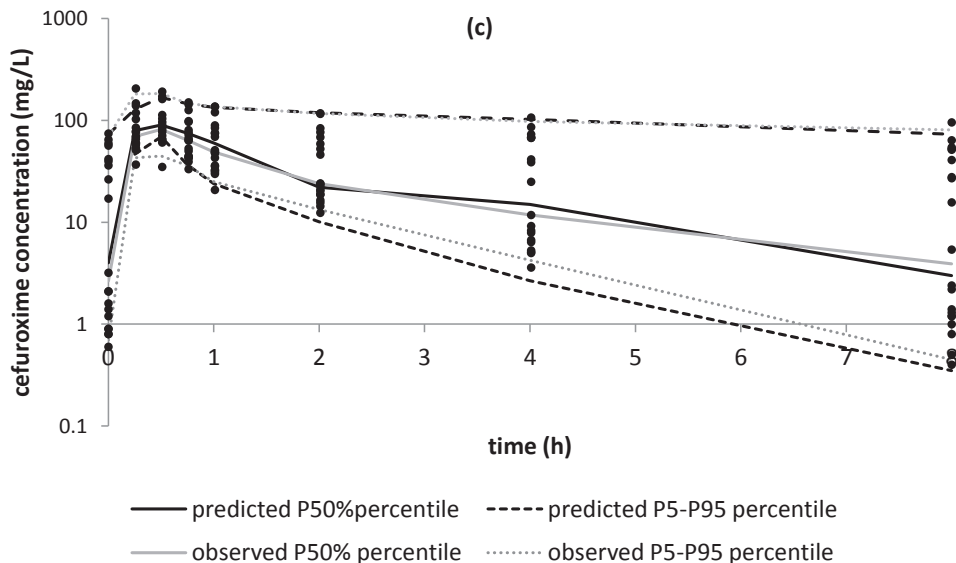
Fig. 1. Diagnostic plots for the final population pharmacokinetic covariate model. (a) population predicted cefuroxime concentrations versus observed concentrations (R^2 0.86). (b) individual predicted cefuroxime concentrations versus observed concentrations (R^2 0.99). The nonlinear regression line of fit is shown by the solid black line, and the line of identity xy is the gray dotted line. (c) visual predictive check generated from a monte carlo simulation (n=1500) and showing that the estimated population pharmacokinetic model has adequate performance. The raw data are shown as black dots.

Table 4: Bootstrap parameter estimates of the final covariate model

	Model		Bootstrap	
	Median	median	95 % confidence interval	
			2.5 %	97.5 %
Fixed effects				
Cl (L/h)	9.0	9.0	8.0	10.1
Vc (L)	10.5	10.5	8.8	12.9
Vp (L)	12.0	12.0	9.3	14.6
Q (L/h)	18.7	18.4	11.8	23.8
Random effects				
BSV (% CV)				
Cl (L/h)	28.0	27.1	19.0	34.6
Vc (L)	23.7	22.0	3.3	33.0
Vp (L)	29.5	26.0	4.8	43.6
Random error				
Proportional (% CV)	10.3	10.4	7.3	13.9
Additive (SD, mg/L)	0.46	0.43	0.01	0.7

Cl = Clearance; Vc = Volume of distribution of the central compartment; Vp = Volume of distribution of the peripheral compartment; Q = Intercompartmental clearance; BSV = between subject variability; CV = coefficient of variation, SD = standard deviation

2.4. Discussion

This is the first paper to investigate the population pharmacokinetics of cefuroxime in critically ill patients. We found that antibiotic clearance was proportional to creatinine clearance, with important variability between patients for antibiotic clearance. Current dosing schemes are not adequate for critically ill patients with a creatinine clearance ≥ 50 mL/min when conservative PK/PD targets are used.

Two pharmacokinetic studies in ambulatory and general ward patients who were treated with cefuroxime have been published before. The first study evaluated patients with a creatinine clearance between 60 and 120 mL/min and reported a mean Vd, of 16.5 L and a clearance of 7.4 L/h [254]. Another study in general ward patients found a typical population value for clearance of 6.0 L/h and also a Vd of 16.5 L [255]. This value for clearance is slightly lower than our findings, most likely because of their study population, which had a lower creatinine clearance than our study population. The values observed for volume of distribution from these studies are lower than the value reported in our study (23.2 L). A larger than normal volume of distribution is one of the typical pathophysiological changes in critically ill patients, a finding reported by multiple pharmacokinetic studies in critically ill patients [93, 140, 157, 256]. These differences from healthy volunteers highlight once again

the importance of performing population pharmacokinetic studies and dosing simulations in our specific patient population.

By performing dosing simulations and investigating the probability of target attainment we have demonstrated that intermittent infusion of 1.5 g cefuroxime 8 hourly will not ensure 90 % probability of target attainment (plasma free concentrations > MIC for at least 65% of the dosing interval) for MIC 8 - The EUCAST breakpoint for cefuroxime for *Escherichia coli* - for patients with a creatinine clearance ≥ 50 mL/min.[235] This problem is exacerbated in patients with higher creatinine clearances such as 100, 200 or even 300 mL/min. These patients have a high probability of underdosing, even for lower and frequently encountered MIC values such as 4, 2 and 1 mg/L.

It is important to point out that there may be other covariates which may also influence plasma concentrations, such as body weight on volume of distribution, or SOFA score. However, only creatinine clearance could be retained in the final covariate model, as none of the other patient characteristics statistically significantly improved the model, and therefore, they could not be included. The reason for this is most likely the relatively small sample size of this study.

Previous research has already demonstrated that patients with augmented renal clearance have a low probability of target attainment[120, 156, 257]. In our study population of 20 patients, 8 patients had a creatinine clearance >150 mL/min, 4 of which were higher than 200 mL/min. Research in critically ill patients shows that higher PK/PD targets may be associated with better outcomes [42, 43]. If one aims to achieve these higher targets such as 100% $fT_{>MIC}$ or even 100% $fT_{>4xMIC}$ other strategies are necessary for all patients without renal dysfunction.

In order to achieve sufficient exposure for an MIC of 8 mg/L, patients with a creatinine clearance ≥ 50 mL/min should be treated with alternate dosing strategies, such as extended or continuous infusion. Patients with creatinine clearances ≥100 mL/min need higher dosages and/or alternate dosing strategies such as extended and continuous infusion. Some patients with very high creatinine clearances (≥ 300 mL) need up to 9 g as a continuous infusion in order to achieve adequate concentrations. However, the clinical superiority of continuous infusions of high doses cefuroxime compared to standard intermittent dosing has yet to be demonstrated. It should also be noted that continuous infusion of high doses

does not guarantee adequate concentrations for all patients with a creatinine clearance of 300 mL/min if the MIC of the micro-organism is 8 mg/L.

This paper has a number of limitations. First, we have not investigated free concentrations or concentrations at the site of infection. Instead, we have measured total drug concentrations with correction for protein binding based on literature [240, 253]. This is an oversimplification, but research has shown that this approach is acceptable for low to moderately protein bound drugs such as cefuroxime although it is not accurate for more highly protein bound drugs [219]. Also, the small cohort of 20 patients could be considered a limitation of this study, given the variability of patient sickness severity. This small cohort may have also prevented other covariates from being shown to be significant and predictive of the variability of pharmacokinetic parameters, such as body weight on volume of distribution. Due to the inclusion criteria of the study, the dose recommendations derived from the data analysis cannot be extrapolated to other critically ill patient populations such as patients with renal replacement therapy or that are obese.

2.5.   Conclusion

In this study in critically ill patients treated with cefuroxime, we found important variability in antibiotic clearance and a larger than normal volume of distribution compared to general ward patients. The results of the dosing simulations show that current dosing regimens of 1.5 g cefuroxime administered 8-hourly as a bolus infusion leads to underdosing for many patients, whereas continuous infusion of higher than normal doses after a loading dose is more likely to achieve pharmacokinetic/pharmacodynamic targets. However, even continuous infusion of high doses (up to 9 g per day) does not guarantee adequate concentrations for all patients with a creatinine clearance of 300 mL/min or higher if the MIC is 8 mg/L.
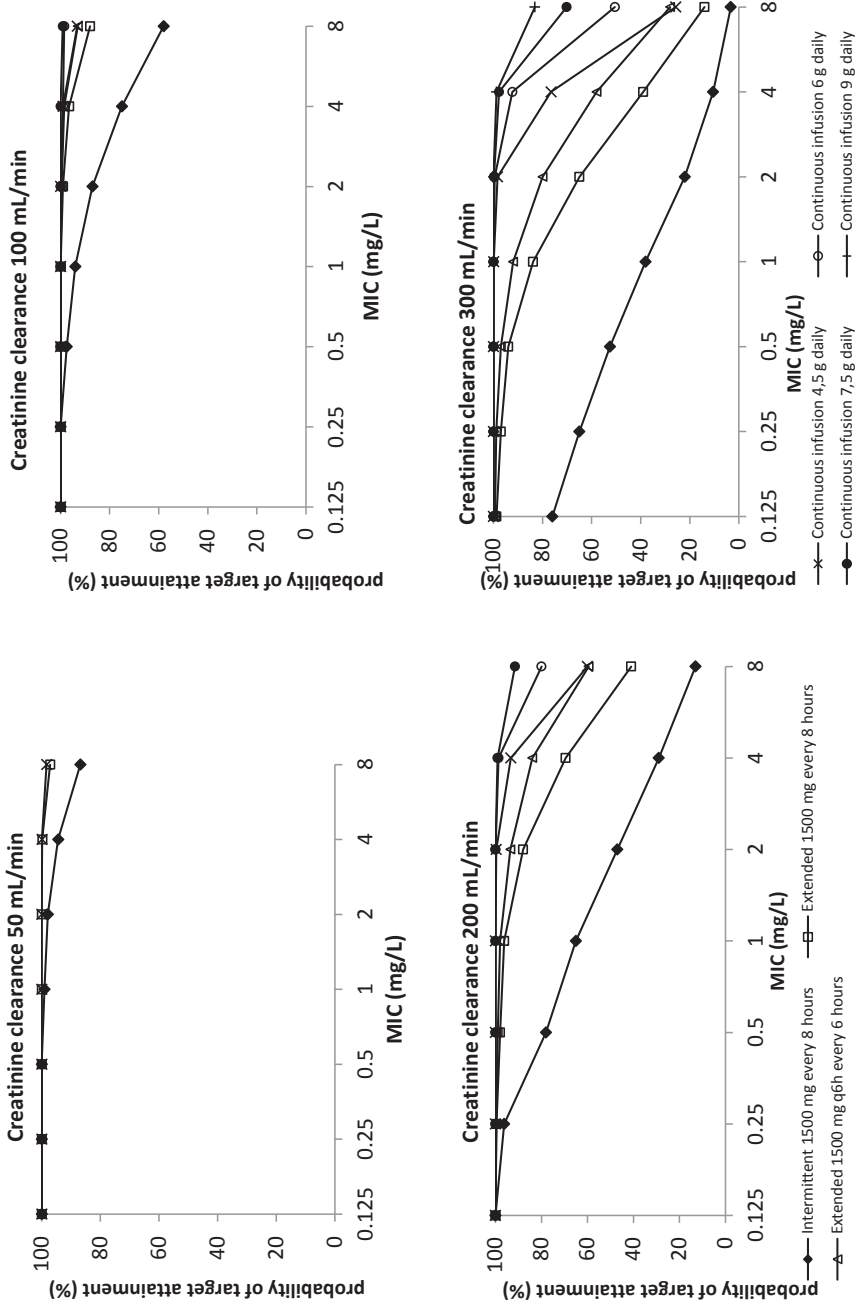
Fig. 2. Probability of target attainment for different dosing strategies for creatinine clearance 50, 100, 200 and 300 mL/min.

3. Population pharmacokinetics and dosing simulations of cefepime in critically ill patients

Authors : Mieke Carlier, Fabio S. Taccone, Majorie Beumier, Lucie Seyler, Fréderic Cotton, Frederique Jacobs, Jason A. Roberts

Abstract

Purpose: The aim of this study was to describe the population pharmacokinetics of cefepime in septic shock patients requiring continuous renal replacement therapy and determine whether current, or alternative dosing regimens can achieve pharmacokinetic/pharmacodynamic (PK/PD) targets.

Methods: In this observational PK study, 62 samples from 13 patients were analyzed using non-linear mixed-effects modeling. Different dosing regimens were evaluated using Monte Carlo simulations with ultrafiltration flow rates (UFR) of 1000, 1500 and 2000 mL/h. The probability of target attainment calculated against a conservative (60 % $T_{>MIC}$) and a higher PK/PD targets (100 % $T_{>MIC}$ against a minimum inhibitory concentration (MIC) of 8 mg/L, which is the clinical susceptibility breakpoint for *Pseudomonas aeruginosa*.

Results: A one-compartment model with between-subject variability (BSV) on clearance and volume of distribution described the data adequately. Ultrafiltration rate was supported as a covariate on both parameters. The typical values for clearance and volume of distribution were 4.4 L/h (BSV 37%) and 40.9 (BSV 20%) L respectively. Dosing simulations showed failure to achieve both a conservative and a higher PK/PD target using a dose of 1g q12h for patients treated with a high UFR (≥1500 mL/h). The dose of 2g q8h or 1g q6h leads to an optimal target attainment for high UFR. One g q8h is optimal for low UFR (≤1000 mL/h).

 Conclusions: We found important variability in PK parameters. The dosing simulations show that a dose of 2g q8h or 1g q6h is needed to ensure rapid achievement of adequate levels if the UFR ≥1500 mL/h, and 1 g q8h for low UFR (UFR ≤ 1000 mL/h).

3.1. Introduction

Septic shock is a leading cause of mortality and morbidity in intensive care units with hospital mortality as high as 40% [258]. Timely and adequate antibiotic therapy is then essential to maximize survival and is therefore highly recommended in the Surviving Sepsis guidelines [23, 59, 259].

β-lactam antibiotics are used as first-line therapy in this setting because of their potent bactericidal activity and wide therapeutic window. These antibiotics are considered to be time-dependent, which means the duration of the dosing interval for which the concentration exceeds the minimum inhibitory concentration (MIC) of the pathogen is the best descriptor of the bacterial killing. *In vitro* and animal pharmacodynamics (PD) models have shown that for cephalosporins, 60-70 % of the time that drug concentrations are above the MIC (%$T_{>MIC}$) between two administrations was associated with maximal killing [260], while retrospective studies in critically ill patients suggest higher targets such as 100% $T_{>MIC}$ might be needed to treat life-threatening infections [42, 43, 46]. However, several studies have shown that the pharmacokinetic (PK) behavior of these hydrophilic antibiotics is profoundly disturbed in critically patients, due to different pathophysiological changes [39]. A higher volume of distribution and either an increased or decreased clearance compared to healthy volunteers has been shown in numerous studies. As such, low concentrations have been reported in sepsis, and may lead to treatment failure and development of antimicrobial resistance [93].

Acute kidney injury is a common complication of sepsis and may lead to accumulation of hydrophilic drugs, which are mainly renally excreted. Although not very common, toxicity from β-lactam antibiotics may occur and is associated with high concentrations [107]. Extracorporeal circuits such as those used for continuous renal replacement therapy (CRRT) may further complicate PK. Indeed, recent studies showed a wide variability in antibiotic concentrations during CRRT, with many patients having low concentrations early in therapy, and accumulation occurring in the next days [107-109]. Unfortunately, there is relatively little clinical data on the drug removal by CRRT; moreover, it is unclear how the specific CRRT settings, such as ultrafiltration flow rate (UFR) and dialysis flow rate influence drug concentrations. Current recommendations on antibiotic dosing during CRRT are based on studies that included a limited sample size of patients who received different types of CRRT [261].

Cefepime is a fourth-generation cephalosporin with a broad spectrum of activity against both gram-positive and gram-negative pathogens including *P. aeruginosa*. Cefepime is commonly used as empirical or directed therapy for a variety of infections in critically ill patients. Adequacy of cefepime dosing during CRRT has previously been evaluated in studies with small cohorts of patients; however, a population PK approach for analysis was not used [262, 263], and therefore these studies could not adequately describe the influence of CRRT settings on cefepime PK. Moreover, these studies sampled after having reached assumed steady state and therefore could not evaluate cefepime PK during the early phase of treatment, where the risk of underdosing is the greatest. Therefore the aim of this study was to describe the population PK of cefepime in septic shock patients requiring CRRT and investigate if pharmacokinetic/pharmacodynamic (PK/PD) targets are achieved with current dosing strategies, as well as investigate the potential advantages of alternative dosing regimens.

3.2. Methods

Patients

In this study, we pooled data from two previously published PK studies, the details of which have been described elsewhere [107, 108]. The first study was a PK study with blood sampling on multiple occasions [108]. The study was conducted according to the principles of the Helsinki Declaration for human research, and was approved by the local ethics committee. An informed consent was obtained from the patient if possible or from a legally authorized representative. The second study reviewed data that had been collected as part of routine treatment. Therefore, the ethics committee waived the need for informed consent because of its retrospective nature [107]. The inclusion criteria of the first study were as follows: age > 18 years; diagnosis of severe sepsis or septic shock according to standard criteria; acute renal failure treated with CRRT; and receiving cefepime. Exclusion criteria were pregnancy, burns and cystic fibrosis. For the second study, there were additional inclusion criteria, namely a residual creatinine clearance (CrCl) <30 mL/minute and at least one therapeutic drug monitoring (TDM) sample taken during the CRRT treatment. An additional exclusion criterion was the use of extracorporeal membrane oxygenation (ECMO) therapy.

Drug administration

The patients received 2 g every 8 or 12 h, based on guidelines for antibiotic dosing in critically ill patients receiving CRRT [261]. The dose was administered as a 30-minute intravenous infusion.

Continuous renal replacement therapy

CRRT was performed according to local practice by insertion of a double-lumen catheter into the subclavian, femoral or internal jugular vein. Continuous veno-venous haemodiafiltration (CVVHDF) or continuous veno-venous haemofiltration (CVVHF) were performed using standard equipment (Prisma or Prismaflex, Gambro Hospal, Bologna, Italy) with a polyacrilonitrile cylinder (AN 69 – Hospal, Meysizeu, France) haemofilter without special coating. Anticoagulation was performed using systemic heparin or citrate within the circuit. Blood flow rate was set around 130 to 150 mL/minute and the ultrafiltration flow rate was adjusted to provide at least 15 to 20 mL/kg/h [107, 108]. CRRT intensity was calculated as dialysate flow rate (mL/kg/h) + ultrafiltration flow rate (mL/kg/h).

Study procedures

In the first study, blood samples were drawn from the arterial line on the day of inclusion, and then every second day during CRRT treatment whenever possible [108]. On each sampling day, blood samples were drawn immediately before antibiotic administration (0 hours), and then 1, 2, 5, and 6 or 12 hours (depending on the antibiotic regimen) after the start of the infusion. The exact sampling times were recorded. In the second study, two blood samples were drawn during the antibiotic elimination phase: 2 h after the end of infusion and just before the next dose administration[107].

Samples were immediately put on ice and sent to the clinical chemistry laboratory, where they were centrifuged at 3000 rpm at 4°C for 10 minutes; the supernatant was then removed and analyzed using a validated high-performance liquid chromatographic technique, as described elsewhere [108].

Additional data were obtained from the medical record and included participant demographics, clinical details, measures of illness severity and CRRT settings.

Pharmacokinetic analysis

The concentration-time data were analyzed using non-linear mixed-effects modeling (NONMEM version 7.3, Globomax LLC, Hanover, USA). A Digital Fortran compiler was used and the runs were executed using Wings for NONMEM (http://wfn.sourceforge.net). The first-order conditional estimation (FOCE) method with interaction was used throughout the model building.

Model development

For the population PK analysis, the plasma concentrations were fitted to one-, two-, or three-compartment linear models using subroutines from the NONMEM library. Between subject variability (BSV) was evaluated using an exponential variability model. Various models for residual unexplained variability (RUV) were also tested.

Model diagnostics

Visual inspection of diagnostic scatter plots and the NONMEM objective function value (OFV) were used to evaluate goodness of fit. Statistical comparison of nested models was undertaken in the NONMEM program on the basis of a $\chi 2$ test of the difference in OFV. A decrease in the OFV of 3.84 units ($p < 0.05$) was considered statistically significant for 1 degree of freedom. Decreases in BSV of one of the parameters of at least 10% were also accepted for inclusion of a more complicated model.

Covariate screening

Covariate model building was performed in a stepwise fashion with forward inclusion based upon the aforementioned model selection criteria for those clinical parameters, which showed correlation with one of the PK parameters. Age, sex, weight, body mass index (BMI), Sequential Organ Failure Assessment (SOFA) score and CRRT settings (blood flow rate, ultrafiltration flow rate, dialysis flow rate and CRRT intensity) were evaluated as covariates. In the case two or more parameters (for example weight and BMI, or ultrafiltration flow rate and CRRT intensity) both improved OFV, the decision to choose one covariate over another was based on the decrease in OFV, comparative improvement in the goodness of fit plots and biological plausibility.

Bootstrap

A non-parametric bootstrap method (n=1000) using NONMEM was used to study the uncertainty of the pharmacokinetic parameter estimates in the final model. From the

bootstrap empirical posterior distribution, we obtained the 95% CI (2.5%–97.5% percentile) for the parameters as described previously [243].

Dosing simulations

Different dosing regimens were evaluated using Monte Carlo simulations of the final covariate model. The ultrafiltration flow rates simulated were 1000, 1500 and 2000 mL/h. One thousand subjects were simulated per dosing strategy and per ultrafiltration flow rate. The simulated dosages were: 1 g every 12 h, 2 g every 12 h, 2 g every 8 h, 1 g every 8 h and 1 g every 6 h. All simulated dosages were intermittent infusions.

For each simulation, the $T_{>MIC}$ was calculated for each simulated subject using linear interpolation. The probability of target attainment (PTA) was obtained by counting the subjects who achieved the PK/PD target against an MIC of 8 mg/L, which is the susceptibility breakpoint of cefepime against *Pseudomonas aeruginosa* [235]. We also calculated the PTA against an MIC of 16 mg/L for the high dose regimens, to evaluate the potential adequacy of dosing against less susceptible strains. Both a conservative PK/PD target, 60 % $T_{>MIC}$ and a higher PK/PD target, 100 % $T_{>MIC}$ were evaluated.

We also determined the probability of subjects achieving a toxic concentration which was defined as a trough concentration exceeding 70 mg/L after 1 week of therapy based on a previous case report which also reviewed the relevant literature [162].

3.3. Results

Patient characteristics

A total of 62 blood samples from 13 patients were used. Eight patients were enrolled in the first study [108]. A second round of sampling was performed in 3 patients. The median number of doses before sampling was one (range 0-3). Five patients were enrolled in the second study [107]. One patient contributed data on 3 occasions. The median number of doses before sampling was 6 (range 0 – 15). Demographic and general clinical characteristics are shown in table 1.

Table 1: Patient characteristics. Data are reported as median (interquartile range) [range]

Age (years)	59 (43-70) [19-77]
Male sex – no (%)	7 (54 %)
Weight (kg)	70 (65-75) [60-110]
BMI (kg/m²)	24.5 (23.9-27.2) [20.5-29.4]
Mechanically ventilated – no (%)	11 (85 %)
Vasopressors – no (%)	10 (77 %)
SOFA score at the start of study	10 (9-14) [4-19]
APACHE II score on admission	20 (14-21) [11-24]
Blood flow rate (mL/min)	150 (140-150) [100-180]
Ultrafiltration rate (mL/h)	1750 (1500-2000) [1000-2000]
CRRT intensity (mL/kg/h)	36 (25-46) [10.5-57.1]
Anticoagulation	
Heparin – no (%)	9 (69 %)
Citrate – no (%)	4 (31 %)

BMI : body mass index, SOFA : sequential organ failure assessment, APACHE : Acute Physiology and Chronic Health Evaluation, CRRT : continuous renal replacement therapy

Pharmacokinetic analysis

The best base model consisted of a one-compartment linear model with zero order with combined additive-proportional residual unknown variability. Between-subject variability was supported on clearance and on volume of distribution. Only RRT intensity and ultrafiltration rate improved the baseline model, but ultrafiltration rate caused the greatest decrease in objective function and we therefore opted to include ultrafiltration rate as a covariate. The model could not be further improved by adding between occasion variability, as the decrease in objective function was not sufficient (2.349). As there was no correlation between weight (ranging between 60 and 110 kg) and volume of distribution ($R^2 = 0.0068$) we found no justification to incorporate weight as a covariate on volume of distribution. The only covariate supported for addition to the baseline model was ultrafiltration flow rate, normalized to the population's median value of 1750 mL/h.

The final model is represented as:

$$TVCL= \theta_1*(UFR/1750) \qquad (1)$$

$$TVV= \theta_2*(UFR/1750) \qquad (2)$$

The typical value of clearance (TVCL) was calculated as a function of ultrafiltration flow rate, normalized to the population's median value of 1750 mL/h. The typical value of volume

of distribution (TVV) was calculated as a function of ultrafiltration flow rate, also normalized to the population's median value of 1750 mL/h.

The typical value for clearance was 4.4 L/h (37 % BSV) and for volume of distribution 40.9 L (20 % BSV). On a L/kg basis, the median value for volume of distribution was 0.66 (interquartile range 0.48-0.73). The coefficient of variation of the exponential residual unexplained variability was 30 % and the standard deviation of the additive residual unexplained variability was 3.4 mg/L.

Figure 1 displays goodness of fit plots and the visual predictive check for the final covariate model and shows that the model describes the measured concentrations adequately. All subsequent dosing simulations were then based on this model.

The values of the parameters for the final models are given in table 2 and include the 95% confidence intervals for the parameters computed from all bootstrap runs.

Dosing simulations

The PTA for an MIC of 8 mg/L and the probability of achieving a toxic concentration for different dosing regimens and different ultrafiltration flow rates are shown in table 3. The PTA against an MIC of 16 mg/L for the high dose regimens is summarized in table 4.

When considering the conservative target of 60 % $T_{>MIC}$, 1 g of cefepime every 12 h will result in adequate concentrations for almost all patients with an UFR of 1000 mL/h, however, this is not the case for patients treated with an UFR of 1500 mL/h and 2000 mL/h (PTA 80 % and 49% respectively). When aiming for 100 % $T_{>MIC}$, this dose does not lead to sufficient PTA for all ultrafiltration flow rates. There is no accumulation to toxic concentrations after one week of treatment, as illustrated in figure 2a and b.

Two g of cefepime every 12 h results in a high PTA when aiming for the conservative target, but still suboptimal when aiming for 100 % $T_{>MIC}$ (PTA 82 % for an UFR of 1500 mL/h and 73 % for an UFR of 2000 mL/h). Moreover, it leads to toxic concentrations in 5% of the patients treated with an UFR of 1000 mL/h.

Two g of cefepime every 8 h results in optimal target attainment for both the conservative, as well as the high target for all ultrafiltration flow rates, however it leads to a significant proportion of patients achieving toxic levels after one week of treatment (up to 30 % for patients treated with an UFR of 1000 mL/h, as shown in figure 2c). One g every 8 h

ensures optimal target attainment for an UFR of 1000 mL/h while minimizing toxicity (figure 2e). However, this dose results in a PTA of only 79 % for patients with an UFR of 2000 mL/h when aiming for 100 % $T_{>MIC}$. One g every 6 h leads to optimal PTA for patients treated with an UFR of 1500 and 2000 mL/h (97 % and 93 % respectively for the target of 100 % $T_{>MIC}$), while minimizing toxicity (figure 2f).
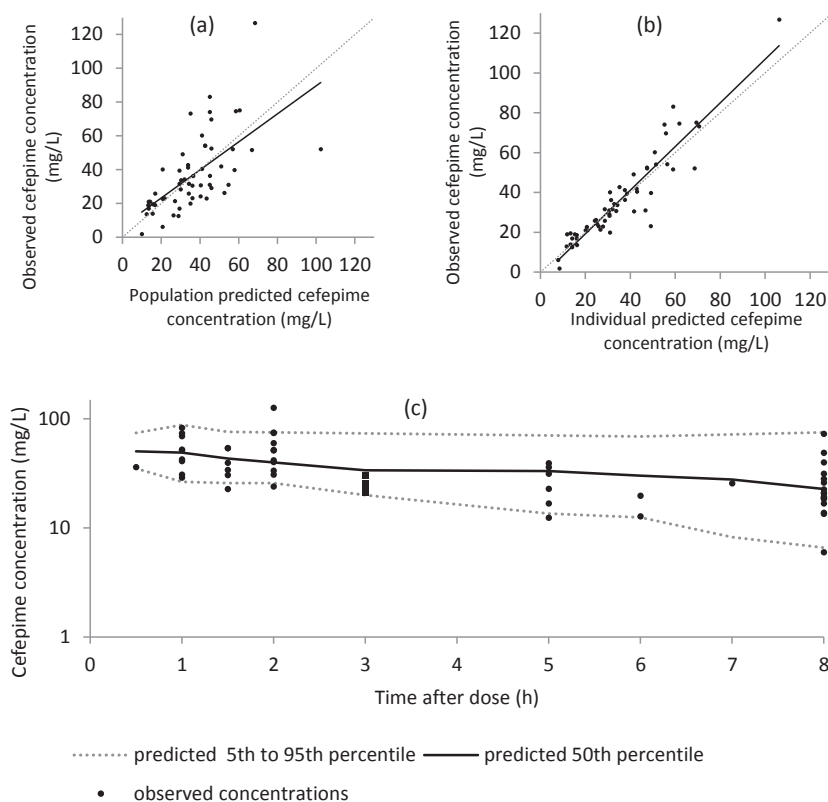
Figure 1

Diagnostic plots for the final population pharmacokinetic covariate model. (a) population predicted cefepime concentrations versus observed concentrations (R^2=0.41). (b) Individual predicted cefepime concentrations versus observed concentrations (R^2=0.87). The non-linear regression line of fit is shown by the black continuous line and the line of identity xy is shown by the grey dotted line. (c) Visual predictive check generated from a Monte Carlo simulation (n=1000) and showing that the population pharmacokinetic model has adequate performance. The raw data are shown as black dots.

Table 2 : Bootstrap parameter estimates of the final covariate model

	Model		**Bootstrap**	
	Mean	Mean	95 % confidence interval	
			2.5 %	97.5 %
Fixed effects				
Cl (L/h)	4.5	4.5	3.6	5.6
V (L)	40.8	40.6	33.4	48.7
Random effects **BSV (% CV)**				
Cl (L/h)	37.7	35.9	17.1	49.5
V (L)	21.2	19.9	0.2	26.8
Random error				
RUV (% CV)	20.4	19.9	10.9	27.3
RUV (SD, mg/L)	3.3	3.3	0.03	5.8

Cl = Clearance, V = Volume of distribution ; BSV = between subject variability; RUV = residual

unexplained variability; CV = coefficient of variation, SD = standard deviation

Table 3: Probability of target attainment for different dosing strategies and different ultrafiltration flow rates

Dose	UFR 1000			UFR 1500			UFR 2000		
	PTA 100% $T_{>MIC}$	PTA 60% $T_{>MIC}$	Probability of toxicity	PTA 100% $T_{>MIC}$	PTA 60% $T_{>MIC}$	Probability of toxicity	PTA 100% $T_{>MIC}$	PTA 60% $T_{>MIC}$	Probability of toxicity
1g q12 h	64 %	95 %	0 %	31 %	80 %	0 %	9 %	51 %	0 %
2g q12 h	89 %	99 %	5 %	82 %	98 %	0 %	73 %	95 %	0 %
1g q8 h	95 %	100%	3 %	87 %	99 %	0 %	77 %	95 %	0 %
2g q8 h	99 %	100 %	30 %	96 %	100 %	9 %	92 %	100 %	3 %
1g q6 h	100	100	10 %	97 %	100	2 %	93 %	100 %	0 %

UFR : ultrafiltration flow rate (mL/h) , PTA : probability of target attainment, q12 h : every 12 h, q8h : every 8 h

Table 4: Probability of target attainment against an MIC of 16 mg/L for the high dosing strategies and different ultrafiltration flow rates

Dose	UFR 1000		UFR 1500		UFR 2000	
	PTA 100% $T_{>MIC}$	PTA 60% $T_{>MIC}$	PTA 100% $T_{>MIC}$	PTA 60% $T_{>MIC}$	PTA 100% $T_{>MIC}$	PTA 60% $T_{>MIC}$
1g q8h	76 %	96 %				
2g q8h			89 %	99 %	76 %	96 %
1g q6h			82 %	96 %	65 %	87 %

UFR : ultrafiltration flow rate (mL/h) , PTA : probability of target attainment, q12 h : every 12 h, q8h : every 8 h
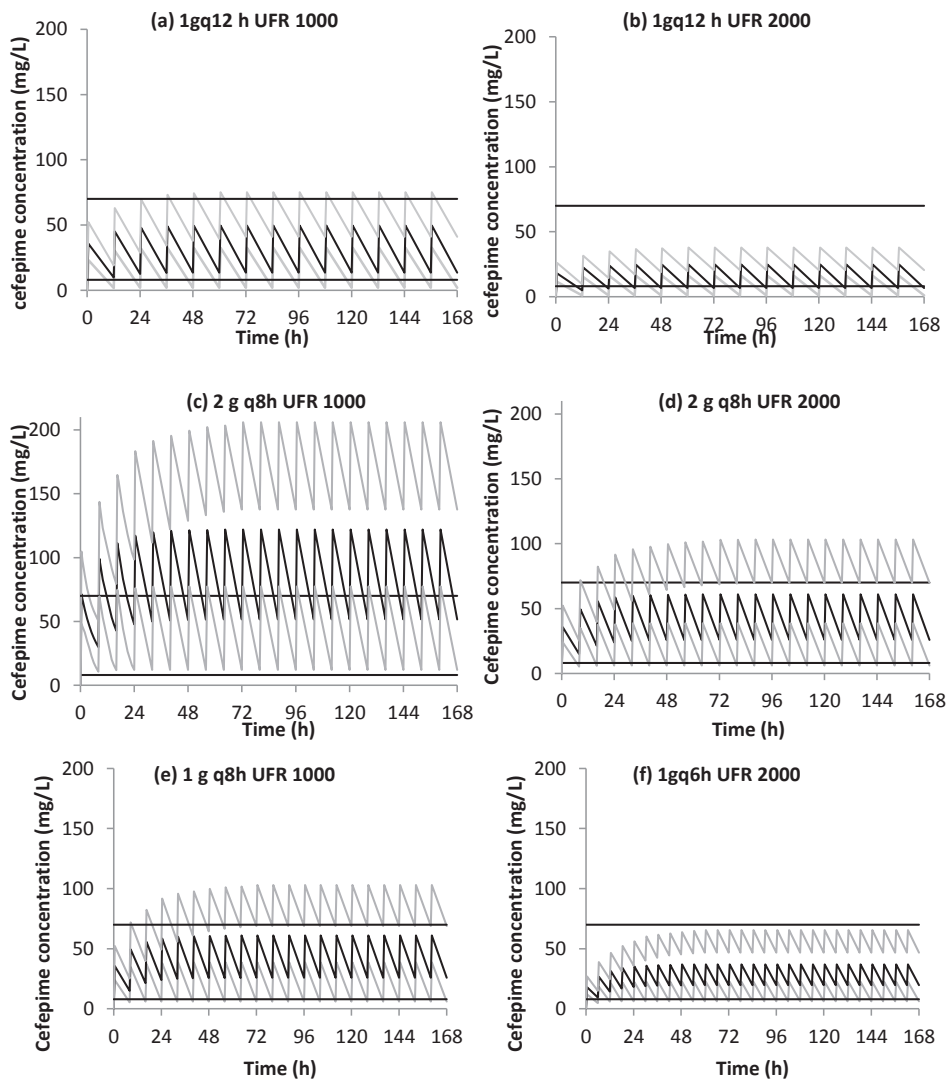
Figure 2: Dosing simulations for cefepime different doses for different ultrafiltration flow rates. Legend: black line : 50 % percentile, grey lines : 2.5 and 97.7 % percentiles. (**a**) cefepime 1 g every 12 hours ultrafiltration flow rate 1000 mL/h. (**b**) cefepime 1 g every 12 hours ultrafiltration flow rate 2000 mL/h. (**c**) cefepime 2 g every 8 hours ultrafiltration flow rate 1000 mL/h. (**d**) cefepime 2 g every 8 hours ultrafiltration flow rate 2000 mL/h. (**e**) cefepime 1 g every 8 hours, ultrafiltration flow rate 1000 mL/h. (**f**) cefepime 1 g every 6 hours, ultrafiltration flow rate 2000 mL/h. UFR : ultrafiltration rate; q12h : every 12 hours; q8h : every 8 hours; q6h : every 6 hours

3.4.   Discussion

This is the first paper to investigate the population PK of cefepime in septic shock patients requiring CRRT. We found that antibiotic clearance was proportional to ultrafiltration flow rate, with important variability between patients for both clearance and volume of distribution. The typical values we found for clearance (4.4 L/h) closely resembled the value for clearance found in a previous study (4.0 L/h) in patients treated with CRRT [263]. The same applies for the volume of distribution, where previous study found a volume of distribution of 0.71 L/kg, and we found a mean value for volume of distribution of 0.64 L/kg [263].

Another study has also been published, which investigated the PK separately for patients treated with CVVH and CVVHDF, found a smaller volume of distribution of 0.46 L/kg for the patients treated with CVVH (which removes solute by convection, and therefore no dialysate is used), and a clearance of only 2.1 L/h [262]. In the patients treated with CVVHDF (which removes solute both by diffusion and convection, and uses both ultrafiltration and dialysis), the mean volume of distribution was 0.34 L/kg and a clearance of 2.8 L/h [262]. However, the mean ultrafiltration flow rate was 960 mL/h in the case of CVVH and 1020 mL/h in the case of CVVHDF. In our study the mean ultrafiltration flow rate of 1673 mL/h was significantly higher than the ultrafiltration flow rates used in this previous study. As ultrafiltration flow rate is a covariate on both volume of distribution and clearance, this may explain the higher typical values for volume of distribution and clearance in our study.

Multiple studies have already investigated cefepime concentrations during CRRT, although not specifically in patients requiring vasopressor support. Two studies report that 1–2 g every 12 h is sufficient to maintain adequate plasma concentrations [262, 263]. One study reported low concentrations during high blood and dialysate flow rate in CVVH [264]. We have found that a dose of 1 g every 12 h, as suggested by a previous PK study, will not achieve 60 % $T_{>MIC}$ exposures for 20 % of the patients treated with an ultrafiltration flow rate of 1500 mL/h (69 % when aiming for 100 % $T_{>MIC}$) when the MIC is 8 mg/L, and up to 50 % when the ultrafiltration flow rate is 2000 mL/h (91 % of the patients when aiming for the higher PK/PD target of 100 % $T_{>MIC}$) [262].

When aiming for the minimum PK/PD target of 60 % $T_{>MIC}$, the recommended dose of 2 g every 12 h will still result in inadequate exposure in 5 % of the patients treated with a high flow ultrafiltration flow rate of 2000 mL/h, and 27 % of the patients when aiming for the higher PK/PD target of 100 % $T_{>MIC}$ [261].

Because of our population PK approach, we were able to investigate the effect of CRRT settings on the cefepime PK and have found that a dose of 2 g every 8 h or 1 g every 6 h leads to an optimal target attainment (100 % $fT_{>MIC}$) whilst minimizing the probability of reaching toxic trough concentrations for patients treated with a high ultrafiltration flow rate (1500 – 2000 mL/min). However, the optimal dose for patients treated with lower ultrafiltration flow rates (1000 mL/h or less) when aiming for the high target was 1g every 8 h. However, even when the dose is adapted to the ultrafiltration flow rate, there is still a huge variability in concentrations between patients, as shown in figure 2, which supports a potential role for therapeutic drug monitoring.

There are a number of limitations of the current analysis we would like to discuss. First, we only investigated total concentrations in blood, while the unbound antibiotic is responsible for the pharmacological effect. However, protein binding for cefepime is low, and therefore the potential effect of changes in protein binding is expected to be limited. Secondly, we only sampled blood, and did not investigate concentrations in the fluids pre and post filter, so we are unable to be more mechanistic with our description of altered pharmacokinetics. Moreover, the study designs of the 2 studies which contributed the data for this population pharmacokinetic model are different and are associated with different limitations. Most of the blood samples from the second study were sampled comparatively late in the antibiotic course and are likely to represent an apparent steady state, while the blood samples from the PK study were sampled much earlier, most likely before steady state was reached. This problem should be overcome through use of the non linear mixed effects modeling used here. However, a potential problem is that the performance of the filter may change over time, something which cannot be taken into account in this retrospective pharmacokinetic analysis because of a lack of data on filter age. Also, the type of filter may affect the pharmacokinetics. This study was conducted using a polyacrylonitrile membrane hemofilter, and as such, our findings apply for this kind of filter only, and not for filters made from other materials, such as polysulfone. Although none of the patients were taken off CRRT during

sampling, it is possible that this happened during the previous days, which may result in some drug accumulation. We did not investigate cefepime associated toxicity in this study. A study on the toxicity of β-lactam antibiotics in critically ill patients found around 30 % neuroworsening in patients treated with cephalosporins, which was similar to those treated with penicillins or carbapenems [35]. However, there was no correlation between cephalosporins concentrations and the risk of neuroworsening while this was found for other β-lactam antibiotics. It is clear that the study of toxicity is difficult, with many confounders, and that setting a specific concentration threshold is also difficult. The threshold we used for toxicity (trough concentration > 70 mg/L after 1 week of treatment) has been synthesized from published reports in the literature. There is limited evidence between concentrations and neurological toxicity, although there is strong biological plausibility. From the available literature it is apparent that cefepime is associated with seizures and this effect is concentration-dependent. The majority of published case reports on cefepime associated toxicity report concentrations around 70 mg/L although concentrations as low as 22 mg/L have been described [265]. A more specific threshold may be defined in the future, but since this is not available now, we set the toxicity threshold at 70 mg/L. Finally, we did not measure residual creatinine clearance, which may also influence antibiotic clearance.

Therefore, larger and better designed studies are needed. However, in absence of these studies, we believe that the findings of this study are relevant as this is the first study reporting on the influence of CRRT settings on cefepime concentrations.

3.5. *Conclusion*

In this study in septic shock patients needing CRRT treated with cefepime, we found important PK variability in antibiotic clearance and volume distribution. The results of the dosing simulations show that a high dose of 2 g every 8 h or 1 g every 6 h is needed when the ultrafiltration rate is 1500 mL/h or more. A lower dose of 1 g every 8 h is optimal when the ultrafiltration rate is 1000 mL/h or less.

4. **Extended versus bolus infusion of meropenem and piperacillin: a pharmacokinetic analysis**

Authors : Jan J. De Waele , Mieke Carlier, Eric Hoste, Pieter Depuydt, Johan Decruyenaere, Steven C. Wallis, Jeffrey Lipman, Jason A. Roberts

Abstract

Background: Extended infusion of beta-lactam antibiotics has been advocated as a method for optimizing antibiotic exposure in critically ill patients. The objective of this study was to compare the pharmacokinetics/pharmacodynamics of extended infusion versus bolus infusion of piperacillin and meropenem in critically ill patients with normal renal function.

Methods: A prospective study of 3-h extended infusion of meropenem and piperacillin in critically ill patients without renal dysfunction. Results from the extended infusion cohort were compared to previously published bolus infusion data in critically ill patients.

Results. Twenty extended infusion patients (15 piperacillin, 5 meropenem) were compared with 13 bolus infusion patients (8 piperacillin, 5 meropenem). The demographic and clinical characteristics between both groups were not statistically different. Significant pharmacokinetic differences were observed in median (interquartile range) C_{max} for both meropenem (extended infusion 17.0 (12.6-21.9) vs. bolus 85.2 (66.7-140.3); p=0.01) and piperacillin (extended infusion 76.2 (57.7-92.6) vs. bolus 240.2 (168.5-275.4); p=0.001). Considerable pharmacokinetic variability existed in each group for both drugs. Compared to bolus infusion, $fT_{>MIC}$ using extended infusion was higher for both drugs: 96% (IQR 71-100%) compared to 77% (IQR 41-93%) for piperacillin (p=0.05) and 82% (IQR 63-89%) compared to 51% (IQR 48-63%) for meropenem (p=0.095); assuming an MIC of 16mg/L and 2mg/L respectively.

Conclusion. This study confirms that extended infusion in critically ill patients result in advantageous pharmacokinetic profiles by increasing the $fT_{>MIC}$ for piperacillin and meropenem. In a significant subpopulation of critically ill patients with normal renal function, a 100% $fT_{>MIC}$ target is not reached, even with 3-hour extended infusions.

4.1.   Introduction.

Broad-spectrum beta-lactam antibiotics are effective against a wide range of pathogens isolated from infected intensive care unit (ICU) patients, and are essential for effective empirical antibiotic therapy of various infections. From a pharmacodynamic point of view, these are time-dependent antibiotics, which means that the time during which the unbound antibiotic concentration exceeds the minimal inhibitory concentration ($fT_{>MIC}$) of the pathogen, determines bacterial killing.

In critically ill patients, pharmacokinetics of beta-lactam antibiotics may differ from healthy volunteers [97]. Lower than expected concentrations have been reported for meropenem, piperacillin, amoxicillin, as well as for cephalosporins [39, 49]. Increased elimination from the circulation, most often due to increased renal clearance and changes in the volume of distribution are often cited as the main causes for this [39]. These lower antibiotic concentrations may lead to a reduction of the $fT_{>MIC}$ in patients treated with beta-lactam antibiotics, increasing the risk of clinical failure of the antibiotic therapy.

To overcome this problem, continuous and extended infusion of beta-lactam antibiotics have been advocated as a method for optimizing beta-lactam antibiotic exposures in critically ill patients [266]. Whilst pharmacokinetic simulation data exist [140, 157, 242, 267-270], actual concentration-time data from critically ill patients supporting use of extended infusions are lacking. The objective of this study was to compare the pharmacokinetics/pharmacodynamics of extended infusion versus bolus infusion of piperacillin and meropenem targeting different $fT_{>MIC}$ targets (100% $fT_{>MIC}$, 50% $fT_{>MIC}$ and 100% $fT_{>4xMIC}$) in critically ill patients with normal renal function.

4.2.   Materials and methods.

Data for the pharmacokinetics of extended infusion piperacillin and meropenem were collected in a prospective pharmacokinetic study performed in the medical and surgical ICU of the Ghent University Hospital, a tertiary care hospital with a total of 50 adult ICU beds. Patients receiving either meropenem (Meronem®, AstraZeneca) or piperacillin/tazobactam (Tazocin®, Pfizer) were included if they did not meet exclusion criteria which included renal dysfunction (defined as an estimated glomerular filtration rate (eGFR) assessed by the Modification of Diet in Renal Disease (MDRD) equation of <80mL/min/1.73 m2), age<18

years, absence of an arterial catheter or absence of informed consent from the patient or the legal representative of the patient.

In the patients receiving extended infusion, the antibiotics were administered according to the extended infusion protocol used at Ghent University Hospital: patients receive a loading dose (1g meropenem or 4g piperacillin) administered over 30 minutes, followed immediately by the first extended infusion dose of either antibiotic (1g meropenem or 4g piperacillin) every 6h for piperacillin and every 8 hours for meropenem. Extended infusion doses are administered over 3 hours using a syringe pump. All antibiotics were administered via a central venous catheter.

Data collected at baseline included demographic data, severity of illness at admission (APACHE-II [271] and SOFA [272] score), and severity of organ dysfunction at study inclusion (SOFA score).

Serial plasma concentrations were obtained between 24-48 hours after the start of therapy at baseline (T0, just prior to initiation of the extended infusion) and after 60 (T1), 120 (T2), 180 (T3), 210 (T4), 240 (T5), 270 (T6), 360 (T7) and 480 (T8) minutes for meropenem; at baseline (T0, just prior to initiation of the extended infusion) and after 60 (T1), 120 (T2), 180 (T3), 210 (T4), 240 (T5), 270 (T6), 300 (T7) and 360 (T8) minutes for piperacillin.

For each sample, 5mL of blood was collected in anticoagulant tubes without separator gel, via the arterial catheter. Specimens were centrifuged at 3000 rpm for 10 min within 30minutes of sampling, and then frozen at minus 80°C. They were shipped to the Burns, Trauma, & Critical Care Research Centre of the University of Queensland, Australia for analysis through a specialized carrier.

Kidney function was described using serum creatinine concentrations and measured 24-h urinary creatinine clearance.

Assuming an MIC_{90} of 16mg/L (piperacillin) and 2mg/L (meropenem) [235], $fT_{>MIC}$ was calculated and a 100% $fT_{>MIC}$ was considered the desired pharmacokinetic/pharmacodynamic target. Secondary PK targets were 50% $fT_{>MIC}$ and 100% $fT_{>4xMIC}$.

Comparison with bolus infusion

Pharmacokinetic results were compared to data from bolus infusion obtained in previous prospective pharmacokinetic studies [140, 157].

Assay

Samples for both the extended infusion and the previously conducted bolus infusion studies were both analyzed at the Burns Trauma and Critical Care Research Centre, The University of Queensland. The plasma concentrations of meropenem and piperacillin were determined by validated High Performance Liquid Chromatography (HPLC) methods based on a published procedure that has been optimized for each drug [168]. Sample preparation was by protein precipitation with acetonitrile and a wash step with dichloromethane. Separations were performed on a Waters X-bridge C18 column (2.1 x 30 mm, 2.5 nm) with an acetonitrile:phosphate buffer mobile phase (pH 2.5 for meropenem, pH 3 for piperacillin). Detection was by UV at 304 nm (meropenem) or 210 nm (piperacillin). The meropenem assay was linear from 0.2 to 100 mg/L with a precision and accuracy <7% at high, medium and low concentrations. The piperacillin assay was linear from 0.5 to 500 mg/L with a precision and accuracy <10% at high, medium and low concentrations.

Pharmacokinetic analysis

The pharmacokinetic values were calculated using non-compartmental methods. The area under the concentration time curve from 0-8 hours (AUC_{0-8}) was calculated using the linear trapezoidal rule. The AUC from 0 to infinity ($AUC_{0-\infty}$) was calculated using AUC_{0-8} and the apparent terminal elimination rate constant (λ_z) which was determined from log-linear least squares regression analysis of concentrations from 2-8 hours (meropenem) or 2-6 hours (piperacillin). Total body clearance (CL_{tot}) was calculated as dose/$AUC_{0-\infty}$. The area under the moment curve from 0-8 hours ($AUMC_{0-8}$) was calculated using the linear trapezoidal rule and AUMC from 0-∞ ($AUC_{0-\infty}$) and λ_z. Mean residence time (MRT) was calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$. The maximum concentration for the dosing period (C_{max}) and the minimum concentration for the dosing period (C_{min}) were the observed values; the apparent volume of distribution during terminal phase (V_z) = CL/λ_z; the half life ($T_{1/2}$) = $\ln(2)/\lambda_z$.

Pharmacodynamic analysis

The f T$_{>MIC}$ was calculated by observing the time during the dosing interval that the log-linear least squares regression analysis of concentrations in the elimination phase intersected the target MICs (16 mg/L for piperacillin and 2 mg/L for meropenem based on EUCAST breakpoints available at www.eucast.org). Observed concentrations were corrected for protein binding (piperacillin 30%; meropenem 2%) [273].

Statistical analysis

The statistical analysis was performed using the statistical software package SPSS 19.0 (SPSS Inc. Chicago, USA). Categorical variables were compared with the Fisher's exact test; continuous variables were analyzed using the Mann Whitney-U test. Data are expressed as median values with interquartile ranges (IQR) for continuous variables, numbers and percentages for categorical variables. All tests were two-tailed, and P<0.05 was considered statistically significant.

Ethics

The study was approved by the Ethics Committee of the Ghent University Hospital (registration number 2009/543) and registered with the European Union Drug Regulating Authorities Clinical Trials (registration number 2008/006825-15). The bolus infusion studies were previously approved by the Ethics Committee of the Royal Brisbane and Women's Hospital, Brisbane, Australia (registration numbers 2005/072 and 2005/028). Written informed consent was obtained from the patient or his/her legal representative.

4.3.   Results.

Patient characteristics.

Twenty patients receiving extended infusion (15 piperacillin/tazobactam and 5 meropenem) were compared to 13 patients receiving bolus infusion. Extended and bolus infusion patients characteristics were similar, and are summarized in table 1.

Table 1. Patient characteristics from extended infusion and bolus infusion groups (data are presented as median (interquartile range).

	Meropenem			Piperacillin		
	Extended infusion (n=5)	Bolus infusion (n=5)	p-value	Extended infusion (n=15)	Bolus infusion (n=8)	p-value
Age (year)	54 (51-60)	55 (48-61)	0.84	60 (52-73)	41 (22-65)	0.19
Height (m)	1.80 (1.74-1.80)	1.70 (1.70-1.80)	0.42	1.72 (1.65-1.79)	1.74 (1.72-1.80)	0.27
Weight (kg)	90 (85-90)	80 (75-85)	0.55	78 (70-82)	83 (75-86)	0.24
SeCr (µmol/L)	44 (43-54)	73 (55-101)	0.10	59 (45-64)	57 (49-69)	0.73
SOFA	5 (3-10)	3 (3-4)	0.22	7 (4-9)	3 (3-5)	0.07

Legend: SOFA – Sequential Organ Failure Assessment; SeCr – Serum creatinine

All patients but one received the standard dose, either 1g meropenem or 4g pipieracillin/tazobactam. One patient received high dose meropenem (2g/8h) according to the same scheme for a central nervous system infection, but this was accounted for in the pharmacokinetic analyses.

Pharmacokinetics results.

Extended infusion patients had significantly lower C_{max} and higher C_{min} values; the area under the concentration-time curve during 8-hour dosing period and mean residence time were also longer in extended infusion patients (table 2). Volume of distribution, the elimination rate constant and total drug clearance were comparable for both meropenem and piperacillin.

Considerable variability was found in both bolus and extended infusion, as illustrated by the wide IQR for all pharmacokinetic variables.

Target attainment

Compared to bolus infusion, $fT_{>MIC}$ was higher using extended infusion for both antibiotics: 96% (IQR 71-100%) compared to 77% (IQR 41-93%) for piperacillin (p = 0.05) , and 82% (IQR 63-89 %) compared to 51 % (IQR 48 – 63 %) for meropenem (p = 0.02) (Figure 1 and 2).

All patients receiving piperacillin as an extended infusion achieved the minimum pharmacokinetic target of 50% $fT_{>MIC}$, whilst only 62.5 % of the patients receiving piperacillin as a bolus infusion achieved this target (p=0.007). This was not statistically significant for meropenem (100 % vs. 60 %, p = 0.4). Only half of the patients on piperacillin as an extended infusion achieved the target of 100% $fT_{>4xMIC}$, versus none of the patients receiving piperacillin as a bolus infusion (p= 0.013). None of the patients receiving meropenem achieved 100% $fT_{>4xMIC}$, regardless of infusion strategy.
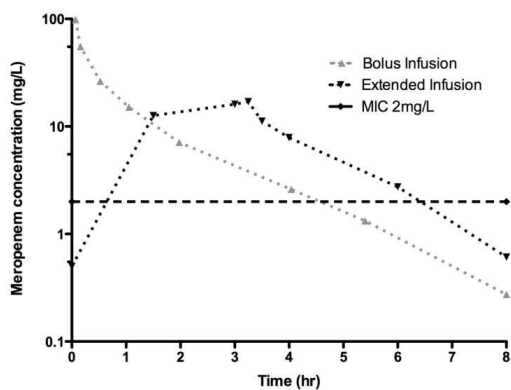
Fig. 1. Median meropenem concentrations in patients treated with extended and bolus infusion.
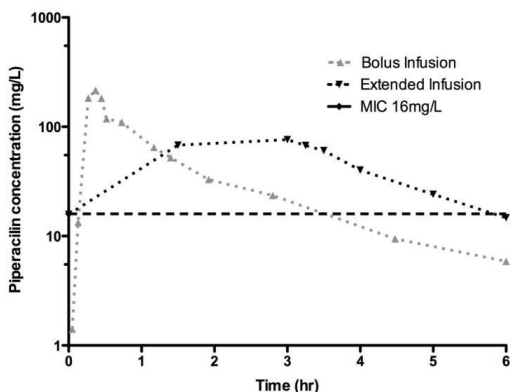
Fig. 2. Median piperacillin concentrations in patients treated with extended and bolus infusion

Table 2. Steady-state pharmacokinetic parameters for meropenem and piperacillin by either extended infusion or bolus infusion .

Data are reported as median (interquartile range)

	Meropenem			Piperacillin		
	Extended infusion (n=5)	Bolus infusion (n=5)	p-value	Extended infusion (n=15)	Bolus infusion (n=8)	p-value
C_{max} (mg/L)	17.0 (12.6-21.9)	85.2 (66.7-140.3)	0.01*	76.2 (57.7-92.6)	240.2 (168.5-275.4)	0.001*
C_{min} (mg/L)	0.6 (0.3-1.4)	0.0 (0.0 – 0.0)	0.03*	14.7 (4.2-25.3)	5.9 (2.7-13.1)	0.21
AUC0-8/0-6 (mg.h/L)	59.6 (41.5-77.5)	63.8 (46.9-66.8)	1.00	290.3 (172.6-324.1)	242.4 (170.9-374.8)	0.39
AUMC0-8/0-6 (mg.h²/L)	183.5 (111.8-227.5)	67.4 (46.5-82.2)	0.03*	808.9 (431.6 – 917.0)	303.4 (234.1-693.0)	0.02*
CL (L/hr)	15.9 (12.8-23.8)	15.7 (14.1-21.3)	0.84	13.2 (10.2-22.7)	16.2 (9.9-22.2)	0.93
MRT	2.9 (2.7-3.0)	1.0 (0.9-1.3)	0.01*	2.7 (2.6-2.8)	1.6 (1.4-1.7)	<0.001*
K_{el} (h-1)	0.7 (0.5-0.7)	0.7 (0.6-1.0)	0.69	0.6 (0.4-0.9)	0.4 (0.4-0.5)	0.33
Vz (L/kg)	0.39 (0.31-0.41)	0.24 (0.23-0.4)	0.69	0.33 (0.25-0.36)	0.36 (0.27-0.72)	0.36
T1/2 (h)	1.0 (1.0-1.5)	1.0 (0.7-1.10)	0.69	1.2 (0.8-1.9)	1.6 (1.5-1.9)	0.36

C_{max}: observed maximum concentration during sampling period; C_{min} : observed minimum concentration during sampling period; AUC0-8: area under the concentration-time curve during 8-hour dosing period; AUMC0-8: area under the moment curve during 8-hour dosing period; MRT: mean residence time; Cl: total clearance; K_{el}: elimination rate constant; T1/2: elimination half-life; Vz: volume of distribution during terminal phase

4.4.   Discussion.

In this study, we observed considerable variability in pharmacokinetics of extended infusion piperacillin and meropenem in critically ill patients that is similar to the variability observed in bolus infusion studies. Although this is one of the first reports of actual concentration-time data of extended infusion in critically ill patients, our findings are in line with earlier reports that have looked at different administration modalities of these antibiotics [140, 157]. This pharmacokinetic variability also translated to PD variability from variable PK/PD target attainment rates. These results suggest that even 3-hour extended infusion TID or QID may not be sufficient to reach a predefined PK/PD target of 100% $fT_{>MIC}$ in the majority of patients.

A previous study by Shea et al., studying 13 hospitalized patients who received a 4-hour extended infusion of piperacillin/tazobactam [269], reported that C_{max} and C_{min} were considerably higher than in the current study. Despite the longer infusion duration, mean C_{max} was 108.2 mg/L and C_{min} 27.6 mg/L, levels that were only reached in less than 25% of the patients in our study. This illustrates that pharmacokinetics in critically ill patients with a median SOFA score of 7 at the moment of sampling, may still be significantly different from hospitalized patients, probably due to the pathophysiological changes of critical illness [274].

Compared to bolus infusion, extended infusion resulted in better PK/PD target attainment rates. $fT_{>MIC}$ was higher for both antibiotics, reaching 82 % $fT_{>MIC}$ for meropenem, and 96 % for piperacillin. Obviously, target attainment rates will be much lower for higher targets such as $fT_{>4xMIC}$, and extended infusion alone may not be sufficient to reach these higher targets. Increasing the dosing frequency or using continuous infusion may be a solution to increase target attainment, but also standard dosages for these antibiotics may be inadequate. It is striking that current dosing schemes do not always result in adequate antibiotic concentrations, which may be explained by the fact that dosing commonly is based on PK data from healthy volunteers or non-critically ill patients. Therefore increasing the dose administered over 24h may also be necessary, irrespective of the infusion strategy chosen.

We also found that increased creatinine clearance is a frequent phenomenon in patients treated with antibiotics. Although the exclusion of kidney dysfunction is an obvious bias, the problem of augmented renal clearance (ARC) cannot be underestimated [110, 275]. Studies

in selected patients such as severe closed head injury have reported incidences of ARC as high as 85% [276]. Using a cut-off of 130mL/min, we have previously reported that 51.6% of patients with apparent normal renal function receiving antibiotic therapy have increased creatinine clearance, and that this was associated with worse clinical outcome [117]. Although renal elimination is by no means the sole determinant of low antibiotic concentrations, it is associated with higher drug clearance and has been associated with low trough concentrations [120, 156, 277]. Because it can be easily measured in clinical practice, it provides an appropriate method to predict those patients at risk of sub-therapeutic antibiotic exposures who are probably at the highest risk of clinical failure. Efforts should be done to more rapidly identify these patients, either using clinical characteristics or biomarkers.

The question remains however if efforts should not concentrate on developing analytical methods for rapid determination of antibiotic concentrations. Currently, HPLC or similar methods are labor intensive, and turnaround times are at least 4h; moreover, laboratories performing these analyses usually do a limited number of runs per week, which limits practical use of therapeutic drug monitoring of beta-lactam antibiotics [168, 169, 178, 224].

Extended infusion of antibiotics is easy to apply in critically ill patients. Most patients have multi-lumen central venous catheters, and the equipment needed (syringes and a syringe pump) is readily available in most ICUs. We prefer the use of syringe pump as infusion pumps require special tubing sets which have high priming volumes, which may result in incomplete or interrupted administration of the antibiotic [278].

Strategies aimed at improving the use of currently available antibiotics are highly relevant, since few new antibiotics are being developed, especially for Gram-negative infections. From this study it can be concluded that antibiotics are used suboptimally in some patients. Although we focused on piperacillin and meropenem, it can be assumed that the same concepts apply for all beta lactam antibiotics. These agents are widely used in ICUs around the world. With the advent of more resistant (or less susceptible) microorganisms this could become even more relevant in the future. Increasing the $fT_{>MIC}$ through extended infusion may therefore be a strategy to counter a gradual increase in MIC. Also, micro-organisms that are borderline resistant could still be treated with beta-lactam antibiotics provided a safe as well as effective concentration of the drug can be reached. Similarly,

applying these strategies may have potential value by decreasing the emergence of antimicrobial resistance.

This study has a number of limitations. First of all, only patients with apparent normal renal function were included, which limits extrapolation of these finding to all ICU patients. Second, the number of patients included in the meropenem group was low, which may explain the lack of statistical significance in the meropenem target attainment rates despite the numerical differences observed. Also, the analysis has been done after 1 to 2 days since initiation of antibiotic therapy, which may miss the problem of insufficient drug concentrations in the very early phase of therapy. Finally, the results of extended infusion were compared to historical control patients treated with bolus infusion at the Burns, Trauma and Critical Care Research Centre in Brisbane, Australia although these patients were part of a prospective intensive pharmacokinetic study enabling suitable comparison to the extended infusion cohort. The extended infusion protocol was implemented at the ICUs of the Ghent University Hospital in 2007 and both the investigators and the clinicians found it unethical to randomize patients between bolus and extended infusion, although this remains a controversial issue and other clinicians may disagree on this topic. The pharmacokinetic characteristics such as clearance and elimination rates were comparable between the patient groups, suggesting that comparison is not inappropriate.

4.5. Conclusions

In conclusion, this study found that pharmacokinetics of piperacillin and meropenem is variable in critically ill patients. Although extended infusion of beta-lactam antibiotics increases the $fT_{>MIC}$, and improves target attainment, a 100% $fT_{>MIC}$ target is not reached in a significant part of critically ill patients. In the future, once patients at risk can be easily identified, other strategies such as extended infusion or continuous infusion based on therapeutic drug monitoring should be used to optimize antibiotic exposure in this subgroup of patients.

5. Does consistent piperacillin dosing result in consistent therapeutic concentrations in critically ill patients? A longitudinal study over an entire antibiotic course

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Abstract

Introduction: Piperacillin plasma concentrations are known to vary between critically ill patients. However, there are no comprehensive data on the variability of antibiotic concentrations within the same patient**.** The purpose of this study was to investigate the adequacy of dosing during an entire antibiotic course of 7 days and secondly, to investigate the variability in antibiotic trough concentrations, both between, as well as within the same patient.

Methods: In critically ill patients with normal renal function, piperacillin trough concentrations were measured daily. Drug assay was performed using ultra high performance liquid chromatography tandem mass spectrometry. The pharmacokinetic/pharmacodynamic (PK/PD) target was 100% $fT_{>MIC}$ of the *Pseudomonas aeruginosa* EUCAST breakpoint. The within- and between-patient variability was calculated as % coefficient of variation (CV).

Results: Eleven patients, treated for pneumonia, were included in this nested prospective observational cohort study; median (range) age was 67 (18-79) years, median (range) weight was 75 (57-90) kg and median BMI 23.5 (22.3-26.4). The median (range) creatinine clearance on day 1 of antibiotic treatment was 102 (62-154) mL/min. Trough concentrations were variable, ranging from 4.9 to 98.0 mg/L. We found a median CV of 40 % for within-patient variability, and a CV of 57% for the between-patient variability. The within-patient variability was inversely correlated with SOFA score (R=0.65, p=0.027) and APACHE II score on admission (R=0.73, p=0.009).

Conclusions: Piperacillin concentrations varied widely both between as well as within the same patient. Within patient variability was inversely correlated with severity of disease.

Consistent dosing of piperacillin/tazobactam does not result in consistent piperacillin concentrations throughout the entire treatment period.

5.1. Introduction:

Infection is a well-recognized but persisting problem in critical care medicine. Antimicrobial therapy is a crucial element in the treatment of severe infections. Currently, timely and appropriate antibiotic therapy plus source control is considered to be the mainstay of treatment [59].

Optimizing antibiotic exposure is important as well, but this is proving to be a considerable challenge with recent data showing that antibiotic concentrations in critically ill patients are highly variable, unpredictable and often sub-optimal, because of the pathophysiological changes in these patients [39, 93, 120]. Administration of the antibiotic as a prolonged or continuous infusion has been proposed as a way to optimize pharmacokinetics and improve patient outcome. However, we have previously shown that even if the antibiotic is administered as an extended infusion, patients with a creatinine clearance > 130 mL/min are still at risk for target non attainment [156].

As creatinine clearance and other pharmacokinetic (PK) determinants may change over time, it can be assumed that antibiotic concentrations are not stable during an entire antibiotic treatment course. To date, there are no comprehensive data available on the variability of antibiotic concentrations during antibiotic treatment within the same patient.

The purpose of this study was therefore to investigate the adequacy of dosing during an entire antibiotic course of 7 days when the antibiotic is administered as an extended infusion and secondly, to investigate the variability in antibiotic trough concentrations, both between patients, as well as within the same patient.

5.2. Methods

Patients

This nested prospective observational cohort study was conducted in the intensive care unit (ICU) of Ghent University Hospital, Belgium, between April 2011 and February 2012. This analysis was done using samples from a randomized controlled trial, which was approved by the Belgian regulatory agency (B67021020250). The trial was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics

Committee of Ghent University Hospital (registration number 2010/814). Written informed consent was obtained from the patient or his/her legal representative. Criteria for inclusion were the need for antibiotic treatment with piperacillin/tazobactam, age of 18 years or older and the presence of an arterial catheter. Patients were excluded in case of pregnancy and/or lactation, allergy to the administered medication, impaired renal function (estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation <80 mL/min), hemoglobin < 7g/dL, do-not–resuscitate orders or if the patient was expected not to survive the first 48h.

The purpose of this study was to investigate the adequacy of dosing during an entire antibiotic course of 7 days when the antibiotic is administered as an extended infusion and secondly, to investigate the variability in antibiotic trough concentrations, both between patients, as well as within the same patient.

For the current analysis we included patients in whom antibiotic concentrations were available for at least 4 consecutive days.

Antibiotic administration

Patients received a loading dose of 4 g/0.5 g piperacillin/tazobactam (Tazocin®, Pfizer), administered over 30 minutes, followed immediately by the first extended infusion dose of 4 g/0.5 g piperacillin/tazobactam every 6h. Extended infusion doses were administered over 3 hours using a syringe pump via a central venous catheter.

Study procedures

The first blood sample was obtained after administration of at least 3 doses, at apparent pharmacokinetic steady-state. One trough sample was taken per day for 7 consecutive days, immediately before administration of the next dose. Nurses were well aware of the importance of accurate sample timing, and timing of this trough sample was very reliable. For each sample, 5 mL of blood was collected in heparin anticoagulant tubes without separator gel, via the arterial catheter. The samples were then sent to the core laboratory, where they were centrifuged and frozen immediately upon arrival at -20°C and were analyzed on the same day. Additional data were obtained from the medical records and included participant demographics, clinical details, measures of illness severity such as the sequential organ failure assessment (SOFA) and the acute physiology and chronic health evaluation II (APACHE II) score, microbiological results and laboratory investigations.

Analytical assay

The plasma concentrations of piperacillin were determined by a validated technique using ultra high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) [279]. Samples were deproteinized using acetonitrile. After centrifugation, a portion of the supernatant was diluted and injected on a Waters BEH C18 column (1.7 μm, 100 mm x 2.1 mm) kept at 50°C. The mobile phase consisted of a gradient elution of water and acetonitrile, both containing 0.1 % formic acid. Compounds were detected with a Waters Acquity TQD mass spectrometer operating in positive electrospray ionization using a compound specific method in the multiple reaction monitoring mode. The assay was linear from 4 to 250 mg/L with an inaccuracy < 8 % and a between-run imprecision < 10 % at high, medium and low concentrations. The measured total concentrations were corrected for protein binding, assuming 30 % protein binding of piperacillin. Creatinine was measured in both serum/plasma and urine using the rate blanked, compensated and uncompensated Jaffe technique, respectively (Modular P and Cobas 6000, Roche Diagnostics GmbH, Mannheim, Germany).

Calculations

The concentrations were inspected for outliers for each individual patient, defined as one value outside 3 times the interquartile range.

The total variance was calculated by dividing the standard deviation of all available samples by the mean of all available trough concentrations. This total variance is the sum of the within-patient variance and the between-patient variance. The within-patient variance was calculated as the mean of all individual variances.

Between-patient standard deviation (SD) can thus be calculated as follows:

$$\text{Between patient SD} = \sqrt{\text{total variance} - \text{within patient variance}}$$

$$\text{Between patient CV (\%)} = \frac{\text{between patient SD x 100 \%}}{\text{mean of all samples}}$$

The within-patient variability coefficient of variation (CV) was calculated by dividing the individual standard deviation (SD) by the mean of this patient's trough concentrations.

Correlations between the within-patient CV and clinical parameters were analyzed using the Pearson correlation coefficient using IBM® SPSS® Statistics 20.0. A P value ≤ 0.05

was considered to be significant. The predefined pharmacokinetic/pharmacodynamic (PK/PD) target was 100% $fT_{>MIC}$ of *Pseudomonas aeruginosa* according to the EUCAST breakpoint (16 mg/L) [235].

5.3. Results

Eleven patients, all treated for pneumonia, were included in the study, and a total of 70 blood samples were used for the current analysis. The median time between hospital admission and start of antibiotic therapy was 8 days (IQR 4-19).

The median (interquartile range, IQR) age was 67 (51-75) years, 82% of the patients were male, with a median (IQR) weight of 75 (67-83) kg, and median BMI of 23.5 (22.3-26.4). The median (IQR) creatinine clearance on day 1 of antibiotic treatment was 93 (88-99) mL/min. The median (IQR) creatinine clearance throughout the study period was 100 (89-136) mL/min. Median (IQR) value for SOFA score on day 1 of the study was 3 (4-6), and 24 (18-30) for APACHE II score on admission.

For 2 patients, only 6 concentrations were available, for one patient 5, and for one patient 4. Antibiotic concentrations varied considerably, both between patients as well as within the same patient. Trough concentrations over the treatment course ranged from 4.9 to 98.0 mg/L.The median (IQR) trough concentration during the first day of treatment was 33.6 (25.5-49.4) mg/L, and during the entire antibiotic treatment 27.0 (15.6-45.3) mg/L.

The boxplot of antibiotic concentrations per day over the 7-day course is shown in figure 1. The black line depicts the PK/PD target of 100 % $fT_{>MIC}$ of *P. aeruginosa* according to EUCAST breakpoints (16 mg/L).
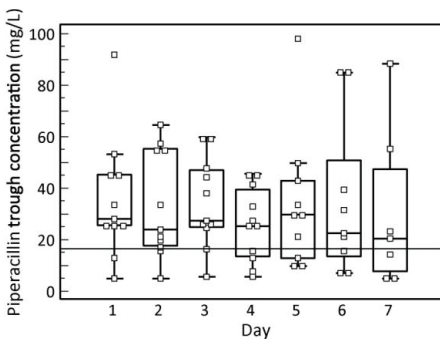
Fig. 1. Box plot of piperacillin concentrations for each day, also showing the individual concentrations (□, individual values). The horizontal black line represents the target

minimum inhibitory concentration (MIC) of *Pseudomonas aeruginosa* according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint (16 mg/L).

The variability within the same patient and between patients is visually depicted in figure 2. The black line depicts the PK/PD target of 100 % $fT_{>MIC}$ 16 mg/L. As shown in this graph, a significant portion of patients (6 out of 11) failed to achieve this predefined PK/PD target at least once throughout the entire 7-day antibiotic course.

One outlier was removed for calculation of the coefficient of variation (CV). The median within-patient CV was 40 %, ranging from 20 to 60%. The between-patient CV was 57 %. The influence of the analytical method (inaccuracy < 8 % and between-run imprecision <10 % at all levels) is negligible.

We found a significant inverse correlation between the within-patient CV and SOFA score on day 1 of study (R=0.65, p=0.027) and between the within patient CV and APACHE II score on admission (R=0.73, p=0.009), indicating that the patients with higher individual variability are the patients who tend to be less severely ill with lower SOFA scores and lower APACHE II scores (figure 3a and 3b). There was no significant correlation between the within-patient CV and weight, age, median creatinine clearance and CV in creatinine clearance. Individual concentrations were significantly correlated with creatinine clearance (p < 0.001), a finding reported in other studies as well [120, 156].
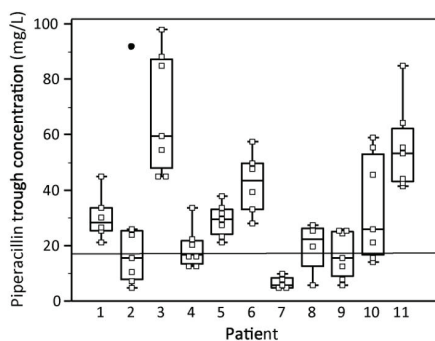
Fig. 2. Box plot of individual patient piperacillin concentrations, also showing the individual concentrations [□, individual values; ●, extreme outlier (outside three times the interquartile range)]. The horizontal black line represents the target minimum inhibitory concentration(MIC) of Pseudomonas aeruginosa according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint (16 mg/L).
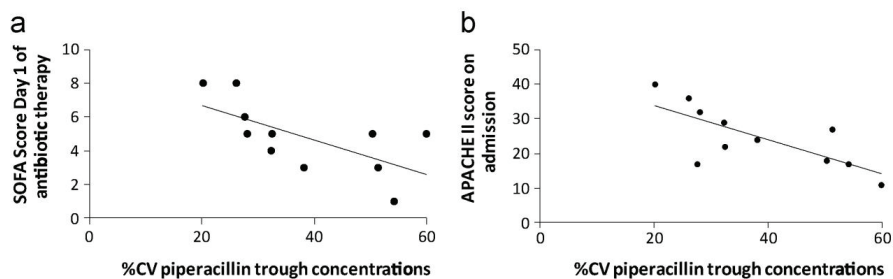
Fig. 3. (a) Coefficient of variation (CV) of individual piperacillin concentrations versus Sequential Organ Failure Assessment (SOFA) score on Day 1 of antibiotic therapy ($R = 0.65$, $P = 0.027$). (b) CV of individual piperacillin concentrations versus Acute Physiology and Chronic Health Evaluation (APACHE) II score on admission ($R = 0.73$, $P = 0.009$).

5.4. *Discussion*

To the best of our knowledge, this report is the first to describe antibiotic concentrations over an entire antibiotic treatment period.

The primary aim of our study was to investigate the adequacy of dosing during an entire antibiotic course of 7 days when the antibiotic is administered as an extended infusion. We have found that many patients (6 out of 11) failed to achieve the PK target of 100 % fT$_{>MIC}$ of *P. aeruginosa* at least once during the entire treatment course. This PK target is high, but in this population 8 out of 11 patients had a prolonged hospital stay of 5 days or more before the start of antibiotic therapy. Therefore these pneumonias were considered to be nosocomial and could be caused by a *Pseudomonas infection*. In case of absence of a causative micro-organism and its sensitivity to the antibiotic, the least sensitive strain should still be covered by the antibiotic dosing regimen.

The second aim of our study was to investigate the variability in antibiotic trough concentrations, both between patients, as well as within-patient. In spite of our strict inclusion criteria (eGFR > 80 mL/min), and relatively narrow range in creatinine clearance (IQR 89-136 mL/min), which should result in a rather homogenous group of patients, we still observed important variability in antibiotic concentrations, both between patients, as well as within-patient. The patients with lower baseline SOFA scores and lower baseline APACHE II scores display the largest within-patient variability.

Our study highlights once again the unpredictability of antibiotic concentrations, which is mostly caused by the altered PK due to pathophysiological changes that occur in critically ill patients as well as therapeutic interventions. More specifically, larger than normal values for volume of distribution and large variations in antibiotic clearance β-lactam antibiotics have been described frequently in critically ill patients, leading to very variable and unpredictable serum concentrations [236, 280, 281]. The finding that the more sick patients had relatively lower variability seems counterintuitive, as it would be expected that less sick patients (with lower SOFA scores) would have less PK variability, and more critically ill patients more variability, because of fluid shifts with third-spacing etc. This is not the first time an association has been made between pharmacokinetic findings and severity of illness scores. Udy et al have found that a modified SOFA score ≤4, is a significant risk factor for developing augmented renal clearance [111]. The reason for this is also not clear.

Because of this variability and unpredictability, therapeutic drug monitoring (TDM) is emerging as a promising tool to guide antibiotic dosing. Roberts et al. demonstrated that 74% out of 236 patients treated with β -lactam antibiotics did not achieve target concentrations [236]. Moreover, several case reports have shown that in difficult situations, such as in patients displaying a complex physiology or with an infection with a microorganism displaying increased resistance, TDM may be useful to guide therapy [256, 275, 282]. However, the literature on TDM for β-lactam dosing is still limited and the exact role of TDM in clinical practice is yet to be determined.

This report is the first to explore the variability of antibiotic concentrations in the same patient over multiple consecutive days. This study suggests that single individual concentrations are not sufficient to predict subsequent concentrations and that more intensive, preferably daily, TDM may be necessary.

This study has a number of limitations. First, we have only included 11 patients in this study, which may not be sufficient to describe the variability present in all ICU patients. Furthermore, given the small sample size of this study, we could not investigate the clinical relevance of target non attainment. Also, this single center study only included patients with normal renal function, which limits extrapolation of these findings to all ICU patients. Finally, we did not investigate free concentrations or concentrations at the site of infection. Instead, we measured total drug concentrations with correction for protein binding based on the

literature. However, research has shown that this is a valid approach for low to moderately protein-bound antibiotics [219].

5.5. Conclusion

In this small group of studied patients with normal kidney function, there was considerable variability in piperacillin concentrations, both between patients, but also within the same patient. Antibiotic concentrations were significantly inversely correlated with creatinine clearance. Patients with lower baseline SOFA and APACHE II scores tended to have greater within-patient variability in antibiotic concentrations. Moreover, many patients with a normal renal function did not achieve the PK/PD target of 100 % $f\text{T}_{>\text{MIC}}$ of *P. aeruginosa* at least once during the treatment course.

6. **Lack of PK/PD target attainment in de-escalated antibiotic therapy in critically ill patients: Less is not always more**

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Abstract:

Introduction: De-escalation of empirical antibiotic therapy is often included in antimicrobial stewardship programs in critically ill patients, but differences in target attainment when switching antibiotics are rarely considered. The primary objective of this study was to compare the fractional target attainment of contemporary dosing of empirical broad-spectrum β-lactam antibiotics and narrower spectrum antibiotics for a number pathogens for which de-escalation may be considered. The secondary objective was to determine whether alternative dosing strategies improve target attainment.

Methods: We performed a simulation study using published population pharmacokinetic (PK) studies in critically ill patients for a number of broad-spectrum β-lactam antibiotics and narrower spectrum antibiotics. Simulations were undertaken using a dataset obtained from critically ill patients with sepsis without absolute renal failure (n=49). The probability of target attainment of antibiotic therapy for different micro-organisms for which de-escalation is applied was analyzed. EUCAST MIC distribution data were used to calculate fractional target attainment.

Results: The probability to achieve therapeutic exposure was lower for the narrower spectrum antibiotics in conventional dosing compared to the broad spectrum alternatives, which could drastically be improved when higher dosages and different modes of administrations are used.

Conclusions: For a selection of microorganisms the probability to achieve therapeutic exposure was overall lower for the narrower spectrum antibiotics using conventional dosing compared to the broad-spectrum antibiotics.

6.1.   Introduction

Provision of antibiotic therapy that is timely and of an appropriate spectrum is one of the mainstays of treatment [12, 20]. This has led to the widespread use of broad-spectrum antibiotic therapy for the empirical treatment of infections. After identification of the causative microorganism, antibiotic therapy is typically adapted to the susceptibility profile of the microorganism, with a preference to change therapy to narrower-spectrum agents in order to decrease selective pressure for resistant pathogens. This process is called antibiotic de-escalation, and considered an important element in antibiotic stewardship programs [54, 283, 284].

Although timing and adequacy of the antibiotic therapy remains crucial, recent data hint at the importance of antibiotic dosing and exposure on clinical outcome [49]. Changes in the physiology of the critically ill alter the pharmacokinetics of β-lactam antibiotics, with many patients being at risk of underdosing [39, 285]. Attainment of pharmacokinetic/pharmacodynamic (PK/PD) targets associated with efficacy is also dependent on the susceptibility of the pathogen and varies across antibiotic classes - an element that is rarely considered [285].

Although de-escalation of antibiotic therapy is a key element in many antibiotic stewardship programs, the possible change in PKPD target attainment in de-escalation has not yet been considered. De-escalation has been associated with improved outcome in many observational (non-randomized) studies, however, these findings may be due to selection bias as de-escalation may be mainly performed in patients who are improving [57, 286] . A recent randomized controlled study performed by Leone et al. found that de-escalation to narrow spectrum antibiotics did not reduce patient intensive care unit (ICU) length of stay and was associated with an increased number of antibiotic days in patients who had been de-escalated. The authors also reported that superinfections were more frequent in patients who were de-escalated, with about half of the superinfections being caused by the same pathogens as the initial infection [68].

Based on these observations, we hypothesized that PK/PD target attainment after de-escalation may be lower than with empiric therapy, even when the pathogen is reported to be susceptible to the de-escalation antibiotic. The primary objective of this study was to compare the probability of achieving PK/PD targets for conventional dosing of empirical

broad-spectrum antibiotics and narrower spectrum antibiotics for a number of pathogens for which de-escalation may occur. The secondary objective was to determine whether PK/PD target attainment could be improved with alternative dosing strategies for both types of antibiotics.

6.2.   Methods

We performed an *in silico* (computer)simulation study using published population pharmacokinetic studies in critically ill patients for a number of broad-spectrum β-lactam antibiotics (meropenem and piperacillin/tazobactam) and narrower spectrum antibiotics often used in de-escalation as reported in recent studies (amoxicillin/clavulanic acid, cefuroxime , flucloxacillin, cefazolin and cefepime) [128, 140, 155, 287-290]. Protein binding was assumed to be 17% for amoxicillin, 33% for cefuroxime and 10 % for cefepime [240]. Protein binding for meropenem is negligible, and the model for piperacillin, flucloxacillin and cefazolin were based on measured free concentrations, so no correction was necessary.

We simulated 30-minute infusions for all antibiotics as intermittent infusion remains the most common method of administration in ICUs [49]. The broad-spectrum empirical antibiotics were also simulated as extended and continuous infusions as these administration techniques are becoming more common as a way to maximize PK/PD target attainment [291]. For amoxicillin/clavulanic acid and piperacillin/tazobactam, doses for the amoxicillin or piperacillin component only were simulated because the PK/PD targets for the β-lactamase inhibitors in these combinations remain unclear. The simulated dosages were derived from the package inserts and are summarized in table 1. PK/PD target attainment of higher dosages and alternative dosing strategies were also investigated for the narrower spectrum antibiotics. The simulated dosages are summarized in table 2.

Table 1: Simulated intravenous dosages of antibiotics

Antibiotic	Dosage simulation
Meropenem	1 g every 8 h as an intermittent infusion
	1 g every 8 h as a 4h extended infusion
	3 g/day as a continuous infusion
Piperacillin	4 g every 8 h as an intermittent infusion
	4 g every 8 h as a 4h extended infusion
	12 g/day as a continuous infusion
	4 g every 6 h as an intermittent infusion
	4 g every 6 h as a 3h extended infusion
	16 g/day as a continuous infusion
Cefepime	1 g every 12 h as an intermittent infusion
	2 g every 12 h as an intermittent infusion for *S. aureus* infections
Amoxicillin	1 g every 6 h as an intermittent infusion
Cefuroxime	1.5 g every 8 h as an intermittent infusion
Flucloxacillin	1 g every 6 h as an intermittent infusion
Cefazolin	1 g every 8 h as an intermittent infusion

Table 2: Simulated dosages for the de-escalation antibiotics using higher dosages and alternative dosing strategies

Antibiotic	Dosage simulation
Amoxicillin	1 g every 4 h as an intermittent infusion
	1 g every 4 h as a 2h extended infusion
	6 g/day as continuous infusion
Cefuroxime	1.5 g every 6 h as an intermittent infusion
	1.5 g every 6 h as a 3h extended infusion
	6 g/day as a continuous infusion
Flucloxacillin	2 g every 6 h as an intermittent infusion
	2 g every 6 h as a 3h extended infusion
	8 g/day as a continuous infusion
Cefazolin	1 g every 6 h as an intermittent infusion
	1 g every 6 h as a 3h extended infusion
	4 g/day as a continuous infusion
Cefepime	2 g every 8 h as an intermittent infusion
	1 g every 4 h as an intermittent infusion
	4 g/day as a continuous infusion

The simulations were performed using NONMEM (version 7.3.0, Globomax LLX, Hanover, USA). A digital FORTRAN complier was used and the runs were executed using Wings for NONMEM (http://wfn.sourceforge.net). For each antibiotic, 1000 Monte Carlo simulations were undertaken using a patient dataset (n=49) with varying creatinine clearances (range 22 - 230 mL/min) using the parameters from the published covariate model. This dataset was obtained from a previous study conducted in a tertiary referral ICU [292]. Patients were

eligible for enrolment if they were between 18-80 years of age and were receiving piperacillin/tazobactam for treatment of sepsis (defined as a presumed or confirmed infection, while manifesting a systemic inflammatory response syndrome). Patients were excluded if they did not have an intra-arterial line, had significant renal impairment (defined by a plasma creatinine concentration > 171 µmol/L or the need for renal replacement therapy); or had a history of allergy to piperacillin or iodine. This therefore represents a convenience sample of critically ill septic patients, without significant renal impairment. The patient characteristics are summarized in table 3.

Table 3: Patient characteristics. Data are reported as median (interquartile range)

Male / female (N)	27/22
Age (years)	46 (33-64)
Height (m)	1.70 (1.63-1.80)
Weight (kg)	84 (73-95)
BMI (kg/m²)	29.4 (25.1-33.3)
Creatinine clearance (mL/min)	105 (74-143)
APACHE II score	17 (14-25)
SOFA score	6 (5-9)
Serum urea concentration (mmol/L)	6.2 (3.9-8.7)
Serum creatinine concentration (µmol/L)	73 (55-97)
Serum albumin concentration (g/L)	21 (20-24)
8 hour creatinine clearance (mL/min)	112 (76-142)
Mechanically ventilated (%)	93.4

BMI: body mass index ; APACHE II**:** Acute Physiology and Chronic Health Evaluation ; SOFA: Sequential Organ Failure Assessment

Using the simulated concentration-time profiles, the time for which the free antibiotic concentration exceeds the minimal inhibitory concentration (fT$_{>MIC}$) was calculated for each simulated subject using linear interpolation. The PK/PD target was set at 40% fT$_{>MIC}$ for carbapenems, 50 % fT$_{>MIC}$ for penicillins, and 65 % fT$_{>MIC}$ for cephalosporins, and this was defined as the conservative PK/PD target, which is the target found to be associated with maximal effect in animal models   [41]. There are almost no data on which targets are needed to treat infections in critically ill patients, however, there are some retrospective studies that have found that higher targets may be needed to treat serious infections in this patient population. Therefore we performed an additional simulation with a higher target of 100 % fT$_{>MIC}$ for all antibiotics [42, 43].

The micro-organisms used in this simulation study were *Escherichia coli, Staphylococcus aureus* and *Streptococcus* spp, *Klebsiella pneumoniae, Haemophilus influenzae, Citrobacter*

freundii, Morganella morganii and *Proteus mirabilis,* as these are micro-organisms for which de-escalation is more commonly performed [55-57, 68, 293].

MIC distribution data for the above pathogens were obtained for each antibiotic from the European Committee for Antimicrobial Susceptibility and Testing (EUCAST) to determine fractional target attainment (FTA) [235]. This identifies the likely success of treatment by comparing the achievement of the PK/PD target against an MIC distribution. Microorganisms with an MIC above the clinical susceptible breakpoint were not included in the FTA calculation because ongoing prescription would not be supported by the susceptibility testing upon which the de-escalation is based.

6.3.   Results

Probability of attainment for the conservative PK/PD target

The results of the simulations for the conservative target are shown in table 4. The FTA for the conservative target for the broad-spectrum antibiotics administered as an intermittent infusion in high doses (piperacillin/tazobactam 4 g every 6 h and meropenem 1 g every 8 h) was > 95 % for all simulations, reaching 100 % when administered as an extended or continuous infusion. The FTA for piperacillin/tazobactam at a lower dose (4 g every 8 h) was slightly lower, with the lowest FTA being 89 % for *K. pneumoniae*, although this increased to 100 % when administered as a continuous or extended infusion.

For the narrower spectrum antibiotics in conventional dosing, the FTA was lower than for the broad-spectrum antibiotics. As shown in table 4, the FTA for amoxicillin/clavulanic acid (1 g every 6 h) ranged from 85 % (*E. coli*) to 100 % depending on the micro-organism. The lowest FTA for cefuroxime (1.5 g every 8 h) was 65 % for *E. coli*. Flucloxacillin (1 g every 6 h), cefepime (2 g every 12 h) and cefazolin (1 g every 8 h) had a FTA of respectively 74, 88 and 90 % against oxacillin-susceptible *S. aureus*.

Probability of target attainment for the higher 100 % $fT_{>MIC}$ target

The FTA for the higher target of 100 % $fT_{>MIC}$ are shown in table 4. For the broad-spectrum antibiotics, only continuous infusion of meropenem and piperacillin/tazobactam (piperacillin doses of 12 and 16 g/day) reached 100% FTA for all studied micro-organisms.

The FTA for meropenem (1 g every 8 h) administered as a 30-minute infusion ranged between 100 % (*Streptococcus* spp.) and 64 % (oxacillin susceptible *S. aureus*), which

increased to 87 % when administered as a 4 h infusion and to 100 % when administered as a continuous infusion.

Similarly, for piperacillin/tazobactam increasing infusion time improved FTA. When administering 4 g every 6 hours, the lowest FTA was 76 % for *K. pneumoniae.* It was 87 % for the 3 h infusion and increased to 100 % for the continuous infusion. In the lower dose, FTA was only 62 % for *K. pneumoniae,* 77 % when administered as a 4 h extended infusion and 100 % as a continuous infusion.

For the de-escalation antibiotics, the FTA was also lower than for the conservative target. The lowest FTA for amoxicillin/clavulanic acid (1 g every 6 h) was 66 % for *E. coli,* and 45 % for cefuroxime using the standard dose of 1.5 g every 8 h (*E. coli)*. The FTA against oxacillin susceptible *S. aureus* for flucloxacillin (1 g every 6 h) and cefepime (2 g every 12 h) was similarly poor with an FTA of 36 % and 69 %, but slightly better for cefazolin (1 g every 8 h) with an FTA of 77 % .

Fractional target attainment when administering higher dosages/alternative modes of administration for the narrower spectrum antibiotics

The FTA using the conservative targets for the higher dosages/ alternative modes of administration are shown in table 4. Increasing the dose for amoxicillin/clavulanic acid from 1 g every 6 h to 1 g every 4 h increased the FTA using the conservative target for *E. coli* from 85 % to 92 %, and to 100 % when an extended or continuous infusion of 6 g was used. Similarly, for cefuroxime, increasing the dose and increasing infusion time improved the FTA from 65 % for *E.coli* (conventional dose of 1.5 g every 8h) to 98 % when administered as a continuous infusion of 6 g. For flucloxacillin, increasing the dose from 1 g every 6 h to 2 g every 6 h as an extended or continuous infusion increased the FTA for oxacillin susceptible *S. aureus* from 74 to 100 %. For cefazolin and cefepime, a continuous infusion of 4 g increased the FTA against oxacillin susceptible *S. aureus* from 88 % (cefepime 2 g every 12 h) and 90 % (cefazolin 1 g every 8 h) to 100 % for both antibiotics .

When using the higher target of 100 % fT$_{>MIC}$, there are large differences in FTA between the broad and narrow spectrum antibiotics (table 4). However, changing the intermittent infusion to a higher dose continuous infusion improved the FTA dramatically. For amoxicillin/clavulanic acid, this improved FTA from 66 % (1 g every 6 h) to 98 % (6 g continuously) for *E. coli*, for cefuroxime from 45 % (1.5 g every 8 h) for *E. coli* to 98 % (6 g

continuously). For flucloxacillin, in order to obtain a high FTA for oxacillin susceptible *S. aureus,* the dose needed to be increased from 1 g every 6 h (FTA 36 %) to 8 g as a continuous infusion (FTA 100 %), and for cefepime and cefazolin respectively from 2 g every 12 h (FTA 69 %) and 1 g every 8 h to 4 g continuously.

Table 4: Fractional target attainment for different antibiotics, microorganisms, dosages and modes of administration using the both the conservative and the high PK/PD target

Antibiotic	Dosing	Oxacillin susceptible S. aureus		Streptococcus spp.		K. pneumoniae		H. influenza		C. freundii		M. Morganii		P. mirabilis		E. coli	
		FTA low (%)	FTA high (%)	FTA low (%)	FTA high (%)	FTA low (%)	FTA high (%)	FTA low (%)	FTA high (%)	FTA low (%)	FTA high (%)	FTA low (%)	FTA high (%)	FTA low (%)	FTA high (%)	FTA low (%)	FTA high (%)
Meropenem	3g CI	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	1g q8h EI	99	87	100	100	100	93	100	93	99	92	99	89	100	92	100	97
	1g q8h II	99	64	100	100	100	72	100	73	99	70	99	66	100	70	100	88
Piperacillin/ tazobactam	16g CI	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	4g q6h EI	100	94	100	100	100	87	100	100	100	88	100	96	100	96	100	91
	4g q6h II	98	87	100	100	95	76	100	100	96	78	99	91	99	91	97	81
	12 CI	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	4g q8h EI	100	88	100	100	100	77	100	100	100	79	100	100	100	92	100	82
	4g q8h II	95	78	100	100	89	62	100	100	90	65	97	83	97	84	92	69
Cefepime	1g q12h II	76	54	100	97	100	99	100	99	100	100	100	100	100	100	100	100
	2g q12h II	88	69	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	2g q8h II	98	90	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	1g q4h II	99	98	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	4g CI	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Amoxicillin/ Clavulanic acid	1g q6h II	96*	83*	100*	100*	90	77	98*	89*	91	74	89*	73*	98	87	85	66
	1g q4h II	99*	92*	100*	100*	95	86	99*	97*	96	86	95*	85*	99	95	92	80
	1g q4h EI	100*	95*	100*	100*	95	90	100*	98*	100	90	100*	89*	100	97	100	85
	6g CI	100*	100*	100*	100*	99	99	100*	100*	99	99	98*	98*	100	100	98	98
Cefuroxime	1.5g q8h II	86	61	100	100	73	53	88	71	66	46			83	64	65	45
	1.5g q6h II	94	81	100	100	84	67	95	84	79	61			92	78	78	60
	1.5g q6h EI	99	90	100	100	95	79	100	92	93	73			99	88	92	72
	6 g CI	100	100	100	100	99	99	100	100	98	98			99	99	98	98

Antibiotic	Dosing	Oxacillin susceptible S. aureus		Streptococcus spp.	K. pneumoniae	H. influenza	C. freundii	M. Morganii	P. mirabilis	E. coli
Flucloxacillin	1g q6h II	74	**36**							
	2g q6h II	87	**54**							
	2g q6h EI	100	**71**							
	8g CI	100	100							
Cefazolin	1g q8h II	90	77							
	1g q6h II	96	**89**							
	1g q6h EI	100	93							
	4g CI	100	100							

FTA low: fractional target attainment using the conservative PK PD target of 40% $fT_{>MIC}$ for carbapenems, 50% $fT_{>MIC}$ for penicillins and 60% $fT_{>MIC}$ for cephalosporins; FTA high: fractional target attainment using the high PK PD target of 100% $fT_{>MIC}$; CI: continuous infusion ; EI: extended infusion ; II: intermittent infusion; q8h: every 8 h; q6h: every 6 h; q4h: every 4 h; * the MIC distribution from amoxicillin was used as no MIC distribution of amoxicillin/clavulanic acid was available

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6.4. Discussion

De-escalation of antibiotic therapy, or changing empirical antibiotic therapy to a narrower spectrum antibiotic, is often advocated to reduce use of broad-spectrum antibiotics in the hospitalized patient [54, 294]. It is generally considered safe and has been associated with improved outcomes in several observational studies and is recommended in the 2013 Surviving Sepsis Campaign guidelines [57, 59, 286]. As such it is often incorporated in antibiotic stewardship programs in critically ill patients, [60, 61] although in clinical practice there seem to be a number of obstacles to use it widely [67]. In observational studies, empirical antibiotics are de-escalated in roughly 15-50% of the patients, depending on the definition used [55, 57, 67, 295].

In this study we have found that for a number of pathogens, the fractional target attainment (FTA) for the empirical broad-spectrum antibiotics meropenem and piperacillin/tazobactam was higher than for the narrower spectrum antibiotics amoxicillin/clavulanic acid, cefuroxime, flucloxacillin, cefazolin and cefepime using conventional dosing. Given that the probability to achieve the PK/PD target for some microorganism/antibiotic combinations is lower for the narrower spectrum alternative, de-escalation, with standard dosing may predispose selected patients to clinical failure and recurrent infection. To the best of our knowledge, this is the first study of its kind that compares the achievement of therapeutic exposure of empiric antibiotic therapy versus de-escalation based on population PK models from critically ill patients. Although there is currently no evidence that subtherapeutic dosing of β-lactam antibiotics leads to treatment failure or to a higher incidence of resistance, this has been shown for other antibiotics. For tobramycin for example, it has been shown that although peak concentration/MIC is associated with effect, for the same area under the curve/MIC value, once daily dosing (with subsequent lower trough concentrations) leads to higher MIC values after 2 weeks of treatment than three times daily dosing [296].

The FTA is dependent on a number of factors and recent insights in PK/PD characteristic in critically ill patients may help us to explain these findings. Because of pathophysiological changes in critically ill patients, such as an increased volume of distribution and augmented renal clearance, standard dosing may not always lead to optimal target attainment [120, 156]. Moreover, it is also dependent on the PK/PD target (40% $fT_{>MIC}$ for carbapenems versus 65% $fT_{>MIC}$ for cephalosporins). Next, the susceptibility of the microorganism plays an

important role. Susceptibility of the same microorganism may vary for different antibiotics, and similarly, the potency of certain antibiotics against different microorganisms may be different, even though all are reported to be susceptible [297]. Moreover, the PK/PD target is currently considered to be fixed, however it has never been investigated if the PK/PD target changes over time. A changing PK/PD target over time, not taken into account by dosing, could also result in treatment failure and emergence of resistance. Finally, an increasing proportion of ICUs are administering meropenem and piperacillin/tazobactam as an extended or continuous infusion, as a way to increase PK/PD target attainment [49, 291]. However, these alternative modes of administration are not used for the narrower spectrum antibiotics, which are still being administered as short infusions with standard doses [49]. This contrasting approach could further increase the gap in PK/PD target attainment between empirical and de-escalation antibiotics.

The findings of our study may partly explain the findings of a recent de-escalation study that could not confirm non-inferiority when comparing de-escalation to continuation of the empirical therapy [68]. Leone et al. found in a non-blinded randomized controlled trial that antibiotic use was higher in de-escalated patients due to an increased number of superinfections, about half of them caused by the same pathogen as the primary infection. This suggests that the antibiotics used in de-escalation arm were less effective in eradicating the infection than the broad-spectrum antibiotics in the comparative arm. In this study, no details regarding dosing were reported [68].

Of the most recent de-escalation studies, only one has mentioned dose and mode of administration of the initial broad-spectrum regimen, but it did not mention these data about the de-escalated antibiotics [55, 56, 68, 293]. Another study mentioned that "the dose and pattern of administration were in accordance with current medical standards" [57]. Dosing may be the key to improve patient outcome, as recent data have demonstrated that there is a correlation between blood concentrations of β-lactam antibiotics and clinical outcome [49]. Future de-escalation studies should ensure that dosing and mode of administration of the narrower spectrum antibiotics are likely to achieve appropriate PK/PD targets.

We could also demonstrate that PK/PD target attainment is drastically improved when higher dosages and different modes of administrations of the de-escalation antibiotics are used. However, it must also be highlighted that blindly increasing the dose in all patients

may give rise to needlessly high concentrations in some of them. Although toxicity of β-lactams is not common, it is severe when it occurs, with seizures from high concentrations being reported previously [162-164]. This wide pharmacokinetic variability suggests that the principle of "one dose fits all" is unlikely to be appropriate in this patient population [35].

There are a number of limitations of the current analysis we would like to discuss. These results are not based on measured concentrations from actual patients. However we have simulated concentrations using population pharmacokinetic models and relevant covariates in critically ill patients. As such the accuracy of the results can be assumed to be acceptable given the same approach was used for simulations with the empiric and de-escalation antibiotic. The patient population simulated were patients who had normal renal function (serum creatinine concentration <171 μmol/L) and did not include patients with acute kidney injury, and therefore these conclusions are only relevant to this patient group. Also, there is little knowledge on which PK/PD target should be aimed for in critically ill patients, as the targets are derived from animal models on day 1 or 2 of antibiotic therapy. Whether or not this PK/PD target changes over time as a result of the changing susceptibility or the adaptive resistance of the pathogen is also a remaining question. Moreover, there is no time dependency of the data. In clinical practice, de-escalation is generally performed when the patient is improving (and therefore the pharmacokinetic issues associated with critical illness may be partly normalized), and with a lower bacterial burden. This cannot be accounted for in the modeling. Finally, only 7 antibiotics were simulated, due to the unavailability of population pharmacokinetic models of other β-lactam antibiotics in critically ill patients, although these are all commonly used agents making these data of significant interest to many ICU clinicians.

6.5.   Conclusion

For a selection of micro-organisms in which de-escalation may be considered, the results of this simulation study show that the probability to achieve the PK/PD target was lower for the narrower spectrum antibiotics amoxicillin/clavulanic acid, cefuroxime, flucloxacillin, cefazolin and cefepime using conventional dosing compared to the broad spectrum antibiotics meropenem and piperacillin/tazobactam. As this may impact clinical outcome parameters, studies that report on the results of de-escalation strategies should accurately report dosing of antibiotics used. Future research should not only be focused on correct dosing of broad-spectrum antibiotics, but also of narrower spectrum antibiotics, where

higher dosages and alternative modes of administration may be needed for patients at risk of not achieving PK/PD targets.

Chapter Six : Optimizing Antibiotic Therapy in Clinical Practice

The sixth chapter of this work summarizes the somewhat more practical studies. The first section discusses 2 stability studies, one for meropenem and another one for amoxicillin/clavulanic, which investigated their stability and potential to be used as a continuous infusion. Section 2 investigates the influence of augmented renal clearance on PK/PD target attainment in critically ill patients treated with meropenem or piperacillin/tazobactam administered as an extended infusion. These data were also collected as part of our TDM study. The last section summarizes the results of our TDM study, performed between April 2011-February 2012, which looked at the effect of TDM-based dose optimization on pharmacokinetic target attainment.

1. **Stability of commonly used antibiotics in the ICU for use as continuous infusion**

1.1 Stability of generic brands of meropenem reconstituted in isotonic saline

Authors : Mieke Carlier, Veronique Stove, Alain G. Verstraete, Jan J. De Waele

Abstract

Background : Meropenem is a relatively unstable compound when dissolved. Currently all available data have been derived from tests on the original product from Astrazeneca, and it is unsure if these data can be extrapolated to the stability of other commercially available vials. The aim of this study was therefore to assess the stability of four different brands of meropenem for use as a prolonged or continuous infusion.

Methods : Commercially available meropenem vials were reconstituted and mixed with 0.9 % sodium chloride to produce solutions with concentrations of 10,20 and 40 mg/mL in polypropylene syringes, which were kept at 25°C. Samples were taken immediately after preparation and up to 12 hours. Solutions retaining >90% of the initial concentration were considered stable.

Results : The stability was concentration dependent. At 25°C, all 10 and 20 mg/mL solutions were stable for 12 hours in 0.9% sodium chloride, while the 40 mg/mL solutions were stable for a maximum of 8 hours. Stability of the different vials of meropenem was comparable for the time period tested (related samples Friedman's two way of analysis of variance by ranks, p=0.282).

Conclusion : All tested commercially available vials of meropenem in a concentration of 10 and 20 mg/mL were stable for 12 hours at 25°C when diluted in 0.9% sodium chloride. The 40 mg/mL solutions were stable for a maximum of 8 hours. This report is the first to show equivalent stability between different commercially available vials of meropenem.

1.1.1 Introduction

Administration of β-lactam antibiotics as a continuous infusion is gaining popularity as a way to optimize pharmacodynamics, as their activity depends on the time the concentration exceeds the minimal inhibitory concentration, with evidence suggesting improved outcomes for critically ill patients. [142, 298, 299]

The poor stability of meropenem has been reported repeatedly when using concentrated solutions at room temperature or at elevated temperatures (≥37°C), which is why continuous infusion over 24 hours has been considered an unacceptable choice for delivery of meropenem. [300-302] However, storage at lower temperature and using less concentrated solutions may be a way to overcome this limitation and may be a viable alternative to administer meropenem as a continuous infusion. [301-305]

Currently almost all available data have been derived from tests on the original product from Astrazeneca, and it is unsure if these data can be extrapolated to the stability of other commercially available vials. In some settings there is a concern how active these generics are, as only bioequivalence tests are necessary in order to be marketed. There has been a report on a stability issue of a cefuroxime generic brand which was discovered because of a significantly increased incidence of post-operative infections in the hospital after switching to the generic cefuroxime. In this case, the generic cefuroxime was not stable and hydrolyzed after only 8 minutes [306] .

Therefore, the aim of this study was to investigate whether the stability of 4 worldwide commercially available vials of meropenem are equivalent.

1.1.2. Materials and methods

Meropenem was obtained as the commercial powder preparation for injection from four different brands worldwide available (Meronem Astrazeneca®, Meropenem Sandoz®, Meropenem Fresenius Kabi® and Meropenem Hospira®). According to the leaflets, the only excipient in all 4 products was sodium carbonate. One gram of Meropenem for Injection I.V. vial delivers 1 g of meropenem and 90.2 mg of sodium as sodium carbonate (3.92 mEq).

Preparation of meropenem solutions

The antibiotic was dissolved as a 10, 20 and 40 mg/mL sterile solution in isotonic saline (Fresenius Kabi, Germany). These concentrations represent the currently approved dose of 0.5 g in 50 mL, 1 g in 50 mL and 2 g in 50 mL of diluents.

Five hundred mg of meropenem was reconstituted with 20 mL of 0.9 % sodium chloride injection according to local practice and was then mixed with 30 mL 0.9% sodium chloride solution in a polypropylene syringe (Becton**,** Dickinson and Company ®) in order to reach a 10 mg/mL solution.

One gram of meropenem was reconstituted with 20 mL of 0.9 % sodium chloride injection according to local practice and was then mixed with 30 mL 0.9% sodium chloride solution in a polypropylene syringe in order to reach a 20 mg/mL solution.

One gram was reconstituted with 20 mL of 0.9 % sodium chloride injection according to local practice and was then mixed with 5 mL 0.9% sodium chloride solution in a polypropylene syringe in order to reach a 40 mg/mL solution.

One polypropylene syringe was made for each combination of meropenem concentration and brand. To minimize analytical imprecision, all samples of the same concentration were analysed in duplicate in the same chromatographic run on 2 separate occasions.

The polypropylene syringes were stored at 25°C. Each solution was sampled immediately after preparation and at 1,3,6,8 and 12 hours.

Sampling procedure

At each sampling time, the syringes were softly shaken before duplicate 200 µL samples were removed from each syringe and immediately frozen at -80°C to await concentration determination (within 2 weeks after collection). Color and clarity were assessed by visual inspection.

Analytical assay

Meropenem concentrations were determined using a high performance liquid chromatography coupled to tandem mass spectrometry operating in the multiple reaction monitoring mode [169].

Because of the high specificity of this detector, there is no influence from degradation products and is therefore stability-indicating.

Chromatographic separation was performed on a Waters Acquity UPLC system using a BEH C_{18} column (1.7 µm, 100 x 2.1 mm) applying a binary gradient elution of water and acetonitrile both containing 0.1 % formic acid. The total runtime was 5.5 minutes.

The extraction procedure was modified, since meropenem was diluted in 0.9% sodium chloride in the current analysis as opposed to serum as noted in the published assay. [169] The total precision for this assay of meropenem in serum was 10.3 % (within run precision 6.2 %, between run precision 8.2 %).

Chromatographic analysis was carried out after diluting the samples to 50 mg/L in water containing meropenem-d_6, in order to reach the linear range of the assay (0.5 – 100 mg/L).

Two meropenem samples taken at each timepoint were independently assayed in duplicate in order to minimize the imprecision, and the average of these concentrations was used for data analysis.

Data analysis

Drug potency was determined at each sampling time as the percentage of the initial meropenem concentration remaining. The solution was considered stable if the percentage of intact meropenem was ≥ 90%.

Related samples Friedman's two way of analysis of variance by ranks was (IBM, Chicago, IL) was used to compare the rate of meropenem degradation between different brands. A p value of ≤ 0.05 was considered statistically significant.

1.1.3. Results

After 12 hours storage at room temperature, all tested brands attained the stability criterion of 90 % intact molecule when meropenem was dissolved as a 10 or 20 mg/mL solution in 0.9% sodium chloride. However, when higher concentrations were used, stability decreased and fell below 90% after 8 hours storage at room temperature, as the percentage intact molecule after 12 hours was 86.5 %. Throughout sampling, solutions were clear and ranged from colorless to slightly yellow.

The results are shown in figures 1 a-c which show the percentage of intact molecule for all tested brands over time for 10, 20 and 40 mg/mL.

The meropenem degradation between the different brands of meropenem was comparable for the time period tested (related samples Friedman's two way of analysis of variance by ranks, p=0.282).

1.1.4. Discussion

At 25°C, all commercially available vials of meropenem in a concentration of 10 and 20 mg/L were stable for 12 hours in 0.9% sodium chloride, and stable for 8 hours in a concentration of 40 mg/L in 0.9% sodium chloride. These results are in line with previous findings from literature. There has been one other report which also investigated the stability of meropenem hospira® in a concentration of 5 mg/mL at different temperatures (25-35°C). These authors found the stability to be time- and temperature dependent, with stability > 8 h if the temperature is ≤ 30°C [307].

This paper is the first to show that the stability of three worldwide commercially available generic brands of meropenem is comparable to that of the original formulation.

For clinical practice, we suggest to divide the daily dose of 3 g (6 g in case of central nervous infections) in 3 solutions of 1 g (or 2 g in the case of central nervous infections) in 50 mL-100 mL isotonic saline. However, it must be noted that there is still much debate about which target is needed for treating serious infections in critically ill patients. In vitro studies and animal studies have show that for carbapenems, a 40 % $fT_{>MIC}$ is sufficient because of the significant post-antibiotic effect [308]. However, limited data from studies in critically ill patients seem to show that higher targets may be beneficial in these patients[42]. Currently, a multi centre study is ongoing comparing intermittent versus bolus infusion for 3 β-lactam antibiotics, including meropenem. The results of the feasibility study show higher plasma concentrations in the continuous infusion group and an improvement clinical cure[142].

This study has a number of limitations. First, we have only tested one lot for each brand, but physicochemical properties are not expected to vary across different lots. Secondly, we did not test stability for longer than 12 hours for the 10 and 20 mg/mL solution. Stability in other solutions, such as 5% glucose has also not been investigated.
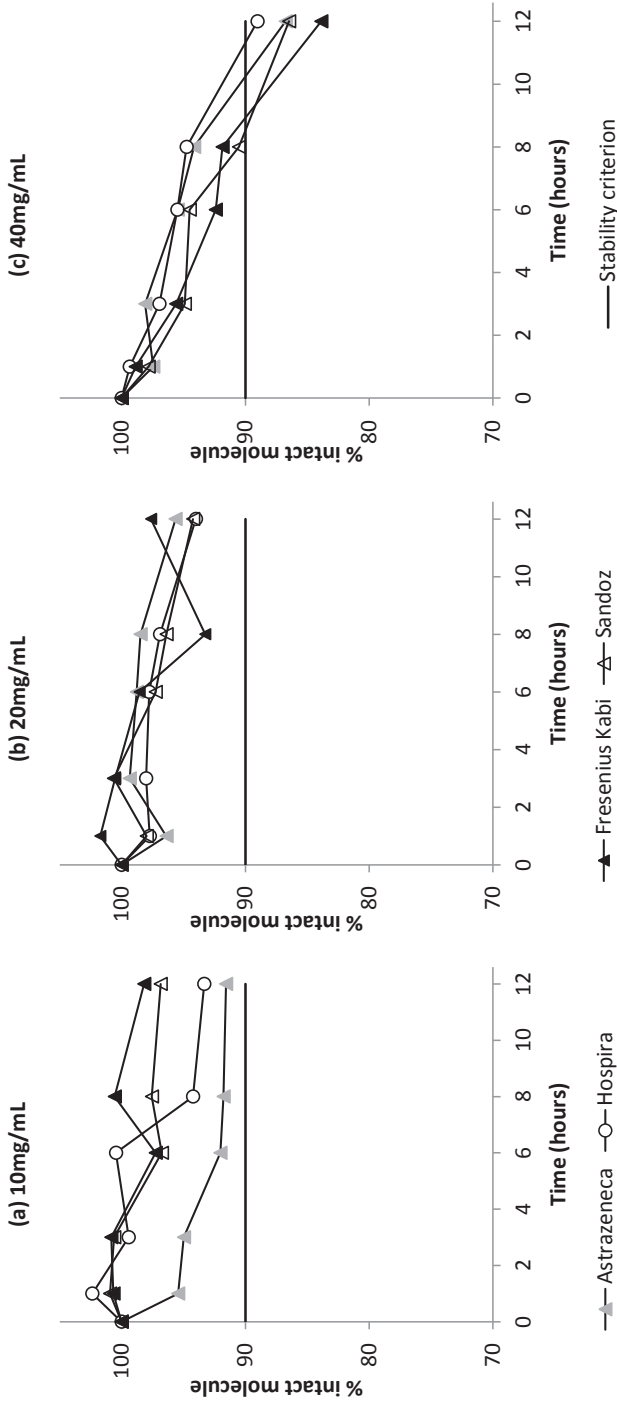
Fig. 1. Stability over time for the 4 tested brands of meropenem in 3 different concentrations. (a) Percentage of intact molecule over time for commercially available vials of meropenem in a concentration of 20 mg/mL. (c) Percentage of intact molecule over time for the 4 commercially available vials of meropenem in a concentration of 40 mg/mL.

1.1.5. Conclusion

Meropenem 10 and 20 mg/mL solutions were stable for 12 hours at 25°C when diluted at 0.9% sodium chloride. The stability of the 40 mg/mL solution in 0.9% sodium chloride decreased to 8 hours at 25°C. The rate of meropenem degradation was similar between the tested brands. Clinicians can safely use these generic forms of meropenem as 8-hour infusions if the concentration is ≤ 40 mg/mL and dissolved in 0.9 % sodium chloride

1.2.   Stability of amoxicillin and clavulanic acid reconstituted in isotonic saline

Authors : Mieke Carlier, Alain G. Verstraete, Jan J. De Waele, Veronique Stove

Abstract

Purpose: Extended or continuous infusions of broad-spectrum β-lactam antibiotics are increasingly used to improve pharmacokinetic target attainment. Concerns over drug stability have limited use of infusions for some β-lactam antibiotics. The purpose of this study was to assess stability of amoxicillin as a 10 g/L solution and amoxicillin/clavulanic acid as a 10/2 g/L solution in 0.9% saline at room temperature (22°C).

Methods: Solutions were prepared in triplicate in physiologic saline in polypropylene infusion bags to a concentration of 10 g/L for amoxicillin and 2 g/L for clavulanic acid. Solutions were sampled immediately after preparation and following up to 12 hours storage at room temperature. The amoxicillin/clavulanic acid solution was sampled immediately after preparation and up to 6 h. Drug concentrations were determined using a stability indicating validated high performance liquid chromatography coupled to tandem mass spectrometry method. The solution was considered stable if less than a 10% reduction in drug concentration occurred during storage.

Results: Amoxicillin was stable for at least 12 hours at room temperature. The combination of amoxicillin and clavulanic acid was less stable because of instability of clavulanic acid. The concentration of clavulanic acid fell below 90% after 3 hours of storage at room temperature.

Conclusions: Amoxicillin/clavulanic acid as a 10 g/L solution in physiologic saline cannot be administered in extended or continuous infusion at room temperature due to limited stability of clavulanic acid.

An increasing proportion of intensive care units use extended or continuous infusion when administering broad-spectrum β-lactam antibiotics. Extended or continuous infusions increase pharmacokinetic/pharmacodynamic target attainment and are associated with improved outcomes for critically ill patients [142, 298]. After identification of a causative microorganism, antimicrobial therapy may be adapted to the susceptibility profile of the microorganism. Use of focused, narrower-spectrum agents reduces selection pressure for resistant pathogens. This process, known as antibiotic de-escalation, is an important component of antibiotic stewardship programs[54, 284]. Among others antibiotics, amoxicillin/clavulanic acid is frequently used in de-escalation from broader spectrum β-lactam antibiotics. Currently extended and continuous infusions of amoxicillin/clavulanic acid are not used. However a recent population pharmacokinetic study of amoxicillin/clavulanic acid in critically ill patients shows that higher dosages and alternate dosing strategies, such as prolonged infusion, may improve pharmacokinetic target attainment [155].

Little is known about the physicochemical stability of amoxicillin and the combination of amoxicillin and clavulanic acid in infusion solutions. Amoxicillin at a concentration of 20 g/L re-constituted in 0.9% saline stored at 20°C in an elastomeric infusion system (Baxter Healthcare Corporation, Deerfield, ILL, USA) remained stable for 48 hours[250]. Significant degradation of clavulanic acid at 4 g/L, in combination with amoxicillin 20 g/L re-constituted in 0.9% saline in polyolefin infusion bags (Macoflex N, MacoPharma, Mouvaux, France) was observed after 3 hours[250, 309]. The product insert for amoxicillin/clavulanic acid, when diluted into 100 mL of 0.9% saline, states the drug is stable for 2-3 hours. However, there is increasing evidence that packet inserts may not truly reflect β-lactam antibiotic stability. This was for example the case for meropenem where the producing companies mentioned stability for 2-3 h for reconstituted meropenem, although multiple investigators found stability for over 8 h when diluted in 0.9 % NaCl, when sufficiently diluted and kept at room temperature[301, 302, 310]. Additionally adsorption of drug onto the inner surface of the plastic container may occur leading to reduction in the delivered drug concentration [311].Therefore testing compatibility and stability for each plastic material-drug combination is required.

The aim of this study was to assess the stability of amoxicillin (10 g/L) solution (Clamoxyl® 1000mg, GlaxoSmithKline Wavre, Belgium) and amoxicillin/clavulanic acid (10/2 g/L) solution (Augmentin® 1000mg/200mg, GlaxoSmithKline Wavre, Belgium) re-constituted in 0.9% saline and stored in a polypropylene infusion bag at room temperature (22°C, RT). Each agent was dissolved in 20 mL of 0.9 % sodium chloride for injection taken from a 100 mL polypropylene infusion bag (*Braun, Diegem, Belgium*) according to local practice. The reconstituted drug was then injected into the remaining 80 mL, from the 100 mL infusion bag, to reach the target concentration. We prepared each antibiotic solution in triplicate and all samples were analyzed five times. The mean concentrations of amoxicillin and clavulanic acid, measured from each sample, was used for analysis.

The polypropylene infusion bags were stored at 22° (standard deviation 0.14°C). The amoxicillin solution was sampled immediately after preparation and after 6, 8 and 12 hours. The amoxicillin/clavulanic acid solution was sampled immediately after preparation and after 1, 2, 3, 4, 5 and 6 hours. At each sampling time point, the infusion bags were gently shaken before a 2 mL aliquot was aspirated with a syringe and immediately assayed. Color and clarity were assessed by visual inspection and pH was measured (HI 8520, Hanna Instruments, Temse, Belgium).

Amoxicillin and clavulanic acid concentrations were determined using a stability indicating high-performance liquid chromatography method coupled to tandem mass spectrometry operating in the multiple reaction monitoring mode based on a previously published method.[181] Briefly, chromatographic separation was performed on a Waters Acquity UPLC system (Waters, Milford, Massachusetts) using a BEH C_{18} column (1.7 µm, 100 x 2.1 mm) using an injection volume of 5 µL. Amoxicillin D_4 (Toronto Research Chemicals, Toronto, ON, Canada) was used as an internal standard. The mobile phase consisted of a mixture of solution A (0.1 % formic acid and 2 mM ammonium acetate in water) and solution B (0.1 % formic acid and 2mM ammonium acetate in methanol). The total run time was 2.5 minutes. Chromatographic analysis was carried out after diluting the samples 200 times (10 µL in 1990 µL water containing 2.5 mg/L amoxicillin D_4). Amoxicillin or clavulanic acid concentration, at each sampling time point, was expressed as a percentage of the initial

drug concentration. We used the, generally accepted, criterion of less than 10% decomposition to define drug stability.[312]

When dissolved without clavulanic acid, amoxicillin was stable for at least 12 hours at RT (figure 1). The concentration of clavulanic acid fell below 90% after only 3 hours storage at RT.   Due to the instability of clavulanic acid at RT, the combination of amoxicillin/clavulanic acid should not be delivered by continuous infusion.
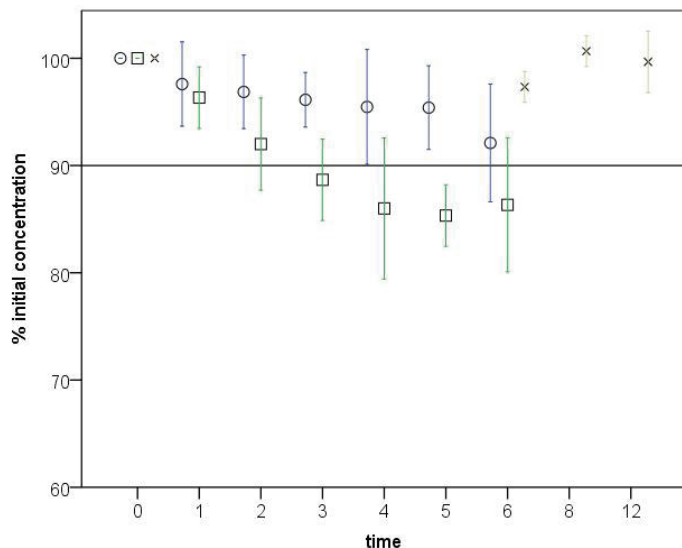
Fig. 1 : Stability of amoxicillin and clavulanic acid over time : mean % intact drug in function of time and type of solution: amoxicillin (amoxicillin/clavulanic acid combination), clavulanic acid (amoxicillin/clavulanic acid combination) and amoxicillin. Error bars : ± 1 standard deviation. Black line : limit of stability (≥90% of the initial concentration). ○ amoxicillin (amoxicillin/clavulanic acid combination) , □ clavulanic acid (amoxicillin/clavulanic acid combination), × amoxicillin

All amoxicillin solutions were clear and colorless. The solutions containing both amoxicillin and clavulanic acid changed from colorless at time 0 to very slightly yellow after 4 hours. The pH for amoxicillin remained constant over the 12 h sampling period (mean 8.76; standard deviation (SD) 0.027). The pH for amoxicillin/clavulanic acid decreased slightly over time from 8.80 (SD 0.017) at time 0 to 8.66 (SD 0.006) after 6 hours (p=0.003).

The results for amoxicillin dissolved alone are in line with previous studies and the packet insert. Amoxicillin, alone, could be administered as a continuous infusion. We found the combination of amoxicillin/clavulanic acid was stable for a maximum of 2 hours. This is similar to previous studies which reported only 4 hour stability of amoxicillin/clavulanic acid at between 20 and 25°C[309]. Amoxicillin/clavulanic acid stability is limited by degradation of clavulanic acid which when dissolved in aqueous solutions is catalyzed by both acids and bases. Clavulanic acid stability is reported to be maximal at a pH of 6.3[313]. The unbuffered amoxicillin/clavulanic acid solution has a pH of 8.80. The stability of clavulanic acid may be increased by acidification of the solution but this approach is not feasible in clinical practice. [314]

This study has a number of limitations. First, we have only tested one lot. However, physicochemical properties are not expected to vary across different lots. Secondly, we did not test stability for longer than 12 h for amoxicillin. Infusion at lower temperatures (via a infusion pump stored in a cold pouch between freezer packs for example) might be a solution to improve stability but was not further evaluated in this method, because this seemed impractical. Finally, we did not test stability in other solvents such as 5% glucose. However, according to the package insert, amoxicillin/clavulanic acid should not be mixed with infusions containing glucose.

2. **Meropenem and piperacillin/tazobactam prescribing in critically ill patients: does augmented renal clearance affect pharmacokinetic/pharmacodynamic target attainment when extended infusions are used**

Authors : Mieke Carlier, Sofie Carrette, Jason A Roberts, Veronique Stove, Alain Verstraete, Eric Hoste, Pieter Depuydt, Johan Decruyenaere, Jeffrey Lipman, Steven C Wallis and Jan J De Waele

Background : Correct antibiotic dosing remains a challenge for the clinician. The aim of this study was to assess the influence of augmented renal clearance on pharmacokinetic/pharmacodynamic target attainment in critically ill patients receiving meropenem or piperacillin/tazobactam, administered as an extended infusion.

Methods : This was a prospective, observational, pharmacokinetic study executed at the medical and surgical intensive care unit at a large academic medical center. Eligible patients were adult patients without renal dysfunction receiving meropenem or piperacillin/tazobactam as an extended infusion. Serial blood samples were collected to describe the antibiotic pharmacokinetics. Urine samples were taken from a 24-hour collection to measure creatinine clearance. Relevant data were drawn from the electronic patient file and the intensive care information system.

Results: We obtained data from 61 patients and observed extensive pharmacokinetic variability. Forty-eight percent of the patients did not achieve the desired pharmacokinetic/pharmacodynamic target (100 % fT$_{>MIC}$), of which almost 80 % had a measured creatinine clearance > 130 mL/min. Multivariate logistic regression demonstrated that high creatinine clearance was an independent predictor of not achieving the pharmacokinetic/pharmacodynamic target. Seven out of nineteen patients (37 %) displaying a creatinine clearance > 130 ml/min did not achieve the minimum pharmacokinetic/pharmacodynamic target of 50 % fT$_{>MIC}$.

Conclusions: In this large patient cohort, we observed significant variability in pharmacokinetic/pharmacodynamic target attainment in critically ill patients. A large

proportion of the patients without renal dysfunction, most of whom displayed a creatinine clearance > 130 mL/min, did not achieve the desired pharmacokinetic/pharmacodynamic target, even with the use of alternative administration methods. Consequently, these patients may be at risk for treatment failure without dose up-titration.

2.1.   Introduction

Infection is a well recognized but persisting problem in critical care medicine. Sepsis alone is the leading cause of mortality in non-cardiac intensive care units, with up to 30 % of patients dying within one month of diagnosis [1, 2]. Adequate antibiotic therapy is one of the mainstays in treatment, with the emphasis on timely administration and appropriateness of the spectrum [24]. Optimizing antibiotic exposure is highly important as well, however, this is proving to be a greater challenge with recent data showing that antibiotic concentrations in critically ill patients are highly variable, unpredictable and commonly sub-optimal [93, 109, 236, 315].

Antibiotic dosing regimens are usually determined in healthy adults with normal physiology or non-critically ill hospitalized patients. Both the volume of distribution and clearance are the key determinants of the pharmacokinetics of a drug. Unfortunately, pathophysiological changes in critically ill patients have profound effect on both [39].

One of these pathophysiological changes is the development of augmented renal clearance (ARC). This is a phenomenon in which renal elimination of circulating molecules – including antibiotics - is enhanced. This, in turn, may lead to sub therapeutic concentrations of time-dependent antibiotics such as β-lactam antibiotics, potentially causing therapeutic failure and selection of antibiotic-resistant pathogens. Critically ill patients are at risk for ARC, because of their pathophysiological disturbances, as well as the clinical interventions administered [110, 316]. The incidence of ARC in critically ill patients is high and varies between 30 and 85 % depending on the studied population and the definition of ARC [276, 317, 318].

One study has demonstrated the relationship between renal clearance and low antibiotic concentrations [120], but the relationship between renal clearance and β-lactam pharmacokinetic/pharmacodynamic characteristics has not been evaluated in a large cohort

of patients. However, various pharmacokinetic modeling and simulation studies have suggested that using extended infusions will prevent low antibiotic exposure. However, this has never been tested in a large cohort of relevant patients with ARC. Therefore, the aim of this study was to assess the influence of renal clearance on pharmacokinetic/pharmacodynamic (PK/PD) target attainment when the antibiotic was administered as an extended infusion. Both the minimum target (50 % $fT_{>MIC}$), as well as the target of 100 % $fT_{>MIC}$ which is considered to have higher bactericidal activity [43] were calculated. Notably this study enrolled patients without renal dysfunction, defined as an estimated glomerular filtration rate (eGFR) assessed by the MDRD equation of <80mL/min.

2.2 Materials and Methods

Inclusion and exclusion criteria

The data used for this analysis were collected in two separate studies performed in the medical and surgical ICU of Ghent University Hospital, a tertiary care hospital with a total of 50 adult ICU beds. Both studies were approved by the Ethics Committee of the Ghent University Hospital (study 1: registration number 2009/543, study 2: 2010/814). Written informed consent was obtained from the patient or his/her legal representative.

Adult patients receiving either meropenem (Meronem®, AstraZeneca) or piperacillin/tazobactam (Tazocin®, Pfizer) were included if they did not meet exclusion criteria which included renal dysfunction (defined as an estimated glomerular filtration rate (eGFR) assessed by the MDRD equation of <80mL/min/1.73 m²), absence of an arterial catheter or absence of informed consent.

Antibiotic administration

Patients received a loading dose (1g meropenem or 4.5 g piperacillin/tazobactam) administered over 30 minutes, followed immediately by the first extended infusion dose of either antibiotic (1g meropenem or 4.5 g piperacillin/tazobactam) every 6h for piperacillin/tazobactam and every 8 hours for meropenem. Extended infusion doses were administered over 3 hours using a syringe pump via a central venous catheter.

Sampling and β-lactam assay

The sampling strategy and β-lactam assay used was different in the studies that contributed patients for this analysis. Twenty patients were included in the first study, and forty-one in the second.

Study 1 (20 patients)

Eight serial plasma concentrations were obtained from each patient between 24-48 hours after the initiation of therapy at baseline and after 1, 1.5, 3, 3.5, 4, 6 and 8 hours for meropenem; at baseline and after 1, 1.5, 3, 3.5, 4, 5 and 6 hours for piperacillin. For each sample, 5mL of blood was collected in heparin anticoagulant tubes without separator gel, via the arterial catheter. Specimens were centrifuged at 3000 rpm for 10 min within 30 minutes of sampling, and then frozen at minus 80°C. They were shipped to the Burns, Trauma & Critical Care Research Centre of the University of Queensland, Australia for analysis by a specialized carrier.

The samples were analysed at the Burns Trauma and Critical Care Research Centre, University of Queensland. The plasma concentrations of meropenem and piperacillin were determined by validated high performance liquid chromatography (HPLC) methods based on a published procedure that has been optimized for each drug [168]. Sample preparation was by protein precipitation with acetonitrile and a wash step with dichloromethane. Separations were performed on a Waters X-bridge C18 column (2.1 x 30 mm, 2.5 μm) with an acetonitrile: phosphate buffer mobile phase (pH 2.5 for meropenem, pH 3 for piperacillin). Detection was by UV at 304 nm (meropenem) or 210 nm (piperacillin). The meropenem assay was linear from 0.2 to 100 mg/L with an imprecision and inaccuracy <7% at high, medium and low concentrations. The piperacillin assay was linear from 0.5 to 500 mg/L with an imprecision and inaccuracy <10% at high, medium and low concentrations. Observed concentrations were corrected for protein binding (piperacillin 30%; meropenem 2%).

Study 2 (41 patients)

Two plasma samples were obtained per patient (mid-dose and trough), after administration of at least 3 doses, to ensure steady-state. For each sample, 5 mL of blood was collected in heparin-anticoagulant tubes without separator gel, via the arterial catheter.

The samples were then sent to the core laboratory of the Dept of Laboratory Medicine at the Ghent University Hospital, where they were centrifuged and frozen immediately upon arrival at minus 20°C and were analyzed on the same day.

These samples were analysed at the toxicology laboratory of the Dept of Laboratory Medicine at the Ghent University hospital. The plasma concentrations of meropenem and piperacillin were determined by validated ultra high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Samples were deproteinized using acetonitrile. After centrifugation, a portion of the supernatant was diluted and injected on a Waters BEH C18 column (1.7 μm, 100 mm x 2.1 mm) kept at 50 °C and a gradient elution of water and acetonitrile, both containing 0.1 % formic acid. Compounds were detected with a Waters Acquity TQD mass spectrometer operating in positive electrospray ionization using a compound specific MRM method. The assay was linear from 2 to 80 mg/L for meropenem, and from 4 to 250 mg/L for piperacillin with an imprecision and inaccuracy < 15 % at high, medium and low concentrations. Observed concentrations were corrected for protein binding (piperacillin 30%; meropenem 2%).

It should be highlighted that the samples in Study 1 and Study 2 were analysed using different assays in two different laboratories. Although a formal inter laboratory validation was not undertaken, both methods have been independently validated according to FDA guidelines. Furthermore, both laboratories monitor the quality of their analysis by using internal quality controls at 3 levels.

Pharmacodynamic analysis

Depending on the study and number of samples available, different methods were used to calculate the fT$_{>MIC}$. When enough samples were available, the fT$_{>MIC}$ was calculated by observing the time during the dosing interval that the log-linear least squares regression analysis intersected the target MICs for *Pseudomonas aeruginosa* (16 mg/L for piperacillin and 2 mg/L for meropenem based on EUCAST breakpoints [235].

In the case when only two concentrations were available per patient, another approach was used. One concentration (C_1) was taken halfway through the dosing interval, the second sample was a trough concentration (C_2). Using these two concentrations, it is possible to calculate the elimination constant (equation 1).

Equation 1 : $C_2 = C_1 - e^{k \cdot t}$

Assuming one compartmental first order kinetics, this is sufficient to calculate the time within the dosing interval where the concentration reaches or drops beneath a certain threshold. In order to investigate if these two approaches are comparable, the $fT_{>MIC}$ for the samples from the first study was calculated using the pharmacodynamic analysis used for the second study. This was performed for validation purpose only and was not used for the analyses.

Measurement of creatinine clearance and calculation of estimates

To calculate a reliable creatinine clearance, urine samples were taken from a 24-hour collection. Creatinine was measured in both serum/plasma and urine using the rate blanked, compensated and uncompensated Jaffe technique, respectively (Modular P and Cobas 6000, Roche Diagnostics GmbH, Mannheim, Germany). The creatinine clearance was calculated as follows :

24 hour creatinine clearance = U_v x U_{cr}/(1440 x S_{cr}), where U_v is the urinary volume (mL), U_{cr} the urinary creatinine concentration (µmol/L) and S_{cr} the serum creatinine concentration (µmol/L).For assessment of ARC a cut-off of creatinine clearance ≥ 130 mL/min was used [120].

Statistical analysis

The statistical analysis was performed using the statistical software package IBM-SPSS statistics version 20.0 (IBM Corp, New York USA). Data are expressed as median values with interquartile ranges (IQR) for continuous variables, numbers and percentages for categorical variables. In order to identify important covariates, multivariate logistic regression analyses (single step, forced entry) were conducted with target attainment 100 % $fT_{>MIC}$ and target attainment 50 % $fT_{>MIC}$ as dependent variable using the variables which gave a p-value of <0.10 in the univariate analysis. In the case of covariates which were closely related (such as weight, height and BMI), the one with the most significant p-value was chosen. Goodness of fit was assessed by the Hosmer-Lemeshow statistic. A receiver operator characteristic (ROC) curve was constructed to examine the sensitivity and specificity.

All tests were two-tailed, and p<0.05 was considered statistically significant.

2.3 Results

Patients

Sixty-one patients were included in the analysis. Patient characteristics on the day of study, and the comparison between the patients who did and did not reach the PK/PD target of both 100% fT$_{>MIC}$ and 50%fT$_{>MIC}$ are shown in table 1. The median (IQR) creatinine clearance from all patients included in the study was 125 (93-173) mL/min ranging from 55 to 310 mL/min.

Validation of the pharmacodynamic analyses

It was found that the results for both methods used for determination of fT$_{>MIC}$ were comparable.

Creatinine clearance and PK target attainment

Sixty-one patients were included in the study. One patient was excluded from the analyses since no urine was collected, as a result of which the creatinine clearance could not be calculated. Six patients treated with meropenem had a trough concentration which was lower than the lower limit of quantification (2 mg/L), which is also the breakpoint MIC of *Pseudomonas aeruginosa*. This implies that these patients did not reach the desired target of 100 % fT$_{>MIC}$, but the exact % fT$_{>MIC}$ could not be calculated, as this is not possible using only one sample. Two patients treated with piperacillin/tazobactam could also not be used for this analysis, because only the trough concentration was available, which is not enough to calculate the exact % fT$_{>MIC}$. These eight patients were included in the analysis using the PK/PD target of 100 % fT$_{>MIC}$, but could not be entered in the analysis using the PK/PD target of 50 % fT$_{>MIC}$

Table 1 : Patient characteristics and comparison between patients who did and did not achieve the PK/PD target of 100 % $fT_{>MIC}$ and 50 % $fT_{>MIC}$. Data are reported as median (interquartile range)

Variable	All patients (n= 60)	PK/PD target (100 % $fT_{>MIC}$) achieved (n= 33/60) (55%)	PK/PD target (100 % $fT_{>MIC}$) not achieved (n=27/60) (45 %)	p-value	PK/PD Target (50 % $fT_{>MIC}$) achieved (n= 43/52) (86 %)	PK/PD target (50 % $fT_{>MIC}$) not achieved (n=7/52) (14 %)	p-value
Male gender (n, %)	51 (85%)	28 (84%)	23 (85%)	0.721	36 (84 %)	7 (100%)	0.330
Age (years)	56 (48-67)	61 (53–73)	51(30-60)	0.016	60 (52-72)	48 (25-67)	0.054
Weight (kg)	78 (69-90)	75 (65-81)	83 (75-90)	0.014	75 (66-85)	85 (75-90)	0.041
Height (m)	1.75 (1.70-1.80)	1.75 (1.67-1.79)	1.79 (1.72-1.80)	0.170	1.74 (1.68-1.80)	1.79 (1.75-1.80)	0.098
BMI	25 (22-28)	24 (22-27)	25 (24-29)	0.084	24 (22-27)	25 (25-28)	0.188
SOFA at the day of study	5 (3-7)	5 (2-8)	5 (3-6)	0.693	5 (3-8)	4 (2-6)	0.358
Serum creatinine concentration (µmol/L)	54 (43-75)	53 (44-79)	56 (41 - 64)	0.623	57 (44-76)	54 (38-59)	0.306
Creatinine clearance (mL/min)		104 (87-123)	165 (138-208)	<0.001	106 (91-143)	215 (190-246)	<0.001
Antibiotic used				0.24			0.515
Meropenem (n, %)	17 (30%)	7/17 (41%)	10/17 (59%)		9/11 (82 %)	2/11 (18 %)	
Piperacillin (n, %)	43 (70%)	25/43 (58%)	18/43 (42%)		33/41 (80 %)	8/41 (20 %)	

SOFA – Sequential Organ Failure Assessment, PK /PD: pharmacokinetic/pharmacodynamic, % $fT_{>MIC}$: time above the minimum inhibitory concentration

Target 100 % $fT_{>MIC}$

Only 33 out of 60 patients (55%), for whom both creatinine clearance and trough concentrations were available, reached the PK/PD target of 100% $fT_{>MIC}$. Patients who did not attain the predefined PK target (100%$fT_{>MIC}$) were younger, had a higher creatinine clearance and a higher weight (table 1). Twenty-nine patients (48 %) had ARC, of which 22 (76 %) did not reach the PK target of 100%$fT_{>MIC}$.

Figure 1 illustrates the $fT_{>MIC}$ for the patients with and without ARC. The mean $fT_{>MIC}$ in patients with and without ARC is shown in figure 2 and was 61% vs. 94% in patients with and without ARC respectively (p<0.001).
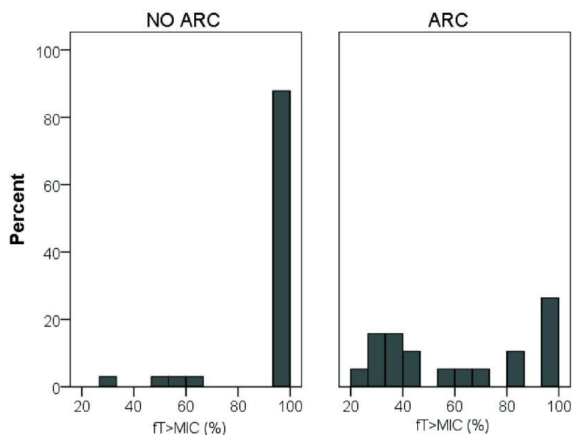
Fig. 1 Histogram *%f*$T_{>MIC}$ for patients with and without ARC
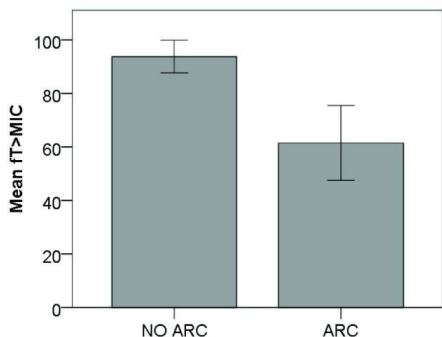
Fig. 2 Mean *% f*$T_{>MIC}$ for patients with and without ARC**.**

The results of the multivariate logistic regression are shown in table 2. As the antibiotic administered was not significantly different between the groups who did and did not achieve the PK/PD target, this was not included in the multivariate analysis (p=0.264). Contrary to creatinine clearance, age and weight were not significant in the multivariate analysis. The area under the ROC-curve was 0.86 (figure 3a), with a sensitivity of 81% and a specificity of 81% for predicting target attainment at 50 % probability.

Table 2 Multivariate regression model with attainment of 100 % $fT_{>MIC}$ as dependent variable

	Attainment of 100 % $fT_{>MIC}$ as dependent variable				
	B	p-value	Exp(B)	95% C.I.for Exp(B)	
				Lower	Upper
Creatinine clearance (ml/min)	-0.028	0.002	0.972	0.955	0.990
Weight (kg)	-0.040	0.114	0.961	0.915	1.010
Age (years)	0.020	0.331	1.020	0.980	1.063
Constant	5.788	0.033	326.34		

As an illustration of the impact of an increase in creatinine clearance, the probability of achieving the PK/PD target of 100% $fT_{>MIC}$ was plotted according to the creatinine clearance using the logistic model for a patient aged 55 years, weighing 75 kg (figure 4).
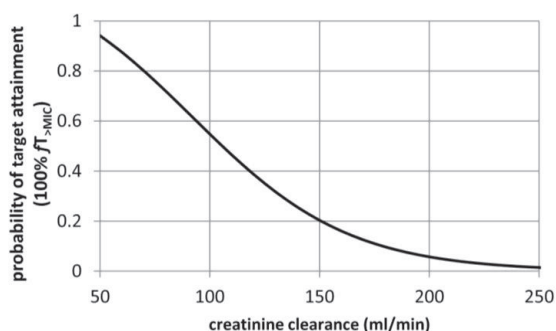
Fig. 4 Predicted probability of 100% $fT_{>MIC}$ target attainment.

Target 50 % $fT_{>MIC}$

Using the data from these 52 patients for whom both creatinine clearance and $fT_{>MIC}$ were available, we found that out of 19 patients displaying ARC, 7 (37 %) did not achieve the lower PK/PD target of 50 % $fT_{>MIC}$(p = 0.002) (table 1)

The results of the multivariate logistic regression analysis are shown in table 3. As the antibiotic administered was not significantly different between the groups who did and did not achieve the PK/PD target, this was not included in the multivariate analysis (p=0.515). The area under the ROC- curve was 0.99, with a sensitivity of 95 % and a specificity of 100% for predicting target attainment at 50 % probability (figure 3b). Only creatinine clearance was found to be significant in the multivariate analysis.

Table 3 Multivariate regression model with attainment of 50 % $fT_{>MIC}$ as dependent variable

	Attainment of 50 % $fT_{>MIC}$ as dependent variable				
	B	p-value	Exp(B)	95% C.I.for Exp(B)	
				Lower	Upper
Creatinine clearance (ml/min)	-0.114	0.045	0.892	0.798	0.997
Weight (kg)	-0.035	0.616	0.965	0.841	1.108
Age (years)	0.005	0.906	1.005	0.926	1.096
Constant	24.07	0.07	2.8×10^{10}		
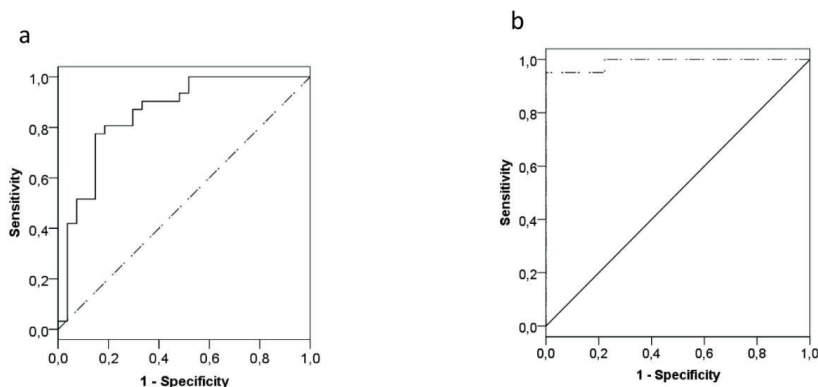
Fig. 3 ROC curves of the binary logistic model. A : 50 $fT_{>MIC}$ target, b : 100 % $fT_{>MIC}$ target

2.4. Discussion

In this large observational PK study, using clinical data from 61 critically ill patients with normal to increased renal function treated with meropenem or piperacillin/tazobactam, we found that ARC was associated with a higher risk of not achieving different PK/PD-targets in critically ill patients, even when administering these drugs by extended infusion. This calls into question the present approach to antibiotic dosing in these patients and supports use of more aggressive dosing strategies to minimize the likelihood of clinical failure.

In patients with apparent normal renal function, the relationship between creatinine clearance and low target attainment may not come as a surprise as previous studies have already demonstrated the correlation between creatinine clearance and clearance of β-lactam antibiotics [92, 158, 241, 319-324]. However, to the best of our knowledge, this study is the first to report the association between creatinine clearance and the lack of attainment of different PK/PD targets including the lower target of 50 % $fT_{>MIC}$ in patients with apparent normal renal function receiving antibiotic therapy administered as an extended infusion. Using trough antibiotic concentrations, Udy et al have demonstrated the association between subtherapeutic β-lactam concentrations and creatinine clearance in select critically ill patients [120]. In the current study we could also investigate other targets as we were able to use data from the entire antibiotic infusion, including the lower PK-target of 50 % $fT_{>MIC}$. We found that - even when the dose was administered as an extended infusion - up to 37% of the patients with ARC did not achieve this minimum PK/PD target - and may thus be at risk for treatment failure.

Controversy exists in contemporary literature which PK target should be aimed for in critically ill patients, as it is not clear which PK/PD target is associated with highest probability of reaching clinical cure. Studies have shown that - depending on the antibiotic - 40 to 70% $fT_{>MIC}$ is necessary to treat infections [40]. However, recent research has shown that achieving higher targets may be associated with a higher probability of reaching clinical cure. In order to maximize the effect of β-lactam antibiotics, it may therefore be necessary to increase the $fT_{>MIC}$ to 100 % or even maintaining the concentration four to five times the MIC for the entire dosage duration [42, 43, 325]. Nevertheless, irrespective of the PK/PD target considered relevant, increasing creatinine clearance is associated with lower target attainments.

Although ARC is a relatively new concept in intensive care medicine, its relevance should not be underestimated. The incidence in critically ill patients is high [276, 317, 318]. Implications for therapy with renally excreted drugs are considerable. Case reports have shown that some patients require up to 6, 8 or even 12 g meropenem per day to reach adequate serum concentrations [275, 282]. The effects of renal clearance are important not only for β-lactam antibiotics, but have also already been described for other antibiotics, such as vancomycin [120, 326].

This study has a number of limitations. First of all, this study did not look at clinical outcomes as the data were drawn from PK studies. Logically, clinical cure and mortality should be investigated in future validation studies of altered antibiotic dosing, although these studies should be even larger than the present study. Secondly, we have described renal function at inclusion using the MDRD which has been shown to underpredict glomerular filtration rate in some critically ill patients [248, 249]. Moreover this study was only a snapshot, and might not be representative for the entire course of treatment as creatinine clearance varies in the course of the disease. Also, this study is a single-center study, which only included patients with apparent normal renal function, which limits extrapolation of these finding to all ICU patients. Finally, we have measured total drug concentrations with correction for protein binding based on literature. This is an oversimplification, but our data show that this approach is acceptable for these two antibiotics, although is not for more highly protein bound drugs.

The findings from this study suggest that an even more sophisticated method of optimization may be necessary in selected patients - patient-tailored antibiotic therapy – which is the adaptation of antibiotic therapy to the need of the individual patient in order to maximize efficacy and minimize toxicity through therapeutic drug monitoring and dose adaptation. Unfortunately, TDM of β-lactam antibiotics is currently challenging with long turn-around times, expensive equipment, logistical problems related to the instability of the antibiotics in the samples and the need for well-trained personnel. Efforts to overcome these limitations, and clinical studies to assess utility in the clinical setting are urgently needed [153].

2.5. Conclusions

In conclusion, this study has demonstrated that in critical care patients receiving meropenem or piperacillin/tazobactam as an extended infusion, creatinine clearance is a key factor in the probability of PK/PD target attainment – irrespective if this is 50 or 100% $f_{T>MIC}$. This study, which excluded patients with renal dysfunction, demonstrated that a specific subset of patients is at risk for PK/PD target non-attainment, more specifically those patients with increased creatinine clearances, even if the dose is administered as an extended infusion, which improves the $f_{T>MIC}$. By means of multivariate logistic regression, it was found that a high creatinine clearance was an independent predictor of not achieving the PK/PD

target, implying that without dose up-titration, these patients are at risk of treatment failure, even when extended infusions are used.

3. **Therapeutic drug monitoring-based dose optimization of piperacillin and meropenem: a randomized controlled trial**

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Abstract

Purpose: There is variability in pharmacokinetics (PK) of antibiotics (AB) in critically ill patients. Therapeutic drug monitoring could overcome this variability and increase PK target attainment. The objective of this study is analyzing the effect of a dose adaption strategy using daily therapeutic drug monitoring on the target attainment.

Methods: This was a prospective, partially blinded, and randomized controlled trial in patients with normal kidney function treated with meropenem (MEM) or piperacillin/tazobactam (PTZ). The intervention group underwent daily therapeutic drug monitoring, with dose adjustment when necessary. The predefined pharmacokinetic/pharmacodynamic (PK/PD) target was 100% $fT_{>4MIC}$. The control group received conventional treatment Primary endpoint was the proportion of patients that reached 100% $fT_{>4MIC}$ and 100% $fT_{>MIC}$ at 72 h.

Results: Forty-one patients (median age was 56) were included in the study. Pneumonia was the primary infectious diagnosis. At baseline 100% $fT_{>4MIC}$ was achieved in 21% of the PTZ patients and in none of the MEM patients; 100% $fT_{>MIC}$ was achieved in 71% of the PTZ patients and 46% of the MEM patients. Eighty-five percent of patients in the intervention group needed dose adaptation, 5 required an additional increase. At 72h, target attainment rates for 100% $fT_{>4MIC}$ and 100% $fT_{>MIC}$ were higher in the intervention group: 58% vs. 16% (p=0.007) and 95% vs. 68% (p= 0.045) respectively.

Conclusions: A strategy of dose adaptation based on daily therapeutic drug monitoring lead to an increase in PK/PD target attainment compared to conventional dosing in critically ill patients with normal kidney function.

3.1.   Introduction

Infections are an important problem in critically ill patients, and an important source of morbidity and mortality in intensive care units (ICUs)[1]. Antimicrobial therapy has emerged as one of the most crucial elements in the treatment of severe infections, and has been studied extensively in recent years[59, 327]. Timely initiation of the antimicrobial agent as well as the appropriate spectrum have shown to be important determinants of clinical success. Antimicrobial therapy in ICU patients most often is based on standard dosing protocols, with little or no attention to the baseline characteristics (e.g. weight) or the altered physiology of the patient that results in changes in pharmacokinetics [328].

Numerous studies [93, 133, 140, 223, 329] have demonstrated that antibiotic plasma concentrations - especially of hydrophilic antibiotics, such as β-lactams - are variable and unpredictable in ICU patients. Increased volume of distribution, changes in protein binding as well as changes in elimination rate from the circulation through the kidney or the use of extracorporeal circuits contribute to this phenomenon, which has important implications [39, 97, 330]. A significant number of patients therefore do not reach pharmacokinetic/pharmacodynamic (PK/PD) targets required for the treatment of severe infections [331, 332].

Several strategies have been proposed to overcome this problem, such as continuous or extended infusion[140, 157]. Recent literature demonstrated higher PK/PD target attainment [156] as well as improved outcomes [298] when extended or continuous infusion strategies are used, and a randomized controlled trial comparing intermittent with continuous infusion resulted in better antibiotic exposure, as well as improved clinical cure in the continuous infusion group [142]. Although this may be an improvement over intermittent dosing, in some patients even higher doses may be required.

There have been multiple reports of patients with augmented renal clearance (ARC) in whom standard dosing is not adequate [120, 282]. Some patients required up to 4 fold increases in dosing for the treatment of severe infections – and often, therapeutic drug monitoring (TDM) was used to guide treatment[120, 275, 281]. Pharmacokinetic studies also confirmed that some patients may require higher doses of β-lactam antibiotics or glycopeptides, especially when aiming for higher PK/PD targets [157, 256, 333, 334].

A more individualized approach using TDM guided antimicrobial therapy with dosing tailored to the altered PK of the patient may be a proper strategy to overcome this variability and the problem of underdosing[335]. Therefore we designed a randomized controlled trial using a TDM based dose-adaptation strategy in patients at risk of underdosing who required therapy with piperacillin/tazobactam (PTZ) and meropenem (MEM) in patients with normal kidney function. We hypothesize that a TDM based approach results in higher attainment of PK/PD targets.

3.2. Methods

Study design

Between April 2011 and February 2012 we conducted a prospective, partially blinded, randomized controlled trial, at the medical and surgical ICU of the Ghent University Hospital. Criteria for inclusion were the need for antibiotic treatment with PTZ and/or MEM, age of 18 years or older and the presence of an arterial catheter. Patients were excluded in case of pregnancy and/or lactation, allergy to the administered medication, impaired renal function (estimated glomerular filtration rate (eGFR) as assessed by the CKD-EPI equation <80 mL/min [28], hemoglobin < 7g/dL, do-not–resuscitate orders or if the patient was expected not to survive the first 48h.

Patients were randomly assigned to the control group, receiving conventional dosing, or the intervention group, subjected to TDM guided dosing which consisted of daily monitoring of the antibiotic plasma concentration, followed by dosing adjustment if the concentration did not meet the predefined target. In the control group antimicrobial concentration was also measured daily, but the treating physician was blinded for the results that were used for comparison only. Total duration of the study was 7 days. Patients were followed up until hospital discharge.

All antibiotics were administered according to the extended infusion protocol used at Ghent University Hospital: patients received a loading dose (1g MEM or 4g PTZ) administered over 30 minutes, followed immediately by the first extended infusion dose of either antibiotic (1g MEM or 4g PTZ) every 6 hours for PTZ and every 8 hours for MEM. Extended infusion doses were administered over 3 hours using a syringe pump. All antibiotics were administered via a central venous catheter.

Target concentrations in the intervention group were in line with previous studies using TDM in critically ill patients. It is traditionally accepted that maintaining concentrations above the minimal inhibitory concentration (MIC) of the causative organisms during 40-70% of the time is adequate. However, recent studies suggest that higher targets are needed in critically ill patients. Given the fact that concentrations 4-5 times greater than the MIC are associated with maximal bactericidal activity[42, 336, 337], the PK/PD target in this study was set at 100% $fT_{>4MIC}$ as in previous studies[120, 236].

Based on actual antibiotic concentrations, dosing of intervention patients then followed a pre-established algorithm (figure 1). Until the MIC of the causative microorganism was known, the epidemiological cutoff (ECOFF) of wild type *Pseudomonas* spp. (16mg/L for PTZ and 2mg/L for MEM) was targeted, and this MIC was used for all calculations in the study as we only investigated the effect of dose adaptation in the first 72 hours – the time it would usually take to have an MIC of the actual infecting organism available. Target trough concentrations were therefore >64mg/L for PTZ and >8mg/L for MEM respectively (>4xMIC). In case of lower concentrations, dosing frequency was increased as a first step in the intervention (4g/0.5g every 4h for PTZ and 1g every 6h for MEM). If MEM concentrations remained below the target, the dose was increased by 50%. If these adjustments failed to reach the targets, no further actions were taken. In patients with trough concentrations > 10xMIC, the antibiotic dose was decreased by 50%, or the dosing frequency reduced if this had been increased in an earlier step.
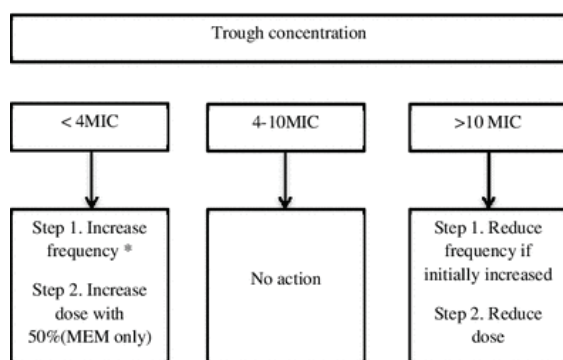
Fig. 1 Therapeutic drug monitoring (TDM)-based dose adaptation strategy. *MIC* Minimal inhibitory concentration, *MEM* meropenem. *Asterisk* see text for details

Endpoints

Target attainment defined as 100% fT$_{>MIC}$ and 100% fT$_{>4MIC}$ within the first 72 hours of treatment were the primary endpoints. The fT$_{>MIC}$ and fT$_{>4MIC}$ at 72h was compared between intervention and control groups, as well as between baseline and at 72h. Although 4 times the MIC was the target of the intervention we wanted also to study the effect of the intervention on a more conservative PK target hence 100% fT$_{>MIC\ was}$ used as an endpoint as well.

Secondary endpoints were absolute values of fT$_{>MIC}$ and fT$_{>4MIC}$,

Clinical response at the end of the study (day 7) was evaluated by two of the investigators. Resolution was defined as disappearance of all signs and symptoms related to infection, improvement was defined as a marked or moderate reduction in the severity and/or number of signs and symptoms of infection and failure was defined as insufficient lessening of the signs and symptoms of infection to qualify as improvement, including death. Response to therapy was also evaluated by bacterial persistence at day 7.

Patient data collection

Relevant data were retrieved from the hospital's Electronic Patient File and the unit's Patient Data Management System and included demographic parameters (gender, age), weight, length, date of hospital/ICU admission and discharge, start and end date of the antibiotic treatment with PTZ/MEM, comorbidities, admission diagnosis, type of infection, Acute Physiology And Chronic Health Evaluation (APACHE)-II score, daily Sequential Organ Failure Assessment (SOFA) score, daily body temperature, urinary output and outcome (survival or death) including cause of death. The following lab results were recorded: white blood cell count, platelet count, fibrinogen, C-reactive protein (CRP), serum creatinine, urinary creatinine and microbiological data.

Study samples

Blood samples were collected daily; during the first 3 days mid-dose (i.e. halfway the dosing interval) and trough samples were obtained, during the last four days of the study only trough concentrations were determined. On the first study day, the first sample (baseline concentration) was drawn after at least 3 completed infusions of the antibiotic.

Twenty-four hour urinary creatinine clearance was measured throughout the study period. The calculated creatinine clearance was corrected for the Body Surface Area (BSA).

Sample analysis

Analysis of the TDM samples was done at the Department of Laboratory Medicine at the Ghent University Hospital. PTZ and MEM concentrations were assayed by validated ultra high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) using oxacillin as an internal standard [33].

Sample preparation consisted of protein precipitation using acetonitrile and subsequent dilution. Five µl was injected onto a BEH C_{18} column (1,7µm, 100mm x 2,1mm) (Waters®), kept at 50°C. The mobile phase consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile at a flow rate of 0.350 mL/min. Separated compounds were detected with the Waters® TQD mass spectrometer, which operated in positive electrospray ionization, using a compound specific MRM method. Runtime was 5.5 minutes. The method was linear between 4 - 250 mg/L for PTZ and 2 - 80mg/L for MEM. Imprecision and inaccuracy were found to be < 15 % at high, medium and low concentrations. Concentrations below the linear range were reported as < 4 mg/L for PTZ and as < 2 mg/L for MEM. System performance was monitored by analyzing 3 internal quality control samples at low, medium and high concentrations in each run.

Pharmacokinetic and pharmocodynamic calculations

One concentration (C_1) was taken halfway through the dosing interval; the second sample was a trough concentration (C_2). Using these two concentrations, it is possible to calculate the elimination constant (equation 1). Equation 1 : $C_2 = C_1 - ek . t$

Assuming one compartmental first order kinetics, this is sufficient to calculate the time within the dosing interval where the concentration drops beneath a certain threshold (1 or 4 x MIC)

Power analysis

Power analysis computed a required sample size of 16 patients per study group, taking into account a one sided test with α = 0.05, β = 0.20 and an expected increase of target

attainment (with trough concentrations of at least 4xMIC as a target) from 50% to 90% of the patients. Considering a dropout rate of 20%, 20 patients per group were projected.

Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics 19.0. Data are expressed as median values with interquartile ranges (IQR) for continuous variables, numbers and percentages for categorical variables. Mann-Whitney U test for comparison of median values and the Friedman test or the Wilcoxon matched-pairs Signed-ranks test were used where appropriate. Proportions were compared using 2x2 tables and the Chi² or Fisher's Exact as appropriate. A p-value of ≤ 0.05 was considered statistically significant.

Ethics

The study was approved by the Ethics Committee of the Ghent University Hospital (registration number 2010/814), and approved by the Belgian regulatory agency (B67021020250). Written informed consent was obtained from the patient or his/her legal representative.

3.3.   Results

Forty-one patients were included in the study, 21 in the intervention group and 20 in the control group. Twenty-eight patients received PTZ: 15 in the intervention group, and 13 in the control group; 13 patients received MEM: 6 in the intervention group and 7 controls.

The majority of the patients were male (n=35, 85%). Characteristics of intervention and control patients were comparable and are summarized in table 1. Most patients were treated for pneumonia (78%), other diagnoses included tracheobronchitis, - peritonitis, and blood stream infection (table 1); 1 patient received antibiotics because of febrile neutropenia.

Table 1 Patient characteristics of the study population

Characteristics	All patients (*n* = 41)	Intervention (*n* = 21)	Control (*n* = 20)	*P* value
Age (years)	56 (46–69)	57 (42–76)	56 (48–64)	0.804
Weight (kg)	76 (67–88)	77 (69–89)	75 (66–88)	0.657
Body mass index (kg/m^2)	25 (22–27)	25 (22–28)	24 (22–25)	0.705
APACHE II score	18 (13–24)	19 (12–24)	17 (13–23)	0.557
Day 1 SOFA score	5 (2–6)	5 (3–6)	5 (2–6)	0.711
Day 1 CrCL (mL/min)	99 (80–135)	130 (92–177)	108 (88–145)	0.291
Day 2 CrCL (mL/min)	115 (82–170)	129 (100–167)	106 (74–175)	0.461
Day 3 CrCL (mL/min)	131 (90–172)	155 (83–182)	110 (90–165)	0.697
Infection characteristics				
Pneumonia	32 (78 %)	16 (80 %)	16 (76 %)	
Tracheobronchitis	2 (%)	1 (5 %)	1 (5 %)	
Peritonitis	5 (12 %)	3 (15 %)	2 (10 %)	
Blood stream infection	1 (2 %)	0 (0 %)	1 (5 %)	
Febrile neutropenia	1 (2 %)	0 (0 %)	1 (5 %)	
Community-acquired infection	3 (7 %)	2 (10 %)	1 (5 %)	
Hospital-acquired infection	38 (93 %)	18 (90 %)	20 (95 %)	

CrCl : creatinine clearance

Forty-three causative microorganisms were cultured from 27 patients; isolates included *E. Coli* (n=7), *Klebsiella pneumoniae* (n=7), *Pseudomonas aeruginosa* (n=6), *Enterobacter cloacae* (n=4), *Staphylococcus aureus* (n=4), *Klebsiella oxytoca* (n=2), *Acinetobacter baumanii* (n=2), *Enterococcus faecalis* (n=2), *Prevotella spp*. (n=2), *Citrobacter spp.* (n=2), *Morganella morganii* (n=2*), Serratia marcescens* (n=2), *Enterobacter aerogenes* (n=1) and *Streptococcus viridans* (n=1). The median MIC was 2 mg/L (IQR 1.5-8) for PTZ and 0.125 (0.125-0.690) mg/L for MEM.

Median antibiotic concentrations before randomization were 30 mg/L (IQR 18-56 mg/L) for PTZ and <2 mg/L (IQR <2-4 mg/L) for MEM.

Baseline target attainment was as follows: 100% $fT_{>4MIC}$ was achieved in 21.4% of the PTZ patients and in none of the MEM patients; 100% $fT_{>MIC}$ was achieved in 71.4% of the PTZ patients and 46.2% of the MEM patients. The median $fT_{>4MIC}$ at baseline was comparable for both antibiotics with 46.5% for PTZ (IQR 18-86.25) and 56.5% for MEM (IQR 15-65). Median baseline $fT_{>MIC}$ was much higher at 100% for both PTZ and MEM.

Patients in the intervention group had numerically lower baseline median concentrations (PTZ 26 mg/L vs. 40 mg/L and MEM <2 vs. 2 mg/L). As a consequence, at baseline fewer intervention patients achieved 100% $fT_{>4MIC}$ (9.5 vs. 20%) and their $fT_{>4MIC}$ is lower (44.5 vs. 58%).

In the intervention group, dose adaptation was necessary in 16 patients (76%); the initial step of increasing the frequency was enough to reach the target of 4xMIC in 69% (11/16) of these patients.

Three patients did not complete the study protocol, and from them target attainment at day 3 could not be calculated. In the remaining 38 patients, the use of a TDM based dose adaptation protocol significantly increased the proportion of patients reaching the PK/PD target within the first 72 hours of treatment: 94.7% of the intervention patients reached 100% $fT_{>MIC}$ in contrast to 68.4% of the control patients (P = 0.045). Also for the target of 100% $fT_{>4MIC}$, attainment rates were higher in the intervention group (57.9% vs. 15.8%, p=0.007). (Figure 2 and 3). No adverse events occurred.
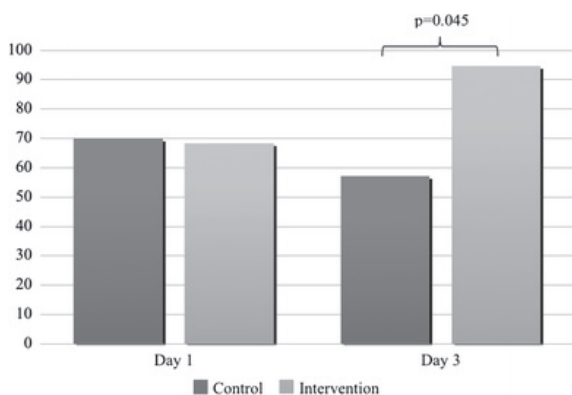
Fig. 2 Percentage of control and intervention patients reaching 100 % $fT_{>MIC}$ at baseline and on day 3. $fT_{>MIC}$ Cumulative percentage of a 24-h period that the free (f) drug concentration exceeded the MIC under steady-state pharmacokinetic conditions
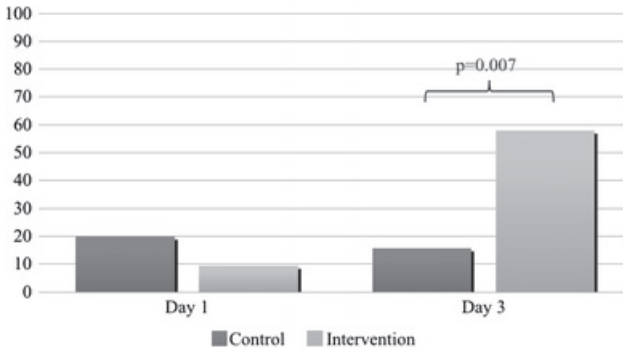
Fig. 3 Percentage of control and intervention patients reaching 100 % $fT_{>4MIC}$ at baseline and on day 3. *>4MIC* Fourfold the MIC

The intervention significantly increased the median $fT_{>4MIC}$ from 44.5% to 86% and 90% on day 2 and 3 respectively (p=0.012)(Fig. 4).
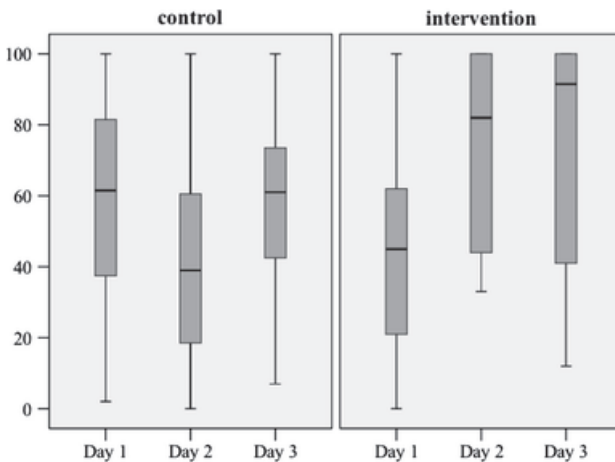
Fig. 4 Boxplots of time above the 4× MIC ($fT_{>4MIC}$) during the first 3 days of treatment in control and intervention patients. *Top, bottom of box* 25 and 75 % percentile, respectively, *dark horizontal line in box* median, *whiskers* minimum and maximum, respectively

Clinical failure was present in 4 patients in the control group, and in 2 in the intervention group (p=0.41); bacterial persistence at day 7 was present in 5 patients in the control group, vs. 1 in the intervention group (p=0.09).

The recovery of organ function during the study was evaluated using the SOFA score in patients who completed the 7-day study protocol (n=15). Median SOFA score changed from 5.5 to 3 in intervention patients (P = 0.093), and from 5 to 4 in the control group (P = 0.575).

Five patients died in the ICU, 4 control patients (20%) and 1 intervention patient (4.8%) (p= 0.18). Hospital and 28-day mortality were also not significantly different with 5 deaths in the control group and 3 in the intervention group (25% vs. 14.3%%, p= 0.45).

3.4. Discussion

In this study we found that daily TDM with dose adaptation resulted in higher median $fT_{>4MIC}$, and a higher proportion of patients attaining both the 100% $fT_{>MIC}$ and 100% $fT_{>4MIC}$ target in patients with normal kidney function. This required doses of 33-100% higher compared to standard dosing regimens.

We also demonstrated that standard dosing – even using extended infusion – does not reach target antibiotic concentrations in all patients, either the 100% $fT_{>MIC}$ or 100% $fT_{>4MIC}$ target. Extended and continuous infusion of β-lactam antibiotics have been found to increase exposure of the microorganism to the antibiotic, which in case of time-dependent antibiotics such as piperacillin and MEM, should theoretically lead to a more efficient antibiotic effect, faster control of the infection and improved outcomes. The literature is scattered with simulation data – most often coming from healthy or non-critically ill patients but all consistently demonstrating that extended or continuous infusion results in improved target attainment rates. Small-scale clinical studies did confirm this for both piperacillin and MEM [140, 157]. However, extended infusion may not be sufficient to overcome the changed physiology of the patient, notably when higher PK targets are used or (borderline) resistant microorganisms are involved, but also in more common settings such as augmented renal clearance (ARC). Taccone et al. recently reported a patient infected with a highly resistant microorganism who needed a daily dose of 12g meropenem to treat the infection [256].

The question remains if our findings apply to all patients in the ICU. This study was performed in patients considered to be at the highest risk of underdosing, i.e. patients with apparent normal renal function. ARC is a frequent finding in this population[316], and for a lot of antibiotics, including piperacillin and MEM drug clearance is largely determined by

renal clearance[39]. ARC has been linked to inadequate antibiotic concentrations [120], and will also have played a role in the current study. Possibly, ARC patients are the best candidates for a TDM based approach to optimize antibiotic exposure. But also other patient categories may be at risk of underdosing. Hites et al. recently reported that obese critically ill patients treated with carbapenems had lower concentrations compared to non-obese patients [280].

This study has a number of limitations. First of all, this study was performed in selected ICU patients and patients with impaired renal function or on renal replacement therapy (RRT) were excluded. Patients on RRT are at particular risk of underdosing when package insert dosing recommendations are followed, and they may indeed also benefit from a TDM based antibiotic dosing approach. Secondly, we only measured total antibiotic concentrations, and not free antibiotic concentrations. Protein binding is limited for piperacillin and almost nil for MEM [123], and therefore the potential effect of changes in protein binding is expected to be limited. Furthermore, the study was not designed or powered to detect any difference in clinical outcome parameters. Finally, we did not include a second step of dose increase in the patients who had inadequate piperacillin concentrations. This would have increased the daily dose to 36g piperacillin and 4.5g of tazobactam, a very high dose of which the PK has never been investigated. As the PK of both compounds are not completely alike, administration of a high dose of PTZ could potentially lead to accumulation of tazobactam and related toxicity.

Potential benefits of a TDM based approach include better outcome because of more appropriate antibiotic concentrations, but also less resistance development and avoidance of toxicity. Although considered safe, β-lactam antibiotics have a number of adverse effects including neurotoxicity, liver damage and bone marrow suppression, and some of these are dose-dependent. TDM may thus not only be helpful to increase efficacy, but also to reduce toxicity.

Although TDM was able to increase target attainment, it should be noted that underdosing remains frequent in the initial phase; TDM may be useful to correct initial underdosing but alternative strategies remain warranted to avoid underdosing in the first 24 hours of therapy. Dose predictions based on PK modeling may offer a solution to counter this.

The literature on TDM based approach for β-lactam dosing is limited [224]and the use of TDM in clinical practice remains controversial [38]. Roberts et al. demonstrated that 74% out of 236 patients treated with β-lactam antibiotics did not reach adequate concentrations, and needed dose adjustment[236]. However, the effect of this was not systematically evaluated; only 21% of the patients were re-sampled, and only 43% of them reached adequate concentrations, confirming our findings. TDM has also proved beneficial in specific populations such as burn patients. Patel et al. found that TDM was able to detect underdosing in up to 60% of the patients[338]. Several case reports have shown that in difficult situations, either patients with a complex physiology or microorganisms with increased resistance to an antibiotic, TDM may indeed be useful to guide therapy[256, 275, 281, 282] [19, 21-23]. This study is however the first to pharmacokinetically confirm that dose adaptation results in better target attainment.

In conclusion, TDM based dose adaptation of β-lactam antibiotic therapy improves antibiotic exposure in critically ill patients with normal renal function. Whether this approach leads to improved outcomes remains to be determined.

Chapter Seven: Discussion and Future Perspectives

Early initiation of antibiotic therapy with an appropriate spectrum after source control is currently the most important clinical intervention to reduce mortality and morbidity in severely infected patients with sepsis and septic shock. To be effective, the antibiotic needs to reach the infected tissue in optimal concentrations. For β-lactam antibiotics, the concentrations have to exceed the minimal inhibitory concentration (MIC) of the microorganism for at least 40-60% of the time, which is considered the minimum pharmacodynamic (PD) target. In recent studies it was suggested that for critically ill patients higher plasma concentrations (100%fT$_{>MIC}$ or even 4 times the MIC) are required for optimal efficacy. However, because of grossly altered pharmacokinetics of hydrophilic antibiotics, dosing remains a significant challenge for the treating physician. Recent research has shown that because of altered pharmacokinetics of hydrophilic antibiotics, current standard dosing – most often based on experiments in healthy volunteers – does not reach even the minimal targets in many critically ill patients, let alone the higher targets mentioned.

Antibiotic dosing that is not optimized for critically ill patients may lead to clinical failure. In this respect, it is interesting to note that two non-inferiority studies of two new β-lactam antibiotics, ceftobiprole and doripenem were stopped prematurely, because of greater mortality in the intervention group, where a greater proportion of patients happened to display augmented renal clearance [339]. In order to improve dosing in critically ill patients, it may be necessary to change current dosing practices from "one dose fits all" to a more individualized patient tailored antibiotic therapy.

The overall objective of this research was to develop tools to individualize antibiotic therapy in critically ill patients. In this respect we have first developed an ultrafast quantification method that allows for fast determination of β-lactam antibiotic concentrations in plasma. We have minimized sample preparation and investigated the pre-analytic phase. In this project we have also confirmed the high variability of antibiotic concentrations, as well as variability over time in within the same patient. We have developed population pharmacokinetic models for amoxicillin/clavulanic acid and

cefuroxime in critically ill patients without acute kidney injury, and a population pharmacokinetic model for cefepime in septic shock patients during continuous renal replacement therapy. We have shown in a simulation study that standard dosing of broad spectrum β-lactam antibiotics leads to better target attainment than standard dosing of narrower spectrum β-lactam antibiotics for a number of organisms in which de-escalation can be performed. Some more practical studies were also performed, such as the stability studies for meropenem and amoxicillin/clavulanic acid. Furthermore, we found that increased creatinine clearance is a risk factor for subtherapeutic conentrations. Finally, we have shown that daily therapeutic drug monitoring leads to improved pharmacokinetic target attainment.

Studies like the ones performed for this thesis have improved our knowledge of the altered pharmacokinetics of many antibiotics. We now have better insights in the determinants of pharmacokinetics in the critically ill. However, there are still many questions that need to be answered before we can truly move from current dosing practice to a more patient tailored antibiotic therapy.

1. Analytical considerations

For this research we have developed an accurate and ultrafast chromatographic method coupled to tandem mass spectrometry. Although this was possible in this tertiary center university hospital, this may not be feasible in smaller centers. Therefore, more research is needed in the analytical field of biosensors and immunoassays, which does not require expensive equipment or highly trained staff. Moreover, if therapeutic drug monitoring would be performed on a routine basis, it should be available at least once a day and preferably 24/7. The best of all cases would be a bedside sample collection device that requires only a small volume of whole blood but immobilizes the proteinaceous and cellular component of the specimen to isolate the free fraction, thus yielding a sample requiring minimal preparation and providing maximal pharmacokinetic information. If such a device were not feasible, research should focus on easy transportation (using for example dried blood spots or other alternative sampling devices) of samples from the hospitals to the reference center. If analysis of β-lactam concentrations were to be performed on a large scale in multiple centers, there will also be a need for certified reference material,

commercial calibrators and controls and proficiency testing schemes in order to ensure quality control.

2. Population pharmacokinetic studies

As critically ill patients are a very heterogeneous group, population pharmacokinetic studies are needed in each subpopulation, such as morbidly obese patients, patients treated with extracorporeal techniques, such as extra corporeal membrane oxygenation (ECMO) and renal replacement therapy (note that a separate study is needed for each mode of clearance, such as sustained low efficiency dialysis (SLED), continuous venoveno hemofiltration (CVVH), continuous venoveno hemodialysis, (CVVHD), continuous venoveno hemodiafiltration (CVVHDF), intermittent hemodialysis, …), burn patients, patients with liver failure etc. The developed population pharmacokinetic models can be used to develop dosing algorithms based on the covariates from the pharmacokinetic model. These algorithms should then be prospectively validated. Research should not only focus on broad spectrum but should also be focused on dosing of narrower spectrum and less frequently used drugs such as amoxicillin, cefuroxime, and even flucloxacillin and penicillin G.

3. Tissue concentrations

In our research we have only investigated total plasma concentrations. However, the bloodstream is not the site of infection is in most cases. It is unclear whether total (or even unbound for that matter) concentrations accurately reflect the concentrations at the site of infection. The required dose to result in optimal tissue concentrations remains unknown.

Studying tissue concentrations is a complex issue. Lung infections are a good example to illustrate the difficulties of studying tissue concentrations. First of all, the location of the proliferating bacteria within the different lung compartments is often uncertain [340]. The infection may affect different sites within the lung (alveoli, interstitium, bronchioles, etc.). Each site has its own diffusion constant, and therefore its own concentrations. Therefore, the specimen to determine the representative drug concentration must be wisely chosen [340]. Possible specimens are : epithelial lining fluid, lung interstitial fluid, alveolar macrophages, blood, lung tissue, bronchial secretions and sputum. Epithelial lining fluid is often considered to be the most representative of the extracellular environment where the most common pulmonary pathogens are located.

However, obtaining a representative specimen is invasive and technically challenging, as epithelial lining fluid is often contaminated with antibiotics released from alveolar macrophages, giving rise to falsely elevated concentrations. Moreover, it is not feasible to sample multiple times within one dosing interval [340].

An emerging technique used to determine the concentration in tissues is microdialysis. A microdialysis probe is inserted into the tissue, and is constantly perfused with physiological fluids (perfusate) with similar composition comparing to the interstitial fluid. The tip of the microdialysis catheter is semi-permeable, which allows the drug to diffuse from the interstitial fluid into the microdialysate. This recovered fluid can then be collected and analysed. This technique is graphically shown in figure 1.
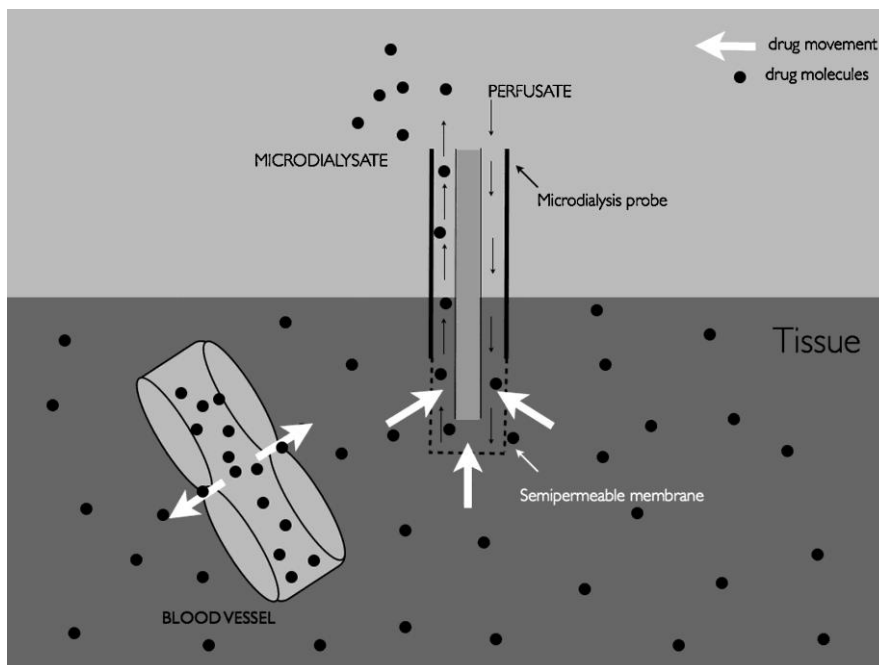
Fig. 1 : Principle of microdialysis (copied from [341]with permission)

Roberts et al have already performed some exploratory studies in a limited number of sepsis patients [133, 140]. Tissue penetration may be more severely impaired in septic shock, which was explored by Joukhadar et al, again in only a limited (n= 6) number of patients, treated with piperacillin in an intermittent infusion [134]. Therefore, future research should more closely investigate concentrations at the site of infection, preferably over multiple days starting from the first day of treatment. These tissue concentrations should be linked to total and free plasma concentrations and other covariates such as renal function, body weight, vasopressor therapy and so on using pharmacokinetic population

analysis, so dosing simulation studies can be performed to investigate which dose results in optimal concentrations.

4. Continuous infusion

It seems that continuous infusion of β-lactam antibiotics is becoming the administration method of choice in critically ill patients. However, it is unclear how this mode of infusion influences tissue penetration. Does continuous infusion improve tissue penetration in septic shock? Does it result in more stable concentrations? This way of infusion may hypothetically lead to more stable tissue concentrations and may therefore prevent the overgrowth of less susceptible organisms and therefore minimize resistance, which should be investigated in prospective studies. Developing methods to improve stability of unstable antibiotics such as amoxicillin/clavulanic acid and imipenem may also be worthwhile, such as cooled infusion syringes.

5. Pharmacokinetic/pharmacodynamic target

Probably the biggest hurdle to overcome before one can truly investigate the impact of TDM on clinical outcome is the PK/PD target, used to decide if dosing adaptations are needed. From *in vitro* and animal models it has become clear that β-lactam antibiotics exert time dependent killing. However, it is not clear whether the target value is 50% $fT_{>MIC}$, or 100% $fT_{>MIC}$ or even 100% $fT_{>4XMIC}$. Moreover, when establishing the optimal PK/PD target, one should not only consider short term outcomes such as clinical cure of the patient, but also long term outcomes, such as the minimization of the emergence of antibiotic resistance, as emerging resistance is an important global issue.

From pre-clinical studies it is clear that the relationship between antibiotic exposure and resistance development is very different from the relationship between bactericidal effect [342]. The magnitude of the threshold for resistance suppression is markedly higher than the threshold needed for optimal clinical success. Therefore, dosing that only aims to optimize bactericidal effect may actually increase resistance formation by selecting less susceptible mutant strains, which is a hypothesis called the mutant selection window and mutant prevention concentration. However, the doses required for achieving concentrations above the mutant prevention concentration are often higher than the maximal

recommended dose, and sometimes not achievable for some antibiotic-pathogen combinations [342].

In order to elucidate the optimal PK/PD target, large multicenter studies are needed in critically ill patients investigating the relationship between concentrations, susceptibility of the microorganism and outcome in patients. Large observational datasets are needed incorporating as much information as possible, such as details on the causative pathogen and its susceptibility, antibiotic concentrations, site of infection, severity of illness scores and other patient characteristics which may all influence outcome. Using this information, a multivariate analysis incorporating these potential confounders can then be performed, investigating whether failure of achieving the PK/PD target attainment is an independent predictor of outcome (clinical failure or the emergence of resistance).

6. How to perform patient tailored antibiotic therapy ?

Once the PK/PD target has been established, studies are needed that investigate how to perform patient tailored antibiotic therapy. Considering the importance of timely antibiotic therapy, the patient should be initiated on a dose based on population pharmacokinetic studies performed in this target population which gives the best prior Bayesian probability of reaching the PK/PD target. Although this dose gives the best probability of reaching the PK/PD target for this population, it does not guarantee that the PK/PD target will be reached in each individual, because of large between subject variability, and therefore, concentrations should subsequently be monitored.

However, there are many questions to be resolved, such as the exact timing of the first sample. There are arguments to be made to wait a sufficient amount of time in order to reach pharmacokinetic steady state (a concept which can be questioned in critically ill patients, as steady state requires stable patients), while others would stress the importance of time, and would sample sooner. Another practical issue is the method of dose adjustment when deemed necessary. Should a generalized dose adjustment method be used (such as: increase the dose by 50% or reduce the frequency by 50%), or should the dose be adjusted using specific software which can estimate patient clearance from the measured concentration and so determine which dosing regimen would most likely result in optimal concentrations (Bayesian forecasting)?

As already mentioned above, these studies should not only be focused on the broad spectrum antibiotics such as meropenem and piperacillin, but also on narrower spectrum drugs, as these are still an important part of our armamentarium against antimicrobial resistance. Focusing research on only the broad spectrum antibiotics may create an illusion of safety for these drugs, and may promote general use of these antibiotics and therefore further driving emergence of resistance.

The ideal design for this study is a randomized controlled trial, where the control group receives the standard dose of antibiotic therapy. If by then, there is sufficient evidence that continuous infusion leads to better outcome, then both arms should receive the antibiotic as a continuous infusion. The intervention group should be started on the dose which is most likely to result in optimal concentration using Bayesian Forecasting. The initial dose should therefore be based on patient characteristics such as weight and renal function. After a few hours a sample can already be taken and sent to the lab. The results should be made available as quickly as is feasible for the lab, and these results should be interpreted using specific dosing software, which predicts individual pharmacokinetic parameters and calculates which dose will most likely result in optimal PK/PD target attainment and takes into account that these are non-steady state conditions. The dose should then be promptly adapted if needed. The probability to achieve a better outcome (clinical cure or prevention of the emergence of resistance) should be compared between both groups. If this study reveals that TDM is a useful intervention, effort should be made to try to reduce the burden of this intervention. Future studies should then be focused on achieving the PK/PD target with less difficulties, such as performing TDM only in the initial phase and then monitoring covariates such as renal function.

7. More and more accurate MIC values

As already mentioned, the likelihood of therapeutic success is based on exposure and susceptibility of the pathogen. Since the MIC values are reported in factors of two, this means that for each level of decreased susceptibility, the PK component of the PK/PD index has to be doubled in order to maintain the pre-defined target PK/PD ratio. Therefore, the susceptibility of the microorganism is a very important parameter to take into account when moving towards more individualized antibiotic therapy.

However, to date, the susceptibility of a microorganism is mostly reported as S(sensitive)/I(intermediary resistant) or R (resistant). These arbitrary values are in PK/PD terms less useful than an actual MIC value, as there is a whole range in MIC values that are considered to be sensitive to a certain antibiotic. The PK/PD target will always be achieved with standard dosing if the MIC value is very low. However, when the MIC value shifts to higher values (closer to the intermediary value, but still reported to be sensitive), it may become more difficult to achieve the PK/PD target using standard dosing. On the other hand, MIC values which are considered intermediary or even resistant, may still be attainable, if higher doses are given and/or using alternative administration techniques, and if the MIC value is only 1 or 2 dilutions higher than the breakpoint MIC. Therefore, MIC values should be preferred over the 3 categories S, I and R.

All currently used methods rely on detecting phenotypic resistance by measuring bacterial growth in presence of the antibiotic being tested. These methods are generally slow, as these require isolation from the clinical sample before testing and require incubation time, and generally take between 24 and 72 h during which broad spectrum empirical is started based on local epidemiology. In the last few years, the knowledge on the molecular basis of antibiotic resistance has widely increased, and therefore novel approaches for rapid detection of bacterial resistance are to be expected, based on polymerase chain reaction (PCR) techniques, mass spectrometry, microarrays, microfluidics, cell lysis-based approaches and whole-genome sequencing, which may also improve outcome and decrease resistance development. However, it is yet to be investigated whether these methods achieve the same level of sensitivity and specificity compared to standard methods and can be available 24/7 with a short turn-around time. Moreover, these methods are currently associated with a significant cost [343].

8. Pharmacoeconomics

Shifting from standard dosing to patient tailored antibiotic therapy will definitely be costly, because of the need for concentration determinations, determination of MIC values, the need for higher doses of antibiotics in some patients and the need for a multidisciplinary team consisting of intensive care physicians, microbiologists, pharmacists and clinical chemists. When a definitive study is undertaken evaluating the impact of patient tailored

antibiotic therapy, one should also perform a cost benefit-analysis, to investigate whether the quantitative benefits of this intervention outweigh its costs.

The costs can be estimated relatively easily. The lab analysis would cost around € 35 per sample, and it is estimated that it takes maximum 0.5 h per TDM to draw the sample, send it to the lab, spin the blood down and to transport the sample to the analyzing laboratory. Assuming an hourly wage of € 40/h, one analysis would cost around € 55 per sample. The cost of one MIC determination using an E-test can be estimated at € 5 consumables and 0.5 h per sample, which would costs around € 25/MIC determination. The costs of this intervention should then be compared to the benefit which may be achieved. This can be evaluated on multiple levels. It can be evaluated on the hospital level, for example by comparing how much antibiotic has been given in the intervention group compared to the control group. There will probably not be a large difference between the 2 groups with respect to antibiotic consumption, as some patients will require higher doses, while others will need less. Moreover, since the introduction of the generics, antibiotics have become a lot cheaper. The purpose of health-economics is to improve the life expectancy, which is expressed in years of life saved (YOLs). The costs of the intervention can then be expressed as the cost to gain 1 YOLs. The conventionally adopted threshold of one YOLs is between € 20 000 to € 40 000 [343]. An intervention with costs less than € 20 000 / YOLs are considered to be very cost-efficient. Ofcourse, this requires very large studies which may be very difficult to conduct. Length of hospital stay, length of ICU stay or number of days free of organ support may be good alternative if such large studies are deemed not feasible.

The value of some other benefits may be more difficult to estimate, for example the potential prevention of the emergence of resistance. It is unclear which costs are associated with this global problem, and how much money can be saved if this process can be prevented. Without question, patients with a resistant infection are more likely to die, have longer ICU stays and need more costly procedures, such as isolation, however, the exact economic burden attributable to these infections is not well known [344].

It is clear that the pharmaco-economic analysis will be an interesting, yet difficult exercise which should look beyond the mere easy to calculate savings on the hospital level such as total dose of antibiotic given to the patient.

9. Conclusion

In this era of emerging resistance and high ICU-infection related mortality and morbidity rates, where very few antibiotics are in development, a rational use of antibiotics has been advocated. Optimized use of antibiotics to improve outcome and reduce antibiotic resistance is therefore the next challenge.

In the heterogeneous population of an intensive care setting, correct antibiotic dosing is problematic because of grossly altered and variable pharmacokinetics, which leads to underdosing in some and overdosing in others. The decreased susceptibility is an additional factor that makes dosing even more problematic. Considering this wide variability of antibiotic concentrations in critically ill patients, individually tailored antibiotic therapy may be a useful strategy to improve outcome, both on short and long term end points. However, before we can truly change to this, more research is needed, both in the analytical field as well as in the clinical field.

Addendum

1. **APACHE II score sheet**

Physiologic variable	+4	+3	+2	+1	0	+1	+2	+3	+4	Points
Temperature (rectal) °C	≥41°	39 to 40.9°		38.5 to 38.9	36 to 38.4°	34 to 35.9°	32 to 33.9°	30 to 31.9°	≤29.9°	
Mean arterial Pressure (mmHg)	≥160	130 to 159	110 to 129		70 to 109		50 to 69		≤49	
Heart Rate (ventricular response)	≥180	140 to 179	110 to 139		70 to 109		55 to 69	40 to 54	≤39	
Respiratory Rate (non-ventilated or ventilated)	≥ 50	35 to 49		25 to 34	12 to 24	10 to 11	6 to 9		≤5	
Oxygenation : A-aDO_2 or PaO_2 (mm Hg) a. FiO_2 ≥ 0.5 record A-aDO_2 b. FIO_2 < 0.5 record PaO_2	≥500	350 to 499	200 to 349		< 200 PO_2>70	PO_2 61 to 70		PO_2 55 to 60	PO_2<55	
Arterial pH (preferred)	≥7.7	7.6 to 7.69		7.5 to 7.59	7.33 to 7.49		7.25 to 7.32	7.15 to 7.24	<7.15	
Serum HCO_3 (venous mEq/L) (not preferred but may use if no ABGs)	≤52	41 to 51.9		32 to 40.9	22 to 31.9		18 to 21.9	15 to 17.9	< 15	
Serum sodium (mEq/L)	≥180	160 to 179	155-159	150-154	130-149		120 to 129	111 to 119	≤110	
Serum Potassium (mEq/L)	≥7	6 to 6.9		5.5 to 5.9	3.5 to 5.4	3 to 3.4	2.5 to 2.9		≤2.5	
Serum Creatinine (mg/dL) Double point score for acute renal failure	≥3.5	2 to 3.4	1.5 to 1.9		0.6 to 1.4		<0.6			
Hematocrit (%)	≥60		50 to 59.9	46 to 49.9	30 to 45.9		20 to 29.9		<20	
White Blood Count (total/mm³)	≥40		20 to 39.9	15 to 19.9	3 to 14.9		1 to 2.9		<1	
Glasgow Coma Score Score = 15 – minus actual GCS										

A. **Total Acute Physiology Score (sum of 12 above points)**
B. **Age Points (years) : ≤ 44 = 0, 45 to 54 = 2, 55 to 64 = 3, 65 to 74 = 5, ≥ 75 = 6**
C. **Chronic Health Points**

1) Cirrhosis of the liver confirmed by biopsy

2) New York Heart Association Class IV

3) Severe COPD - Hypercapnia, home O2 use, or pulmonary hypertension

4) On regular dialysis

5) Immunocompromised = mmunosuppression from chemotherapy, radiation therapy, long-term or recent high-dose steroids, immunodeficiency (eg, leukemia, lymphoma, AIDS)

None : 0 points
Non-Surgical : 5 points
Emergent operation : 5 points
Elective operation : 2 points

Total APACHE II score = A+B+C

2. **SOFA score sheet**

Organ system	1	2	3	4
Respiratory PaPO$_2$/Fi0$_2$ (mmHg)	<400	<300	<200	<100
Hematologic Platelets/nL	< 150	<100	<50	<20
Hepatic Bilirubin, mg/dL (μmol/L)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (> 204)
Cardiovascula Hypotension	MAP<70 mmHg	Dopamine ≤ 5 or dobutamine (any dose)	Dopamine > 5 or epinephrine ≤0.1 or norepinephrine ≤0.1	Dopamine > 15 epinephrine > 0.1 or norepinephrine > 0.1
Neurologic Glasgow Coma Score	13-14	10-12	6-9	<6
Renal Creatinine (mg/dL) Urine output	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440) < 500 mL/day	> 5.0 (>440) < 200 mL/day

Abbreviations

AKI : acute kidney injury

APACHE II : Acute Physiology and Chronic Health Evaluation II

ARC : augmented renal clearance

AUC : area under the curve

BMI : body mass index

BSV : between subject variability

CrCL : creatinine clearance

CL : clearance

C_{max} : maximum concentration

CPE : carbapenemase producing Enterobacteriaceae

CRRT : continuous renal replacement therapy

CV : coefficient of variation

ECMO : extracorporeal membrane oxygenation

EMA : European medicine agency

ESBL : extended spectrum beta lactamases

EUCAST : European committee on antimicrobial susceptibility testing

FDA : food and drug administration

$fT_{>MIC}$: percentage of the dosing interval for which the free antibiotic concentration exceeds the MIC

ICU: intensive care unit

IQR : interquartile range

LC : liquid chromatography

LLOQ : lower limit of quantification

MEM : meropenem

MIC : minimal inhibitory concentration

MRSA : methicillin resistant staphylococcus aureus

MS/MS: tandem mass spectrometry

MSSA : methicillin susceptile staphylococcus aureus

NONMEM : non linear mixed effect modeling

PCR : polymerase chain reaction

PD : pharmacodynamic

PK : pharmacokinetic

PTZ : piperacillin/tazobactam

Q : intercompartmental clearance

QCL : low concentration quality control

QCM : medium concentration quality control

QCH : high concentration quality control

RIFLE : Risk Injury Failure Loss End-Stage Kidney Disease

RUV : residual unexplained variability

SD : standard deviation

SIRS : systemic inflammatory response system

SLED : sustained low efficiency dialysis

SOFA : sequential organ failure assessment

TDM : therapeutic drug monitoring

ULOQ : upper limit of quantification

UV: ultraviolet

VRE : vancomycin resistant enterococcus

V_c : volume of the central compartment

V_d : volume of distribution

V_p : volume of the peripheral compartment

YOLs : years of life saved

Curriculum Vitae

Personal particulars

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Education

Secondary:	2000-2005 KA Assebroek (Assebroek, Bruges)
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	2011-present: Master of Science in Clinical Chemistry, Microbiology and Immunology, Ghent University
Doctorate	2011-2015: PhD program promoted by Prof. Jan De Waele and Prof. Veronique Stove; Ghent University, Faculty of Medicine, Department of Clinical Chemistry, Microbiology and Immunology & Department of Critical Care Medicine. Title: Patient Tailored Antibiotic Therapy in Critically Ill Patients

Attended Scientific Meetings

International Association of Therapeutic Drug Monitoring and Clinical Toxicology Conference 2011, Stuttgart, Germany (poster);

European Society on Intensive Care Medicine 2012, Lisbon, Portugal;

European Conference on Clinical Microbiology and Infectious Diseases 2013, Berlin, Germany (poster and oral presentation);

Interscience Conference on Antimicrobial Agents and Chemotherapy 2013, Denver (poster);

European Society on Intensive Care Medicine 2013, Paris, France;

European Conference on Clinical Microbiology and Infectious Diseases 2014, Barcelona (poster and oral presentation);

Postgraduate Technical Workshop Advanced Antimicrobial Pharmacokinetic and Pharmacodynamic Modelling and Simulation 2014, Liverpool, United Kingdom;

ESCMID Conference on Reviving Old Antibiotics 2014, Vienna, Austria;

Conference Update on Antibiotic Therapy in the ICU, Rome, 2014;

European Conference on Clinical Microbiology and Infectious Diseases 2015, Copenhagen (poster);

Interscience Conference on Antimicrobial Agents and Chemotherapy 2015, San Diego (poster)

Grants

Grant for a pre-doctoral mandate from the Research Foundation Flanders (October 2011-October 2015)

Grant for a research stay abroad from the Research Foundation Flanders (Brisbane, Australia, January 2013-April 2013)

Grant for an international conference from the Research Foundation Flanders (ICAAC, Denver, September 2013)

Attendance grant for an international conference from ESCMID (ESCMID Reviving Old Antibiotics, Vienna, October 2014)

Grant for an international conference from the Research Foundation Flanders (ICAAC, San Diego, September 2015)

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