Drug Monitoring und Toxikologie/ Drug Monitoring and Toxicology

Redaktion: W. Steimer

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Personalised beta-lactam therapy: basic principles and practical approach

Grundprinzipien und Praxis der personalisierten Therapie mit Beta-Lactamen

DOI 10.1515/labmed-2016-0050
Received July 4, 2016; accepted November 4, 2016

Abstract: Bacterial infections are potentially life-threatening diseases requiring effective antibiotic treatment right from the outset to achieve a favourable prognosis. Therapeutic success depends on the susceptibility of the bacterial pathogen, determined by the minimum inhibitory concentration (MIC), and the concentration of the antibiotic at the focus of infection, which is influenced by drug metabolism and pharmacokinetic (PK) factors. Beta-lactams are time-dependent antibiotics. Bacterial killing correlates with the duration of the drug concentration above the MIC of the pathogen. Critical illness is associated with major PK changes. This may lead to unexpected drug concentrations and unpredictable dose requirements differing significantly from standard dosages. Emerging dosing strategies are therefore based on PK/pharmacodynamic (PD) principles. Therapeutic drug monitoring (TDM) is increasingly playing a key role in antibiotic treatment optimisation in general and in beta-lactam therapy, in particular, notably in severely ill patients. Furthermore, evidence of the superiority of continuous beta-lactam infusions over shorter administration regimens is growing. Target drug concentrations have to be defined, considering MIC values especially in pathogens with limited susceptibility. For reliable TDM results, correct pre-analytical sample handling is indispensable. Personalised, TDM-guided therapy currently offers the most promising approach to assuring that beta-lactam treatment is effective, especially in critically ill patients.

Keywords: administration; adverse effects; anti-bacterial agent; antibiosis; antibiotic therapy; antibiotic; beta-lactam; clearance; critical care; critically ill; distribution; dosage; dose; dosing; drug monitoring; drug therapy; individualised; pharmacodynamics; pharmacokinetics; resistance; TDM; toxicity; volume.

Zusammenfassung: Bakterielle Infektionen sind potenziell lebensbedrohliche Erkrankungen. Deren Prognose hängt wesentlich von einer frühzeitigen und wirksamen Antibiotikatherapie ab. Die Wirksamkeit wird durch die Empfindlichkeit des bakteriellen Erregers gegenüber den eingesetzten Antiinfektiva, ausgedrückt als Minimale Hemmkonzentration (MHK), sowie die am Infektionsort erreichte Antibiotikakonzentration bestimmt, die hauptsächlich durch Metabolisierung und pharmakokinetische Faktoren beeinflusst wird. Die Wirkung der Beta-Lactame ist zeitabhängig. Sie korreliert mit der Dauer der MHK-Überschreitung des Erregers. Kritische Erkrankungen gehen mit ausgeprägten pharmakokinetischen Veränderungen einher. Diese können zu unerwartet niedrigen/hohen Medikamentenkonzentrationen führen sowie zu nicht vorhersagbarem Dosisbedarf, der von der Standarddosierung deutlich abweichen kann. Moderne Dosisoptimierungsstrategien orientieren sich daher zunehmend an pharmakokinetisch/pharmakodynamischen (PK/PD) Modellen. Zunehmend spielt das TDM eine Schlüsselrolle in der Optimierung der antiinfektiven Behandlung im Allgemeinen sowie im Besonderen der Beta-Lactam-Therapie vornehmlich schwerstkranker Patienten. Darüber hinaus verdichtet sich die Datenlage hinsichtlich einer Überlegenheit der kontinuierlichen

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Infusion von Beta-Lactamen verglichen mit kürzeren Verabreichungsformen. Vor allem für Erreger mit eingeschränkter Empfindlichkeit müssen therapeutische Konzentrationsziele definiert werden. Für verlässliche TDM-Resultate ist eine korrekte Präanalytik entscheidend. Die personalisierte, TDM-geführte Therapie stellt gegenwärtig den erfolgversprechendsten Ansatz für eine wirksame Beta-Lactam-Behandlung insbesondere der kritisch kranken Patienten dar.

Schlüsselwörter: Antibiose; Antibiotikum; antibiotische Therapie; Ausscheidung, Elemination, Beta-Lactam; Darreichung; Dosierung; Dosis; Elimination; individualisiert; Intensivmedizin; kritisch krank; medikamentöse Therapie; Nebenwirkung; Pharmakodynamik; Pharmakokinetik; Resistenz; Spiegelbestimmung; TDM; Toxizität; Verteilung; Volumen.

Introduction and clinical issue

Beta-lactams represent the most commonly used antibiotics worldwide today [1, 2]. A beta-lactam ring is an element of the chemical core structure of carbapenems, cephalosporins, monobactams, and penicillins. The ring is involved in the inhibition of bacterial cell wall synthesis by interfering with peptidoglycan synthesis. Fundamentally, beta-lactams act bactericidally on Gram-positives, Gram-negatives and anaerobics. The beta-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam also contain the beta-lactam ring, however are devoid of anti-bacterial activity (except sulbactam). They protect the beta-lactams from being inactivated when administered as a combination preparation. There are at least two major causes of resistance to beta-lactams: bacterial beta-lactamases inactivating the beta-lactam ring, as well as altered penicillin binding proteins to which beta-lactams can no longer effectively bind.

Most notably in the setting of intensive care, antimicrobial therapy is facing the problem of antibiotic resistance in emerging strains as well as a lack in the development of new agents [3]. There is a need for new drugs, particularly in the treatment of severe Gram-negative infections. The conditions of sepsis and septic shock are associated with high mortality rates. The incidence of severe sepsis, quantified in a prevalence study in Germany, was demonstrated as being 76–110 newly diagnosed cases per 100,000 inhabitants [4].

The successful treatment of septic shock demands immediate and adequate antimicrobial therapy [5, 6]. This must be initiated as soon as possible and include a fitting antimicrobial agent, chosen either empirically or in accordance with the resistance of the identified pathogen. In an observational multicentre trial on the impact of infection management guidelines, source control and timely antibiotic therapy revealed a potential improvement in survival if source control occurs within 6 h of the onset of sepsis [7].

Fuelled by the lack of novel drug development, a growing fundamental awareness of inadequate dosing of the antibiotics currently available to certain patient groups is leading to greater interest in alternative dosing strategies. The pharmacokinetics (PK) of antimicrobials in critically ill patients is highly variable in comparison with other hospitalised patients [8]. A review of the literature on beta-lactam PK in ICU patients with infection revealed marked PK heterogeneity in the volume of distribution and drug clearance by a factor of more than two [9, 10]. The unpredictable PK led the authors to conclude that optimised approaches considering a minimum inhibitory concentration (MIC)-dependent drug concentration target, drug monitoring, and PK/pharmacodynamics (PD) of the individual patient promise potential improvements in outcome for such patients [8, 11].

The term “antimicrobial pharmacodynamics” (PD) describes the effect of an antimicrobial agent on microorganisms, relative to the agent’s concentration. The PD of beta-lactam antibiotics is time-dependent. The drug effect correlates best with the length of time that concentrations of the antimicrobial agents exceed the MIC of the microorganism. Other antibiotics, such as fluoroquinolones and aminoglycosides, have concentration-dependent PD. Their antimicrobial effect correlates best with the peak concentration/MIC ratio and/or the area under the concentration-time curve/MIC ratio [12].

PK aspects describe the time course of antimicrobial drug concentrations in the body. Following intravenous administration of a drug bolus, a peak-free drug concentration will result as a function of the drug quantity administered, the distribution volume, and plasma protein binding. Peak concentration will decrease depending on drug elimination (renal, non-renal), as well as drug distribution volume. In order to optimise antimicrobial efficacy, several dosing regimens have been investigated that utilise the PD attributes of said antimicrobial agents. With respect to time-dependent agents such as beta-lactams, extended administration (e.g. 3–4 h) and continuous infusion were compared with traditional intermittent infusion (over 30 min) [11].

Implementing PK and PD principles in clinical practice may lead to the greatest possible bacterial killing. The toxic effects of antimicrobial agents, such as seizures or central nervous system pathology in beta-lactam therapy,

as well as the development of bacterial resistance, may be reduced by avoiding sub-therapeutic dosing regimens. Nonetheless, the overall positive effects promise a significant improvement in patient outcome.

Clinical case

The following case acts as an illustration of a typical complicated clinical situation that may be solved with consistent diagnostics and the consequential adjustment to therapy.

A 38-year-old woman (body mass 75 kg, height 165 cm) with pneumococcal meningitis (1813 cells/µL) caused by mastoiditis was transferred for decompressive hemicraniectomy to relieve increasing intracranial pressure despite conservative treatment. Procalcitonin (PCT) on admission was 4.9 µg/L (normal range ≤ 0.1 µg/L). Antimicrobial treatment comprised intravenous 2 g of ceftriaxone b.i.d together with 8 mg of dexamethasone q.i.d for 96 h. Screening for beta-lactam resistance of the *Streptococcus pneumoniae* isolate by an agar diffusion method with 1 µg oxacillin disk revealed a zone diameter of 24 mm indicating full susceptibility. Decompressive hemicraniectomy was performed on day 4 after the diagnosis of pneumococcal meningitis. A heparin perfusor was started on day 7 to treat cerebral septic venous sinus thrombosis. Also on day 7, her body temperature increased up to 39.2 °C, despite antipyretic measures. PCT had now dropped to 0.2 µg/L. Blood cultures were taken, which were found to be clear of bacterial pathogens. Investigation of the liquor yielded 59 cells/µL and a lactate of 7.3 mmol/L, indicating insufficient antibiotic treatment. Microbiological culture of liquor did not reveal any bacterial growth. Antimicrobial treatment was switched to 2 g of meropenem t.i.d and 1 g of vancomycin b.i.d. Over the course of the following days, vancomycin blood levels always remained below 5 mg/L (target value between 15 and 20 mg/L) despite increasing the dose to 2 g b.i.d. Her body temperature remained elevated at 38.5 °C. Nosocomial pneumonia was ruled out. Her leucocyte count rose to 20.8×10^3/µL, PCT was now 0.1 µg/L. It was noticed on day 13 that vancomycin and heparin were being administered over a y-site. If heparin and vancomycin are administered through the same line, a concentration-dependent acid-base reaction may lead to precipitation and inactivation of vancomycin [13]. However, administration of heparin and vancomycin through separate lines had no effect on the vancomycin levels. Initial measurement of her meropenem level on day 15 revealed a serum concentration of only 0.3 mg/L (target for continuous infusion 32–80 mg/L, Table 1). It was found that her fluid turnover had increased from day 9 with a urine output of up to 6.9 L on day 11. Vancomycin was stopped. Meropenem continuous infusion (8 g over 24 h) was started simultaneously with another 2 g meropenem loading dose. About 18 h later, her meropenem level had increased to 14.7 mg/L and her leucocyte count and body temperature began to normalise. This short case highlights the importance of detailed therapeutic drug monitoring (TDM) for time-dependent antimicrobial agents, as the drug levels in critically ill patients may be influenced by a number of factors simultaneously (fluid turnover, volume of distribution, interaction of different drugs, etc.).

Table 1: Defined target values for piperacillin, meropenem, and cefotaxime.

Substance	MIC, mg/L	Target tissue, MHK × 4	MIN_{plasma}, MIC tissue × 4	MAX_{plasma}, MIC tissue × 10
Piperacillin	16	64	96	240
Meropenem	4	16	16	40
Cefotaxime	8	32	32	80

If EUCAST provides no breakpoint [14], CLSI values may be used in place [15]. A maximum killing effect is achieved with a four-fold MIC, the four-fold MIC being defined as MIN-Target in infected tissue. The maximum target concentration is defined as 10-fold MIC. For piperacillin, the target plasma concentration is additionally multiplied by a factor of 1.5, as tissue (lung) concentration was found to be around 36%–57% of the plasma concentration [9].

Microbiological issues

Prior to initiating any empirical treatment, it is crucial to obtain both sufficient and adequate material to perform microbiological investigations as the correct identification of the pathogen and susceptibility testing is essential to the success of any targeted therapy. Converting from initial empirical broad-spectrum treatment to a more targeted agent or regimen with a narrower bacterial spectrum lowers antibiotic pressure on uninvolved colonising bacteria. This can reduce the risk of subsequent secondary disease.

Collected material should be sent to the microbiology laboratory immediately for further processing and analysis. Storage and refrigeration of specimens (e.g. respiratory secretions or urine) need to be minimised to avoid false-negative or false-positive results. Blood cultures must be taken in accordance with quality standards for diagnostic procedures in microbiology and infectious diseases [16, 17] and kept at room temperature until loaded into an

automated blood culture incubator. The drawn volume of 60 mL of blood has to be divided among three pairs of blood culture flasks at once (a pair comprising an aerobic and an anaerobic bottle) [18]. The procedure of preparing sequential blood cultures with 30-min intervals for up to several hours is time-consuming and is often neglected. However, if a catheter-associated infection is suspected, one additional pair of blood cultures is taken from the catheter and three pairs from a peripheral site. The difference in duration needed to obtain positive results from the catheter and peripheral blood cultures (time to positivity) will serve as an aid in deciding whether a catheter-associated infection is probable or not.

After successfully identifying the bacterial pathogen, susceptibility testing is performed [14]. It determines the MIC, which is defined as the lowest concentration of an antibiotic preventing visible growth during a defined time period in a broth dilution system. The MIC is based on a doubling-dilution system (e.g. 0.5, 1, 2, 4 mg/L) [19]. The categorisation of results into susceptible (S), intermediate susceptible (I), and resistant (R) is done according to the breakpoint tables for the interpretation of MICs, provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [14]. A pathogen with an MIC below a defined concentration (breakpoint) is considered "sensitive" to the drug in question, which is in turn associated with a high likelihood of therapeutic success. On the contrary, if the determined MIC is above a defined concentration, then the bacterial pathogen is "resistant" to the drug in question, which is associated with a high likelihood of therapeutic failure.

Maximum bacterial killing for Enterobacteriaceae, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, is attained at around 4 times the MIC [20–22] and could be the PK target. Additional killing effects are negligible at concentrations above this level.

However, this statement refers to concentrations strictly in blood stream infections. If another compartment, unlike the blood circulation, contains the focus of infection, tissue penetration of the antibiotic agent needs to be taken into consideration. Valid information concerning this issue is limited but it is reasonable to achieve plasma concentrations which are higher than 4 times the MIC.

Pharmacokinetics and drug concentration target

Successful medical treatment concepts maximise/optimise the intended therapeutic effect and at the same time minimise the likelihood of adverse reactions. Drug therapy, including antibacterial treatment, is most effective when optimum-free drug target concentrations are attained at the site of action. Bioavailability, distribution, elimination, and metabolism of the drug as well as the susceptibility of the target may strongly influence dose-concentration and dose-effect relationships, respectively.

Drug therapy and pharmacokinetics in clinically stable patients

Drugs with close dose-concentration relationship in stable patients

Many drugs used in everyday patient care (not critically ill patients) have a wide therapeutic range. They are commonly given in clinically stable patients and exhibit only a limited inter-individual variability in drug metabolism, PKs, and PDs. Body mass, body surface area, gender, age, and renal function have to be considered only roughly. In certain clinical situations, e.g. in stable renal failure, correction formulae and appropriate nomograms may be of assistance in dose adaptation. The therapeutic effect of such drugs is mostly clinically evident. Furthermore, therapy can easily be optimised where necessary through dose adjustment, in accordance with patient characteristics and the observable clinical symptoms. Treatment is commonly effective and safe.

Some of these drugs, including beta-lactams and all antibiotics, have only silent PD indices and no immediate, clinically apparent effect. During the initial phase of an infection, the attending physician is unable to recognise whether or not treatment is effective, at least over a limited period of time: antibiotic treatment may take days until fever response or a decline in inflammatory markers such as C-reactive protein, procalcitonin, or IL-6 can be ascertained. In addition, administered antipyretics may influence the natural fever course; thus, compromising the diagnostic value of fever response as an indicator of therapy response. Based on results from dose-finding studies (translated into manufacturers' recommendations) and individual medical expertise, however, an attending physician can expect therapeutic success *a priori*.

In rare cases of treatment failure, the stable clinical status of the patient allows the attending physician to switch antibiotic medication (in accordance with microbiological test results, if available) without taking

inadequate risks. Therapy optimisation in this patient group is commonly not time-sensitive: in anything other than exceptional cases (Table 2), TDM in these patients would be of only limited value and simply cause unnecessary costs.

Drugs with poor dose-concentration relationship in stable patients

There is a second, smaller group of drugs, for which TDM is recommended irrespective of patient stability. A summary of common TDM indications is presented in Table 2.

As far as antibiotics are concerned, this second smaller class of drugs mainly includes vancomycin and aminoglycosides. Such drugs demonstrate a poor dose-effect correlation, which is in most cases the result of an insufficient relationship between the dose and plasma concentrations or area under the curve (AUC). They may also possess potential, relevant side effects, and a low therapeutic index, in which the concentrations with therapeutic effect and unwanted toxicity lie close to each other. The factors of poor dose-effect correlation and/or low therapeutic index in combination with the poor clinical visibility of efficacy and adverse side effects point to a high degree of therapy-related risk and are, hence, a strong indication for the implementation of TDM measures. This is also the case in patients with stable disease [23–33].

Pharmacokinetics in critically ill patients

Approved dosing regimens

Dose-finding studies with the objective of obtaining drug approval are frequently performed with healthy individuals or with a limited spectrum of non-critically ill patients.

Table 2: Common indications for therapeutic drug monitoring.

Insufficient dose-effect correlation of the drug
Low therapeutic index
Therapeutic effect clinically not (promptly) provable
Lacking therapeutic effect
Suspected overdose (adverse side effects)
Insufficient patient compliance
Drug administration error
Disorders with altered drug absorption, distribution, excretion, and metabolism

Resulting “standard” dosing regimens are not validated with respect to critically ill patients and limit their applicability in this setting [34–38].

Unpredictable pharmacokinetic/pharmacodynamic changes

Critically ill patients with severe systemic infections develop pronounced pathophysiological and pathobiochemical changes, which may deeply influence PK variables (volume of distribution, additional compartments, and excretion) and metabolic processes [38–44]. They may suffer from organ dysfunctions already in existence prior to the acute infection. Furthermore, medical interventions (infusions, blood transfusions, renal replacement therapy, and cardiovascular drugs) may considerably alter the PK system [45–49]. Consequently, the clinical/PK situation of critically ill patients differs strongly from the conditions presented by non-critically ill patients, in whom dose-concentration and dose-effect relationships may be severely compromised or even annulled. Moreover, clinical, PK, and metabolic conditions are unstable and change over time. In addition, it is quite likely that patients are administered a combination of medications rather than a single drug. Thus, drug-drug interactions also have to be considered.

Accordingly, standard drug doses in such patients lead to unpredictable concentrations both in plasma and at the site of infection. Simple adjustment factors obtained from correction formulae or nomograms prove unreliable and insufficient as a result of this complexity. Standard dose-related drug therapy in critically ill patients frequently ends up being either ineffective or toxic [34, 37, 49–52].

Reasonable use of TDM

Fundamentally, treatment management in critically ill patients may follow visible clinical signs [48]. However, treatment optimisation in critical patients is particularly time-sensitive and does not permit any clinical assessment of efficacy or toxicity that can take days. The diagnostic signs of response to therapy, or the lack thereof, frequently do not occur during the critical phase of therapeutic decision-making. Clinical endpoints for timely dose correction may well arise too late and thus endanger patient safety.

This situation is virtually paradigmatic for the antimicrobial treatment of patients with severe systemic infections. Alternative early diagnostic/prognostic indicators

are therefore required. This group of patients is often confronted with not only one but a number of the indications summarised in Table 2. TDM is, therefore, a reasonable measure in the surveillance of antibiotic drug concentrations and a prerequisite for rational dose adjustment [35, 48–50, 53].

As a result of the frequently observed increased volumes of distribution and augmented drug clearances in critically ill patients, dosage adjustment to counter insufficient plasma levels often requires a dosage exceeding approved standards [35, 37, 50, 54]. However, this dosage increase, although prescribed for the benefit of the patient, may be interpreted as off-label use and may place the attending physician in a potentially legally questionable or difficult position.

Drug administration mode and pharmacokinetic impact

Pursuant to the recommendations of the manufacturer, beta-lactams are commonly administered as intermittent bolus injections. Time-course studies reveal wide fluctuations in plasma concentrations, especially in drugs with short half-lives. Pre-dose drug concentrations lie typically below efficacious target concentrations, while observed peak concentrations are often or mostly higher than needed (also under steady-state conditions). It should be recalled that concentrations of antimicrobials greater than 4 times MIC do not have any further therapeutic advantage with respect to time-dependent bacterial killing.

Reducing the infusion rate and extending infusion time (while maintaining the dosage) lowers peak and raises pre-dose minimum levels. It may thus prolong the time above target concentration and improve clinical patient outcome. The longer the infusion time during a dosing interval, the higher the minimum plasma concentration is (decisive for efficacy). The maximum trough concentration attainable with a given dose is achieved when the antibiotic agent is administered continuously over the entire dosing interval. Hence, continuous infusion might be the administration mode of choice for PK reasons. De Waele et al. [38] performed a convincing PK analysis using the data obtained during the DALI multi-centre study including 343 critically ill patients from 68 ICUs [50]. In the hypothetical situation of empirical dosing, they demonstrated the use of intermittent bolus administration (compared to extended and continuous infusion) to be the main determinant of target non-attainment.

Despite convincing theoretical evidence, clinical trials have been unable to prove the superiority of prolonged infusions over bolus administration with respect to clinical outcome [55–59].

One might ask oneself what an explanation for the outstanding clinical proof of concept could be. Some of the comparative studies revealed methodological shortcomings in their study design, such as low patient numbers and insufficient statistical power. Striking points were the lack of TDM application, inconsistent therapeutic endpoints, and selection of enrolled patients, the use of inconsistent doses or of standard drug dosages, regardless of the higher dose requirements of critically ill patients [60].

Consequently, apart from the necessity for a greater number of patients to be included, future study designs comparing different drug administration modes need to be complemented by TDM-guided dosage-adjustment procedures [61, 62].

The use of continuous infusions has an additional valuable advantage. Optimum sampling times for TDM in patients with intermittent bolus administrations or different extended infusion times cannot be defined consistently and need standardisation, which is currently lacking. In patients treated with continuous infusions, TDM samples may be drawn at any time, at least under steady-state conditions. Underdosing is directly recognisable and dosage adjustment is uncomplicated.

Dosage adjustment

Once the appropriate antibiotic has been selected, a therapeutic target concentration or individual therapeutic range has to be defined, dependent on the expected or identified pathogen, its antibiotic susceptibility, and the type and site of infection (see above). Beta-lactams are categorised as “time-dependent” antibiotics. As such, beta-lactams need to be present in concentrations of at least the MIC or higher. “Hit early and hard” is still mandatory in the antibiotic treatment of the critically ill patients. Appropriate and early dosing is decisive. High loading doses shorten the time to reach effective antibiotic plasma concentrations [36, 37, 63].

Intermittent bolus injection of beta-lactams in severely ill patients with normal kidney function has been shown to produce often insufficient plasma concentrations for more than 50% of the dosing interval [38, 41, 64].

This is considered critical with respect to bacterial regrowth, therapy failure, and the development of antibiotic resistance [37, 50, 65]. To increase the dosage of the single bolus to an extent that sufficient plasma concentrations (≥ MIC) are achieved throughout the dosing

interval would lead to extreme peak concentrations and AUC values. Although adverse effects, such as allergic reactions, gastrointestinal disturbances, colitis, or elevated liver enzymes are dose-independent (or any dose-dependency is at least questionable), nephrotoxic or neurotoxic adverse reactions may be dose- or concentration-related, respectively [52, 66–73]. Very high peak values potentially trigger complications relating to toxicity.

For the reasons indicated above, intensive care physicians increasingly prefer prolonged dosing schemes implementing extended or continuous infusion [39, 40, 54, 60, 74]. Most importantly, continuous drug administration lowers potentially toxic peak concentrations and AUCs, raises ineffective sub-therapeutic concentrations, and allows constant plasma levels to remain permanently above MIC. Furthermore, sampling for TDM, PK assessment, and dosage adjustment can be carried out more easily and accurately. Whereas antibacterial agents such as imipenem are chemically not stable enough, substances like piperacillin may be administered as 24-h infusions.

With respect to the question as to whether there are simple rules for dosage adjustment, most beta-lactams are eliminated by the kidneys primarily following non-saturable first-order elimination kinetics. In such drugs, steady-state dose increases lead to a proportional rise in plasma concentrations, that is, a double dose doubles the plasma concentration. The results of dose escalation may again be verified by TDM. (Only few beta-lactams demonstrate a relevant percentage of saturable, non-renal zero-order clearance. In such drugs, in a plasma concentration range above the Michaelis constant, smaller dosage increases may lead to steep, non-proportional plasma concentration rises.)

Owing to the changing PKs in severely ill patients, repeated concentration monitoring is also recommended after target concentrations have been achieved. Improvement in the patient's clinical status often influences the PK parameters and may require further dose adjustment. [75]. In conclusion, drug monitoring at regular intervals has to be complemented by concentration control when particular clinical changes occur (high volume/infusion demand, surgical intervention, therapy discontinued for diagnostic reasons).

Analytics and pre-analytics

Analyte stability

Optimum antibiotic therapy requires elaborate TDM. Great emphasis must be placed on pre-analytical and analytical procedures with respect to accuracy, precision, and turnaround times.

The labile beta-lactam ring in penicillins and other beta-lactam antibiotics is characterised by its marked susceptibility to various nucleophiles, acid-base reagents, metal ions, oxidising agents, or even solvents such as water and alcohol, as well as dihydropeptidase-1 (DHP1) [76]. The stability, or lack thereof, in the beta-lactams is fundamentally linked to their antimicrobial activity and bacterial resistance, which has been demonstrated in a number of structure-activity relationship studies [76–78]. Therefore, knowledge of the respective in vivo and in vitro stability of the beta-lactams is essential to the success of TDM. The sampling, transport, processing, and analysis of these samples have to be performed fast enough to avoid degradation (cooling may be necessary). The instability of the beta-lactams in whole blood as well as in plasma, extracted supernatant, and in cooled or frozen samples has been examined by different groups [79–84]. A sensitive beta-lactam is meropenem; it was observed to be stable for between 3 and 6 h in acidified solution at room temperature [79, 80]. Stability was tested in the recovery of ±10% of the target value. The samples need to be frozen at − 80 °C if long-term storage is necessary: storage at − 20 °C induces substantial degradation after ≥7 days for piperacillin, cefepim, and meropenem (90 days, 23% recovery). However, no substantial changes were observed for any of the above-mentioned analytes when stored at − 80 °C for up to 180 days [83]. There are only a few publications on the quantification and stability of imipenem in human plasma, in which morpholinosulfonic acid and renal DHP-1 inhibitor were used [85, 86].

The use of dried blood spot (DBS) assays has been discussed as a tool to overcome stability problems. Some DBS assays for antibiotic drugs have already been published; ertapenem, linezelid, and ceftriaxon in DBS have been demonstrated as remaining stable for months in contrast to plasma or serum stabilities [86–89].

Sampling time

Empirical and/or targeted therapy has to be started instantly using continuous infusion and an additional loading dose. Commonly, when no loading dose is administered, blood sampling is recommended to take place four to five half-lives after attaining steady-state conditions [20–22]. Depending on the patient's clinical status, TDM may prove reasonable within the first 24 h, to ensure early efficacy, and subsequently 2–3 times weekly, until the patient is clinically stable or therapy is discontinued.

Owing to the changing PK conditions in severely ill patients, repeated TDM is also recommended when target concentrations have been achieved, after dose change, as well as after every clinical event or intervention that potentially influences the PK conditions of the patient.

In patients with continuous infusion, blood may be collected at any point in time during the dosing interval, as drug levels remain largely constant. Wong et al. [90] demonstrated in an international survey that most centres using beta-lactam intermittent bolus administration sampled trough concentrations. In patients treated with so-called extended infusions of varying duration, it is not possible to define a single optimum sampling time. Standardisation is required and to date lacking.

Measurement method, feasibility, quality assessment

Microbiological in vitro assays are used to determine the biological activity, e.g. PD equivalence of generic intravenous antibiotics [91]. Currently, there are no immunoassays available for TDM of beta-lactams. Due to the flexibility, sensitivity, and specificity of chromatography with UV or mass detection, these techniques represent the methods predominantly in use to determine beta-lactam concentrations, even though they require well-trained operators. Currently, there are still limitations in automation, a dearth of commercially available kits, calibrators, and quality controls. Furthermore, there is still a lack of quality assessment ring trials (proficiency testing) for beta-lactams. To date, INSTAND, as well as UK NEQAS (a German and a British provider of proficiency testing), covers only the following antimicrobial analytes: amikacin, flucytosine, gentamicin, teicoplanin, tobramycin, vancomycin, voriconazole, posaconazole, itraconazole, and hydroxy-itraconazole. The German provider RFB currently covers even fewer antimicrobial agents in its programme, respectively.

Most of the published methods are not feasible for use in daily routine determination, owing to the simultaneous determination of a large number of beta-lactams. Some assays have large turnaround times, which may exceed the described 6-h stability of meropenem, particularly if a larger number of samples have to be determined [82]. The methods and stabilities of published beta-lactam assays are illustrated in Table 3. Stability decreases on storage at room temperature or after extraction in acidified solutions. In some cases, stability increased through the use of a stabilisation reagent [81, 82].

Future chromatography-based TDM methods may also run in 2 min or less, such as current ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) assays, e.g. for immunosuppressant drugs [93]. In most cases, time-consuming multiple-drug assays are not needed. Multiple analyte methods may prove more useful when screening in food and water [94, 95].

Nevertheless, clinicians require same-day results of TDM. Therefore, the incorporation of UPLC-MS/MS or HPLC-UV (ultraviolet) systems into a laboratory-based

Table 3: Consumption data of the beta-lactams at the University Medical Center in Göttingen, methods and stabilities of published beta-lactam assays.

AB-group	Beta-lactam	Percent of total AB Consumption, %	Stability at 4 °C	Analytical methods	Reference
Natural penicillin	Benzylpenicillin (Penicillin G)	0.9	> 12 h, 36 h, 48 h	HPLC-UV LC-MS/MS	[82] [79, 80]
Penillinase-resist. penicillin	Flucloxacillin	2.9	> 24 h	LC-MS/MS	[92]
Aminopenicillin	Ampicillin	0.6			
Aminopenicillin + β-LI	Ampicillin/sulbactam	6.7			
Ureidopenicillin	Piperacillin	0.1	> 12 h	HPLC-UV	[82]
Ureidopenicillin + β-LI	Piperacillin/tazobactam	10.5			
Ceph. G. 1	Cefazolin	2.3	> 144 h	HPLC-UV	[79]
Ceph. G. 2	Cefuroxime	5.3			
Ceph. G. 3a	Cefotaxime	1.1	> 12 h, > 24 h	HPLC-UV	[82, 84]
Ceph. G. 3a	Ceftriaxone	5.3	> 12 h, > 160 h	HPLC-UV	[82, 84]
Ceph. G. 3b	Ceftazidime	1.1	> 12 h	HPLC-UV	[82]
Ceph. G. 4	Cefepime	0.04	> 12 h	HPLC-UV	[82]
Carbapenem	Meropenem	10.1	> 12 h, > 24 h > 36 h, > 8 h	HPLC-UV LC-MS/MS	[82, 84] [79, 80]
Carbapenem	Imipenem	0.6	> 12 h	HPLC-UV	[82]

automated system will be the next step in the successful integration of TDM and other drugs into routine clinical practice.

Several chromatographic assays with UV [81, 96–99] or mass spectrometric detection [81, 85, 87, 88, 92] are currently in use. The methods employed are all in-house methods validated in accordance with several different guidelines, e.g. Valistat, CLSI C62-A, EMA guidelines or other in-house validation guidelines [100–102].

Total and free analyte concentration

Most of the published methods use plasma in TDM. Protein precipitations, as well as liquid-liquid extraction procedures, have been described for sample preparation. In most procedures, both bound and free concentrations of beta-lactams can be determined. The determination of free concentrations is useful in drugs with a high degree of protein binding, particularly in individual cases in which protein concentrations strongly deviate from the norm [96, 103–105]. The measurement of unbound concentrations is just a question of assay sensitivity, as to whether the unbound fraction can be isolated using ultrafiltration centrifugal filters. It has to be mentioned that binding capacity changes rapidly; therefore, the ultra-centrifugation steps have to be performed very soon after the sample has been drawn [106]. Microdialysis is a further catheter-based sampling method to monitor in vivo free-tissue drug concentrations [92, 107, 108]. Protein binding and tissue penetration of the beta-lactams play a rather subordinate role in comparison with the other factors of influence. The determination of plasma concentrations in these antibiotics seems to be the best practical method for monitoring treatment efficacy.

Practical therapeutic approach in critically ill patients

A simplified scheme to schedule diagnostics and anti-infective treatment in critically ill patients could include the following:

Having obtained sufficient material for microbiological investigation, empirical therapy must be initiated instantly using continuous infusion and an additional loading dose, to ensure/enable sufficient drug concentrations from the outset (Figure 1). Empirical antibiotic treatment is based on the most probable bacterial pathogen associated with the suspected infection as well as any local resistance, which finally determines the drug target

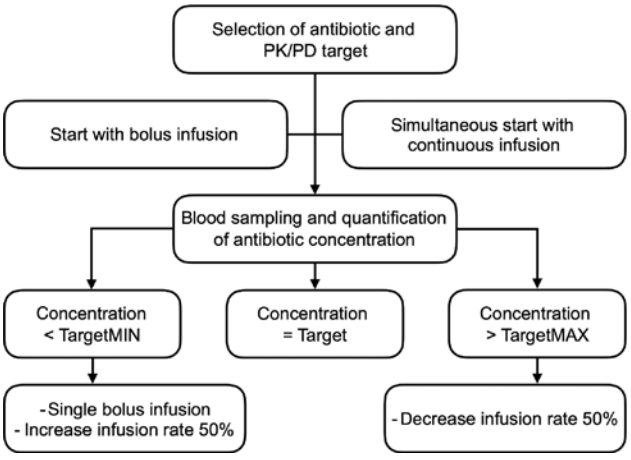

Figure 1: Flow chart describing the administration of beta-lactam antibiotics with continuous administration, TDM monitoring, and dose correction.
PK/PD, pharmacokinetic/pharmacodynamics; TargetMIN, lowest appropriate concentration in blood; Target, range of appropriate concentration in blood; TargetMAX, highest appropriate concentration in blood.

concentrations in plasma. The suggested dose adjustment (Figure 1) is one possibility, actually practiced in our ICUs.

The described simple dose adjustment algorithm is easy to implement. There are actually no data available supporting a specific dose adjustment regime. This topic should be addressed in further trials. However, there is potential for refinement of dose adjustment algorithm. Wong et al. (88) reported about several different dosing adjustment regimes reported by five different ICUs. They concluded a change by 25%–50%. Scaglione et al. described an algorithm for an intermittent dosing regime and suggest an increase of 25% (3/day–4/day dosing).

Targeted therapy must follow as soon as possible, assuming the pathogen is identified correctly and in accordance with the determined resistance pattern. The target drug concentration can then be derived from the MIC of said identified species (4 times MIC).

Depending on the patient's clinical status, TDM needs to have been initiated already within the first 24 h, and subsequently 2–3 times weekly, until the patient is clinically stable or therapy is discontinued. Owing to the changing PK conditions in severely ill patients, repeated TDM is also recommended when target concentrations have been achieved, as well as when marked changes in clinical status occur.

In beta-lactams following non-saturable first-order elimination kinetics (which applies to most beta-lactams), steady-state dose increases give rise to a proportional increase in drug plasma concentration. The results of dose adjustment will, of course, require verification by TDM.

Conclusion and future prospects

Beta-lactams are widely used, highly effective antibiotic agents and of particular importance in the intensive care setting. Especially in severely ill patients, however, the PK/PD characteristics differ significantly from the norm, are unpredictable, and need an individualised dosing approach based on TDM to avoid therapeutic failure. There is a growing understanding of this challenge at least in maximum care hospitals, and beta-lactam measurement is increasingly available.

A number of relevant questions still remain to be answered and currently lacking standards must be set. Considering the effort necessary in labour-intensive beta-lactam monitoring and PK individualisation, it has to be clarified as to which group of patients will profit from such measures. We must also address the questions as to which mode of drug administration (bolus; extended or continuous infusion) is the most effective and what the optimum standardised TDM sampling times for substitute drug administration modes could be.

Furthermore, recent technical developments already underway can reduce the manual effort, the demands placed on staff knowledge and training, as well as turnaround times in TDM. Chromatography-based TDM methods can now run in under 3 min. Pre-analytical sample preparation is increasingly automated without the involvement of specialised technicians. In addition, LC/MS systems are on the verge of their integration into laboratory automation systems. This will allow TDM to be performed around the clock.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. FS and TP contributed equally to this work.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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