Clinical pharmacology of antimicrobials: towards optimized treatment of infectious diseases in hospitalized patients

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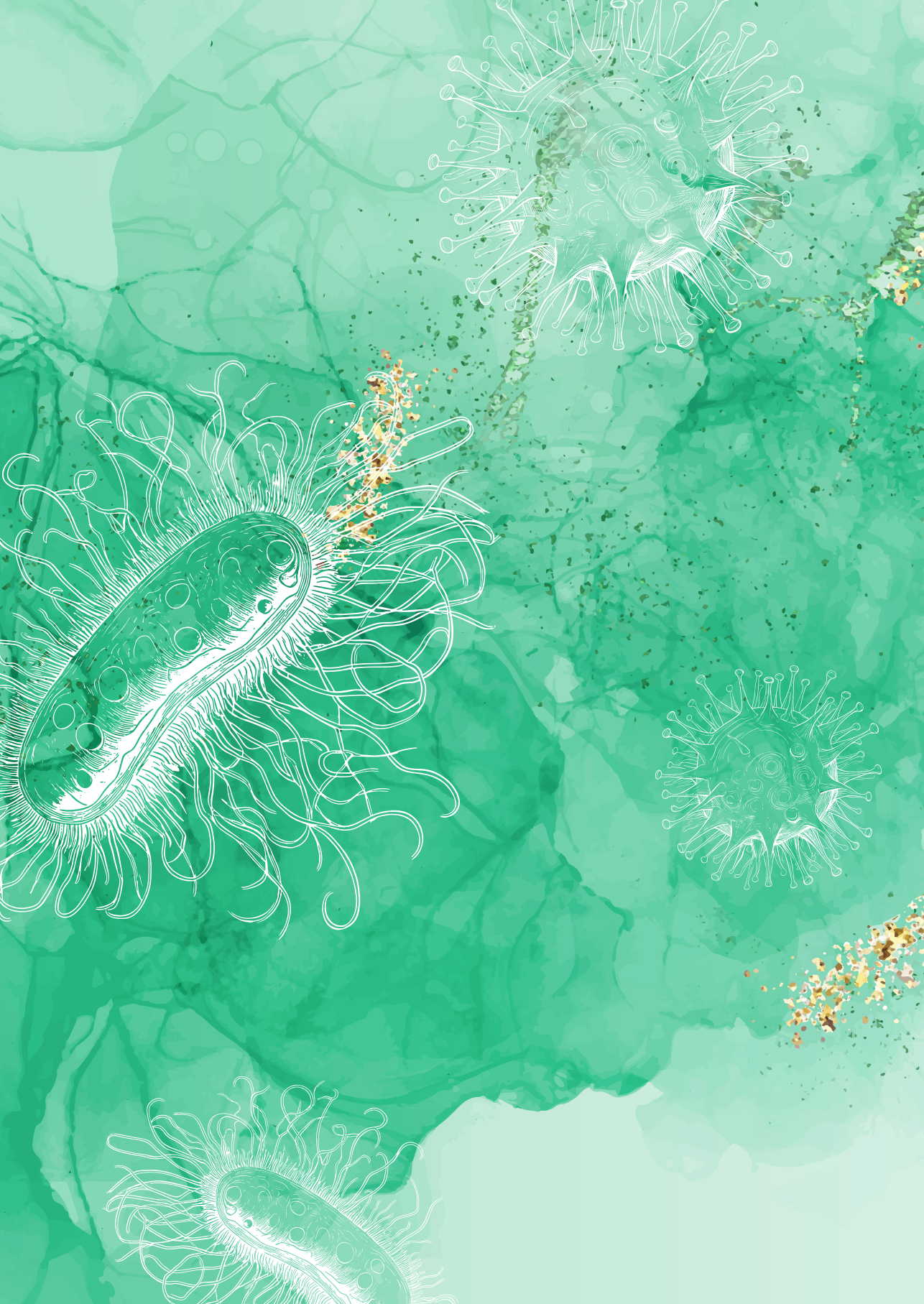

General discussion and future perspectives

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This thesis focuses on optimizing antimicrobial treatment for specific hospitalized patient populations. Population pharmacokinetic modeling was utilized to improve antimicrobial dosing regimens. Pharmacodynamic studies were conducted to determine the impact of antimicrobials on pathogens and the human body. In this chapter, the most important conclusions of this thesis are discussed, and perspectives for future studies are presented. Each part of this discussion begins with a summarizing statement.

It is essential to measure the right antimicrobial concentrations.

The initial step to conduct dose optimization studies, is to accurately measure antibiotic concentrations in relation to the time of administration, and to correlate these measurements with the concentrations at the site of infection. In this thesis, the primary method we used to measure antibiotic concentrations was liquid chromatography tandem mass spectrometry (LC-MS/MS). LC-MS/MS is a sensitive and specific method for measuring the concentrations (free and protein-bound) of antimicrobials in various biological fluids (matrices).[1,2] However, to use these measurements to optimize dosing regimens it is important to measure the right concentration at the right time in the right matrix.

One factor herein is influence of protein binding. This is important as only the unbound antibiotics exhibit antibacterial activity. Especially for highly protein-bound antimicrobials it is essential to take the unbound fraction into account to estimate the effective concentration at the site of infection. In **Chapter 3**, unbound ceftriaxone concentrations were measured after ultrafiltration. There are uncertainties about the optimal use of ultrafiltration as a technique for isolating the unbound fraction from the protein-bound fraction.[3] A recent study discovered that the difference in temperature during ultrafiltration (20°C vs 37°C) had a significant impact on the binding properties of flucloxacillin in a population pharmacokinetic model.[4] Therefore, it is essential to validate methods that use ultrafiltration for separation by comparing them with alternative methods such as microdialysis.

Furthermore, modeling the protein binding is not an alternative for measuring the unbound concentrations. In **Chapter 3**, we developed a model to estimate the unbound fraction of ceftriaxone using the total and free concentrations of ceftriaxone. Since then, several other studies have investigated the protein binding of ceftriaxone with the goal of predicting the unbound concentration based on total concentration. [5,6] However, adequately predicting in-vivo unbound

concentrations based on total ceftriaxone concentrations remains difficult due to the influence of various factors, including patient characteristics, disease state, and concurrent drug use. Therefore, measurements of the unbound fraction remain necessary in PK/PD studies of antimicrobials with high protein binding.

It has been suggested that the extended half-life of ceftriaxone, compared to other antimicrobial agents, is due to its high protein binding.[5] However, this correlation does not hold true for flucloxacillin, which also has high protein binding, but a similar half-life compared to other penicillins with low protein binding. An explanation could be a combination of the difference in active tubular secretion and the difference in binding affinity to serum albumin or alpha-1-acid glycoprotein or maximal binding capacity. To explore the impact of protein binding more studies should be conducted to investigate the role of protein binding, binding capacity, and binding strength of antimicrobials to predict the impact of protein binding on the pharmacokinetics of highly protein-bound antimicrobials.

Another factor that needs to be considered is the concentration of unbound and active antibiotic at the site of the infection. The antibiotic used to treat these infections should be able to penetrate the infected tissues adequately to reach therapeutic antibacterial concentrations[7] This is complicated by the fact that tissue structure, vascular permeability and blood flow to the infection site change during the acute phase of infection. An example is the blood-prostate barrier for hydrophilic molecules. This tissue is highly penetrable during the acute phase of an infection, such as acute prostatitis. During the later stages of treatment, inflammation is limited, leading to a decrease in antimicrobial diffusion, especially of hydrophilic molecules such as beta-lactam antibiotics.[8,9] Future studies that aim at optimizing dosing regimens by connecting tissue end serum concentrations should therefore also consider variation in penetration over time.

Additionally, there are significant variations in tissue penetration among different classes of antibiotics. For example, beta-lactam concentrations in tissues are often between 10-30% of the serum concentrations, while fluoroquinolones accumulate in tissues, resulting in higher concentrations compared to serum.[10,11] This can be partially attributed to drug characteristics such as lipophilicity, molecular weight, and ionization at physiological pH. But it also depends on active transport mechanisms. For antimicrobials with limited tissue penetration, it is essential to thoroughly validate the analysis and sample preparation methods

used to determine tissue concentrations. As the antimicrobial concentrations in other matrices, such as blood, are higher, contamination of the target tissue (e.g., blood or urine in prostate tissue) might lead to an overestimation of tissue concentrations.

As viruses replicate intracellularly, it is essential for most antiviral treatments to act inside the target cell.[12] However, it is challenging to determine the extent to which an administered dose will penetrate the target cell and whether this will be sufficient to produce a pharmacologic effect. A significant amount of intracellular pharmacokinetic research for antivirals targeting HIV and COVID-19 is conducted using peripheral blood mononuclear cells (PBMCs). PBMCs can be extracted from blood samples, and their intracellular concentration can be measured. However, peripheral blood mononuclear cells (PBMCs) are not the primary target cells of respiratory viruses like SARS-CoV-2.[13] Therefore, testing antiviral compounds using PBMCs is not sufficient to establish intracellular activity against SARS-CoV-2. Especially because in vitro studies have shown significant variations in measured EC50 values for remdesivir in different cell lines.[14] This may be due to the transmembrane transport of remdesivir and GS-441524, or the efficiency of intracellular phosphorylation. To determine the best dosing regimen for remdesivir and GS-441524, it is crucial to develop measurements that can assess the in human intracellular exposure in the primary target cells of SARS-CoV-2.

Development of population pharmacokinetic models can be optimized by combining studies with dense sampling schedules with routinely collected data.

After measuring the antimicrobial concentrations, the next step in optimizing antimicrobial dosing is population pharmacokinetic modeling. Population pharmacokinetic models are simplified representations of the actual physiological processes in humans, with different levels of accuracy and complexity. Every model, therefore, has its limitations. By optimizing data collection and the pharmacological modeling process, we can develop a model that closely represents reality. In **Chapter 2**, the data was collected as part of routine therapeutic drug monitoring (TDM) of trimethoprim-sulfamethoxazole in a large hospital-based population to develop the model. In **Chapter 3,** we utilized a large intensive care population with a sparse sampling schedule, while in **Chapter 5**, we focused on a small population of hospitalized COVID-19 patients with a dense sampling schedule. All of these methods had their strengths and limitations.

In an ideal scenario, one would measure a real-time concentration-time curve in a large sample of patients to accurately describe the pharmacokinetics and capture all covariates that could influence the model.[15] However, this is rarely feasible in a clinical setting. To accurately predict the pharmacokinetics of antimicrobials, we recommend first building a base model using a small, well-defined population with a dense sampling time schedule. This initial model can be used to determine the best time schedule for limited sampling in a clinical setting. A larger population with more diverse characteristics should be sampled with the limited sampling schedule to assess the impact of covariates. Ideally, the study includes patients with normal baseline characteristics as well as those with divergent characteristics. This makes it possible to assess the influence of these characteristics more accurately. Factors such as the processing of blood samples (e.g. different methods of separating unbound from protein-bound fraction) and variation between different analysis methods (e.g. LC-MSMS or historic HPLC methods) do not interfere with the interpretation of the results. Certain modeling tools are available to integrate dense data with sparse data, for example, by utilizing the PRIOR subroutine in NONMEM.[16]

However, even when optimal modeling strategies are used to establish pharmacokinetic models, inter-individual variation in the parameter estimates often persists. This could be the result of unmeasured variables, sampling errors, or complex interactions between measured variables. To evaluate these interactions, machine learning tools and artificial intelligence could be utilized to enhance the quality of future pharmacokinetic models.[17]

The last step is to utilize the pharmacokinetic model to optimize the dosing regimens. In addition to adjusting the dose or dosing interval, alternative strategies can be employed to optimize dosing regimens. These include continuous intravenous infusion of antimicrobials, a priori model-informed precision dosing, a posteriori therapeutic drug monitoring schedule, and adjusting the dose to alter the ratio between the drug and metabolite. Information about the pharmacodynamic targets of the antimicrobial and its metabolites is essential to choose the optimal dosing regimen.

An MIC or EC50 alone is insufficient as pharmacodynamic endpoint for PK/PD studies.

Exploring the impact of antimicrobial exposure on clinical outcomes is complex. For example, the in-vitro target concentration for antimicrobials does

not always correspond with optimal clinical outcomes. The MIC or EC50 are frequently used in clinical studies investigating antimicrobial agents. However, there is considerable variation within an MIC measurement itself. The EUCAST considers an MIC of 1.0 mg/L to be best interpreted as a value between 0.5 mg/L and 2.0 mg/L.[18,19] This should be taken into account when aiming for a target concentration based on a measured MIC. Clinical breakpoints are currently recommended, suggesting that all pathogens with an MIC below this breakpoint are considered susceptible. Dosing simulations are often performed based on this "worst case" breakpoint. However, this might lead to overexposure, as most of the pathogens have MICs far below the cut-off value.[20] For the EC50 of COVID-19 therapies, there is considerable variation based on the cell lines used, making the translation of in-vitro EC50 to clinically relevant plasma concentrations for antiviral drugs complicated.[14,21] Personalized dose optimization studies should ideally include the actual MIC value for antibiotics and EC50 measurements in clinically relevant cell types for antivirals.

The MIC is the minimal concentration that results in the inhibition of growth in-vitro, and the EC50 the concentration that leads to 50% of the maximal effect. These concentrations do not directly correspond with the maximal antimicrobial effect (Emax).[22] Changes in antimicrobial exposure over time in vivo should also be considered when studying the optimal exposure, as this may differ between dosing regimens (e.g., continuous versus intermittent infusion). Furthermore, translating the MIC or EC50 directly to dosing regimens does not consider factors such as the penetration of antimicrobials into target tissue, the post antibiotic effect and the pharmacological effect of metabolites.[10,23,24]

Other factors to consider are the various phases a pathogen can go through and the influence of the environment of the infection. For instance, bacteria can enter a stationary phase, extend to the intracellular space and form biofilms, especially on implant devices.[25,26] Bacteria in the stationary phase hardly grow, which limits the effectiveness of most antibiotics.[27] Also, most commonly used antimicrobials have lower penetration into biofilm.[28,29] The different phases of bacteria and the environment of infection are not always addressed in PK/PD studies, but they are essential to assess the effectiveness of therapy and need for combination therapy with antimicrobials such as rifampicin.[30] Population pharmacokinetic models combined with models describing bacterial replication and growth in different situations (e.g., patients with infected implants) are necessary to develop dosing regimens tailored to individual needs.

Time is also a crucial factor to consider when evaluating the pharmacodynamic effect of an antimicrobial. In patients with sepsis, the timeliness of effective treatment has been associated with the clinical effectiveness of antimicrobial therapy[31,32] Therefore, dosing regimens for sepsis are designed to achieve effective concentrations as rapidly as possible. This can be achieved by incorporating a loading dose into continuous infusion regimens[33] Additionally, for viral infections such as COVID-19 and influenza, there is only a brief window of opportunity for antiviral therapy.[34-36] This occurs during the phase of infection when viral replication is at its peak, often within the first few days of infection. As described in **Chapter 9**, there is a need for biomarkers that can characterize the infection period when antiviral therapy is most effective, in order to optimize therapy.

In addition to predicting whether a patient is in the optimal period for treatment initiation, alternative pharmacodynamic markers could be considered to assess the effectiveness of antimicrobial therapy or its potential toxicity. For example, it is important to also consider alternative markers for antimicrobial effect such as the minimal concentration to prevent the induction of resistance and the minimal bactericidal concentration.[37,38] Changes in laboratory values (for example procalcitonin and C-reactive protein) could be evaluated as early pharmacodynamic markers to measure treatment effect before clinical signs of treatment effect become visible.[39] For the development of acute kidney injury, alternative markers that predict AKI as a result of pharmacological therapy prior to the increase in creatinine are needed.[40] More research is needed before these markers can be used in clinical practice.

Optimized dosing regimens based on PK/PD studies need validation in clinical studies.

The use of clinically relevant endpoints that are objective, reproducible, and have a high internal and external validity is needed to select the optimal dosing regimen of an antimcrobial.[41] However, studying the impact of dosing regimens on clinical outcomes is complex because large sample sizes are required to determine if an alternative dosing regimen is superior to the approved dosing regimen. Therefore, dosing regimens from population pharmacokinetic models are rarely prospectively validated. Nonetheless, these studies are essential for establishing the clinical benefits of optimized dosing regimens.

For example, continuous infusion of beta-lactam antibiotics is superior to intermittent administration based on target attainment. However, this does not always lead to improved clinical outcomes for all patient populations.[42] Another example is model-informed precision dosing, this was expected to increase the effectiveness of beta-lactam antimicrobials by achieving higher percentage of target attainment. However, in a multi-center trial, this approach did not lead to improved clinical outcomes.[43] On the other hand, for antimicrobials such as vancomycin and aminoglycosides, TDM has been shown to improve outcomes and is therefore recommended in current guidelines.[44,45]

To facilitate studies aiming to provide clinical evidence, it is essential to clearly estimate the dose-response effect. It is important to consider both pharmacokinetic and pharmacodynamic variation, as well as the PK/PD index.[46] As described in **Chapter 2**, there is no consensus on whether cotrimoxazole works via Cmax>MIC or T>MIC.[47,48] Additionally, even when the PK/PD index is known, there is still controversy about the optimal target concentration. For beta-lactam antibiotics, multiple targets ranging from 40% fT>MIC up to 100% 6 times fT>MIC have been suggested.[49,50] A fT>MIC of 100% has been associated with better outcomes than fT>MIC below 100% in critically ill patients and patients with serious infections.[50-52] For prevention of resistance, 4 times fT>MIC of 100% has been suggested as the optimal target.[52,53] However, there is limited clinical information available for other endpoints, populations, individual drugs and different indications. To improve the selection of the best dosing regimen, the ideal target range should first be established through in-vitro and animal studies, and then translated to humans.

When the optimal theoretical target range is established, it is necessary to conduct studies that differentiate between the theoretical target and clinical effectiveness. Observational studies that measure steady-state concentrations could help determine the optimal clinical target range for the infection and the toxicity threshold.[50,54,55] Once the optimal target range is known, the most effective dosing regimen can be determined using population pharmacokinetic modelling.[56,57]

When determining the effectiveness of optimized dosing regimens, the selection of the clinical endpoint is important. It is not always easy to select clinically relevant endpoints in infectious disease studies.[41] In studies investigating soft-tissue infections, the use of antimicrobials in combination with the host immune response is almost always sufficient to cure an infection, making mortality an

impractical endpoint.[58] On the other hand, in critically ill patients mortality occurs frequent, but could also be influenced by various other factors related to critical illness, beyond the impact of the antimicrobial on the infection. Other clinically relevant pharmacodynamic endpoints include discontinuation due to adverse reactions, development of pathogen resistance, impact on the microbiome, or impact on quality of life.

Preferably, randomized clinical trials are conducted to implement the regimen and determine whether alternative dosing regimens are superior in efficacy, less toxic, or more practical. However, conducting clinical trials is often not feasible. Optimized dosing regimens should still be incorporated in to clinical practice. Implementation or observational studies of patients treated with the optimized dosing regimens are therefore crucial. Preferably, data should be collected from multiple hospitals both before and after the implementation of the new dosing regimen.

Incorporation of optimized dosing regimens in treatment guidelines could also help with implementation. For example, the EUCAST has updated their recommended dosing schedules, which now include different doses for pathogens with intermediate susceptibility.[18,59] For several cephalosporins and carbapenems, these recommended regimens involve doubling the same dose without changing the dosing interval. Recommending continuous infusion or more frequent administration of smaller doses would be preferred from a PK/PD standpoint.

Retrospective evaluations are suitable to detect potential drug-drug interactions in clinical practice.

For the past few decades, most beta-lactam antibiotics have been considered relatively safe in terms of drug-drug interactions.[60] However, information about interactions is still emerging (e.g., **Chapter 7**). Since most of the beta-lactam antibiotics were registered approximately 50 years ago, thorough exploration of drug-drug interactions was not performed as part of the registration process. For example, flucloxacillin interactions with other drugs were initially only described in case reports.[61] In vitro and human studies have confirmed the influence of flucloxacillin on the pregnane x receptor (PXR). The activation of PXR led to the upregulation of CYP enzymes and p-glycoprotein[61,62]

It is important to investigate potential signs of interactions with molecules that are registered without adequate in vitro data and drug-drug interaction studies. The case described in **Chapter 8** is an example. Clinicians should consider drug interactions as a potential cause when unexpected pharmacological responses to drugs occur in combination with other drugs. Retrospective evaluation of plasma concentrations or dose adjustments can help to provide evidence of drug-drug interactions. However, in vitro verification is necessary to understand the mechanisms underlying these interactions, and human drug-drug interaction studies are required to assess the magnitude of the effect[63]

Data collected during routine care is suitable to detect adverse effects of antimicrobials and to establish risk factors

Monitoring adverse effects of antimicrobials is of utmost importance. Years of clinical use have demonstrated that the majority of commonly used antimicrobials are relatively safe. However, signals regarding safety concerns are still emerging. Two examples of such risks include the potential for hypokalemia with high-dose beta-lactam antibiotics, as outlined in **chapter 6**, and crystal-induced acute kidney injury in patients receiving high doses of amoxicillin.[64-66] These adverse effects were only documented decades after the introduction of the antimicrobials. Signals from clinical practice are important for identifying adverse effects. Cohort studies, case-control studies, and disproportionality analysis can be used to further investigate the adverse event. Cohort studies can be used to calculate the incidence of adverse effects and identify risk factors, or to establish the relationship between serum concentrations and adverse effects.

However, assessing adverse effects in patients treated with antimicrobials is often complicated. For example, if there are neurological complications, it is possible that the impact of beta-lactam antimicrobials on the GABA receptor led to neurotoxicity.[67] However, it could also result from an underlying neurological disorder, other potentially neurotoxic drugs, or manifestations of the infection.[20,55,67] Another challenging adverse event to study is the association between antibiotic concentrations and nephrotoxicity. High concentrations of beta-lactam antibiotics have been linked to nephrotoxicity.[55,68] However, renal clearance is the primary route of elimination for most beta-lactam antibiotics. It is not always clear whether nephrotoxicity is caused by high concentrations of beta-lactam antibiotics or if high concentrations are caused by reduced renal clearance due to the infection or other causes.[55,69,70] Therefore, novel methods to explore the mechanisms of these adverse events are needed. The use of

biomarkers could help determine the impact of antimicrobials on specific processes. Additionally, comparison studies could be conducted to assess different antimicrobials in the same patient population, in order to distinguish between drug adverse events and disease effects.

Research in clinical practice is essential for optimization of the use of antimicrobials.

Integrating research into routine care offers several benefits. Healthcare providers are best suited to identify the research gaps that impact daily practice. Clinical research conducted in routine care settings can enhance the generalizability and applicability of research findings, as it mirrors real-world settings and populations. Furthermore, research in routine care can enhance the implementation of new findings and optimized dosing regimens.[71] The following situations are suitable for pharmacological research

First, most of the dosing regimens for registered antimicrobials have been established based on historical data and have not been adequately evaluated in special populations.[72] Using TDM results or blood samples that are either routinely collected or collected at additional sampling moments can be used to measure antimicrobial concentrations. Pharmacokinetic modeling can then assess whether the pharmacokinetics differ in special populations and if they require alternative dosing regimens.

Secondly, when new antimicrobial therapies are introduced, they are often only studied in clinical trials with strict inclusion and exclusion criteria. The population in which they are used in clinical practice is often larger and includes a wide variety of patients with different clinical characteristics.[73] Since it is not feasible for registration holders to conduct clinical studies in every patient population, it is important to gather this data through real-world studies.[74] For instance, the introduction of new antimicrobials, such as remdesivir, should be followed by multicenter real-world studies to validate the results of the initial clinical trials, assess the effects in subgroups not included in the trial population, optimize dosing regimens in the target population, and select the optimal patients for antimicrobial therapy. To conduct these studies, it is preferable to document outcomes in a structured manner during treatment. An alternative approach involves retrospective data collection, where a clinical data collector extracts outcome information from electronic patient files.[75]

Third, if an observation is made in clinical practice, similar cases should be explored. For example, the low posaconazole concentrations in **Chapter 7**, a retrospective evaluation can help determine if the observation has previously occurred and study the magnitude of the effect. Furthermore, a retrospective assessment of an adverse effect such as hypokalemia associated with flucloxacillin in **Chapter 6** can aid in identifying risk factors and identifying patients who would benefit from more thorough monitoring.

Routinely collected data is suitable, but not ideal, for performing clinical pharmacological research

Retrospective research using routinely collected healthcare data presents a significant challenge due to the quality of documentation in the electronic patient records. Electronic patient records have not been optimized to be used for (retrospective) research, as a significant portion of them are comprised of unstructured data.[76] One method to overcome these limitations is to manually review all patient files. However, this process is time-consuming and requires a lot of resources.

In this thesis, data was collected in three chapters using a validated electronic clinical data collector capable of aggregating data from both structured fields and unstructured text.[77] The use of a clinical data collector allows for the rapid selection of patients with specific characteristics, such as those receiving concomitant posaconazole and flucloxacillin, or for collecting data from a targeted population, such as hospitalized COVID-19 patients. In the future, developments such as artificial intelligence should enhance the quality of clinical data collectors.[78] Efforts should also be directed towards improving the quality and structure of documentation in electronic patient records.

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