Simone H.J. van den Elsen

Therapeutic Drug Monitoring in Tuberculosis Treatment

The use of alternative matrices and sampling strategies

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The use of alternative matrices and sampling strategies

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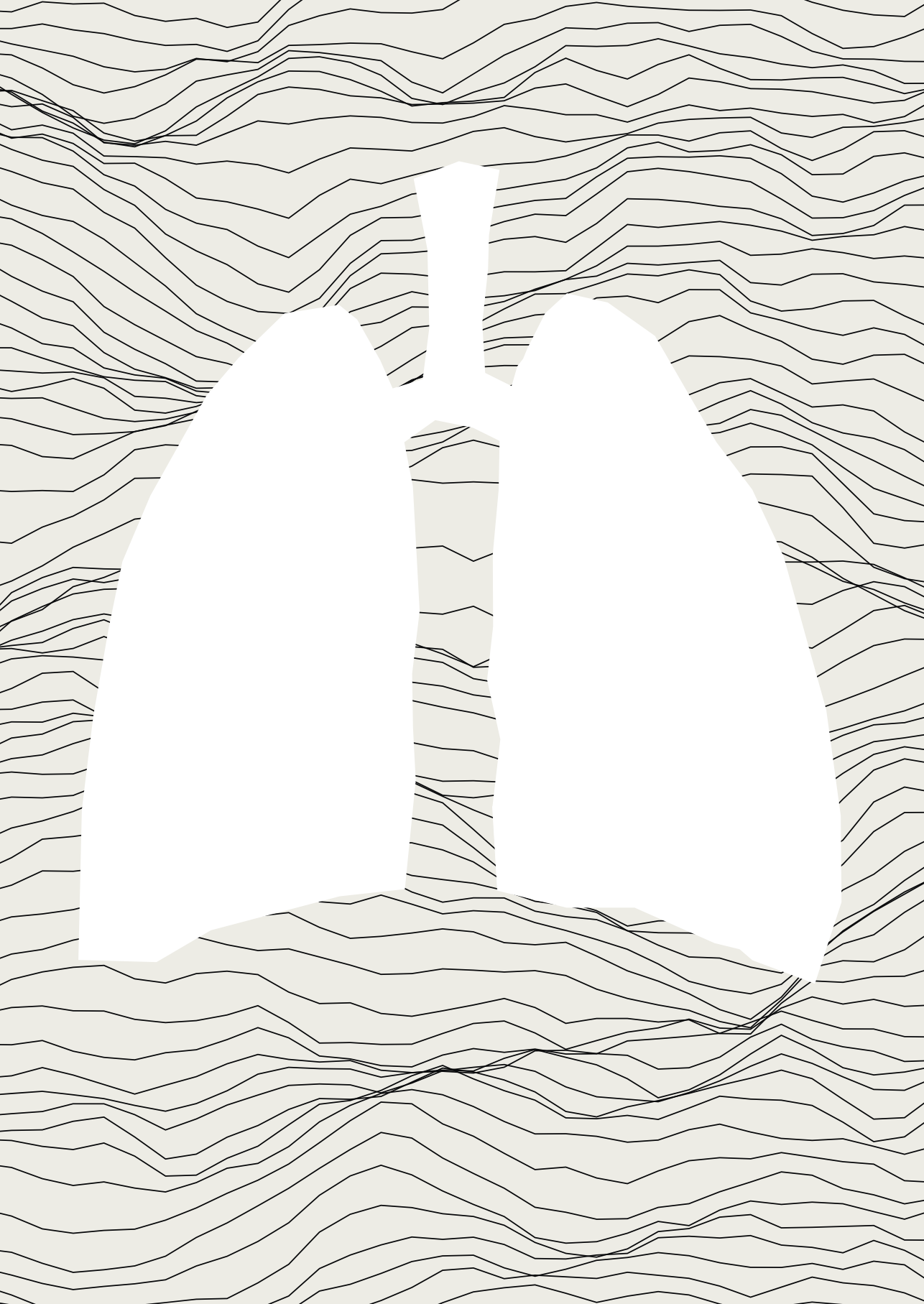

Chapter

1

Introduction and scope of the thesis

Tuberculosis (TB) is an infectious disease caused by slowly replicating bacteria belonging to the group of *Mycobacterium tuberculosis* complex bacteria. TB infection predominantly spreads by inhaled small airborne droplets containing the *M. tuberculosis* bacterium. These airborne droplets or aerosols are primarily produced by individuals with pulmonary TB by coughing. Typical symptoms of active pulmonary TB are a persistent cough, fever, night sweats, fatigue, and weight loss. Some patients, especially those with an immunosuppressed status, may have less pronounced symptoms. Chest-radiography, sputum smear microscopy, culture-based methods, and rapid molecular tests are used to detect and diagnose TB.

Classification of TB

Most individuals infected by inhaling TB bacilli from patients with active pulmonary TB do not fall ill. The majority either fights off the TB bacilli by their host defences including their mucosal and mucociliary protection, or by their effective immune system [1]. Others develop a latent TB infection, where *M. tuberculosis* is present in the body in low numbers, but is dormant and not active. Latent TB infection can progress into active TB disease when the bacillary burden is overwhelming, or when immune defences are compromised [2]. Active TB disease can be located in the lung (pulmonary TB), but also in other parts of the body, such as lymph nodes, central nervous system, bones, as well as the intestinal and urogenital systems (extra-pulmonary TB). Furthermore, there is the one-of-a-kind infection called miliary TB. The infection is then all-over, widely spread across the body, and therefore in general has a worse prognosis than pulmonary TB [3,4]. Another classification of TB infections is based on the drug susceptibility pattern of the involved *M. tuberculosis* strain. Drug-susceptible TB (DS-TB) is sensitive to all first-line drugs including rifampicin, isoniazid, pyrazinamide, and ethambutol. Mono-resistant TB is resistant to one drug only, e.g. rifampicin-resistant TB (RR-TB) or isoniazid-resistant TB. Multi-drug resistant TB (MDR-TB) is resistant to at least rifampicin and isoniazid, while extensively drug-resistant TB (XDR-TB) is additionally resistant to one fluoroquinolone and one injectable second-line drug.

TB epidemic

Although TB is not endemic in the Netherlands (806 cases in 2018), the global burden of TB remains extensive. TB is the worldwide leading cause of death caused by a single infectious agent. In 2018, an estimated 10 million people developed TB and 1.45 million patients died due to TB [5]. Antibiotic resistance is a major concern. Approximately 500,000 people developed RR-TB in 2018 and 78% of this group was additionally resistant to isoniazid, thus having MDR-TB [5]. In 2014, the World Health Organization (WHO) set ambitious targets in the End TB Strategy [6]. The aim is to reduce the annual number of TB deaths with 95% and the TB incidence with 90% in 2035, using the year 2015 as comparator. The current global decrease in both incidence and mortality is not fast enough to reach these targets by 2035 [5].

Treatment of TB

The current DS-TB treatment has been established in the 1980's and has not changed since [7]. It starts with an intensive phase using rifampicin, isoniazid, pyrazinamide, and ethambutol for 2 months to reduce the bacterial load, followed by a continuation phase with only rifampicin and isoniazid during 4 months to kill the persistent survivors [8]. Treatment success rates of this first-line regimen are considered to be relatively high, even under programmatic conditions (85% in 2017) [5]. Nevertheless, treatment failure and acquired drug resistance are present-day problems due to inappropriate drug management, incompliance, and suboptimal drug exposures [9–11]. The recommended treatment regimen for MDR-TB (also used for RR-TB) has been changing over the last five years. Previously, the second-line anti-TB drugs used in MDR-TB treatment were organized in four groups with decreasing preference; the fluoroquinolones, second-line injectable agents, other core second-line agents, and add-on agents [12]. The grouping of the second-line drugs was revised by the WHO in 2018 (Table 1) in response to a meta-analysis on the association between the use of certain anti-TB drugs and positive treatment outcomes as well as the growing preference for an all-oral regimen [13–15].

Table 1. Present grouping of second-line drugs in MDR-TB treatment [14].

Group A: Include all three (if possible)	Levofloxacin or moxifloxacin
	Bedaquiline
	Linezolid
Group B: Add one or both (if possible)	Clofazimine
	Cycloserine or terizidone
Group C: Complete regimen with one or more (if required)	Ethambutol
	Delamanid
	Pyrazinamide
	Imipenem-cilastatin or meropenem
	Amikacin (or streptomycin)
	Ethionamide or protionamide
	p-aminosalicylic acid

The MDR-TB regimen contains at least four drugs that the involved *M. tuberculosis* strain is susceptible for; and treatment duration is 9 to 18-20 months. Culture methods (e.g. Mycobacteria Growth Indicator Tube system), molecular testing (e.g. Xpert MTB/RIF) or line probe assays are able to determine bacterial susceptibility; these tests guide the treating physician in compiling an adequate drug regimen. Meanwhile, a standardized shorter regimen of only 9 to 12 months was evaluated in MDR/RR-TB patients who have not been treated before and in whom resistance to fluoroquinolones

and second-line injectable agents was excluded. This shorter regimen was found to be non-inferior to longer treatment regimens [16,17]. However, the patient population eligible for this shorter regimen is small, in the Netherlands for instance only around 50% of the MDR-TB patients[18], and therefore the applicability is limited [19]. The current global success rate of MDR-TB treatment is 56% which is unacceptably low [5]. The response to TB therapy is observed using clinical monitoring, laboratory tests, and frequent sputum smear and culture analysis. If at least 2 consecutive sputum samples of a patient with pulmonary TB are free from *M. tuberculosis* in culture tests, one month apart, it is defined as sputum conversion. Fast sputum conversion is a sign of response to treatment, but a patient is not cured yet after conversion and relapses are common. Additionally, patients with TB are closely monitored for medication adherence, side effects (especially in case of more toxic second-line drugs), and comorbidities.

Individualized approach using therapeutic drug monitoring

TB treatment is fairly standardized because a high level of individualisation is expensive and consequently unfeasible due to the high global burden centred in low-resource countries. The TB treatment regimens and drug dosages are described in detail in guidelines [8,20]. However, every patient has unique characteristics, for instance TB presentation, body composition, pharmacokinetic parameters, comorbidities, concomitant medication, immune defences, etcetera. Additional inter-individual variation is introduced by highly variable susceptibility patterns of the involved TB strains. Thus, one dose does not fit all and a more individualized approach would be an asset to fight TB in its most efficient way [21,22].

One method to individualize TB therapy is to use therapeutic drug monitoring (TDM). TDM uses drug concentrations analysed in blood samples to determine the optimal dose for one particular individual using pharmacokinetic/pharmacodynamic knowledge. In general, it is only useful to perform TDM if the clinical effect is related to the drug concentration or exposure; together with a narrow therapeutic window; pharmacokinetic variability; and a difficult to monitor clinical effect [23]. For antibiotic drugs in specific, the pharmacological effect is not only related to drug concentrations, but also to the susceptibility of the involved bacterial strain, a feature that is defined as minimal inhibitory concentration (MIC). The efficacy of some antibiotic drugs increases with higher peak concentrations. These drugs act concentration-dependant (e.g. amikacin) and their efficacy is best described by the ratio of peak concentration and MIC (C_{max}/MIC), while the efficacy of time- dependant antimicrobial agents (e.g. meropenem) is related to the percentage of time the free drug concentration is above the MIC (%fT>MIC) [24]. However, most drugs are concentration- as well as time-dependant (e.g. fluoroquinolones, rifampicin) and therefore the ratio of area under the concentration time curve and MIC (AUC/MIC) is the best predictor for efficacy [24,25].

Drug resistance of anti-TB drugs is emerging and there is increasing evidence that some host factors are associated with inadequate drug exposure. Therefore, TDM is increasingly recommended in guidelines for specific patient populations [20,26–28]. Inter-individual pharmacokinetic variability is described for many anti-TB drugs and it could cause suboptimal drug exposures which in turn could lead to acquired drug resistance as well as treatment failure [29–31]. Moreover, some second-line TB drugs are rather toxic and can cause significant side effects, especially when used for a longer period of time. For linezolid, TDM can be used to minimise toxicity while still maintaining an adequate drug exposure [32]. Additionally, the effect of drug-drug interactions can be monitored and corrected for by using TDM, e.g. rifampicin in combination with moxifloxacin [33].

Ideally, TDM is performed shortly after starting treatment, but clearly at steady state conditions, to determine the adequate dosages and to identify the subset of patients at risk, e.g. slow responders, as soon as possible. Presently, the general implementation of TDM in TB treatment is slow because it is considered laborious, time-consuming and expensive [34]. Traditionally, TDM is performed using plasma or serum samples, although alternative matrices such as saliva, dried blood spots and urine have been studied because of their ease and potentials for home-based sampling [34,35]. Moreover, to perform adequate TDM, multiple samples are required to determine the %T>MIC, C_{max}, or AUC used for dose optimisation. Limited sampling strategies (LSS) are able to estimate the AUC using one to three optimally timed samples and therefore could reduce the burden for patients and health care personnel [36–38]. Centralized TDM is a method to concentrate parts of the TDM process in one experienced location to increase the availability and quality of TDM and decrease the challenges for small healthcare facilities. The use of alternative matrices, sampling strategies, and centralized TDM could reduce the burden, organisational efforts as well as the costs of TDM and therefore could overcome the present objections for more frequent TDM in TB treatment.

AIM OF THIS THESIS

This thesis aims to evaluate alternative matrices and sampling strategies for TDM of multiple anti-TB drugs. More specifically, this thesis focuses on TDM using saliva samples, limited sampling strategies, as well as centralized TDM to enhance the feasibility of TDM. The ultimate goal of these innovative techniques is to stimulate performing TDM of anti-TB drugs, particularly in TB endemic countries, to improve worldwide treatment outcomes and proceed towards the elimination of TB.

OUTLINE OF THE THESIS

In Chapter 2 we provide an overview of the available literature on concentrations of anti-TB drugs in saliva and blood. In addition, this systematic review will help to identify knowledge gaps to be targeted for future research and investigates the potentials for saliva-based TDM.

Due to the scarcity of data on plasma to saliva drug penetration in TB patients, the objective is to perform a prospective, observational cohort study of concentrations of various anti-TB drugs in saliva of patients with TB. Saliva-serum or saliva-plasma ratios are evaluated and the feasibility of salivary TDM is discussed for each drug. In Chapter 3a we investigate the first-line drugs rifampicin and isoniazid, in Chapter 3b we focus on the group A second-line drugs moxifloxacin and linezolid, whereas in Chapter 3c the second-line injectable agent amikacin is studied. To be able to collect saliva samples of infectious TB patients in the prospective study without infection hazard, a safe sampling method is developed to sterilize the saliva samples after collection and before processing for analysis (Chapter 3d).

To be able to accurately monitor drug exposure with minimal burden for patients and caregivers, there is an urgent need for population pharmacokinetic models and LSS for moxifloxacin (Chapter 4a) and levofloxacin (Chapter 4b). Two different methods being the Bayesian approach and multiple linear regression, each with their own (dis)advantages, are used to develop these LSS. The predictive performances of the population pharmacokinetic models and the LSS are evaluated in these chapters as well.

Evidence to support TDM beyond target attainment is limited. Clinical trials are urgently needed, but funding is scarce [26,39]. An alternative approach using a case-control design, which significantly reduces costs, may help to provide the first evidence and attract funding for a randomized controlled trial. In Chapter 5 the design of a multicenter observational study is proposed. This study will evaluate the feasibility of concentrating the sample analysis and dosing advice in a central facility (centralized TDM) and secondary the impact of TDM of fluoroquinolones on treatment outcomes of MDR-TB patients.

To complete this thesis, a general discussion on the use of saliva as matrix for TDM, LSS, and centralized TDM, including future perspectives of TDM in TB treatment will provide guidance for future research.

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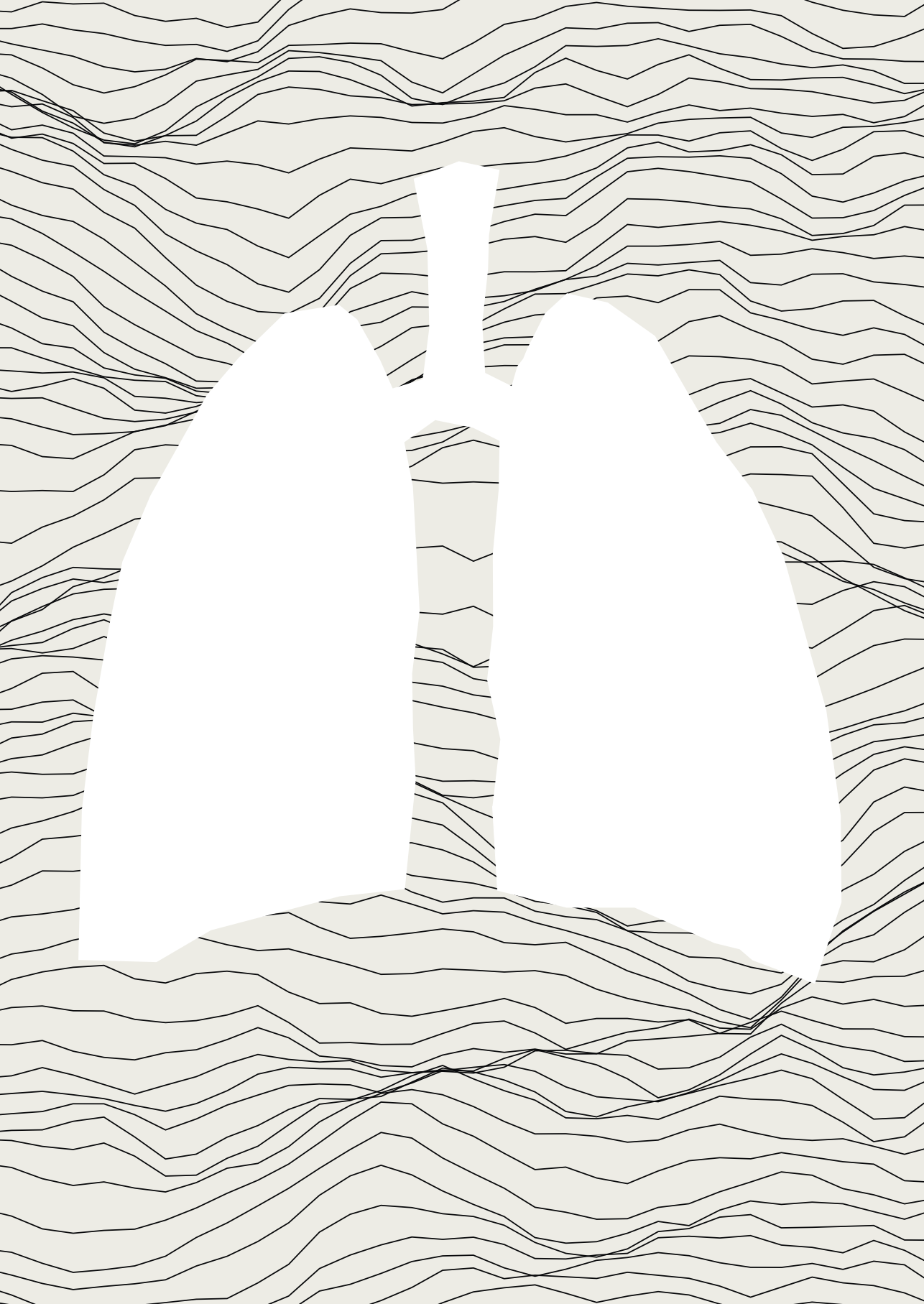

Chapter

2

Systematic Review of Salivary versus Blood Concentrations of Antituberculosis Drugs and Their Potential for Salivary Therapeutic Drug Monitoring

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ABSTRACT

Background: Therapeutic drug monitoring is useful in the treatment of tuberculosis to assure adequate exposure, minimise antibiotic resistance and reduce toxicity. Salivary therapeutic drug monitoring could reduce the risks, burden and costs of blood-based therapeutic drug monitoring. This systematic review compared human pharmacokinetics of antituberculosis drugs in saliva and blood to determine if salivary therapeutic drug monitoring could be a promising alternative.

Methods: On December 2, 2016, PubMed and Institute for Scientific Information Web of Knowledge were searched for pharmacokinetic studies reporting human salivary and blood concentrations of antituberculosis drugs. Data on study population, study design, analytical method, salivary Cmax, salivary area under the time-concentration curve, plasma/serum Cmax, plasma/serum area under the time-concentration curve and saliva-plasma or saliva-serum ratio were extracted. All included articles were assessed for risk of bias.

Results: In total, 42 studies were included in this systematic review. For the majority of antituberculosis drugs, including the first-line drugs ethambutol and pyrazinamide, no pharmacokinetic studies in saliva were found. For amikacin, pharmacokinetic studies without saliva-plasma or saliva-serum ratios were found.

Conclusions: For gatifloxacin and linezolid, salivary therapeutic drug monitoring is likely possible due to a narrow range of saliva-plasma and saliva-serum ratios. For isoniazid, rifampicin, moxifloxacin, ofloxacin, and clarithromycin, salivary therapeutic drug monitoring might be possible; however, a large variability in saliva-plasma and saliva-serum ratios was observed. Unfortunately, salivary therapeutic drug monitoring is probably not possible for doripenem and amoxicillin/clavulanate, as a result of very low salivary drug concentrations.

INTRODUCTION

Tuberculosis (TB) is an infectious disease that is still a huge problem worldwide, although it is curable with antibiotics. In 2015, approximately 10.4 million people worldwide had TB for the first time, including 480,000 patients with multi-drug resistant tuberculosis (MDR-TB) [1]. MDR-TB is caused by strains of *Mycobacterium tuberculosis* resistant to at least first-line drugs isoniazid and rifampicin. Drug-susceptible TB is treated with a standard combination of isoniazid, rifampicin, ethambutol, and pyrazinamide during 2 months followed by 4 months of only isoniazid and rifampicin [2]. The treatment of MDR-TB consists of a combination of at least 5 antibiotics that are likely to be effective [3].

Therapeutic drug monitoring (TDM) can be used to assure adequate exposure, minimise antibiotic resistance, and reduce side effects [4]. TDM is, however, not a part of the standard TB treatment according to the World Health Organization (WHO) guidelines. Subtherapeutic drug concentrations cause decreased cure rates and can induce antibiotic resistance [5,6]. On the other hand, too high concentrations of some anti-TB drugs can lead to serious toxicity [4,7]. In addition, pharmacokinetics of anti-TB drugs show large interindividual variability [8]. Thus applying TDM in TB therapy could be helpful to achieve therapeutic drug concentrations in an early stage of treatment.

Although blood samples have been routinely used for TDM, venipuncture is an invasive procedure with increased risks of infection, local hematoma, and pain at the puncture site [9,10]. Also, pain-related fear plays a major role for patients [9]. In addition, venipuncture is rather expensive because it requires qualified staff and appropriate materials [9,10]. Blood sampling is undesirable for some patient groups because of limited blood supply (e.g. neonates), less accessible veins (e.g. elderly), or religious objections [9]. Because of these disadvantages, alternatives to regular blood sampling (e.g. saliva) are being studied. Oral fluid is a mixture of saliva secreted by all glands present in the oral cavity [11]. The terms saliva and oral fluid are used interchangeably in literature.

Saliva sampling is less complicated compared with taking blood samples and reduces costs [10,12]. An economic study about saliva collection in children found 58% savings with the saliva sampling procedure alone compared with blood sampling, caused by a shorter sampling time and less expensive materials [13]. If parents were collecting saliva samples instead of medical staff, the savings could increase up to 90% [13]. Collecting saliva samples is also experienced as more comfortable by patients [9,12,14]. For certain patient groups, such as children, elderly, and people with disabilities, saliva sampling is a preferred method [10,12,14]. Stimulated saliva samples can be taken

by chewing on absorbent cotton rolls, paraffin or after applying citric acid under the tongue. For nonstimulated saliva samples, the passive drooling technique is regularly used.

Dried blood spot (DBS) sampling is another less invasive method. However, DBS sampling can be painful, is more complicated, and has higher failure rates than saliva sampling [15] The drug concentrations in DBS are influenced by the haematocrit value and spot volume [16] In addition, free (unbound) drug concentrations are not determinable in DBS [16], whereas salivary concentrations generally represent the free (unbound) drug concentrations [14,17].

Distribution of drugs from blood to saliva generally occurs by passive diffusion. Protein binding, negative log of acid dissociation constant (pKa), molecular mass, lipid solubility, and chemical stability in saliva are physicochemical properties of drugs that influence the salivary drug concentration. Salivary pH value, salivary flow rate, and some diseases of the oral cavity are physiological properties that determine drug penetration into saliva [12,18]. Actively stimulating saliva flow will increase the excretion of bicarbonate and therefore can influence the drug distribution and concentration in saliva [11,14].
Generally, concentrations in saliva reflect the free (unbound) drug concentration in plasma at a certain ratio [14,17]. The saliva-plasma ratio can be determined not only by calculating the mean saliva-plasma ratio of all chosen time points but also by using the area under the time-concentration curve (AUC) values of the time-concentration curves in saliva and plasma. For some anti-TB drugs saliva-plasma or saliva-serum ratios are studied, but a clear overview of the comparison of salivary to blood-based TDM for anti-TB drugs is not available.
The aim of this systematic review was to investigate whether TDM of anti-TB drugs using saliva samples is feasible, and if so to determine for which drugs it should be optimized.

MATERIALS AND METHODS

A protocol of this systematic review was registered at PROSPERO with registration number CRD42017051749 and available through www.crd.york.ac.uk/prospero/display_record.asp?ID=CRD42017051749. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was used for this review [19].

For this review, the first-line and second-line anti-TB drugs were selected from the WHO guidelines [2,3]. Ertapenem, faropenem, doripenem, ofloxacin, and clarithromycin were added to this list.

PubMed and Institute for Scientific Information (ISI) Web of Knowledge searches were performed on December 2, 2016. The keywords used for this systematic search were: (isoniazid OR rifampicin OR pyrazinamide OR ethambutol OR levofloxacin OR moxifloxacin OR gatifloxacin OR amikacin OR capreomycin OR kanamycin OR streptomycin OR ethionamide OR prothionamide OR cycloserine OR terizidone OR linezolid OR clofazimine OR bedaquiline OR delamanid OR para-aminosalicylic acid OR imipenem/cilastatin OR imipenem OR cilastatin OR meropenem OR amoxicillin/clavulanate OR amoxicillin OR clavulanate OR thiacetazone OR ertapenem OR faropenem OR doripenem OR ofloxacin OR clarithromycin) AND saliva AND (pharmacokinetics OR saliva-plasma ratio OR saliva-serum ratio OR TDM OR penetration OR distribution OR drug concentration). No limitation of publication date was used. A second reviewer checked the reproducibility of the search using the stated keywords.

After duplicate articles were removed, titles and abstracts were screened for eligibility and selected manuscripts were read by 2 independent reviewers. Exclusion factors were as follows: no human study, no anti-TB drug concentration was measured in saliva or plasma/serum, and if the manuscript was a review article. Primary references of the excluded reviews were checked and included if the study was relevant and obtainable.

Data extraction of the included articles was performed by 1 person. A reviewer independently checked the data extraction afterward. Data on study population, study design, saliva sampling method, analytical method, peak concentration (Cmax) in saliva, AUC in saliva, Cmax in plasma or serum, AUC in plasma or serum, and saliva-plasma or saliva-serum ratio were extracted from the included articles. Authors of included articles were contacted if numerical Cmax values were missing, although a time-concentration curve was stated.

If the article contained a time-concentration curve of the drug, but no numerical Cmax value was available, the Cmax was estimated using the graph. If AUC values of both saliva and plasma or serum were given, the ratio was manually calculated by dividing the salivary AUC by the plasma or serum AUC. The saliva-plasma or saliva-serum ratio was calculated (1/plasma-saliva ratio or 1/serum-saliva ratio respectively), if the

article only mentioned the plasma-saliva or serum-saliva ratio. All calculated ratios and estimated Cmax values were marked in the table.

As no validated tool for risk of bias assessment of pharmacokinetic studies is available, we used the Risk Of Bias In Non-randomised Studies - of Interventions (ROBINS-I) tool [20]. This tool was validated for nonrandomized intervention studies. Changes were made in the confounding section to make the tool more suitable for pharmacokinetic studies. The assessment was checked by a second reviewer.

RESULTS

A total of 162 records were found in the PubMed (n=108) and ISI Web of Knowledge (n=54) search (Figure 1). After duplicates were removed a number of 129 articles remained, of which 58 were classified as not relevant based on title and abstract. After full-text assessment, 30 records were excluded. One article, Ichihara *et al.* [21], was included after searching the references of the excluded review articles. Overall, 42 articles were included in this systematic review.

No articles concerning salivary pharmacokinetics of first-line anti-TB drugs ethambutol, pyrazinamide and second line anti-TB drugs levofloxacin, capreomycin, kanamycin, streptomycin, ethionamide, prothionamide, cycloserine, terizidone, clofazimine, bedaquiline, delamanid, para-aminosalicylic acid, imipenem/cilastatin, meropenem, thiacetazone, ertapenem or faropenem were found in the systematic search.
Study populations of the included articles were composed of healthy volunteers, patients with TB, children, neonates, or patients with numerous diseases and ranged from studies as few as 2 to as many as 80 participants. For each anti-TB drug, variable dosage regimes were administered, and multiple saliva sampling methods as well as several analytical methods were used (Table 1).

Figure 1. Results of searches and study selection. Using the search terms, 162 records were found, 71 of which were assessed as relevant. After full-text assessment, 30 articles were excluded. A total of 42 articles were included in this systematic review.

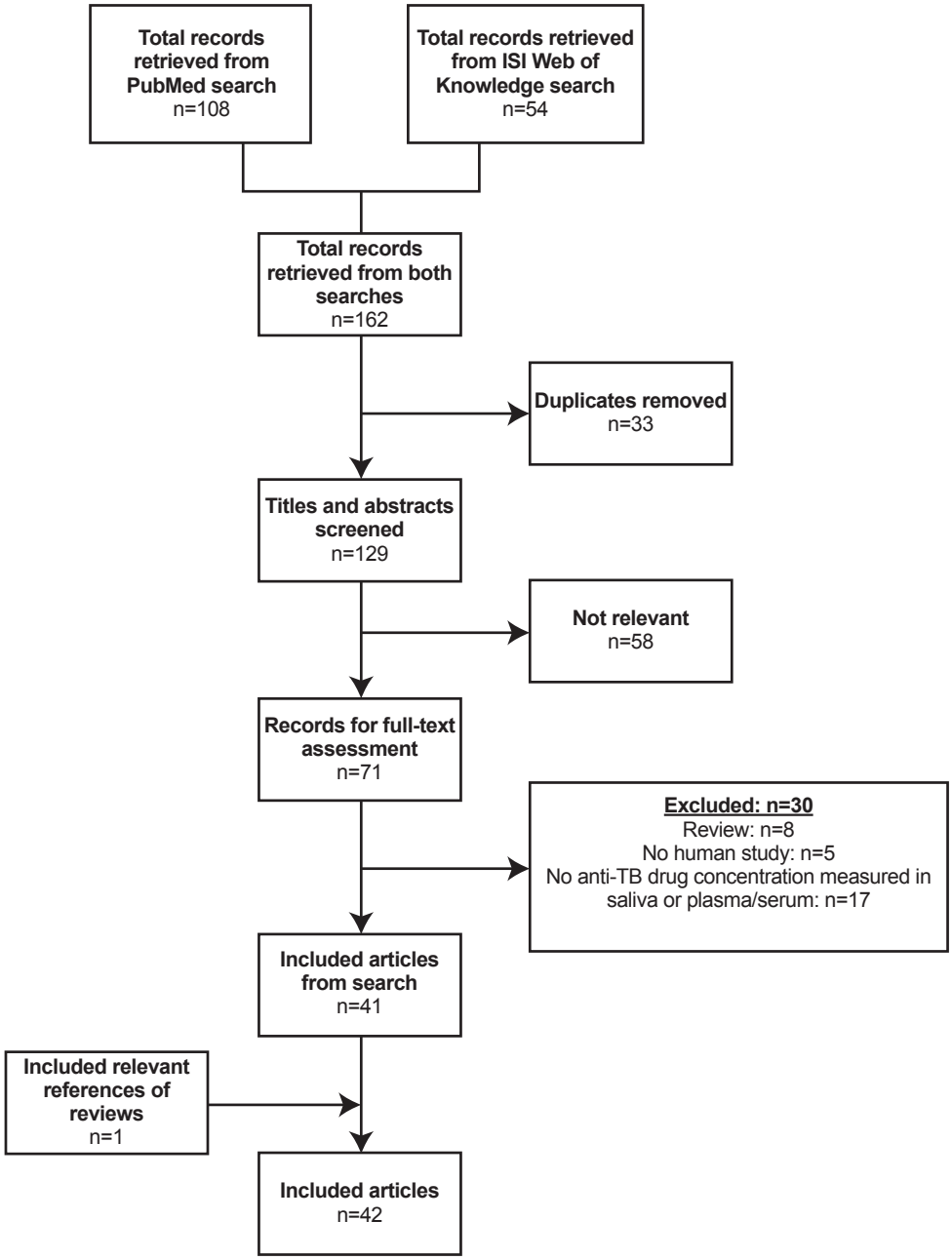


Table 1. Data of included pharmacokinetic studies comparing salivary and blood anti-TB drug peak concentrations, values of AUC, and the saliva-plasma or saliva-serum ratio in humans.

Drug	Study	Study population	Study design	Dose	Saliva sampling method	Analytical method	Saliva Cmax (µg/mL) and AUC (µg·h/mL)	Plasma or serum Cmax (µg/mL) and AUC (µg·h/mL)	Saliva-plasma or saliva-serum ratio	Characteristics of ratio
Isoniazid	Brown et al. [24]	HV; N=5	Open-label cross-over	300 mg, single dose	S; unflavoured chewing gum	UV (saliva), Ehrlich reagent and UV (plasma)	Cmax: 1.70±0.10 $AUC_{0\text{-}24h}$: 8.96±0.37 $AUC_{0\text{-}inf}$: 10.06±0.43	Plasma Cmax: 4.50±0.20 Plasma $AUC_{0\text{-}24h}$: 65.50±6.82 Plasma $AUC_{0\text{-}inf}$: 65.90±6.67	0.14 0.14● 0.15●	Conc $AUC_{0\text{-}24h}$ $AUC_{0\text{-}inf}$
	Gurumurthy et al. [31]	PTB and ITB patients; N=30	Open-label	300 mg, single dose	S; unflavoured chewing gum	UV	Cmax: Slow acetylators: 7.6 (5.4-13.2) Rapid acetylators: 6.0 (4.8-7.4) AUC: Slow acetylators: 37 (20-58) Rapid acetylators: 17 (12-22)	Serum Cmax: Slow acetylators: 7.8 (4.8-15.0) Rapid acetylators: 5.9 (4.6-8.7) Serum AUC: Slow acetylators: 39 (21-62) Rapid acetylators: 18 (11-27)	Slow acetylators: 0.95● Rapid acetylators: 0.94●	AUC
	Hutchings et al. [79]	Patients with various diseases; N=22	Open-label	200 mg, single dose	S; chewing teflon tape	HPLC-UV	Cmax: Slow acetylators: 2.5# Rapid acetylators: 2.3# AUC: ND	Plasma Cmax: Slow acetylators: 2.0# Rapid acetylators: 1.7# Plasma AUC: ND	-	-
	Suryawati et al. [40]	HV; N=8	Open-label	10 mg/kg, single dose	ND	UV	Cmax: ND $AUC_{0\text{-}inf}$: 31.88±9.57	Serum Cmax: ND Serum $AUC_{0\text{-}inf}$: 38.66±10.53	0.80±0.05 Elimination: 0.81±0.05 Absorption: 1.09±0.29	$AUC_{0\text{-}inf}$ Conc
Rifampicin	Gurumurthy et al. [31]	PTB and ITB patients; N=30	Open-label	10 mg/kg, single dose	S; unflavoured chewing gum	Plate diffusion assay with *Staphylococcus aureus*	Cmax: 0.9 AUC: ND	Serum Cmax: 8.5 Serum AUC: ND	0.07-0.13	Conc
	Orisakwe et al. [32]	HV; N=5	Open-label cross-over	600 mg, single dose	S; chewing gum	UV	Cmax: 12.8±0.33 $AUC_{0\text{-}24h}$: 63.6±1.4 $AUC_{0\text{-}inf}$: 68.1±1.8	Plasma Cmax: 17.8±1.04 Plasma $AUC_{0\text{-}24h}$: 95.5±2.2 Plasma $AUC_{0\text{-}inf}$: 103.6±3.6	0.67● 0.66●	$AUC_{0\text{-}24h}$ $AUC_{0\text{-}inf}$
	Ezejiofor et al. [30]	HV; N=5	Open-label cross-over	600 mg, single dose	S; unflavoured chewing gum	UV	Cmax: 9.00±0.70 $AUC_{0\text{-}24h}$: 68.85±5.48 $AUC_{0\text{-}inf}$: 72.18±8.18	Plasma Cmax: 16.00±2.12 Plasma $AUC_{0\text{-}24h}$: 485.60±62.57 Plasma $AUC_{0\text{-}inf}$: 505.60±77.13	0.15 0.14● 0.14●	Conc $AUC_{0\text{-}24h}$ $AUC_{0\text{-}inf}$

Drug	Study	Study population	Study design	Dose	Saliva sampling method	Analytical method	Saliva Cmax (µg/mL) and AUC (µg·h/mL)	Plasma or serum Cmax (µg/mL) and AUC (µg·h/mL)	Saliva-plasma or saliva-serum ratio	Characteristics of ratio
Rifampicin	Darouiche et al. [29]	HV; N=5	Open-label	600 mg, for 4 days	ND	HPLC-UV	Cmax: ND Highest measured conc at 2 h: 0.42±0.12	Serum Cmax: ND Highest measured serum conc at 5 h: 10.65±4.55	-	-
							AUC: ND	Serum AUC: ND		
	McCracken et al. [80]	Children (6-58 months old) with impetigo or cellulitis; N=38	Open-label	10 mg/kg, single dose	Capillary pipettes	Agar disk diffusion micro-method with *Sarcina lutea*	Cmax: ND Median conc at t=2 h: Suspension: 1.7 (0.54-7.2) Suspension in apple sauce: 1.6 (0.48-4.0) Powder in applesauce: 2.4 (0.85-3.8)	Serum Cmax: ND Highest measured serum conc at 1 h: Suspension: 10.7±0.81 Suspension in applesauce: 8.9±1.29 Powder in applesauce: 11.5±2.3	-	-
							AUC: ND	Serum AUC: Suspension: 56 Suspension in applesauce: 38 Powder in applesauce: 57		
	Murthy et al. [28]	PTB patients; N=20	Open-label	450/600 mg, single dose	Wide, capped bottle	RP-HPLC-EC	Cmax: 450 mg: 0.84±0.21 600 mg: 1.23±0.17	Serum Cmax: ND Highest measured serum conc at t=3 h: 450 mg: 7.99±1.98 600 mg: 12.18±1.92	600 mg: 0.1 450 mg: 0.11-0.31	Conc
							AUC: 450 mg: 10.59±4.36 600 mg: 15.13±2.81	Serum AUC: ND		
	Orisakwe et al. [33]	Male HV; N=6	Open-label	600 mg, single dose	ND	UV	Cmax: 11.6±4.9	Plasma Cmax: 17.8±5.1	0.53● 0.52●	AUC_{0-24h} AUC_{0-inf}
							AUC_{0-24h}: 49.68±9 AUC_{0-inf}: 50.01±11	Plasma AUC_{0-24h}: 94.15±18 Plasma AUC_{0-inf}: 96.76±12		
Moxifloxacin	Burkhardt et al. [38]	Male, Caucasian HV; N=12	Double-blind, randomised cross-over	400 mg, for 7 days	S; Salivette	HPLC-Fluor	Cmax: Day 1:3.6# Day 7: 4.8#	Serum Cmax: Day 1: 3.10±0.60 Day 7: 3.98±1.10	t>2 h: 0.8	Conc
							AUC: ND	Serum AUC_{0-12h}: Day 1: 28.2±4.1 Day 7: 39.5±6.6 Serum AUC_{0-inf}: Day 1: 35.6±6.5		
	Müller et al. [37]	Male HV; N=13	Randomised, open-label cross-over	400 mg, single dose p.o and i.v.	S; Salivette	HPLC-Fluor	Cmax: p.o.: 3.6±1.0 i.v.: 5.1±1.4	Plasma Cmax: p.o.: 3.2±0.6 i.v.: 3.7±0.7	0.83±0.20 p.o.: 0.88● i.v.: 0.93●	AUC_{0-12h} AUC_{0-12h}

Table 1. Continued.

Drug	Study	Study population	Study design	Dose	Saliva sampling method	Analytical method	Saliva Cmax (µg/mL) and AUC (µg·h/mL)	Plasma or serum Cmax (µg/mL) and AUC (µg·h/mL)	Saliva-plasma or saliva-serum ratio	Characteristics of ratio
Moxifloxacin				(during 60 min)			AUC_{0-12h}: p.o.: 17.6±2.7 i.v.: 21.4±5.0	Plasma AUC_{0-12h}: p.o.: 19.8±1.5 i.v.: 22.9±11.1		
	Stass et al. [36]	Male, Caucasian HV; N=39	Double-blind, randomised cross-over and group comparison	50-800 mg, single dose	S; chew on cotton roll	HPLC-Fluor	Cmax: 50 mg: 0.31±1.55 100 mg: 0.84±1.74 200 mg: 1.62±1.44	Plasma Cmax: 50 mg: 0.29±1.25 100 mg: 0.59±1.21 200 mg: 1.16±1.35 400 mg: 2.50±1.31 600 mg: 3.19±1.19 800 mg: 4.73±1.16	50 mg: 0.72● 100 mg: 0.97● 200 mg: 0.91●	AUC_{0-inf}
							AUC_{0-inf}: 50 mg: 2.81±1.40 100 mg: 8.27±1.54 200 mg: 14.0±1.29	Plasma AUC_{0-inf}: 50 mg: 3.88±1.13 100 mg: 8.51±1.21 200 mg: 15.4±1.20 400 mg: 26.9±1.18 600 mg: 39.9±1.11 800 mg: 59.9±1.24		
	Burkhardt et al. [35]	Male patients with SCI and decubitus ulcer; N=4	Open-label	400 mg, single dose	S; Salivette	HPLC-Fluor	Cmax: 1.4±0.4	Serum Cmax: 4.4±2.7	0.45 0.31●	Conc AUC_{0-8h}
							AUC_{0-8h}: 4.7±3.0	Serum AUC_{0-8h}: 15.0±9.7		
	Kumar et al. [34]	HV; N=24	Open-label	400 mg, single dose	S; unflavoured chewing gum	RP-HPLC-Fluor	Cmax : ND	Plasma Cmax : ND	0.54	Conc
							AUC : ND	Plasma AUC : ND		
Ofloxacin	Kozjek et al. [44]	Male HV; N=6	Randomised parallel group	400 mg, single dose	NS	RP-HPLC-Fluor	Cmax: 1.71±0.44	Plasma Cmax: 3.66±0.72	0.43±0.02 0.36±0.07 0.455	Conc AUC Corr
							AUC: 6.41±1.08	Plasma AUC: 18.22±2.52		
	Koizumi et al. [41]	Patients with chronic respiratory tract infections; N=18	Open-label	300 mg, single dose	Sterile glass dishes	RP-HPLC-Fluor	Cmax: 4.53±0.75	Serum Cmax: 4.25±0.41	T=0-4 h: <1 T=4-8 h: increases from <1 to >1 T=8-16 h: >1 T=16 h: 1.14±0.11 1.22●	Conc AUC
							AUC: 63.0±8.9	Serum AUC: 51.5±5.7		
	Warlich et al. [45]	HV; N=6	Open-label	200 mg b.i.d., for 3 days	S; chewing parafilm	RP-HPLC-Fluor	Cmax: 2.07±0.38	Serum Cmax: 2.96±0.30	0.61±0.03 0.606	Conc AUC_{0-12h}
							AUC_{0-12h}: 10.8±0.8	Serum AUC_{0-12h}: 17.8±0.5		

Drug	Study	Study population	Study design	Dose	Saliva sampling method	Analytical method	Saliva Cmax (µg/mL) and AUC (µg·h/mL)	Plasma or serum Cmax (µg/mL) and AUC (µg·h/mL)	Saliva-plasma or saliva-serum ratio	Characteristics of ratio
Ofloxacin	Leigh et al. [46]	HV; N=11	Open-label	200 mg b.i.d., for 3.5 days	NS	Micro-biological assay with *Bacillus subtilis*	Cmax: 1st dose: 1.9±0.7 7th dose: 2.6±0.7	Serum Cmax: 1st dose: 2.7±0.7 7th dose: 3.4±0.5	0.78 1st dose: 0.64● 7th dose: 0.74●	Corr AUC_{0-8h} AUC_{0-inf}
							AUC_{0-8h}: 1st dose:8.9±3.1 7th dose:12.9±4.5 AUC_{0-inf}: 1st dose:14.8±5.0 7th dose: 20.7±8.5	Serum AUC_{0-8h}: 1st dose: 13.9±3 7th dose: 17.5±3.6 Serum AUC_{0-inf}: 1st dose: 23.0±5.3 7th dose: 28.2±7.4	1st dose: 0.64● 7th dose: 0.73●	
	Immanuel et al. [47]	Male HV; N=7	Open-label	600/800 mg, single dose	S; unflavoured chewing gum	RP-HPLC-Fluor	Cmax: 600 mg: 4.1 800 mg: 4.2	Plasma Cmax: 600 mg: 8.0 (7.4-8.6) 800 mg: 9.8 (8.2-11.4)	600 mg: 0.40-0.57 800 mg: 0.40-0.56	Conc
							AUC_{0-24h}: 600 mg: 29.7 800 mg: 40.2	Plasma AUC_{0-24h}: 600 mg: 60.8 (54.2–67.4) 800 mg: 85.3 (69.4–101.2) Plasma AUC_{0-inf}: 600 mg: 67.9 (60.9–74.9) 800 mg: 93.1 (79.7–106.5)	600 mg: 0.49● 800 mg: 0.47●	AUC_{0-24h}
	Miya et al. [81]	PTB or NSCLC patients; N=12	Open-label	200 mg t.i.d., for at least 7 days	ND	HPLC-Fluor	Cmax: ND Conc at day 3, t=2 h: 3.36±2.23	Serum Cmax: ND Serum conc at day 3, t=2 h: 3.15±1.52	-	-
							AUC: ND	Serum AUC: ND		
	Ohkubo et al. [27]	Male HV; N=4	Open-label	100/200 mg, single dose	S; chewing parafilm	HPLC-UV	Cmax: 100 mg: 0.5133-0.7333 200 mg: 0.9442-2.0530	Serum Cmax: 100 mg: 0.7682-1.1785 200 mg: 1.8792-3.0890	0.508 100 mg: 0.42-0.71 200 mg: 0.40-0.63	Corr AUC_{0-6h}
							AUC_{0-6h}: 100 mg: 1.7368-2.4653 200 mg: 3.8850-6.5199	Serum AUC_{0-6h}: 100 mg: 2.8755-4.6179 200 mg: 7.0148-10.0860		
	Fujita et al. [25]	Patients with infections or antibiotic prophylaxis and HV; N=80	Open-label	100 mg alt. d.– 200 mg t.i.d., (depending on renal function), for 5 days	ND	Paper disk method with *Bacillus subtilis* and *Escherichia coli*	Cmax: ND	Serum Cmax: ND	0.9969	Corr
							AUC: ND	Serum AUC: ND		

Table 1. Continued.

Drug	Study	Study population	Study design	Dose	Saliva sampling method	Analytical method	Saliva Cmax (µg/mL) and AUC (µg·h/mL)	Plasma or serum Cmax (µg/mL) and AUC (µg·h/mL)	Saliva-plasma or saliva-serum ratio	Characteristics of ratio
Ofloxacin	Edlund et al. [48]	Gastric surgery patients; N=20	Open-label	400 mg, single dose	Sterile glass tubes	Agar-well diffusion method with *Escherichia coli*	No Cmax Detected in 40% of samples of day 2 Conc: 0.1-0.7	Serum Cmax: 3.6±1.7	-	-
							AUC: ND	Serum $AUC_{0\text{-}inf}$: 47.3±28.3		
	Ichihara et al. [21]	Male HV; N=19	Open-label	100/300/600 mg single dose	ND	RP-HPLC-UV (serum), paper disk-plate method with *Bacillus subtilis* or *Escherichia coli* (serum and saliva)	Cmax: ND Highest measured conc of single doses: 100 mg: 0.77±0.17 at 2 h 300 mg: 2.51±0.24 at 2 h 300 mg fasting: 3.02±1.20 at 1 h 600 mg: 4.44±0.79 at 3 h	Serum Cmax of single doses: 100 mg: 0.95±0.17 300 mg: 2.65±0.41 300 mg fasting: 3.86±0.85 600 mg: 6.64±0.76	0.655	Corr
							AUC: ND	Serum $AUC_{0\text{-}24h}$ of single doses: 100 mg: 6.02±1.05 300 mg: 21.70±2.63 300 mg fasting: 29.38±4.74 600 mg: 68.40±7.61		
	Tsubakihara et al. [49]	Patients with renal failure; N=12 (6 HD, 6 non-HD)	Open-label	100 mg, single dose	ND	Paper disk method with *Bacillus subtilis* and *Escherichia coli*	Cmax: Non-HD: 1.32 HD: ND	Serum Cmax: Non-HD: 1.68 HD: ND	Non-HD: 0.75 HD: 1.07 Non-HD: 0.61●	Corr AUC
							AUC: Non-HD: 64.29 HD: ND	Serum AUC: Non-HD: 105.23 HD: ND		

Drug	Study	Study population	Study design	Dose	Saliva sampling method	Analytical method	Saliva Cmax (µg/mL) and AUC (µg·h/mL)	Plasma or serum Cmax (µg/mL) and AUC (µg·h/mL)	Saliva-plasma or saliva-serum ratio	Characteristics of ratio
Gatifloxacin	Nakashima et al. [53]	Male, Asian HV; N=30	Open-label	100/200/400/600 mg, single dose 300 mg b.i.d., for 6.5 days	NS	RP-HPLC-Fluor	Cmax: 200 mg: 1.55±0.51 400 mg: 3.05±0.74	Serum Cmax: 100 mg: 0.873±0.187 200 mg: 1.71±0.35 400 mg: 3.35±0.55 600 mg: 5.41±1.13 Serum 300 mg b.i.d.: Day 1: 2.77±0.54 Day 4: 3.45±0.63 Day 7: 3.36±0.46	0.81	Corr
							AUC: ND	Serum $AUC_{0\text{-}inf}$: 100 mg: 7.00±1.36 200 mg: 14.5±2.6 400 mg: 32.4±4.1 600 mg: 53.5±2.6		
	Mignot et al. [54]	Male, Caucasian HV; N=36	Double-blind, randomised, placebo controlled	400/600 mg, single dose and for 10 days	NS	HPLC-Fluor	Cmax: 400 mg, day 1: 3.2# 600 mg, day 1: 7.0#	Plasma Cmax: 400 mg: Day 1: 3.682±0.75 Day 15: 4.226±1.283 600 mg: Day 1: 5.266±1.237 Day 15: 5.811±1.043	About 1	Conc
							AUC: ND	Plasma $AUC_{0\text{-}inf}$: 400 mg day 1:30.871±4.390 600 mg day 1: 51.728±7.625 Plasma $AUC_{0\text{-}24h}$: 400 mg day 15: 34.409±5.740 600 mg day 15: 61.763±10.198		
Amikacin	Masumi et al. [39]	Neonates (2- and 12-days old); N=2	Open-label	3.0-6.0 mg/kg i.v.	ND	Paper disk method with *Bacillus subtilis*	Cmax: ND	Serum Cmax: ND	-	-
							AUC: ND	Serum AUC: ND		
	Biasini et al. [23]	Children with CF and	Open-label	10 mg/kg i.v. injection	ND	ND	Cmax: ND	Serum Cmax: ND	-	-

Table 1. Continued.

Drug	Study	Study population	Study design	Dose	Saliva sampling method	Analytical method	Saliva Cmax (µg/mL) and AUC (µg·h/mL)	Plasma or serum Cmax (µg/mL) and AUC (µg·h/mL)	Saliva-plasma or saliva-serum ratio	Characteristics of ratio
Amikacin		pneumonia; N= ND					AUC: ND	Serum AUC: ND		
Linezolid	Bolhuis et al. [51]	MDR-TB patients (5 African, 1 Caucasian, 1 Asian); N=7	Open-label	300 mg b.i.d. at steady state	S; Salivette	HPLC-MS/MS	Cmax: 10.1 (8.2-10.7)	Serum Cmax: 10.9 (6.8-11.5)	0.97	Conc serum-saliva
							AUC_{0-12h}: 62.1 (50.5–89.2)	Serum AUC_{0-12h}: 63.9 (47.8–83.8)	1.03•	Conc saliva-serum
									0.97•	AUC_{0-12h}
									1.05	Corr serum-saliva
									0.95•	Corr saliva-serum
	Hara et al. [82]	HV; N=4	Open-label	600 mg, single dose	S; Salivette	HPLC-UV	Cmax: ND Highest measured mean conc at t=3 h: 7.1-17.0	Plasma Cmax: ND Highest measured mean plasma conc at t=3 h: 10.4-14.1	-	-
							AUC: ND	Plasma AUC: ND		
Amoxicillin/ clavulanate	Goddard et al. [26]	Male HV; N=8	Double-blind, randomised, placebo-controlled cross-over	750 mg (amoxicillin), for 5 days	ND	Bioassay with *Sarcina lutea*	Not detected	Plasma Cmax: 14.56 (11.03-18.1)	-	-
							AUC: ND	Plasma AUC_{0-4h}: 24.4 (21.1–27.6) Plasma AUC_{0-inf}: 25.9 (21.8–30.1)		
	Ortiz et al. [62]	HV; N=26	Open-label, randomised, cross-over	500 mg (amoxicillin), single dose	ND	RP-HPLC-UV	Not detected	Plasma Cmax: H. Pylori -: 51.9 (29.0-74.8) H. Pylori +: 41.7 (23.3-60.0)	-	-
							AUC: ND	Plasma AUC_{0-2h}: H. Pylori -: 1587.7 (1208.2-1967.2) H. Pylori + : 1203.3 (989.3-1417.3) Plasma AUC_{0-inf}: H. Pylori -: 1755.1 (1394.0-2116.2) H. Pylori +: 1358.4 (1135.4-1581.4)		

Drug	Study	Study population	Study design	Dose	Saliva sampling method	Analytical method	Saliva Cmax (µg/mL) and AUC (µg·h/mL)	Plasma or serum Cmax (µg/mL) and AUC (µg·h/mL)	Saliva-plasma or saliva-serum ratio	Characteristics of ratio
Amoxicillin/ clavulanate	Ginsburg et al. [61]	Children (4-54 months old) with AOM; N=24	Open-label, cross-over	15 and 25 mg/kg (amoxicillin), single dose	Capillary pipettes	Micro-method with *Sarcina lutea*	Cmax: ND Highest measured conc at t=2h: 15 mg/kg: 0.3 (0-0.36) Detected in 50% of samples 25 mg/kg: 0.17 (0-0.4) Detected in 70% of samples	Serum Cmax: ND Highest measured serum conc at t=1 h: 15 mg/kg: Fasting: 5.4±0.76 Fed: 3.2±0.48 25 mg/kg: Fasting: 8.9±1.4 Fed: 7.9±1.7	-	-
							AUC: ND	Serum AUC: 15 mg/kg, fasting: 16 15 mg/kg, fed: 14 25 mg/kg, fasting: 24 25 mg/kg, fed: 24		
	Baglie et al. [22]	HV; N=20	Open-label, randomized cross-over	875 mg (amoxicillin), single dose	NS; sterile glass tubes	RP-LC-ESI-MS (plasma), RP-HPLC-UV (saliva)	Cmax: Amoxil®: 6.37±3.63 Amoxicillin EMS®: 6.23±4.89	Plasma Cmax: Amoxil®: 14.37±6.01 Amoxicillin EMS®: 16.94±6.39	Amoxil®: 0.47● Amoxicillin EMS®: 0.34●	$AUC_{0\text{-}8h}$
							$AUC_{0\text{-}8h}$: Amoxil®: 22.83±13.92 Amoxicillin EMS®: 18.78±14.62 $AUC_{0\text{-}inf}$: Amoxil®: 26.29±14.27 Amoxicillin EMS®: 18.50±15.06	Plasma $AUC_{0\text{-}8h}$: Amoxil®: 48.28±20.00 Amoxicillin EMS®: 55.10±14.25 Plasma $AUC_{0\text{-}inf}$: Amoxil®: 47.62±18.42 Amoxicillin EMS®: 54.14±12.38	Amoxil®: 0.55● Amoxicillin EMS®: 0.34●	$AUC_{0\text{-}inf}$
	Wüst et al. [60]	HV; N=10	Open-label	750 mg (amoxicillin), single dose	ND	Agar diffusion method with *Bacilllus subtilis*	Cmax : ND Conc at est Tmax (2 h): 0.03±0.01	Serum Cmax : ND Serum conc at est Tmax (2 h): 7.16±2.53	-	-
							AUC: ND	Serum AUC: ND		
Doripenem	Burian et al. [59]	Male HV; N=6	Open-label	500 mg i.v. in 1 h, single dose	ND	UHPLC-MS/MS	Cmax: 0.5±0.2	Plasma Cmax: 15.3±6.0	0.04±0.03 0.03●	$AUC_{0\text{-}inf}$ $AUC_{0\text{-}8h}$
							$AUC_{0\text{-}8h}$: 0.9±0.5 $AUC_{0\text{-}inf}$: 1.0±0.5	Plasma $AUC_{0\text{-}8h}$: 26.0±9.9 Plasma $AUC_{0\text{-}inf}$: 26.3±10.1		
Clarithro-mycin	Fassbender et al. [83]	HV; N=10	Randomised, cross-over	500 mg b.i.d., for 3 days	S; chewing on cotton roll	RP-HPLC-coulometric detection	Cmax at steady state: Day 3: 1.9# Highest measured conc: Day 1 at 4 h: 1.06±0.7 Day 3 at 4 h: 1.87±1.3	Serum Cmax: Day 1: 2.1±0.7 Day 3: 2.3±1.0	-	-

Table 1. Continued.

Drug	Study	Study population	Study design	Dose	Saliva sampling method	Analytical method	Saliva Cmax (µg/mL) and AUC (µg·h/mL)	Plasma or serum Cmax (µg/mL) and AUC (µg·h/mL)	Saliva-plasma or saliva-serum ratio	Characteristics of ratio
Clarithromycin							AUC: ND	Serum $AUC_{0\text{-}inf}$: Day 1: 15.3±4.8 Day 3: 27.9±12.4		
	Kees et al. [50]	Male HV; N=12	Open-label, randomised, cross-over	500 mg q.d./250 mg b.i.d., for 5 days	NS; dental tampon	HPLC-EC	Cmax: 500 mg q.d.: Day 1: 0.89±0.32 Day 5: 1.06±0.38 250 mg b.i.d.: Day 1: 0.31±0.15 Day 5: 0.29±0.07	Serum Cmax: 500 mg q.d.: Day 1: 2.10±0.49 Day 5: 2.33±0.58 250 mg b.i.d.: Day 1: 0.94±0.33 Day 5: 1.23±0.37	0.25-0.40	Conc
							AUC: ND	Serum $AUC_{0\text{-}12h}$: 250 mg b.i.d., day 1: 5.21±1.31 Serum $AUC_{0\text{-}inf}$: 500 mg q.d., day 1: 15.63±4.46 250 mg b.i.d., day 1: 5.80±1.31 Serum AUC_{ss}: 500 mg q.d., day 5: 18.32±4.77 250 mg b.i.d., day 5: 7.85±2.00		
	Burkhardt et al. [38]	Male, Causasian HV; N=12	Double-blind, randomised, cross-over	500 mg b.i.d., for 7 days	S; Salivette	HPLC-EC	Cmax: Day 1: 0.9# Day 7: 1.6#	Serum Cmax: Day 1: 1.76±0.51 Day 7: 2.41±0.81	Around 0.5	Conc
							AUC: ND	Serum $AUC_{0\text{-}12h}$: Day 1: 10.6±2.51 Day 7: 18.0±5.0 $AUC_{0\text{-}inf}$: Day 1: 12.6±3.34		
	Bolhuis et al. [51]	MDR-TB patients (5 African, 1 Caucasian, 1 Asian); N=7	Open-label	250 mg at steady state	S; Salivette	HPLC-MS/MS	Cmax: 2.8 (2.0-3.4)	Serum Cmax: 1.7 (1.3-2.7)	3.07	Conc serum-saliva
							$AUC_{0\text{-}12h}$: 10.7 (9.4–12.1)	Serum $AUC_{0\text{-}12h}$: 8.2 (6.2–12.2)	0.33●	Conc saliva-serum
									1.30●	$AUC_{0\text{-}12h}$
									2.67	Corr serum-saliva
									0.37●	Corr saliva-serum

Drug	Study	Study population	Study design	Dose	Saliva sampling method	Analytical method	Saliva Cmax (µg/mL) and AUC (µg·h/mL)	Plasma or serum Cmax (µg/mL) and AUC (µg·h/mL)	Saliva-plasma or saliva-serum ratio	Characteristics of ratio
Clarithro-mycin	Goddard et al. [26]	Male HV; N=8	Double-blind, randomised, placebo-controlled, cross-over	500 mg, for 5 days	ND	Bioassay with *Sarcina lutea*	Cmax: 3.87 (3.03-4.72)	Plasma Cmax: 5.39 (4.54-6.23)	0.75●	AUC_{0-4h}
							AUC_{0-4h}: 9.48 (7.56–11.41)	Plasma AUC_{0-4h}: 12.7 (11.5–13.9) Plasma AUC_{0-inf}: 29.5 (20.2–38.8)		
	Edlund et al. [52]	HV; N=10	Double-blind, randomised	500 mg b.i.d., for 10 days	NS; glass tubes	Agar plate diffusion method with *Bacillus subtilis*	Cmax: Day 1: 2.38 (0.78-4.58) Day 10: 4.29 (2.67-7.39)	Plasma Cmax: Day 1: 2.98 (1.74-4.94) Day 10: 3.87 (2.23-7.41)	Day 1: 0.73● Day 10: 0.99●	AUC_{0-10h}
							AUC_{0-10h}: Day 1: 13.3 (5.2-28.4) Day 10: 27.4 (20.2-35.9)	Plasma AUC_{0-10h}: Day 1: 18.1 (9.8-27.8) Day 10: 27.8 (18.8-42.8)		
	Wüst et al. [60]	HV; N=10	Open-label	500 mg, single dose	ND	Agar diffusion method with *Micrococcus luteus*	Cmax: ND Conc at estimated Tmax (2 h): 2.72±0.87	Serum Cmax: ND Serum conc at estimated Tmax (2 h): 4.04±1.14	-	-
							AUC: ND	Serum AUC: ND		
	Morihana et al. [84]	Male HV; N=3	Open-label	300 mg, single dose	NS	Paper disk method with *Micrococcus luteus*	Cmax: 1.93457	Serum Cmax: 1.48624	0.95●	AUC
							AUC: 17.7031	Serum AUC: 18.584		

*The legend of the graph in the article referred to the upper curve as a result of a 400-mg dose. We assumed this was a mistake; therefore the Cmax values of 400 mg and 600 mg are exchanged. Authors of the article were contacted, but did not respond.

estimated value

● calculated value

alt. d., every other day; AOM, acute otitis media; AUC, area under the time-concentration curve; b.i.d., twice a day; Cmax, peak concentration; conc, concentration; corr, slope of correlation of saliva and plasma or serum; EC, electro-chemical; fluor, fluorescence; HD, haemodialysis; HPLC, high-performance liquid chromatography; HV, healthy volunteers; ITB, intestinal TB; i.v., intravenous; ND, not defined; NS, non-stimulated; NSCLC, non-small cell lung cancer; p.o., per oral; PTB, pulmonary TB; q.d., once a day; RP, reversed phase; S, stimulated; SCI, spinal cord injury; SP, spectrophotometry; t.i.d., three times a day; Tmax, time of peak concentration; UV, ultraviolet-visible spectrophotometry.

All included articles were assessed for risk of bias. Baglie *et al.* [22], Biasini *et al*. [23], Brown *et al.* [24], Fujita *et al*. [25], Goddard *et al*. [26] and Ohkubo *et al*. [27] were considered at a serious risk of bias (Table 2). This means that the studies have some serious problems with bias for a nonrandomized study [20]. Baglie *et al.* [22] and Brown *et al.* [24] both used different analytical methods for saliva and plasma. This could have introduced bias in the measurement of outcomes. Fujita *et al.* [25] and Biasini *et al.* [23] were judged at a serious risk of bias because important information, for instance, the sampling or analytical procedure, was scarcely described. Fujita *et al.* [25] did not mention any validation of the analytical method, whereas Biasini *et al.* [23] provided too little information about the analytical procedures to estimate the risk of bias. Goddard *et al.* [26] did not use paired sampling for all time points. Ohkubo *et al.* [27] sampled saliva after tooth brushing. This could have contaminated the samples with blood. All other studies were estimated at a moderate risk of bias, meaning the study provides evidence for a nonrandomized study but is not comparable with a well-performed randomized trial [20].

Table 2. Results of risk of bias assessment of included articles using Risk Of Bias in Nonrandomized Studies of Interventions (ROBINS-I) tool.

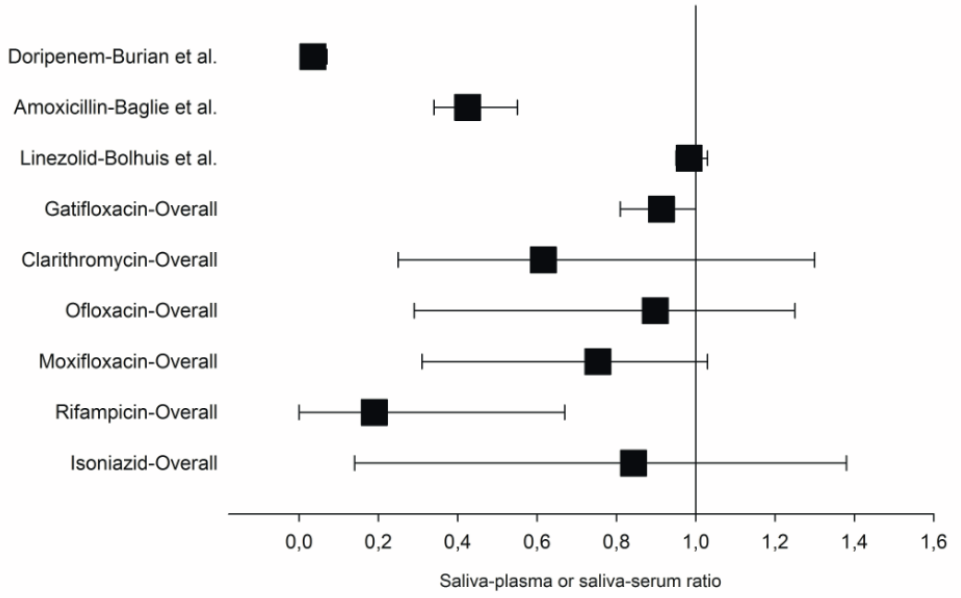
Study	Confounding	Selection of participants	Classification of interventions	Deviations from interventions	Missing data	Measurement of outcomes	Selection of reported result	Overall
Baglie et al.	+	+	+	+	+	-	+/-	-
Biasini et al.	-	+	+	+	-	?	+/-	-
Bolhuis et al.	+	+	+	+	+	+	+/-	+/-
Brown et al.	+	+	+	+	+	-	+/-	-
Burian et al.	+	+	+	+	+	+	+/-	+/-
Burkhardt et al. 2006	+	+	+	+	+	+	+/-	+/-
Burkhardt et al. 2002	+	+	+	+	+	+	+/-	+/-
Darouiche et al.	+	+	+	+	+	+	+/-	+/-
Edlund et al. 2000	+	+	+	+	+	+	+/-	+/-
Edlund et al. 1998	+	+	+	+	+	+	+/-	+/-
Ezejiofor et al.	+	+	+	+	+	+	+/-	+/-
Fassbender et al.	+	+	+	+	+	+	+/-	+/-
Fujita et al.	-	+	+	+	+	+	+/-	-
Ginsburg et al.	+	+	+	+	+	+	+/-	+/-
Goddard et al.	-	+	+	+	+	+	+/-	-
Gurumurthy et al.	+	+	+	+	+	+	+/-	+/-
Hara et al.	+	+	+	+	+	+	+/-	+/-

Table 2. Continued

Study	Confounding	Selection of participants	Classification of interventions	Deviations from interventions	Missing data	Measurement of outcomes	Selection of reported result	Overall
Hutchings et al.	+	+	+	+	+	+	+/-	+/-
Ichihara et al.	+	+	+	+	+/-	+	+/-	+/-
Immanuel et al.	+	+	+	+	+	+	+/-	+/-
Kees et al.	+	+	+	+	+	+	+/-	+/-
Koizumi et al.	+	+	+	+	+	+	+/-	+/-
Kozjek et al.	+	+	+	+	+	+	+/-	+/-
Kumar et al.	+	+	+	+	+	+	+/-	+/-
Leigh et al.	+	+	+	+	+	+	+/-	+/-
Masumi et al.	+	+	+	+	+	+	+/-	+/-
McCracken et al.	+	+	+	+	+	+	+/-	+/-
Mignot et al.	+	+	+	+	+	+	+/-	+/-
Miya et al.	+	+	+	+	+	+	+/-	+/-
Morihana et al.	+	+	+	+	+	+	+/-	+/-
Müller et al.	+	+	+	+	+	+	+/-	+/-
Murthy et al.	+	+	+	+	+	+	+/-	+/-
Nakashima et al.	+	+	+	+	+	+	+/-	+/-
Ohkubo et al.	-	+	+	+	+	+	+/-	-
Orisakwe et al. 2004	+	+	+	+	+	+	+/-	+/-
Orisakwe et al. 1996	+	+	+	+	+	+	+/-	+/-
Ortiz et al.	+	+	+	+	+	+	+/-	+/-
Stass et al.	+	+	+	+	+	+	+/-	+/-
Suryawati et al.	+	+	+	+	+	+	+/-	+/-
Tsubakihara et al.	+	+	+	+	+	+	+/-	+/-
Warlich et al.	+	+	+	+	+	+	+/-	+/-
Wüst et al.	+	+	+	+	+	+	+/-	+/-

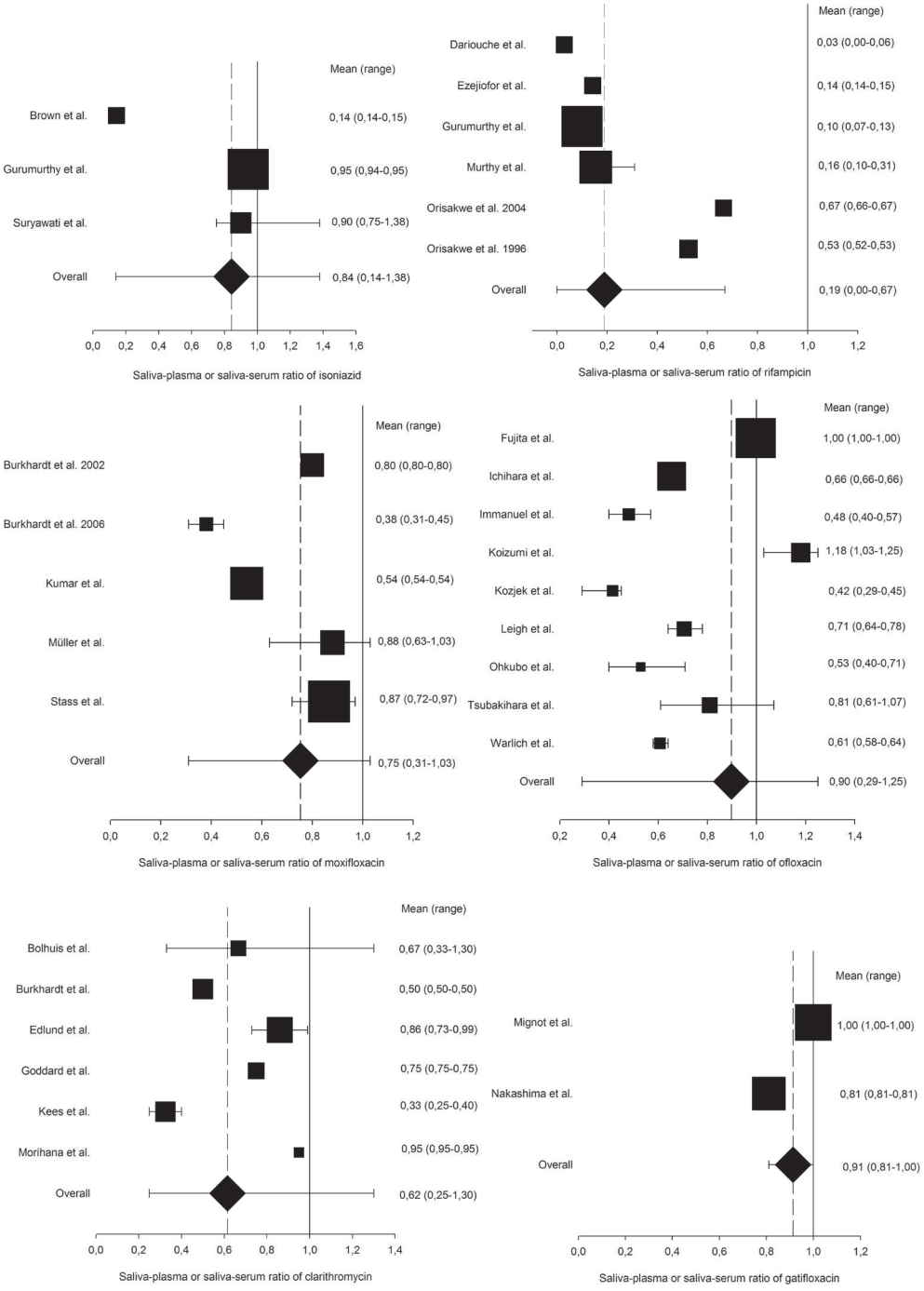
Low risk of bias (+), moderate risk of bias (+/-), serious risk of bias (-), and no information (?).

Figure 2. Saliva-plasma or saliva-serum ratio of anti-TB drugs. The weighted mean and range of saliva-plasma or saliva-serum ratio was displayed per drug. Mean (range) of doripenem: 0.04 (0.01-0.07), amoxicillin: 0.43 (0.34-0.55), linezolid: 0.98 (0.95-1.03), gatifloxacin: 0.91 (0.81-1.00), clarithromycin: 0.62 (0.25-1.30), ofloxacin: 0.90 (0.29-1.25), moxifloxacin: 0.75 (0.31-1.03), rifampicin: 0.19 (0.00-0.67) and isoniazid: 0.84 (0.14-1.38). For doripenem, amoxicillin and linezolid, only 1 study with a saliva-plasma or saliva-serum ratio was included. For the other drugs, the numbers of included studies were as follows: gatifloxacin (n=2), clarithromycin (n=6), ofloxacin (n=9), moxifloxacin (n=5), rifampicin (n=6), and isoniazid (n=3).



In general, a large variability in saliva-plasma and saliva-serum was observed for isoniazid, rifampicin, moxifloxacin, ofloxacin, and clarithromycin (Figures 2 and 3). The saliva-plasma and saliva-serum ratios of rifampicin clustered in 2 groups: Murthy and Kumar [28], Darouiche *et al.* [29], Ezejiofor *et al.* [30], and Gurumurthy *et al.* [31] with ratios of 0.1-0.2, in contrast to Orisakwe *et al.* [32] and Orisakwe and Ofoefule[33] with ratios around 0.6. A similar clustering effect was seen with moxifloxacin. Kumar *et al.* [34] and Burkhardt *et al.* [35] reported saliva-plasma and saliva-serum ratios of 0.4-0.6, whereas Stass *et al.* [36], Müller *et al.* [37], and Burkhardt *et al.* [38] found ratios of 0.8-0.9. Isoniazid, ofloxacin, and clarithromycin showed an overall large diversity of reported saliva-plasma and saliva-serum ratios. For gatifloxacin, linezolid, and doripenem relatively small ranges of saliva-plasma and saliva-serum ratios were found.

Figure 3. Saliva-plasma or saliva-serum ratios of anti-TB drugs. Top left: isoniazid, top right: rifampicin, middle left: moxifloxacin, middle right: ofloxacin, bottom left: clarithromycin, and bottom right: gatifloxacin. As per drug, the saliva-plasma or saliva-serum ratios of the included articles were displayed as weighted mean with range. In addition, the overall mean and range was determined for each drug. All numerical values of mean and range were presented to the right of the graphs.



All included studies of amoxicillin/clavulanate administered only amoxicillin instead of the combination with clavulanate that is used in TB treatment. The small range of saliva-plasma ratios for amoxicillin is distorted. In fact, all studies, except Baglie *et al.* [22], reported a very low or even no detectable salivary concentration of amoxicillin, indicating a saliva-plasma or saliva-serum ratio of close to 0. By contrast, Baglie *et al.* [22] reported amoxicillin quantifiable salivary Cmax and AUC values as well as a saliva-plasma ratio of 0.34-0.55. The 2 included studies of amikacin, Masumi *et al.* [39] and Biasini *et al.* [23], did not report any saliva-plasma or saliva-serum ratio.

Several studies reported a time-dependent saliva-plasma of saliva-serum ratio. Suryawati and Santoso [40] reported a rifampicin saliva-serum ratio of 1.09±0.29 during the absorption phase and 0.81±0.05 during the elimination phase. For moxifloxacin, Burkhardt *et al.* [38] and Müller *et al.* [37] observed a saliva-plasma or saliva-serum ratio higher than 1 during the first 2 hours after administration. Thereafter, the ratio declined to below 1. A time-dependent saliva-serum ratio was also found for ofloxacin by Koizumi *et al.* [41]. During the first 4 hours after administration, the saliva-serum ratio was below 1, and during the following 4 hours, the ratio increased to above 1 and remained above 1 during 8-16 hours after administration. After 16 hours, a mean saliva-serum ratio of 1.14 was measured.

DISCUSSION

In this systematic review, we aimed to investigate whether TDM of anti-TB drugs using saliva samples is feasible. We found this to be likely possible for linezolid and gatifloxacin, whereas possible for isoniazid, rifampicin, ofloxacin, moxifloxacin, and clarithromycin. For other anti-TB drugs, either too few data were available, or the drugs seemed unlikely to be feasible for salivary TDM.
The review was strengthened by the inclusion of all WHO-approved anti-TB drugs as well as ertapenem, faropenem, and doripenem because interest in using these other carbapenems as part of anti-TB treatment has increased [42]. Ofloxacin and clarithromycin were still included, despite the WHO recommendation to not use these drugs [3]. In specific situations, ofloxacin and clarithromycin might be useful to treat difficult cases [43]. The information gained from this systematic review could also be applied to other infectious diseases.

Isoniazid [24,31,40], moxifloxacin [34-38], ofloxacin [21,25,27,41,44-49], and clarithromycin [26,38,50-52] showed varying saliva-plasma and saliva-serum ratios. The same issue applied to rifampicin, although rifampicin showed some low saliva-plasma and saliva-serum ratios that could complicate the detection of the drug in saliva for low-dosage regimes. A wide range of saliva-plasma and saliva-serum ratios

is especially caused by highly varying mean ratios across studies, not by wide ranges of study-specific ratios. A wide range of saliva-plasma and saliva-serum ratios could be caused by differences in study population, dose, saliva sampling method, and analytical method between the studies. The influences of these factors on the saliva-plasma and saliva-serum ratio are hard to determine because of the great variation of these factors among the included studies. Salivary TDM of these 5 anti-TB drugs may be possible; however, 1 workable saliva-plasma or saliva-serum ratio is required (Table 3). For instance, if the saliva-plasma ratio of isoniazid of 0.14 as found by Brown *et al.* [24] is applied to predict AUC values in blood using salivary AUC, the calculated AUC in blood will be almost 7 times higher than if the ratio of Gurumurthy *et al.* [31] (0.95) or of Suryawati and Santoso[40] (0.90) is used. These substantial differences could have an effect on the dosing recommendations based on such TDM results. However, the quality of Brown *et al.* [24 was unclear, as the study was classified as at a serious risk of bias.

For gatifloxacin and linezolid, salivary TDM is likely possible, because of the narrow range of saliva-serum and saliva-plasma ratios [51,53,54]. An additional study of gatifloxacin, preferably in patients with TB, should be performed to confirm the reported findings because pharmacokinetic parameters could significantly differ in patients with TB using several anti-TB drugs compared with healthy volunteers. However, in 2006, the US Food and Drug Administration (FDA) officially warned that gatifloxacin is associated with an elevated risk of dysglycemia [55,56]. So, gatifloxacin might be replaced in TB treatment by other fluoroquinolones, such as moxifloxacin or levofloxacin, in the future. Additional studies of linezolid using other dosages are necessary to rule out any dose dependency of the saliva-serum ratio and to complete the salivary pharmacokinetic profile of linezolid.

For doripenem and amoxicillin/clavulanate, salivary TDM is probably not possible because of very low salivary drug concentrations (Table 3). Both doripenem and amoxicillin are hydrophilic drugs and this complicates passage through membranes [57,58]. This problem could also apply to the other carbapenems. More studies comparing doripenem concentrations in blood and saliva are needed to confirm the results of Burian *et al.* [59] and to rule out any dose dependency. Nearly all studies regarding amoxicillin/clavulanate reported undetectable amoxicillin concentrations in saliva [26,60-62]. Only Baglie *et al.* [22] reported a substantial salivary concentration of amoxicillin and a saliva-plasma ratio. A possible reason is that this study administered the highest dose of all included studies. Besides, the variant results of Baglie *et al.* [22] could also be explained by the serious risk of bias.

Table 3. Summary of salivary TDM potentials of all anti-TB drugs.

Group	Anti-TB drug	Conclusion	Comments
First-line drugs	Isoniazid	Maybe possible	Wide range of saliva-plasma and saliva-serum ratios.
	Rifampicin	Maybe possible	Wide range of saliva-plasma and saliva-serum ratios. Some low ratios reported.
	Ethambutol	No data	Studies needed.
	Pyrazinamide	No data	Studies needed.
Group A: Fluoroquinolones	Levofloxacin	No data	Studies needed.
	Moxifloxacin	Maybe possible	Wide range of saliva-plasma and saliva-serum ratios.
	Gatifloxacin	Likely possible	Promising saliva-plasma and saliva-serum ratios. Additional study in patients with TB needed.
Group B: Second-line injectable agents	Amikacin	No data	Studies needed. Included studies did measure salivary concentrations, but no Cmax, AUC or saliva-plasma or saliva-serum ratio was reported.
	Capreomycin	No data	Studies needed.
	Kanamycin	No data	Studies needed.
	Streptomycin	No data	Studies needed.
Group C: Other core second-line agents	Ethionamide	No data	Studies needed.
	Prothionamide	No data	Studies needed.
	Cycloserine	No data	Studies needed.
	Terizidone	No data	Studies needed.
	Linezolid	Likely possible	Promising saliva-serum ratios. More studies with other dosage regimes needed.
	Clofazimine	No data	Studies needed.
Group D1: add-on agents	Pyrazinamide Ethambutol High dose isoniazid	See first-line drugs	See first-line drugs.
Group D2: add-on agents	Bedaquiline	No data	Studies needed.
	Delamanid	No data	Studies needed.
Group D3: add-on agents	p-aminosalicylic acid	No data	Studies needed.
	Imipenem/cilastatin	No data	Studies needed.
	Meropenem	No data	Studies needed.
	Amoxicillin/ clavulanate	Probably not possible	Low or undetectable drug concentrations in saliva, probably due to low lipophilicity.
	Thioacetazone	No data	Studies needed.
Other	Ofloxacin	Maybe possible	Wide range of saliva-plasma and saliva-serum ratios.
	Clarithromycin	Maybe possible	Wide range of saliva-plasma and saliva-serum ratios.
	Ertapenem	No data	Studies needed.
	Doripenem	Probably not possible	Low saliva-plasma ratio, probably due to low lipophilicity. More studies with other dosage regimes needed.
	Faropenem	No data	Studies needed.

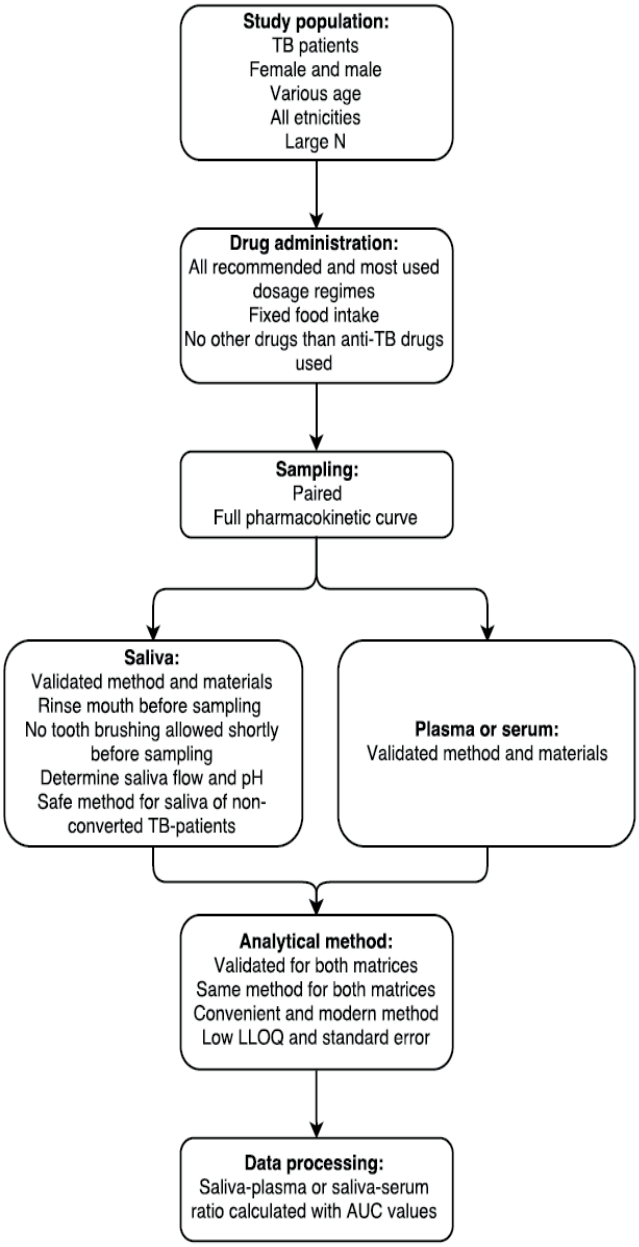
The conclusion of this systematic review is displayed per anti-TB drug using "No data", "Probably not possible", "Maybe possible" and "Likely possible". Besides, comments are added to clarify these conclusions.

More information is needed about the salivary pharmacokinetics of amikacin, since no saliva-plasma or saliva-serum ratios or salivary AUC values are reported in the analysed articles [23,39].
For many anti-TB drugs, salivary pharmacokinetic information is lacking, even for first-line drugs pyrazinamide and ethambutol (Table 3). As the incidence of drug-susceptible TB is significantly greater than the incidence of MDR-TB, the first-line drugs have to prioritised in future studies of salivary TDM. Especially, for pyrazinamide, more information about the pharmacokinetic parameters in saliva versus blood is important, as it is part of the MDR-TB regimen [3]. Besides pyrazinamide is one of the few anti-TB drugs for which low serum concentrations are associated with poor treatment outcomes [63,64]. The priority of second-line drugs should be ranked according to the grouping system of WHO as shown in Table 3. Anti-TB drugs in group A are considered the most beneficial in MDR-TB treatment and will be often used, while group D2 and D3 contain add-on anti-TB drugs that will be less frequently prescribed.

Obviously, more pharmacokinetic studies comparing anti-TB drug concentrations in saliva and plasma or serum are needed before salivary TDM could be implemented in the treatment of TB. To overcome the observed variability in saliva-plasma and saliva-serum ratios, large study populations and comparable study designs, study populations, dosage regimes, saliva sampling methods (stimulated versus nonstimulated), and analytical methods should be used in future studies.
An ideal design for this kind of study is proposed in Figure 4 to assist and advise all future researchers. Most important factors are inclusion of patients with TB, paired sampling, validation, salivary flow, salivary pH, and saliva-plasma or saliva-serum ratios calculated using AUC values.

A limitation of this systematic review is that many studies included healthy volunteers instead of patients with TB. It is hard to extrapolate the findings of these studies to the clinic because the effect of TB on the salivary pharmacokinetics is unknown. Furthermore, almost none of the included studies reported the saliva flow and pH, although both can influence the salivary drug concentration [12,18]. The salivary flow and pH values were not included in this review because of a lack of information. In future studies of salivary pharmacokinetics, salivary flow and pH should be measured to provide a complete profile. Besides, risk of bias assessment of the included articles was problematic because no tool is validated for pharmacokinetic studies. The ROBINS-I tool was not used in its validated structure as a result of changes in the confounding section. A validated and appropriate tool for the risk of bias assessment of pharmacokinetic studies is needed to assess the quality of these studies.

Figure 4. Ideal study design for pharmacokinetic studies comparing anti-TB drug concentrations in saliva and plasma or serum. LLOQ, lower limit of quantification; N, number.



Overall, our review found predictable saliva-plasma or saliva-serum ratios of less than 1. However, 3 studies of isoniazid and moxifloxacin reported saliva-plasma or saliva-serum ratios with values of above 1 during the absorption phase [37,38,41]. A high ratio during the absorption phase could be explained by drug adhesion to the oral mucosa [38]. Normally, this effect is averted by rinsing the mouth with water before sampling, but this precaution was not reported in the 2 moxifloxacin studies [37,38]. An active transport system across the salivary epithelium can also cause a high concentration in saliva [37]. However, this seems unlikely because not all studies of isoniazid and moxifloxacin reported this high saliva-plasma or saliva-serum ratios.

In the future, many TB endemic settings may benefit from TDM with saliva samples, particularly if the saliva sample collection is standardized and sample analysis is optimized. For instance, salivary TDM would allow patients the option to sample themselves at any location and afterward bring their saliva samples to a local health post. Importantly, for first-line drugs isoniazid and rifampicin, several analytical methods using ultraviolet-visible (UV-VIS) spectrophotometry have been used in several studies [65-67]. In addition, for ethambutol [68], moxifloxacin [69], levofloxacin [70], ofloxacin [71], para-aminosalicylic acid [72], amoxicillin/clavulanate [73], and imipenem/cilastatin [74] UV-VIS spectrophotometry methods were described in literature. Remarkably, 1 analytical method that determines isoniazid, rifampicin, and pyrazinamide simultaneously with a UV-VIS spectrophotometer was published [75]. After validation in both blood and saliva, these UV-VIS methods could easily be implemented in referral laboratories of more resource limited settings because of their relative simplicity and lower costs. Of caution, however, before implementing salivary TDM, the chemical stability of anti-TB drugs in saliva should be thoroughly studied to determine the necessity for rapid sample analysis. Isoniazid, for instance, is known to be unstable in both saliva and blood [76,77].

Furthermore, the eventuality of *Mycobacterium tuberculosis* being culturable from the saliva of nonconverted patients with TB is an extra factor that must be taken into account. The sampling method should be thoroughly designed and tested in advance to create a safe technique for the investigators working with the saliva samples and all other people involved. A recent study found that membrane filtration (pore size 0.22 μm) is suitable for decontamination of saliva samples containing *M. tuberculosis* [78]. However, before membrane filtration can be implemented in salivary TDM, recovery testing should rule out any adhesion of the drug to the membranes.

CONCLUSION

In this systematic review, we summarised the current knowledge about the salivary and blood concentrations of anti-TB drugs and their saliva-plasma or saliva-serum ratio in humans and determined for which anti-TB drugs salivary TDM should be further investigated either in basic pharmacokinetic studies or in larger validation cohorts.

Unfortunately, for most anti-TB drugs, salivary pharmacokinetic information is entirely lacking. For these drugs, such as pyrazinamide, pharmacokinetic studies comparing drug concentrations in saliva and blood are needed. For amikacin, pharmacokinetic studies using saliva samples were found but without saliva-plasma or saliva-serum ratios. Salivary TDM is likely possible for gatifloxacin and linezolid, because of their promising, narrow-ranged saliva-plasma and saliva-serum ratios. It may be possible for isoniazid, rifampicin, moxifloxacin, ofloxacin, and clarithromycin, but because of the wide range of saliva-plasma and saliva-serum ratios, further well-designed pharmacokinetic studies in patients with TB would be recommended. TDM with salivary samples is probably not feasible for doripenem and amoxicillin/clavulanate because of very low salivary concentrations. Overall, it seems worthwhile to further explore saliva as potential matrix for TDM, especially for children.

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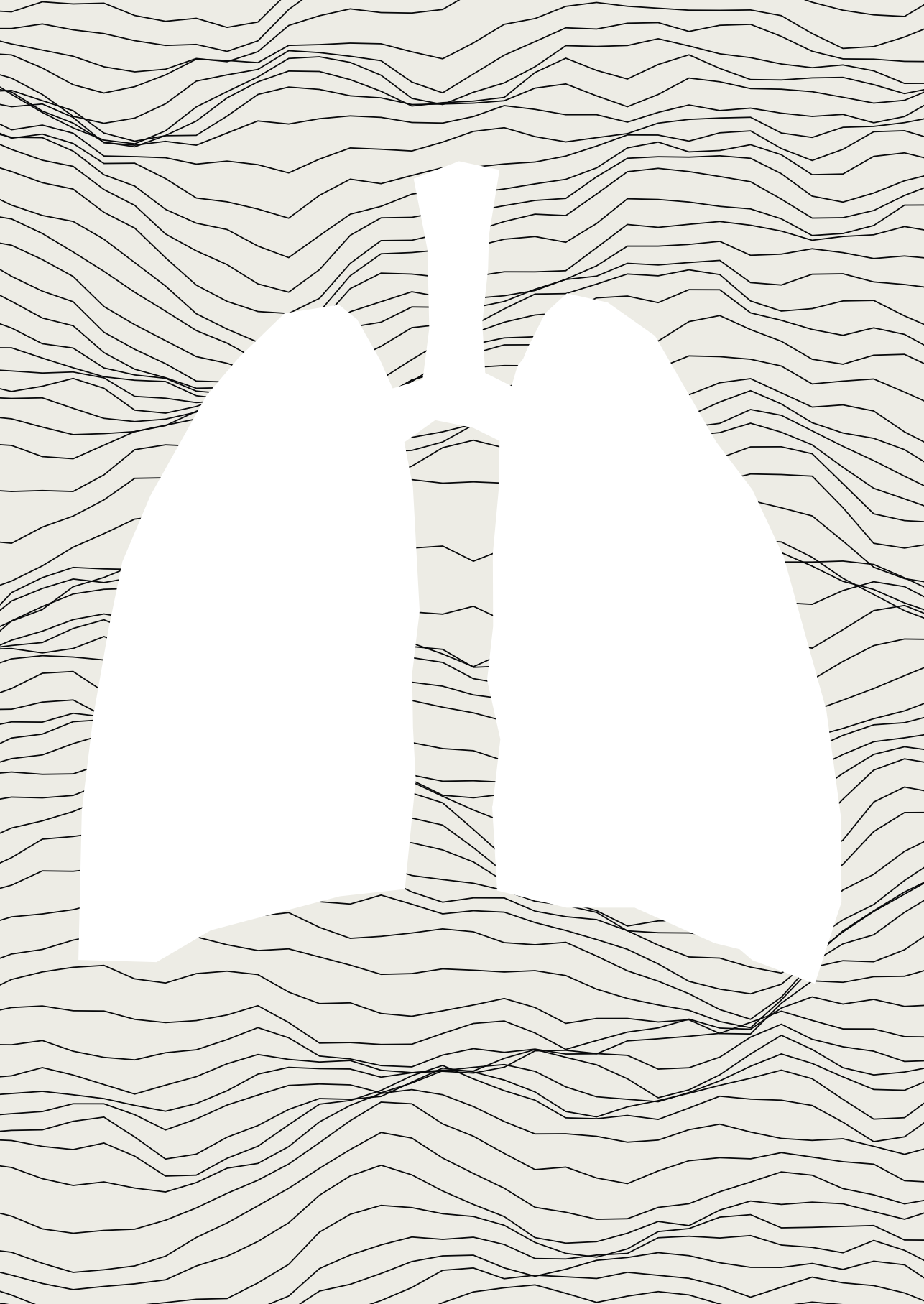

Chapter

3a

Dose Optimisation of First-line Tuberculosis Drugs using Therapeutic Drug Monitoring in Saliva: Feasible for Rifampicin, not for Isoniazid.

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Manuscript in preparation.

To the Editor:

The persisting worldwide burden of tuberculosis (TB) is worrisome. In 2018, an estimated 10 million individuals developed TB and 1.45 million deceased [1]. The increase in drug resistance is an important point of concern. Resistance can be acquired by inappropriate drug management, non-compliance, and insufficient drug exposure [2,3]. The last is frequently described for the first-line TB drugs rifampicin and isoniazid due to large inter-individual pharmacokinetic variability [3]. Therapeutic drug monitoring (TDM) can be used to verify drug exposure and adjust individual drug dosages if needed [4]. The efficacy of rifampicin and isoniazid is associated with the ratio of the steady-state area under the concentration-time curve from 0-24 h to minimal inhibitory concentration (AUC_{0-24}/MIC) with a target value of >271 for rifampicin and >567 for isoniazid [5,6]. Traditional TDM uses plasma or serum samples, whereas other matrices like dried blood spot and saliva have been recommended as alternatives suitable for programmatic use [4,7]. Collecting saliva samples is non-invasive and simple with the perspective of home-based self-sampling [8]. Salivary concentrations of rifampicin and isoniazid have been studied before, but highly variable saliva-serum concentration ratios across studies were observed.[8] Moreover, none of these studies assessed the feasibility of TDM using saliva samples. Therefore, the aim of this prospective study was to evaluate the feasibility of saliva instead of serum samples for TDM of rifampicin and isoniazid in patients with TB.

Adult patients with TB admitted at the Tuberculosis Center Beatrixoord in Haren, the Netherlands, who were treated with rifampicin or isoniazid and had routine TDM for rifampicin or isoniazid were eligible for inclusion. All patients provided informed consent. This study was approved by the ethical review board of the University Medical Center Groningen (IRB 2016/069) and registered at Clinicaltrials.gov (NCT03080012). All samples were taken after at least 14 days of treatment (steady-state) and stored at -80 °C pending analysis. Saliva and serum samples were collected simultaneously according to the routine TDM schedule which usually included samples drawn before, and 0.5, 1, 2, 3, 4, and 6 hours after drug intake. Two different methods of saliva collection were used. The Salivette (Sarstedt, Nümbrecht, Germany) was utilized for sputum culture negative patients. Membrane filtration was applied to the samples of sputum culture positive patients to minimize infection hazard [9,10]. The recovery of both sampling methods was determined for rifampicin and isoniazid at concentrations of 1 and 7 mg/L as described [11]. Rifampicin recovery at 1 mg/L was 64% (coefficient of variation [CV], 9%) using the Salivette and 67% (5%) using membrane filtration, while at 7 mg/L recovery was 102% (2%) and 99% (8%), respectively. For isoniazid, recovery (CV) at 1 mg/L was 77% (8%) using the Salivette and 68% (4%) using membrane filtration, whereas at 7 mg/L recovery was 91% (1%) and 88% (3%). After analysis, the salivary drug concentrations were corrected for the recovery of the applied sampling method.

The pH of each saliva sample was determined by two independent researchers using pH indicator strips (range 4.0-7.0 and 2.0-9.0, Merck KGaA, Darmstadt, Germany). Saliva and serum samples were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods [12,13]. The method for rifampicin was recently updated and validated using another internal standard ([$^2H_8$]-rifampicin). Cross-validation in saliva was successfully performed for both drugs. Bias and precision of spiked pooled saliva met the pre-set criteria of <20% for lower limit of quantification (LLOQ; rifampicin 0.1 mg/L, isoniazid 0.2 mg/L) as well as <15% for low (rifampicin 0.5 mg/L, isoniazid 0.4 mg/L), medium (rifampicin 5.0 mg/L, isoniazid 4.0 mg/L), and high (rifampicin 8.0 mg/L, isoniazid 6.4 mg/L) concentrations. Saliva-serum ratios were calculated using the paired drug concentrations for each time point as well as the non-compartmental AUC_{0-24} (MWPharm version 3.82, Mediware, Groningen, The Netherlands) in both matrices. The saliva-serum concentration ratios were evaluated using Passing Bablok regression and Bland-Altman plots (Analyze-it 4.81; Analyze-it Software Ltd., Leeds, United Kingdom). C_{max} was defined as highest observed drug concentration and T_{max} as time of C_{max}. Intra-individual variation was assessed using the CV (%) of the saliva-serum ratios within one pharmacokinetic curve, while inter-individual variation was calculated as CV (%) of the mean saliva-serum ratios of all curves.

Table 1. Patient characteristics, non-compartmental pharmacokinetic (PK) parameters (C_{max}, T_{max}, AUC_{0-24}) in serum and saliva, salivary pH, as well as saliva-serum ratios. Presented as median (interquartile range), unless stated otherwise.

	Rifampicin (n=11)	**Isoniazid (n=8)**
Study population		
Male [n(%)]	9 (82%)	6 (75%)
Age (years)	34 (25-54)	54 (49-58)
Bodyweight (kg)	69 (58-71)	68 (57-72)
Creatinine concentration (µmol/L)	62 (51-72)	65 (49-75)
Dose (mg/kg)	10.2 (8.5-12.3)	5.4 (4.2-6.5)
Serum PK		
C_{max} (mg/L)	8.70 (5.99-12.12)	3.50 (1.65-4.75)
T_{max} (h)	2 (2-3)	2 (1-2)
AUC_{0-24} (mg*h/L)	38.01 (34.44-76.50)	17.83 (7.80-20.74)
Saliva PK		
C_{max} (mg/L)	1.21 (1.08-1.35)	1.57 (0.93-2.75)
T_{max} (h)	3 (2-4)	1 (1-2)
AUC_{0-24} (mg*h/L)	5.88 (5.08-7.94)	7.62 (7.28-11.73)
Salivary pH	6.1 (5.5-7.0)	6.1 (5.8-6.8)
Saliva-serum ratio		
Paired concentration ratio	0.126 (0.109-0.154)	0.763 (0.413-1.158)
Inter-individual variation [CV(%)]	21.5%	48.3%
Intra-individual variation [mean (range) of CV (%)]	17.2% (7.4%-24.0%)	22.3% (9.2%-36.5%)
AUC_{0-24} ratio	0.154 (0.127-0.162)	0.824 (0.492-1.200)

Characteristics of the study population, pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-24}) in both matrices, and saliva-serum ratios are shown in Table 1.
Penetration of rifampicin into saliva was low and slightly delayed. This resulted in undetectable salivary concentrations, when collected before drug intake, 0.5 h, or 1 h after drug intake. Saliva and serum concentrations (>1 h after drug administration) correlated well with a regression line of saliva concentration=0.074+0.112*serum concentration (95% confidence interval [CI] of intercept -0.0311 to 0.161; 95% CI slope 0.087 to 0.138; r=0.803). Bland-Altman analysis led to a mean (95% CI) saliva-serum concentration ratio of 0.13 (0.12-0.14) with SD of 0.04. The AUC_{0-24} saliva-serum ratio was slightly higher, but comparable (Table 1). Inter- and intra-individual variation were both approximately 20%.
Isoniazid saliva-serum ratios were much higher than for rifampicin as can be explained by the difference in protein binding (10% versus 90%). Passing-Bablok regression resulted in a regression line of saliva concentration=-0.055+0.812*serum concentration (95% CI intercept -0.556 to 0.460; 95% CI slope 0.185 to 1.244; r=0.889). The Bland-Altman analysis showed a mean (95% CI) saliva-serum concentration ratio of 0.80 (0.65-0.95) with SD of 0.46. Intra-individual variation was 22.3%, while inter-individual variation was relatively large (48.3%) which could suggest that isoniazid penetration into saliva is influenced by other factors. Salivary pH was not related to the saliva-serum ratios of isoniazid and rifampicin.

A limitation of this study is the lack of data on salivary flow and protein binding. Both could introduce variation in the saliva-serum ratios [8]. However, we aimed to evaluate the feasibility of salivary TDM and consider it unfeasible if protein binding and salivary flow have to be determined in each patient. Moreover, no influence of salivary pH on saliva-serum ratios was detected, whereas salivary pH is related to salivary flow [8].
Despite this limitation, we propose that rifampicin AUC_{0-24} in serum can be satisfactorily predicted using the AUC_{0-24} in saliva applying a correction factor of 6.5 and used for AUC_{0-24} guided dose optimization in patients with TB. The sampling burden can be reduced by collecting samples only at 2, 3, 4, and 6 hours after drug intake, since the other salivary rifampicin concentrations (0, 0.5, and 1 h) were undetectable. Simple HPLC-UV methods [14] are available in TB endemic areas, but usually not LC-MS/MS. Additional testing is recommended to determine if these analytical techniques are also able to assess low rifampicin concentrations in saliva.
The results of isoniazid are less encouraging. Based on the findings in this study, we would not recommend TDM of isoniazid in saliva. The major cause of the large variation of isoniazid saliva-serum ratios remains unclear, as is the case with moxifloxacin [10]. A future study could focus on the identification of acetylator phenotype using saliva samples. Unfortunately, our sample size was too small to distinguish three groups with different drug clearance rates and we did not perform NAT2 genotyping.

In general, we conclude that TDM for isoniazid using saliva samples will not be an equivalent alternative to traditional TDM as already shown for moxifloxacin [10] and amikacin [15], but it can be useful in home screening of rifampicin drug exposure in patients with TB as has been established for linezolid [10] and levofloxacin [11].

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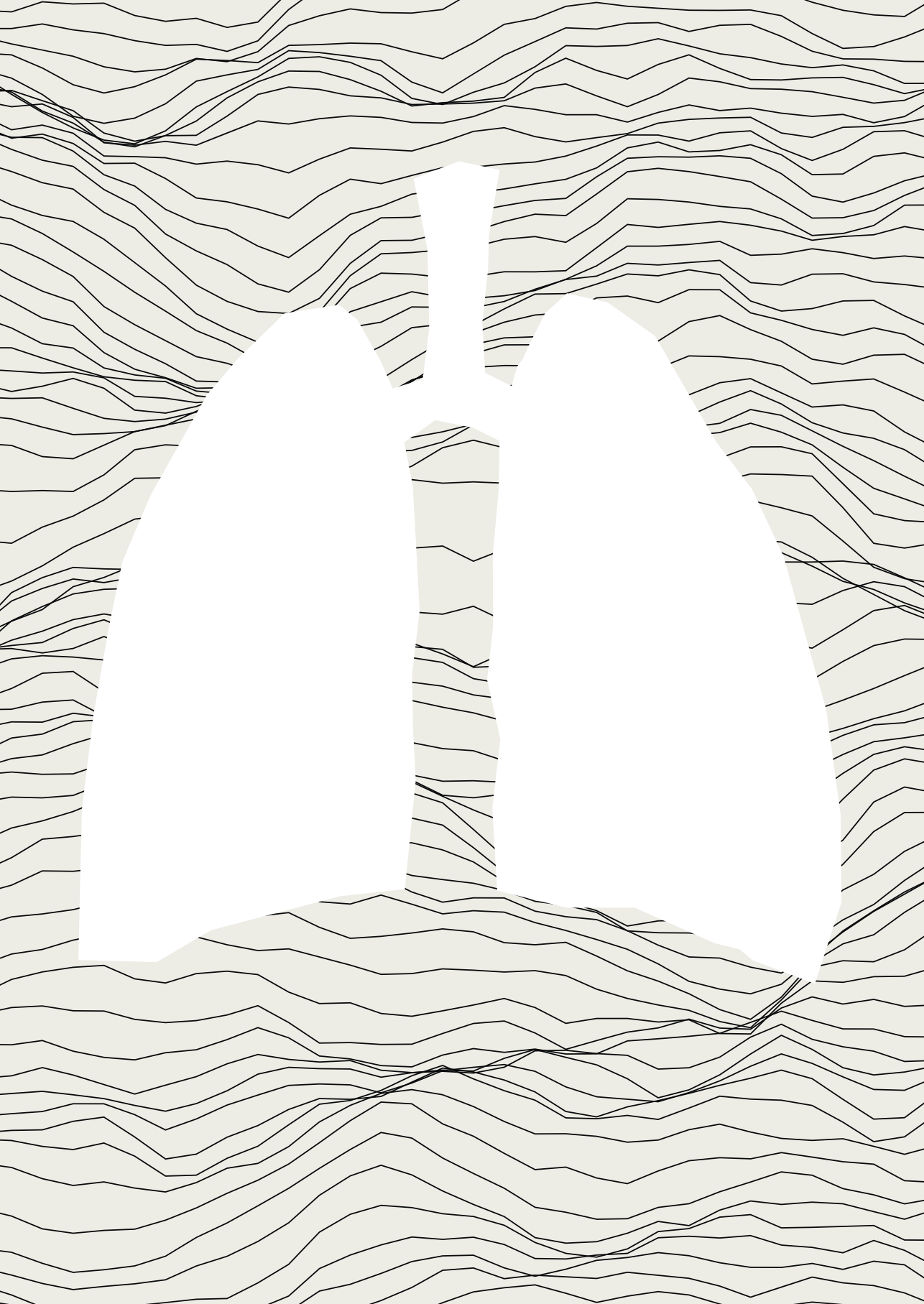

Chapter

3b

Therapeutic Drug Monitoring using Saliva as Matrix: an Opportunity for Linezolid, but Challenge for Moxifloxacin

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To the Editor:

The World Health Organization (WHO) has listed moxifloxacin and linezolid among the preferred "Group A" drugs in the treatment of multidrug-resistant tuberculosis (MDR-TB).[1] Therapeutic drug monitoring (TDM) could potentially optimize MDR-TB therapy, since moxifloxacin and linezolid show large pharmacokinetic variability.[1–4] TDM of moxifloxacin focuses on identifying patients with low drug exposure who are at risk of treatment failure and acquired fluoroquinolone resistance.[5, 6] Alternatively, TDM of linezolid strives to reduce toxicity while ensuring an adequate drug exposure because of its narrow therapeutic index.[1, 3, 7]

TDM is typically performed using plasma or serum samples, but other biological matrices can be considered as alternatives (e.g. saliva).[8] A benefit of saliva is the easy and non-invasive nature of sampling. Especially in high TB burden areas the option for sampling at home would be advantageous. Penetration of moxifloxacin into saliva has typically been studied in healthy volunteers, but has never been evaluated for the purpose of TDM in MDR-TB patients.[9] Only one study described linezolid concentrations in saliva and found that saliva is a suitable matrix for TDM in MDR-TB patients and that salivary concentrations can be translated to serum concentrations without the need of a correction factor.[7] The aim of this prospective study was to explore the feasibility of saliva-based TDM of moxifloxacin and to determine if earlier results of linezolid in saliva of MDR-TB patients could be confirmed.

Hospitalized adult TB patients in the Tuberculosis Center Beatrixoord (Haren, The Netherlands), who had moxifloxacin or linezolid as part of their TB treatment and had routine TDM using blood samples were eligible for inclusion. All participants signed informed consent. This study was registered at Clinicaltrials.gov (NCT03080012) and approved by the ethical review committee of the University Medical Center Groningen (IRB 2016/069).

After at least 14 days of treatment, saliva samples were taken simultaneously with plasma (moxifloxacin) or serum (linezolid) according to routine TDM schedule which generally included a sample before and 1, 2, 3, 4, and 8 h after drug administration. All samples were stored at -80^{0}C until analysis.

To collect saliva samples, patients were asked to chew on a cotton roll after rinsing their mouth with water. Subsequently, the samples were processed using one of the following methods. Salivette (Sarstedt, Nümbrecht, Germany) in combination with centrifugation was used for non-contagious patients. Membrane filtration was applied to the saliva samples of the TB patients who still had *Mycobacterium tuberculosis* bacilli in their sputum to minimize infection hazard.[10] Salivary pH values were determined by two independent observers using pH indicator strips with pH range 4.0-7.0 and 2.0-9.0 (Merck KGaA, Darmstadt, Germany), because it could influence drug penetration into saliva.[9] Recovery of both sampling methods was determined similarly to Ghimire *et al* [11], except

that moxifloxacin was tested at 1 and 3 mg/L and linezolid at 2 and 20 mg/L. Recovery was comparable for low and high concentrations. Using the Salivette, recovery was 48% (coefficient of variation [CV] 6%) for moxifloxacin or 95% (CV 3%) for linezolid and via membrane filtration 48% (CV 6%) or 98% (CV 3%), respectively. After analysis the salivary concentrations were corrected for recovery accordingly.

All samples were analysed using an updated version of our previously published liquid chromatography tandem mass spectrometry (LC-MS/MS) method.[12, 13] The LC-MS/MS method of linezolid was already cross-validated for saliva.[7] Cross-validation between plasma and saliva was performed for moxifloxacin at low (1 mg/L), medium (2 mg/L), and high (4 mg/L) concentrations as well as at lower limit of quantification (LLOQ; 0.05 mg/L). All concentration levels met the pre-set criteria for accuracy and precision (bias and CV <15%; at LLOQ both <20%).

Area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) in saliva and plasma/serum was calculated using noncompartmental pharmacokinetic analysis (MWPharm version 3.82, Mediware, Groningen, The Netherlands). C_{max} was defined as highest observed concentration and T_{max} as corresponding time of C_{max}. Two different saliva-plasma/serum ratios were calculated; one used the paired drug concentrations, while the other compared AUC_{0-24} in both matrices. Passing-Bablok regression and Bland-Altman plots (Analyze-it 4.81; Analyze-it Software Ltd., Leeds, United Kingdom) were used to analyse results.

Table 1. Characteristics of the linezolid and moxifloxacin study populations, pharmacokinetic (PK) parameters in serum/plasma and saliva, and saliva-serum/plasma ratios using paired concentrations as well as AUC_{0-24}.

	Linezolid (n=7)	**Moxifloxacin (n=15)**
Study population		
Male [n(%)]	6 (86%)	11 (73%)
Age (years)	44 (37-55)	34 (25-55)
Bodyweight (kg)	67.1 (60.5-68.4)	67.1 (57.5-70.5)
Creatinine concentration (μmol/L)	73 (72-90)	(72 (63-90)
Dose (mg/kg)	8.85 (7.42-9.93)	5.96 (5.68-7.08)
Serum/plasma PK[a]		
C_{max} (mg/L)	12.45 (8.84-15.78)	2.28 (1.62-2.80)
T_{max} (h)	3 (2-4)	2 (1-2)
AUC_{0-24} (mg*h/L)	119.4 (116.2-128.2)	21.3 (15.8-31.0)
Saliva PK		
C_{max} (mg/L)	7.93 (7.55-12.38)	3.20 (2.51-4.25)
T_{max} (h)	3 (2-3)	2 (1-2)
AUC_{0-24} (mg*h/L)	93.6 (91.7-108.0)	21.3 (13.7-28.3)
Saliva-serum/plasma ratio[a]		
Paired concentration ratio	0.76 (0.64-0.85)	1.00 (0.68-1.35)
AUC_{0-24} ratio	0.81 (0.74-0.88)	0.89 (0.61-1.14)

All parameters are presented as median (interquartile range) unless stated otherwise.
[a] Serum for linezolid and plasma for moxifloxacin.

Patient characteristics, pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-24}), and saliva-serum/plasma ratios are shown in Table 1. All patients on linezolid did also receive moxifloxacin.

Individual linezolid concentration-time curves in saliva versus serum were similarly shaped and T_{max} in saliva was not delayed, which suggested that penetration of linezolid into saliva is fast. Passing-Bablok analysis showed a linear regression line of saliva concentration = 0.389 + 0.680*serum concentration with 95% confidence interval (CI) of intercept -0.14 to 1.06; 95% CI of slope 0.60 to 0.76, r=0.954, and p=0.519. Bland-Altman demonstrated a mean (95% CI) saliva-serum concentration ratio of 0.76 (0.70-0.82). In general, the linezolid saliva-serum paired concentration ratio was considerably constant at 0.6-0.8 (range 0.25-1.29). Saliva-serum AUC_{0-24} ratios were even less variable with a median of 0.81 (range 0.54-0.96). However, we found a lower saliva-serum ratio than before,[7] which could be caused by differences in sampling method, processing or storage. Because linezolid efficacy is related to the ratio of AUC_{0-24} to minimal inhibitory concentration (AUC_{0-24}/MIC), it is recommended to collect multiple saliva samples to calculate AUC_{0-24} in saliva and afterwards translate to plasma AUC_{0-24} using a correction factor of 1.2. Based on these results, salivary TDM of linezolid indeed might be feasible and is ready for testing in a high burdened TB setting. Although LC-MS/MS was used in our study, HPLC-UV could be a suitable alternative in less resourced settings. Simple point-of-care tests in saliva, centralized drug analysis, stability studies for transport at room temperature conditions, and cross-validation of existing analytical methods in saliva may improve feasibility.[8]

Moxifloxacin paired saliva-plasma concentration ratios were highly variable with a range of 0.15-2.81 (median 1.00) which does not favour saliva as a sampling matrix for TDM. Passing-Bablok showed a linear relation of saliva concentration = -0.620 + 1.49*serum concentration, 95% CI intercept -0.97 to -0.33, 95% CI slope 1.32 to 1.74, r=0.796, and p=0.103. As moxifloxacin saliva-plasma concentration ratios were not normally distributed according to Shapiro-Wilk test (p=0.0003), Bland-Altman analysis could not be used. Unfortunately, saliva-plasma AUC_{0-24} ratios showed similar results (range 0.30-2.00), but the underlying cause remains unclear. Both inter-individual as well as intra-individual variation was observed. No effect of salivary pH on saliva-plasma ratios of moxifloxacin could be detected.

A limitation of our study was that we did not measure the unbound concentrations. Therefore, variation in protein binding could have affected the saliva-plasma ratios. Interestingly, salivary concentrations higher than plasma concentrations were observed suggesting possibilities of active transport in addition to passive diffusion.[9] Moxifloxacin also shows excellent penetration into diseased lung tissue with a median free-tissue/free-serum ratio of 3.2.[14] It would be interesting to investigate whether salivary concentrations are related to tissue concentrations at the site of infection and

if penetration is driven by similar mechanisms. If closely related, it might be possible to determine infection site moxifloxacin concentrations without the need of invasive tissue sampling. Clearly, it is the free drug concentrations at the site of action that is predictive of treatment efficacy, while plasma moxifloxacin concentrations serve not more than proxy markers.

Salivary TDM could be an alternative method for traditional linezolid TDM using plasma or serum, and future studies can focus on improving the feasibility. However, for moxifloxacin our data does not support saliva as suitable matrix for TDM using the described method. Future studies should investigate moxifloxacin protein binding, salivary flow, and transport mechanisms to gain more insight in the feasibility of moxifloxacin TDM in saliva. As shown before for amikacin [15], saliva will likely not be a universal but only a selective matrix for TDM of anti-TB drugs.

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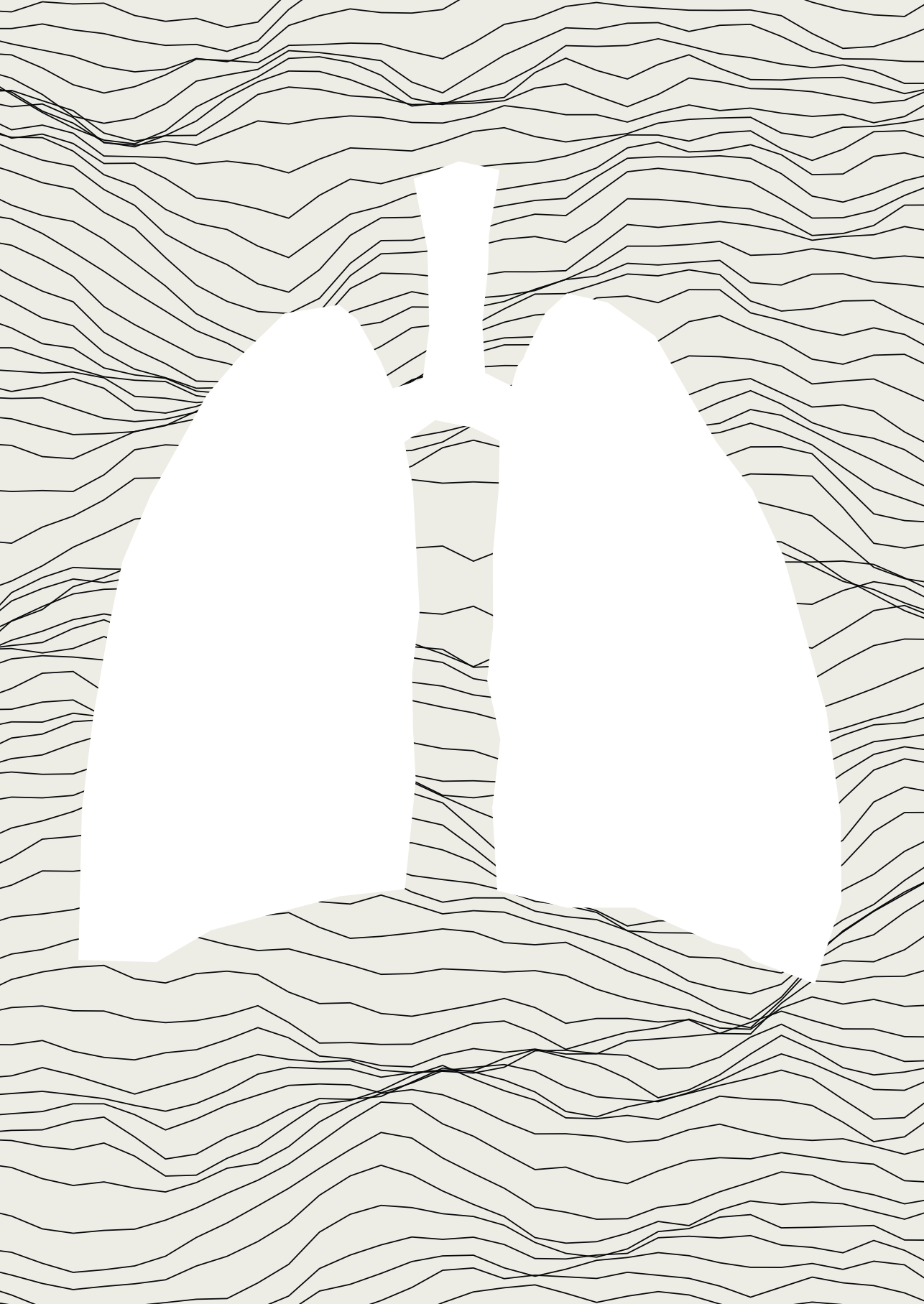

Chapter

3c

Lack of Penetration of Amikacin into Saliva of Tuberculosis Patients

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To the Editor,

In the 2016 update of the World Health Organization treatment guideline of drug-resistant tuberculosis (TB), a shorter multidrug-resistant TB regimen was opposed because of its higher treatment outcomes [1]. However, therapeutic drug monitoring (TDM) is an excellent method to improve clinical outcomes as well and its practise is on the rise [2]. A well-known side effect of group B injectable anti-TB drugs (e.g. amikacin) is ototoxicity [3]. TDM could also be a solution to minimise side-effects by lowering the drug exposure [4]. In the study of Altena *et al.* [5], TDM was practised using the ratio of peak concentration (C_{max}) to minimal inhibitory concentration (MIC) and this resulted in a reduction in patients with hearing loss. Saliva is considered as an alternative matrix for TDM because it is easy, non-invasive and more patient friendly to sample [6]. Studies found a limited penetration of gentamycin and tobramycin into saliva [7], while detectable levels of amikacin in saliva of neonates were reported [8]. Given the low penetration of aminoglycosides into saliva and interest in C_{max} for TDM of amikacin, our objective was to study whether the salivary C_{max} of amikacin is measurable and useful in salivary TDM.

TB patients from the Tuberculosis Center Beatrixoord (Haren, The Netherlands) who were 18 years or older, used amikacin as part of their TB treatment and in whom TDM using blood samples was routinely performed, were eligible for inclusion. Written informed consent was obtained. This study was approved by the Ethical Review Committee of University Medical Centre Groningen (IRB 2016/069) and registered at Clinicaltrials.gov (NCT03080012).
Conventional TDM was part of routine treatment. Salivary samples were taken simultaneously with blood samples before and 1,2,3,4 and 8 h after administration.
After rinsing their mouth with water, the patients chewed on two cotton rolls (Orbis Dental, Münster, Germany) for 2 min. The cotton rolls were each placed in a 5-ml syringe connected to a membrane filter with pore size ≤0.22 μm (Millex-GP; Merck Milipore, Carrigtwohill, Ireland). Membrane filtration was used to decontaminate the saliva samples for laboratory safety reasons, as saliva of infectious TB patients contains *Mycobacterium tuberculosis* [9]. Saliva and serum samples were stored at -20°C until analysis. The recovery of the described saliva sampling method was determined in five-fold using solutions of amikacin in pooled saliva of 5 mg/L and 20 mg/L.
Both saliva and blood samples were analysed with a calibrated particle-enhanced turbidimetric inhibition immunoassay (Architect; Abbott Diagnostics, Lake Forest, IL, USA) using the amikacin reagent kit 6L3520 (Multigent; Abbott Diagnostics). The lower limit of quantification (LLOQ) of the analytical method was 2.0 mg/L. Quality control samples of amikacin in pooled saliva were prepared at concentrations of 5 mg/L and 20 mg/L.

In total, six TB patients (five males and one female) with a median (interquartile range) age of 47 (32-59) years, body weight of 65.4 kg (57.3-76.2), creatinine clearance of 95 mL/min/1.73 m^2 (69-106) and amikacin dose of 7.19 mg/kg bodyweight (6.44-7.30) were included in this study. All *M. tuberculosis* isolates had MIC values of 1.0 mg/L. The recovery of the saliva sampling method was determined at 42.9% with a coefficient of variation of 9.2%. The amikacin concentrations of the quality control samples were within an acceptable range of error (6.5-9.8%). The amikacin concentrations, including C_{max}, were not detectable in the saliva samples of the patients and did not exceed the LLOQ of 2.0 mg/L, whereas amikacin could be quantified in all serum samples (Figure 1).

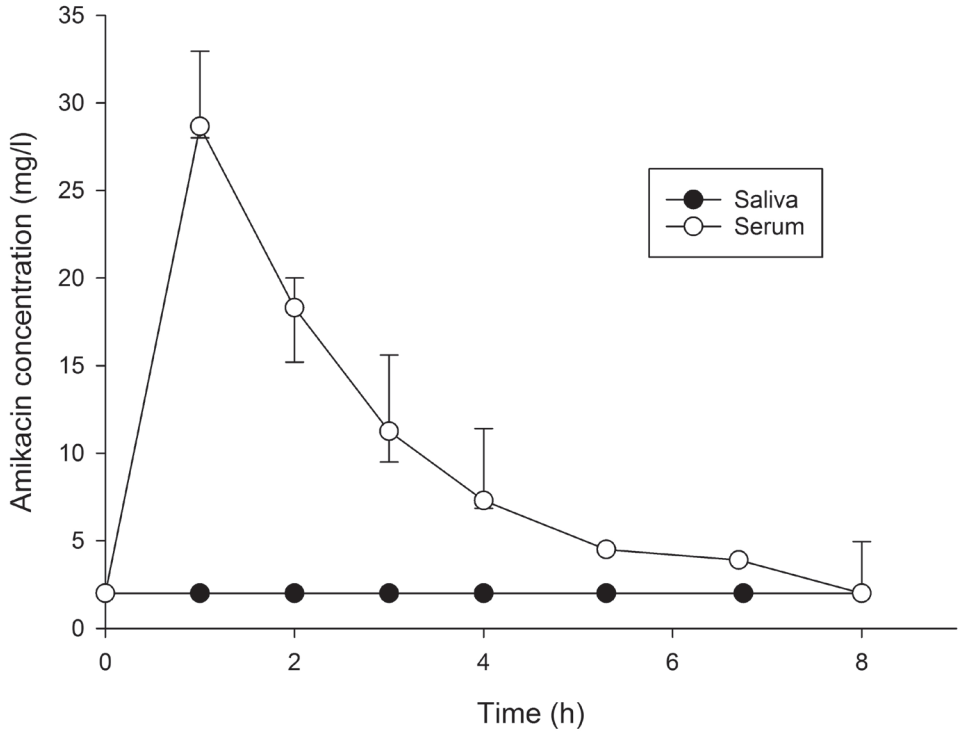

Figure 1. Median (interquartile range) amikacin concentration–time curves in serum and saliva of tuberculosis patients (n=6).

Low penetration of amikacin into saliva could be explained by its physicochemical properties as it is a polycationic and highly polar compound. Amikacin cannot easily diffuse across membranes, such as in the salivary gland [6]. This effect also applies to other aminoglycosides and encourages the use of aerosolised administration [7].

A limitation of this study is the relatively high LLOQ of the immunoassay used. Other analytical methods with lower LLOQ values, such as liquid chromatography-tandem mass spectrometry, have been validated but are not available for TDM in high TB burden countries with limited resources [10]. In addition, the recovery of the sampling method was low due to adhesion of amikacin to the membrane filter or sampling material. Due to a high LLOQ and low recovery, we were able to measure salivary amikacin concentrations >5 mg/L. As median serum C_{max} concentrations were 28.75(28-33) mg/L, we were able to quantify saliva/serum ratios up to 0.18. These low ratios are generally considered to be unsuitable for salivary TDM, unless a sensitive analytical method is used and other factors influencing variability in saliva penetration are absent.

In conclusion, the robust design using a full concentration-time curve enabled us to conclude that amikacin C_{max} concentrations were not measurable in saliva and the concept of simple salivary TDM of amikacin using immunoassay appeared not feasible.

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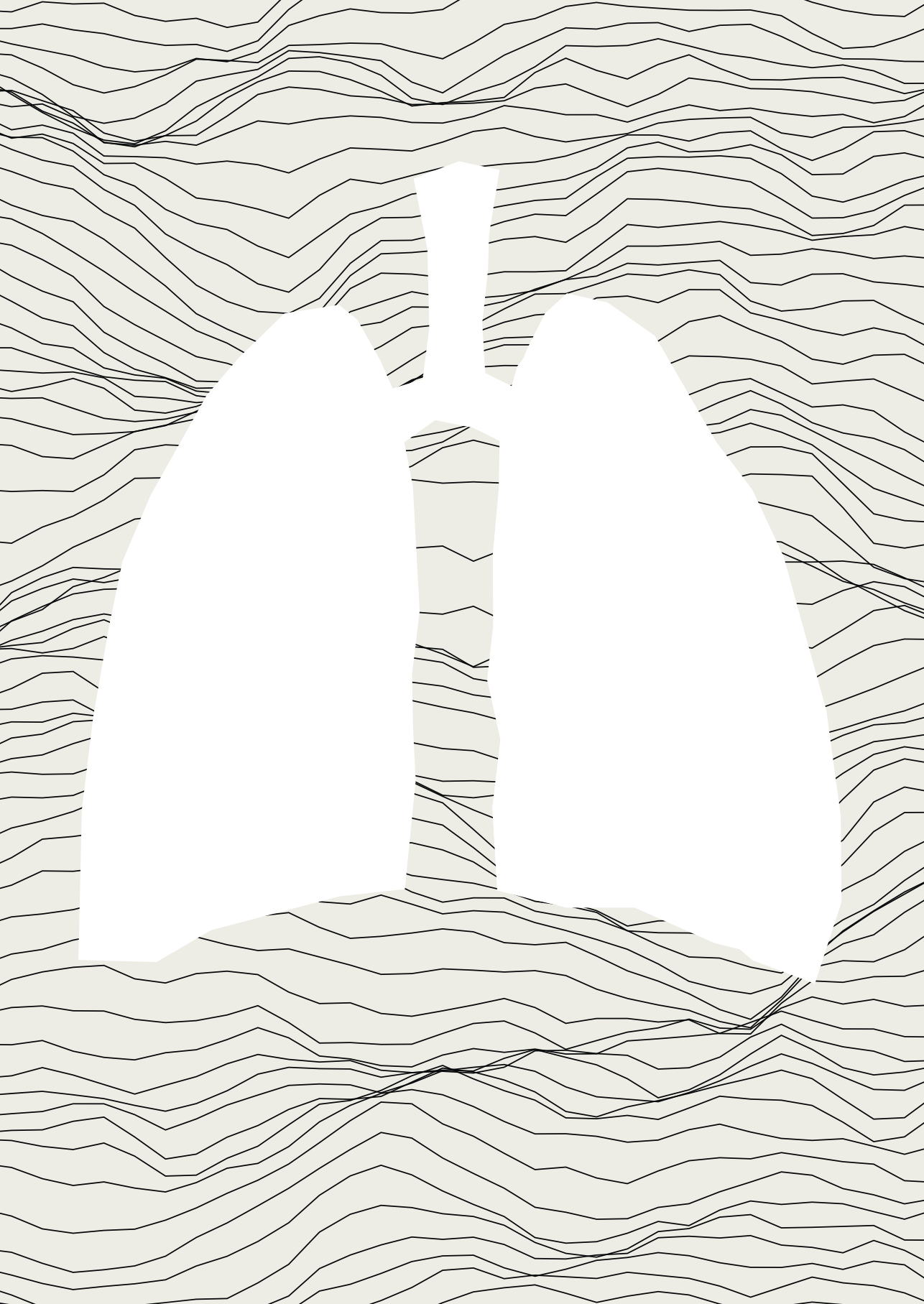

Chapter

3d

Membrane Filtration is Suitable for Reliable Elimination of Mycobacterium tuberculosis from Saliva for Therapeutic Drug Monitoring

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Tuberculosis (TB) remains an infectious disease of worldwide concern. Therapeutic drug monitoring (TDM) of blood could be helpful in optimising TB treatment, as anti-TB drug exposure shows interpatient variability [1]. TDM in saliva instead of blood is currently being studied as more practical alternative, since saliva sampling is noninvasive and more acceptable to patients [2,3]. Along with the growing interest in the pharmacokinetics of anti-TB drugs, TDM is increasingly used in daily routine practice. However, saliva of infectious TB patients contains *Mycobacterium tuberculosis* and TDM sample analysis usually does not take place in a biosafety level 3 laboratory. A quantitative study found a mean bacterial load of $7x10^4$ (range, $1x10^2$ to $6x10^5$) CFU/mL in saliva of infectious TB patients [4]. Laboratory-acquired TB infections should be prevented by applying biosafety measures when working with *M. tuberculosis*-containing saliva samples [5]. Therefore, saliva samples from TB patients require sterilisation prior to laboratory processing (e.g. centrifugation). Unfortunately, decontamination by heat sterilisation is not possible because of thermal instability of drugs. The objective of this experiment was to test whether membrane filtration is able to reliably decontaminate a solution containing *M. tuberculosis*.

Five *M. tuberculosis* strains (Table 1) were incubated in Mycobacteria Growth Indicator Tubes (MGITs; Becton, Dickinson and Company, United States) after the addition of 0.8 mL of oleic acid, albumin, dextrose, and catalase as a growth supplement. For each strain, 2.0 mL of the culture fluid containing at least 10^5 to 10^6 CFU/mL was filtered in duplicate using a polyvinylidene fluoride membrane filter with pore size of 0.22 µm and diameter of 33 mm (Millex-GV; Merck Milipore, Ireland). The filtrate was inoculated into a new MGIT tube with culture fluid. For each strain, 0.5 ml of the culture fluid containing at least 10^5 to 10^6 CFU/mL was also inoculated in a new MGIT tube as a positive control. All tubes were incubated at 36.5°C for 55 days in the BACTEC MGIT 960 system (Becton, Dickinson and Company, United States). No mycobacterial growth was observed in the MGITs inoculated with filtrate, while all of the control tubes were positive within two weeks (Table 1).

Table 1. Growth of five strains of *M. tuberculosis* in positive-control samples and filtrates (in duplicate; A and B).

Strain	Species	Drug resistance	No. of growth units		
			Positive control	Filtrate A	Filtrate B
1	*M. tuberculosis* complex	Sensitive	7037	0	0
2	*M. tuberculosis*	Isoniazid, rifampicin	18216	0	0
3	*M. tuberculosis*	Rifampicin	20413	0	0
4	*M. tuberculosis*	Sensitive	26757	0	0
H37Rv	*M. tuberculosis*	Sensitive	22776	0	0

This is the first description of membrane filtration of *M. tuberculosis*-containing fluids for sterilisation purposes in the process of TDM. No mycobacterial growth was measured in any of the filtrates. The membrane filter therefore successfully filtered all bacteria of multiple *M. tuberculosis* strains from culture fluids. We found no difference among the five strains in the number of growth units in the filtrates. It is not possible to test all *M. tuberculosis* isolates received at a mycobacteria laboratory, but according to this experiment, variation in the feasibility of membrane filtration between different strains is not likely. Membrane filtration of solutions with a larger bacterial load than tested here requires further investigation, as sterilisation cannot be assured by only this experiment. However, the bacterial load of saliva of TB patients is usually not as large as tested in this experiment [4]. Because of the satisfying results obtained with culture fluids with large bacterial loads, we conclude that membrane filtration is suitable for the decontamination of salivary TDM samples from infectious TB patients.

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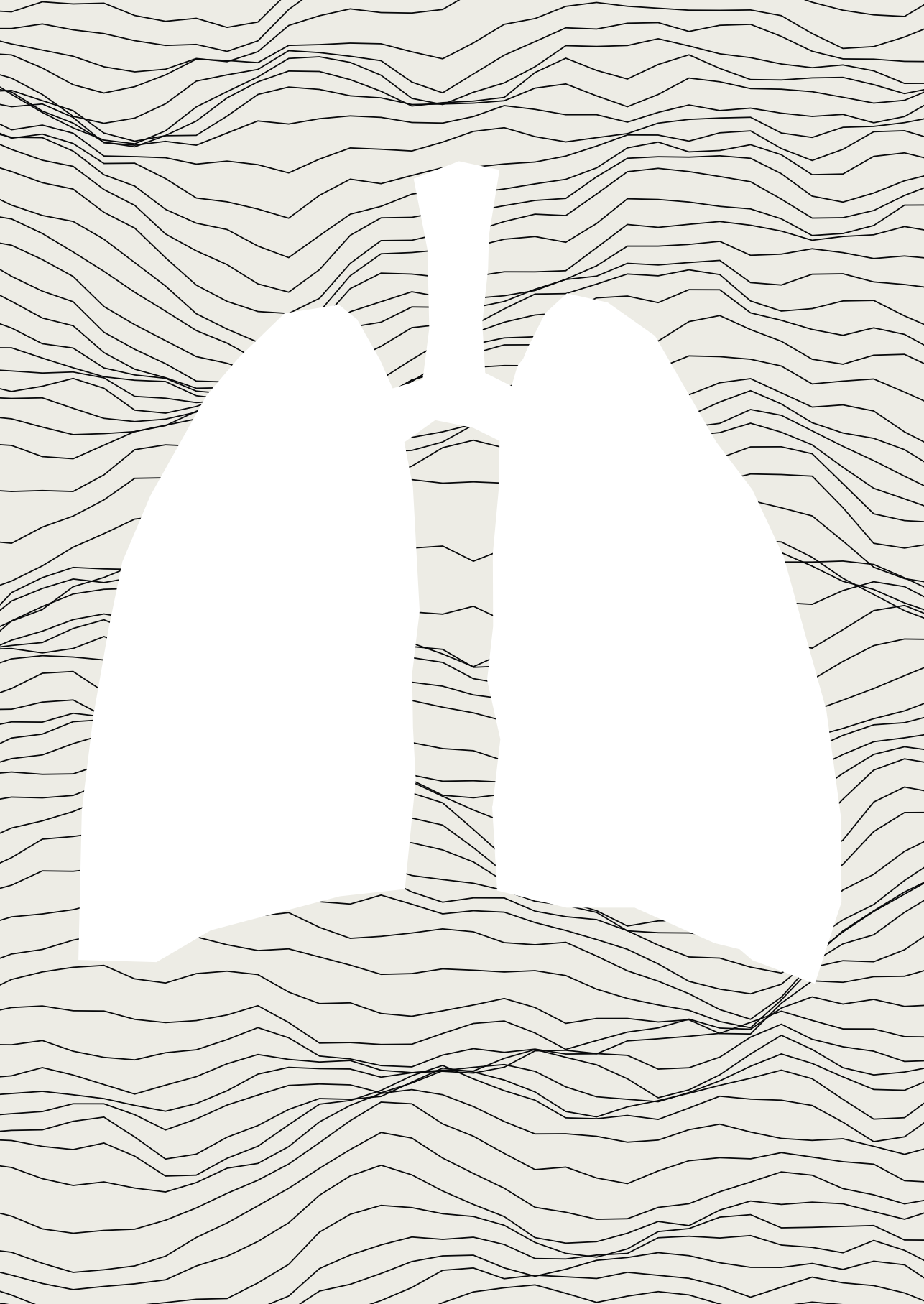

Chapter

4a

Limited Sampling Strategies Using Linear Regression and the Bayesian Approach for Therapeutic Drug Monitoring of Moxifloxacin in Tuberculosis Patients.

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ABSTRACT

Therapeutic drug monitoring (TDM) of moxifloxacin is recommended to improve response to tuberculosis treatment and reduce acquired drug resistance. Limited sampling strategies (LSSs) are able to reduce the burden of TDM by using a small number of appropriately timed samples to estimate the parameter of interest; the area under the concentration-time curve. This study aimed to develop LSSs for moxifloxacin alone (MFX) and together with rifampicin (MFX+RIF) in tuberculosis (TB) patients.

Population pharmacokinetic (popPK) models were developed for MFX (*n*=77) and MFX+RIF (*n*=24). In addition, LSSs using Bayesian approach and multiple linear regression were developed. Jackknife analysis was used for internal validation of the popPK models and multiple linear regression LSSs. Clinically feasible LSSs (one to three samples, 6-h timespan postdose, and 1-h interval) were tested.

Moxifloxacin exposure was slightly underestimated in the one-compartment models of MFX (mean -5.1%, standard error [SE] 0.8%) and MFX+RIF (mean -10%, SE 2.5%). The Bayesian LSSs for MFX and MFX+RIF (both 0 and 6 h) slightly underestimated drug exposure (MFX mean -4.8%, SE 1.3%; MFX+RIF mean -5.5%, SE 3.1%). The multiple linear regression LSS for MFX (0 and 4 h) and MFX+RIF (1 and 6 h), showed a mean overestimation of 0.2% (SE 1.3%) and 0.9% (SE 2.1%), respectively.

LSSs were successfully developed using the Bayesian approach (MFX and MFX+RIF; 0 and 6 h) and multiple linear regression (MFX 0 and 4 h; MFX+RIF, 1 and 6 h). These LSSs can be implemented in clinical practice to facilitate TDM of moxifloxacin in TB patients.

INTRODUCTION

Each year, the global tuberculosis (TB) incidence declines with approximately 2%, while by 2020 an annual 4 to 5% decline is strived for by the World Health Organization (WHO) [1]. Multidrug-resistant TB (MDR-TB) remains a major problem, with an estimated number of 458,000 cases in 2017 [1]. Currently, the worldwide success rate of MDR-TB treatment is 55% and this is considered low compared to a success rate of 85% for drug-susceptible TB (DS-TB) [1].

Moxifloxacin (MFX), a fluoroquinolone, is one of the most important drugs for the treatment of MDR-TB [2], but it has also been used as an alternative to first-line anti-TB drugs if not well tolerated or suggested to include in case of isoniazid resistance [3–5]. In general, the toxicity profile of moxifloxacin is rather mild, though it includes concentration-dependent corrected QT interval prolongation and, rarely, tendinopathy [6–9]. A clinically relevant drug-drug interaction is the combination of moxifloxacin with rifampicin, since these two drugs can be used concomitantly in TB treatment. Rifampicin (RIF) lowers the moxifloxacin area under the concentration-time curve of 0 to 24 h ($AUC_{0\text{-}24}$) with approximately 30% by inducing phase II metabolising enzymes (glucuronosyltransferase and sulfotransferase) [10–12].

The efficacy of fluoroquinolones is related to the ratio of $AUC_{0\text{-}24}$ to minimal inhibitory concentration ($AUC_{0\text{-}24}$/MIC) [13,14]. The fluoroquinolone exposure is effective against Gram-negative bacteria at an $AUC_{0\text{-}24}$/MIC ratio of >100 to 125 and against Gram-positive species at an $AUC_{0\text{-}24}$/MIC ratio of >25 to 30 [13,15,16]. An *in vitro* moxifloxacin exposure of unbound (*f*)$AUC_{0\text{-}24}$/MIC ratio of >53 was able to substantially decrease the total population of *M. tuberculosis* by >3 $\log_{10}$ CFU/mL as well as suppress emergence of drug resistance, while an *f*$AUC_{0\text{-}24}$/MIC ratio of >102 completely killed the fluoroquinolone-sensitive population of *M. tuberculosis* without observing the development of drug resistance [17]. Approximately 50% of moxifloxacin is assumed to be protein bound, although protein binding is highly variable between individuals and might be concentration dependent [13,16,18,19]. Corresponding with *f*$AUC_{0\text{-}24}$/MIC ratio of >53 and a fraction unbound of 0.5, the target total (bound and unbound) $AUC_{0\text{-}24}$/MIC ratio of >100 to 125 is regularly used in TB, because individual data of protein binding is often lacking [18,20,21]. In case of a proven susceptibility for moxifloxacin while lacking a MIC value of the strain, the target $AUC_{0\text{-}24}$ is generally set at >50 to 65 mg·h/L based on a critical concentration of 0.5 mg/L [22,23].

Therapeutic drug monitoring (TDM) is recommended by the American Thoracic Society for all second-line drugs, including moxifloxacin [24,25]. It is important to monitor the moxifloxacin exposure in TB patients to determine an individualized dose,

because of substantial interindividual pharmacokinetic variability and relevant drug-drug interactions with the risk of treatment failure and developing drug resistance [18,26–28]. However, routine TDM to estimate the AUC_{0-24} requiring frequent blood sampling is time-consuming, a burden for patients and health care professionals, and expensive. Optimising the sampling schedule by developing a limited sampling strategy (LSS) could overcome these difficulties with TDM in TB treatment [29].

There are two main methods to develop an LSS; the Bayesian approach and multiple linear regression [30]. The advantages of the Bayesian approach are the flexible timing of samples as the population pharmacokinetic model can correct for deviations and that it takes a number of parameters into account for example sex, age, and kidney function, leading to a more accurate estimation of AUC_{0-24}. The advantage of multiple linear regression-based LSSs is that these do not require modelling software and AUC_{0-24} can be easily estimated using only an equation and the measurement of drug concentrations. The disadvantage is that samples must be taken exactly according to the predefined schedule and the population of interest should be comparable because patient characteristics are not included in the equations to estimate drug exposure [30].

Pranger *et al.* described a LSS for moxifloxacin for the first time using t=4 and 14 h postdose samples [21]. This sampling strategy can be considered unpractical to be used in daily practice. Magis-Escurra *et al.* described LSSs to simultaneously estimate the AUC_{0-24} of all first-line drugs, together with moxifloxacin (t=1, 4, and 6 h or t=2, 4, and 6 h), but did not differentiate between patients using moxifloxacin alone and moxifloxacin in combination with rifampicin [20]. Therefore the influence of the drug-drug interaction between moxifloxacin and rifampicin, namely, an increased moxifloxacin clearance, was not taken into account in these LSSs.

Therefore, the aim of this study was to develop and validate two population pharmacokinetic models of moxifloxacin (alone and with rifampicin), along with clinically feasible LSSs using the Bayesian approach, as well as multiple linear regression, for the purpose of TDM of moxifloxacin in TB patients.

MATERIALS AND METHODS

Study population

This study used three databases. Database 1 consisted of retrospective data of routine TDM in 67 tuberculosis patients treated at Tuberculosis Center Beatrixoord, University Medical Center Groningen, Groningen,The Netherlands and was collected between January 2006 and May 2017, partly published earlier [18]. All patients received moxifloxacin (with or without rifampicin) as part of their daily TB treatment and pharmacokinetic curves were obtained as part of routine TDM care. Each patient was only included once. Various sampling schedules were used, but most profiles included t=0, and 1, 2, 3, 4, and 8 h postdose samples. Pharmacokinetic profiles consisting of less than three data points were excluded. The second database included data of 25 TB patients participating in a clinical study in Thessaloniki, Greece [31]. After at least 12 days of treatment with moxifloxacin with or without rifampicin, blood samples were collected at t=0, and at 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 h after drug intake. The third database consisted of pharmacokinetic data of nine Brazilian TB patients receiving 400 mg moxifloxacin (no rifampicin) daily in an early bactericidal activity study [14]. At day 5, blood samples were collected at t=0, and at 1, 2, 4, 8, 12, 18 and 24 h after drug intake.

As steady state is reached within 3 to 5 days of treatment with moxifloxacin, all data were collected during steady-state conditions [11]. In general, no informed consent was required, due to the retrospective nature of the study.

The total study population was split in two groups - patients that received moxifloxacin alone (MFX) and patients that received moxifloxacin together with rifampicin (MFX+RIF) - because of the pharmacokinetic drug-drug interaction between rifampicin and moxifloxacin [10]. Since sample collection in the MFX+RIF group was performed after a median number of days on rifampicin treatment of 35 (interquartile range [IQR] 13 to 87 days), maximum enzyme induction by rifampicin was expected to be reached in most patients [32].

Patient characteristics of both groups were tested for significant differences, median (IQR) using the Mann-Whitney U test and number (%) using the Fisher exact test in IBM SPS Statistics (version 23; IBM Corp., Armonk, NY). P values <0.05 were considered significant.

Population pharmacokinetic model

For each group, MFX and MFX+RIF, a population pharmacokinetic model was developed using the iterative two-stage Bayesian procedure of the KinPop module of MWPharm (version 3.82; Mediware, The Netherlands). Since the pharmacokinetics of moxifloxacin have been described with one compartment [14,21], as well as two-compartment models [33,34], both types were evaluated. The population

pharmacokinetic parameters of the models were assumed to be log normally distributed, with a residual error and concentration-dependent standard deviation (SD; SD=0.1+0.1*C, where C is the moxifloxacin concentration in mg/L). Because the bioavailability (F) of moxifloxacin is almost complete [11] and pharmacokinetic data following intravenous administration was not available, F was fixed at 1 in the analysis and pharmacokinetic parameters are presented relative to F. Moxifloxacin is mainly metabolised in the liver by glucuronosyltransferase and sulfotransferase (ca. 80%) [11]. Only total body clearance (CL), the sum of metabolic and renal clearance, was included in the model development because it was not possible to determine renal clearance due to a small range of creatinine clearance values in our data set.

We started the analysis with a single default one compartment model for both MFX and MFX+RIF developed by Pranger *et al* using a very similar methodology [21]. This study found comparable pharmacokinetic parameters of MFX and MFX+RIF, although likely due to a small sample size. Two default two-compartment models were used, one for MFX and one for MFX+RIF [33,35]. Modelling was started with all parameters fixed, and Akaike Information Criterion (AIC) was used to evaluate the model [36]. Subsequently, one by one parameters were Bayesian estimated and each step was evaluated by calculation of the AIC. A reduction of the AIC with at least three points was regarded as a significant improvement of the model [37]. One-compartment models included the parameters CL, volume of distribution (V), and absorption rate constant (K_a). Two-compartment models included the parameters K_a, CL, the intercompartmental clearance (CL_{12}), the central volume of distribution (V_1), the volume of distribution of the second compartment (V_2), and the lag time for absorption (T_{lag}). Afterwards, T_{lag} was added to the best performing one-compartment model and evaluated for goodness of fit as well because of oral intake of moxifloxacin. The default two-compartment models already included T_{lag}. The final models of MFX and MFX+RIF were chosen based on AIC values.

The final models were internally validated using 11 different (n-7) sub models for MFX and 12 (n-2) sub models for MFX+RIF, each leaving out randomly chosen pharmacokinetic curves. All pharmacokinetic curves were excluded once (jackknife analysis). The Bayesian fitted $AUC_{0\text{-}24}$ of each left out curve ($AUC_{0\text{-}24,fit}$) was compared to the $AUC_{0\text{-}24}$ calculated with the trapezoidal rule ($AUC_{0\text{-}24,ref}$) using a Bland-Altman plot and Passing Bablok regression (Analyse-it 4.81; Analyse-it Software Ltd., Leeds, United Kingdom). In the calculation of $AUC_{0\text{-}24,ref}$, moxifloxacin concentrations at t=0 and 24 h after drug intake were assumed to be equal due to steady-state conditions. The C_{max} (mg/L) was defined as the highest observed moxifloxacin concentration and T_{max} (h) as the time at which C_{max} occurred. Noncompartmental parameters (i.e. $AUC_{0\text{-}24,ref}$, dose-corrected $AUC_{0\text{-}24,ref}$ to the standard dose of 400 mg, C_{max}, T_{max}) and population pharmacokinetic model parameters of the MFX and MFX+RIF group were compared and tested for significant differences using the Mann-Whitney U test.

LSS using Bayesian approach

Using the Bayesian approach, we performed two separate analyses to develop LSSs; one for MFX and one for MFX+RIF. Using Monte Carlo simulation in MWPharm, 1,000 virtual pharmacokinetic profiles were created to represent the pharmacokinetic data used in the development of the LSS. The reference patient for the Monte Carlo simulation was selected based on representative pharmacokinetic data and patient characteristics. For MFX, a 36-year-old male with a bodyweight of 57 kg, a height of 1.60 m, a body mass index (BMI) of 22.2 kg/m^2, a serum creatinine of 74 μmol/L, and a moxifloxacin dose of 7.0 mg/kg was chosen. For MFX+RIF, a 56-year-old male with a bodyweight of 56 kg, a height of 1.63 m, a BMI of 21.1 kg/m^2, a serum creatinine of 80 μmol/L, and a moxifloxacin dose of 7.1 mg/kg was selected. The LSSs were optimised using the steady-state $AUC_{0\text{-}24}$. Only clinically feasible LSSs using one to three samples between 0 and 6 h post-dose and a sample interval of 1 h were tested. The LSSs were evaluated using acceptance criteria for precision and bias (RMSE<15%, MPE<5%) [18]. For both MFX and MFX+RIF, one LSS was chosen for internal validation based on performance, as well as clinical feasibility. The $AUC_{0\text{-}24}$ estimated with the chosen LSS ($AUC_{0\text{-}24,est}$) was compared with $AUC_{0\text{-}24,ref}$ using a Bland-Altman plot and Passing Bablok regression. In addition, the performance of a LSS using 2 and 6 h postdose samples was evaluated because this LSS is frequently used for TDM of anti-TB drugs [38].

LSS using multiple linear regression

Two separate analyses (MFX and MFX+RIF) using multiple linear regression were performed.

Only clinically suitable LSSs (one to three samples, 0 to 6 h postdose, and sample interval of 1 h) were included in the analysis. Each analysis excluded the pharmacokinetic curves without data at the selected time points of the LSS, resulting in a variable number of included curves (N). Multiple linear regression in Microsoft Office Excel 2010 was used to evaluate the correlation of moxifloxacin concentrations at the chosen time points of the LSS and $AUC_{0\text{-}24,ref}$. The acceptance criteria (RMSE<15%, MPE<5%) were applied to each LSS [18]. Internal validation using 11 different (n-6) subanalyses for MFX and 14 (n-1) subanalyses for MFX+RIF was used to evaluate the performance of the LSSs. Each subanalysis excluded randomly chosen profiles, and all profiles were excluded once (jackknife analysis). Agreement of $AUC_{0\text{-}24,est}$ and $AUC_{0\text{-}24,ref}$ was tested using a Bland-Altman plot and Passing Bablok regression.

RESULTS

Study population

The group with moxifloxacin alone (MFX) included pharmacokinetic profiles of 77 TB patients and the group with moxifloxacin together with rifampicin (MFX+RIF) included profiles of 24 TB patients (Figure 1). The baseline characteristics sex, age, and height were significantly different (P<0.05) between these two groups (Table 1). Additionally, the $AUC_{0\text{-}24}$ calculated with the trapezoidal rule ($AUC_{0\text{-}24,ref}$) was significantly lower, and time of peak concentration (T_{max}) was significantly earlier in the MFX+RIF group (P<0.05, Table 2). Several abnormal pharmacokinetic curves (e.g., delayed absorption or single aberrant data point) were observed in both the MFX and MFX+RIF group.

Table 1. Patient characteristics of the study population. Data is presented as median (IQR) unless otherwise stated. BMI: body mass index. NA: not applicable.

	Median (IQR)		
Parameter	**MFX (n=77)**	**MFX+RIF (n=24)**	***P***
Male, no. (%)	47 (61.0)	21 (87.5)	0.023[a]
Age (yr)	33 (25-41)	48 (36-62)	<0.001[b]
Height (m)	1.65 (1.59-1.74)	1.72 (1.64-1.76)	0.047[b]
Weight (kg)	58.0 (52.5-68.2)	55.5 (52.3-63.9)	0.500[b]
Dose (mg/kg bodyweight)	7.0 (5.9-8.1)	7.3 (6.4-7.7)	0.629[b]
BMI (kg/m^2)	21.2 (19.3-23.5)	20.1 (17.6-22.7)	0.053[b]
Serum creatinine (µmol/L)	71 (59-83)	73 (63-91)	0.752[b]
No. of samples/curve	7 (6-8)	10 (7-10)	<0.001[b]
Days on rifampicin treatment at time of sampling	NA	35 (13-87)	NA

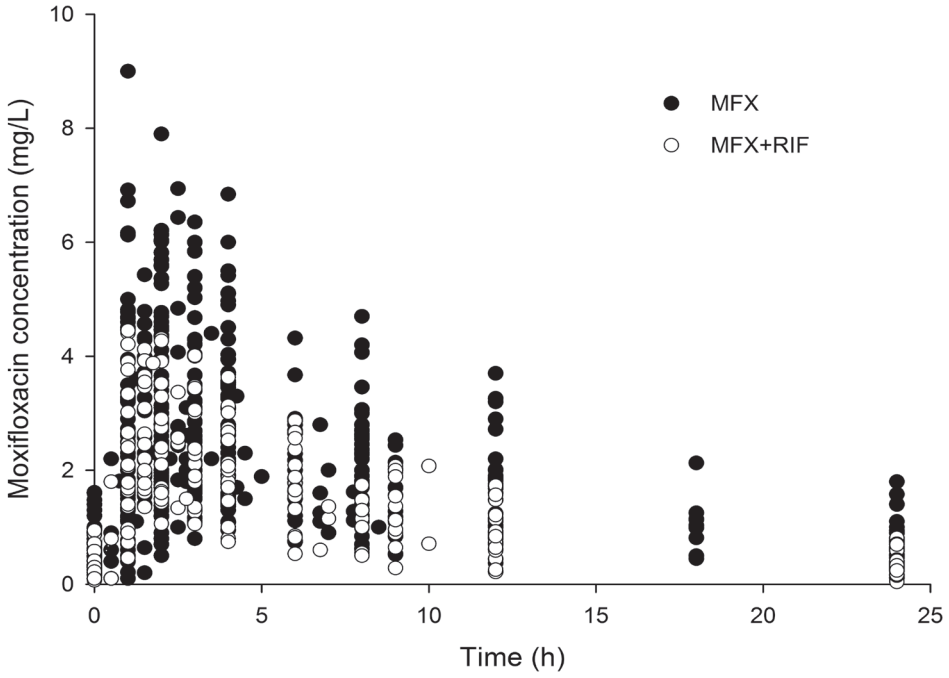
[a] Fisher exact test
[b] Mann-Whitney U test

Table 2. Non-compartmental parameters of MFX and MFX+RIF

	Median (IQR)		
Parameter	**MFX (n=77)**	**MFX+RIF (n=24)**	***P*[a]**
$AUC_{0\text{-}24,ref}$ (mg·h/L)	34.0 (25.2-49.2)	25.5 (20.4-31.6)	0.006
Dose-corrected $AUC_{0\text{-}24,ref}$ (mg·h/L, per 400 mg)	30.8 (24.7-40.3)	25.5 (19.1-31.3)	0.014
C_{max} (mg/L)	3.00 (2.27-4.64)	2.83 (2.25-3.90)	0.407
T_{max} (h)	2 (1-3)	1.5 (1-2)	0.018

[a] Mann-Whitney U test

Figure 1. Moxifloxacin concentrations of the pharmacokinetic curves of MFX (n=77) and MFX+RIF (n=24).



Population pharmacokinetic model

For both MFX and MFX+RIF, an one-compartment model with lag time resulted in the lowest Akaike Information Criterion (AIC) values and described the data best (Table 3). Two-compartment models were not favourable for either MFX or MFX+RIF. A statistical comparison of the pharmacokinetic parameters of the MFX versus MFX+RIF model is provided in Table 4. The total body clearance (CL) was higher, and the lag time (T_{lag}) was shorter in the MFX+RIF model ($P<0.05$).

Table 3. Starting parameters of the default one-compartment and two-compartment models of MFX and MFX+RIF together with the parameters of the final models based on AIC.

Parameter	Mean ± SD[a]			
	Default model MFX	**Final model MFX**	**Default model MFX+RIF**	**Final model MFX+RIF**
One compartment				
CL/F (L/h)	18.500±8.600	14.655±5.683	18.500±8.600	19.898±8.800
V/F (L/kg bodyweight)	3.000±0.7000	2.7467±1.0077	3.000±0.7000	2.8264±0.6902
K_a (/h)	1.1500±1.1600	6.2904±4.8164	1.1500±1.1600	7.3755±6.8205
T_{lag} (h)	NA	0.8769±0.2357	NA	0.7460±0.1093
AIC	5564	903	1361	236
Two compartments				
CL/F (L/h)	11.800±0.740	13.428±5.494	49.100±2.550	18.108±8.570
CL_{12}/F (L/h)	5.620±1.080	5.620±1.080	3.150±0.800	3.150±0.800
V_1/F (L/kg bodyweight)	2.5300±0.0800	2.4898±1.0838	2.8400±0.1500	2.7004±0.7535
V_2/F (L/kg bodyweight)	0.6900±0.1300	0.6900±0.1300	0.8900±0.1900	0.8900±0.1900
K_a (/h)	16.7000±2.9200	3.2774±2.9422	2.3200±0.5600	6.2314±9.0508
T_{lag} (h)	0.4600±0.0800	0.7940±0.3720	0.6000±0.0700	0.7312±0.1995
AIC	11892	940	2995	249

[a] Values are represented as means ± the SD, except AIC.

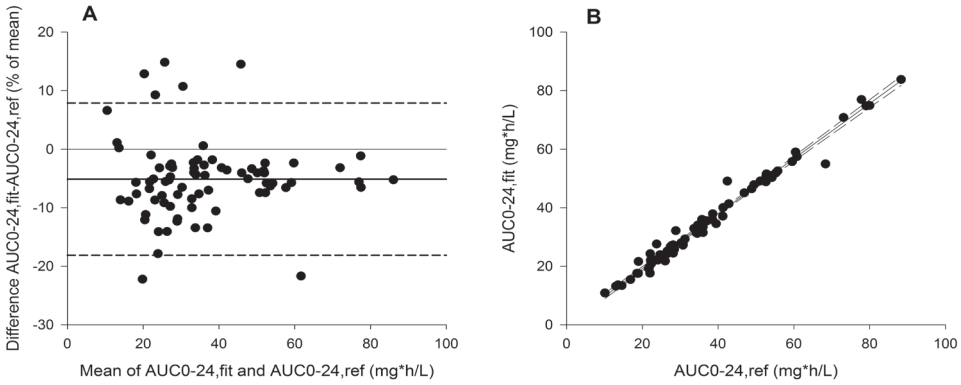
Table 4. Comparison of pharmacokinetic parameters of the population pharmacokinetic model of MFX versus MFX+RIF. Geometric mean±SD.

Parameter	Geometric mean ± SD		
	MFX (n=77)	**MFX+RIF (n=24)**	***P*[a]**
CL/F (L/h)	14.655±5.683	19.898±8.800	0.004
V/F (L/kg bodyweight)	2.7467±1.0077	2.8264±0.6902	0.534
K_a (/h)	6.2904±4.8164	7.3755±6.8205	0.231
T_{lag} (h)	0.8769±0.2357	0.7460±0.1093	<0.001

[a] Mann-Whitney U test

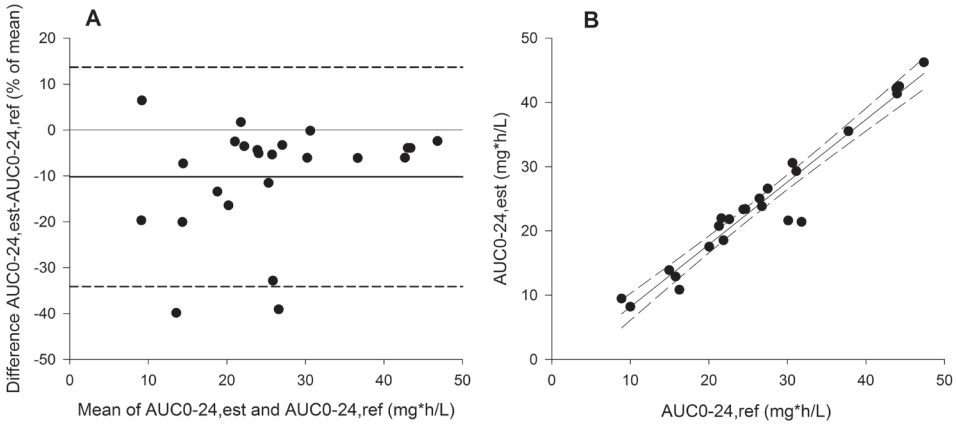
Internal validation of the two models resulted in a mean underestimation of AUC_{0-24} of -5.1% (standard error [SE] 0.8%) in the MFX model and a mean underestimation of -10% (SE 2.5%) in the MFX+RIF model (Figure 2A and Figure 3A). In the validation of the MFX model, an r^2 of 0.98, a y-axis intercept of -0.3 (95% confidence interval [CI] = -1.1 to 0.5), and a slope of 0.96 (95% CI = 0.94 to 0.98) were found in the Passing Bablok regression (Figure 2B). For the MFX+RIF model, an r^2 of 0.94, a y-axis intercept of -1.0 (95% CI = -4.1 to 0.9), and a slope of 0.98 (95% CI = 0.92 to 1.07) was found in the Passing Bablok regression (Figure 3B).

Figure 2. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n-7) of population pharmacokinetic model of MFX (n=77).



4a

Figure 3. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n-2) of population pharmacokinetic model of MFX+RIF (n=24).



LSS using the Bayesian approach

The best performing LSSs of MFX and MFX+RIF are shown in Table 5 and Table 6, including mean prediction error (MPE), root mean-squared error (RMSE), and r^2 to evaluate the performance of the LSSs. The performance of the LSS using t=2 and 6 h samples was evaluated as well because this strategy is currently used in many health facilities for TDM of anti-TB drugs [38]. Not all strategies met the preset acceptance criteria (RMSE<15%, MPE<5%) [21]. Low r^2 values were observed that were caused by high interindividual variability in performance of the LSSs.

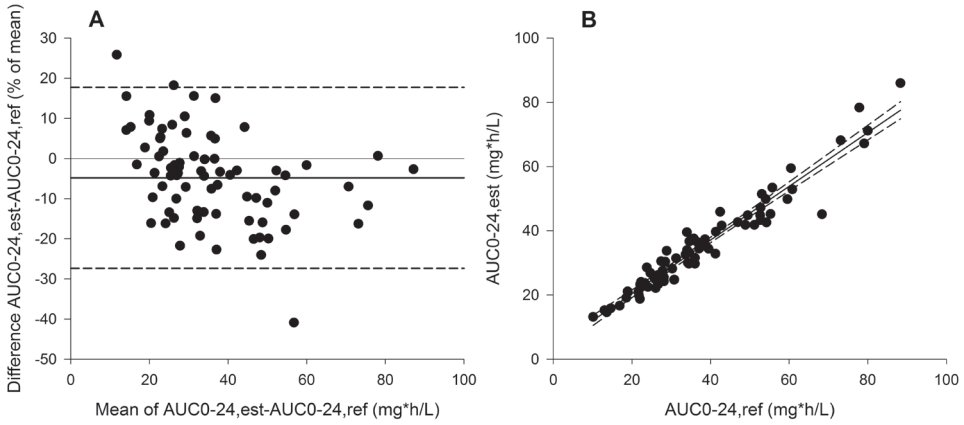
For the MFX model, an LSS using t=0 and 6 h samples was chosen for further evaluation (RSME=15.17%, MPE= 2.42%, r^2=0.874), because it required one sample less than the three-sample strategies, while RMSE was only slightly above 15%. The internal

validation showed a mean underestimation of -4.8% (SE 1.3%). However, low AUC_{0-24} values were more frequently overestimated in contrast to an AUC_{0-24} of >40 mg*h/L mainly being underestimated by the LSS (Figure 4A). The Passing Bablok regression showed an r^2 of 0.94, a y-axis intercept of 3.4 (95% CI = 1.6 to 4.9), and a slope of 0.85 (95% CI = 0.80 to 0.91) (Figure 4B).

Table 5. LSSs of moxifloxacin without rifampicin using the Bayesian approach.

Sampling time point (h)			MPE (%)	RMSE (%)	r^2
5			2.69	24.64	0.659
6			1.74	22.00	0.726
2	6		-2.20	20.83	0.742
0	5		2.84	15.82	0.864
0	6		2.42	15.17	0.874
0	4	6	0.97	13.22	0.883
0	5	6	1.03	12.97	0.888

Figure 4. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation of Bayesian LSS (t=0 and 6 h) of MFX (n=77).

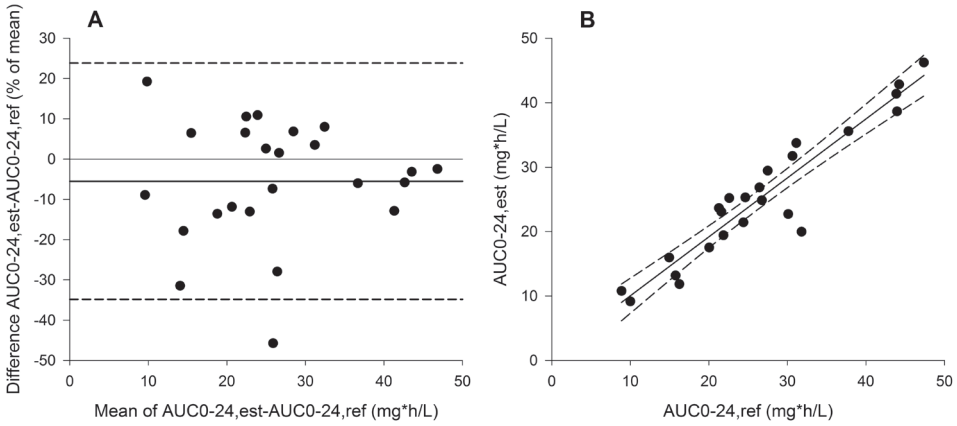


For the MFX+RIF model, an LSS using t=0 h and 6 h samples was chosen for further evaluation (RSME=15.81%, MPE= 2.35%, r^2=0.885) because of the benefit of requiring only two samples while performance in terms of RSME and MPE remained acceptable. The internal validation showed a mean underestimation of -5.5% (SE 3.1%) in the Bland-Altman plot and an r^2 of 0.90, a y-axis intercept of -1.3 (95% CI = -4.4 to 2.8), and a slope of 1.0 (95% CI = 0.88 to 1.10) in the Passing Bablok regression (Figure 5).

Table 6. LSSs of moxifloxacin with rifampicin using the Bayesian approach.

Sampling time point (h)			MPE (%)	RMSE (%)	r^2
5			-1.97	22.35	0.768
6			-0.79	19.22	0.826
2	6		-2.89	18.38	0.832
0	5		1.88	16.67	0.877
0	6		2.35	15.81	0.885
0	4	6	1.06	14.10	0.907
0	5	6	0.79	13.73	0.912

Figure 5. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation of Bayesian LSS (t=0 and 6 h) of MFX+RIF (n=24).



LSS using multiple linear regression

Tables 7 and 8 show the best-performing LSSs for MFX and MFX+RIF. The performance of the frequently used LSS using t=2 and 6 h samples was evaluated as well and included in the tables. None of the MFX LSSs met the acceptance criteria (RMSE<15%, MPE<5%) as bias was above 5% for all combinations. For MFX+RIF, the two three-sample strategies and LSS using t=1 and 6 h samples met the acceptance criteria.

The MFX LSS using t=0 and 4 h samples (RSME=9.25%, MPE= 6.85%, r^2=0.957) had a performance comparable to the three-sample strategies while being more clinically feasible and therefore was chosen for further evaluation. In contrast to the Bayesian LSSs for MFX and MFX+RIF, a t=0 and 6 h strategy was not feasible using a multiple linear regression approach as its performance was substantially worse (RMSE=12.01, MPE=9.43, r^2=0.905) than the LSS using t=0 and 4 h samples. Internal validation of this t=0 and 4 h LSS for MFX showed a mean overestimation of 0.2%

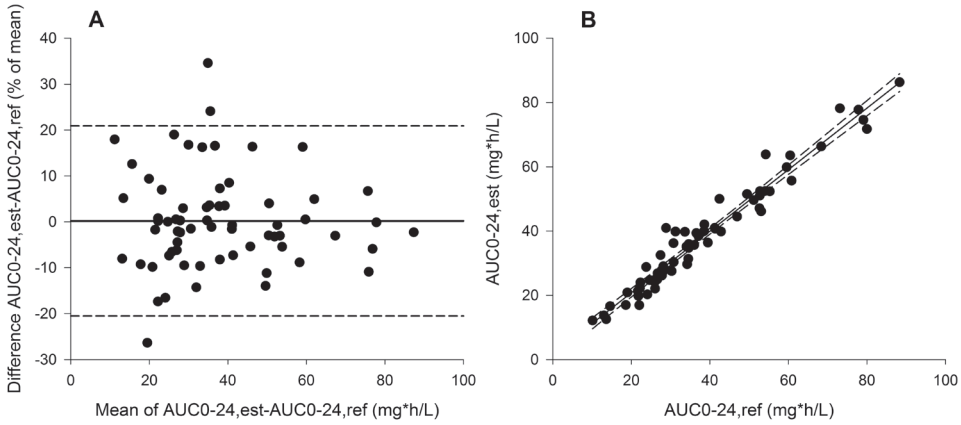
(SE 1.3%) in the Bland-Altman plot and an r^2 of 0.95, a y-axis intercept of 0.1 (95% CI = -2.1 to 1.6), and a slope of 0.99 (95% CI = 0.95 to 1.06) in the Passing Bablok regression (Figure 6).

Table 7. LSSs of moxifloxacin without RIF using linear regression. N: number of included curves.

Sampling time point (h)			Equation[a]	N	MPE (%)	RMSE (%)	r^2
4			$AUC_{0\text{-}24,est}$ = 3.47+12.32*C4	66	12.68	17.02	0.862
6			$AUC_{0\text{-}24,est}$ = 2.27+15.01*C6	22	14.85	16.89	0.822
2	6		$AUC_{0\text{-}24,est}$ = -1.44+3.55*C2+11.24*C6	22	10.02	12.27	0.901
0	3		$AUC_{0\text{-}24,est}$ = 3.61+28.67*C0+5.38*C3	53	10.08	13.36	0.917
0	4		$AUC_{0\text{-}24,est}$ = 1.10+20.76*C0+8.68*C4	66	6.85	9.42	0.957
0	2	4	$AUC_{0\text{-}24,est}$ = 1.10+20.37*C0+0.92*C2+7.71*C4	65	6.91	9.25	0.958
0	1	4	$AUC_{0\text{-}24,est}$ = 1.00+21.06*C0+0.66*C1+8.02*C4	63	7.07	9.23	0.958

[a] C0, C1, etc., are moxifloxacin concentrations at t=0 h, t=1 h, etc.

Figure 6. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n-6) of LSS using multiple linear regression (t=0 and 4 h) of MFX (n=66).



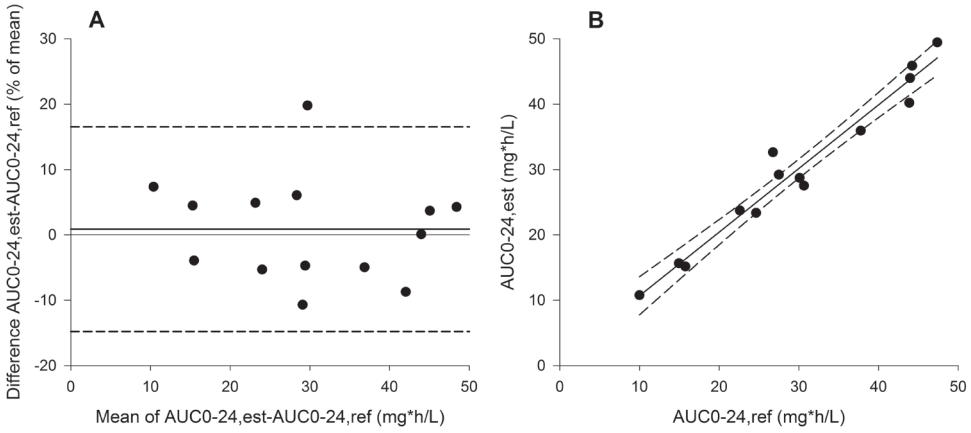
For MFX+RIF, the LSS using t=1 and 6 h samples (RSME=6.09%, MPE= 4.83%, r^2=0.971) was chosen for further evaluation, because of clinical suitability in addition to good performance (RMSE<15%, MPE<5%). Internal validation showed a mean overestimation of 0.9% (SE 2.1%) in the Bland-Altman plot and an r^2 of 0.96, a y-axis intercept of -0.2 (95% CI = -4.9 to 2.3), and a slope of 1.02 (95% CI = 0.88 to 1.15) in the Passing Bablok regression (Figure 7).

Table 8. LSSs of MFX+RIF using multiple linear regression. N: number of included curves.

Sampling time point (h)			Equation[a]	N	MPE (%)	RMSE (%)	r^2
3			$AUC_{0\text{-}24,\,est}$ =-2.76+13.28*C3	18	8.27	11.10	0.907
6			$AUC_{0\text{-}24,\,est}$ = 0.95+16.44*C6	16	6.93	8.87	0.941
2	6		$AUC_{0\text{-}24,\,est}$ = 0.08+1.21*C2+15.02*C6	13	6.23	7.88	0.945
0	6		$AUC_{0\text{-}24,\,est}$ = 1.38+7.40*C0+14.05*C6	16	5.85	6.99	0.960
1	6		$AUC_{0\text{-}24,\,est}$ = 1.43+0.22*C1+16.25*C6	14	4.83	6.09	0.971
0	3	6	$AUC_{0\text{-}24,\,est}$ = 1.20+10.66*C0-0.39*C3+13.52*C6	15	4.85	5.31	0.977
0	2	6	$AUC_{0\text{-}24,\,est}$ = 0.46+9.99*C0+0.13*C2+13.39*C6	13	4.20	4.66	0.978

[a] C0, C1, etc., are moxifloxacin concentrations at t=0 h, t=1 h, etc.

Figure 7. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n-1) of LSS using multiple linear regression (t=1 and 6 h) of MFX+RIF (n=14).



DISCUSSION

In this study, we successfully developed a population pharmacokinetic model for moxifloxacin alone and in combination with rifampicin. Furthermore, we developed and validated sampling strategies using the Bayesian approach (MFX and MFX+RIF t=0 and 6 h) and multiple linear regression (MFX t=0 and 4 h; MFX+RIF t=1 and 6 h) for both groups as well.

It was decided to develop two separate population pharmacokinetic models, and therefore also separate LSSs, for moxifloxacin alone and in combination with rifampicin after observing a significant effect of rifampicin on the pharmacokinetics of moxifloxacin. The population pharmacokinetic model of MFX+RIF showed an approximately 35% higher total body clearance of moxifloxacin compared to the MFX pharmacokinetic model (Table 4). This was to be expected as rifampicin enhances metabolism of moxifloxacin and increases in total body clearance of 45 to 50% have been reported by others [10,39]. As a result of this drug-drug interaction, pharmacokinetic profiles of MFX+RIF showed reduced moxifloxacin concentrations and 25% lower median moxifloxacin $AUC_{0\text{-}24}$ values after administration of a similar dose (Figure 1, Table 2). The latter is confirmed by a significant -17% difference in dose-corrected $AUC_{0\text{-}24,ref}$ between the MFX and MFX+RIF group (Table 2). The decrease in moxifloxacin exposure by rifampicin was estimated at 30% in previous studies [10,12,39], although others found nonsignificant or smaller decreases in moxifloxacin $AUC_{0\text{-}24}$ [21,31]. In this study we observed only a slightly smaller effect of rifampicin on the total body clearance and exposure than previously reported. This might be explained by the possibility that maximal enzyme induction was not yet achieved at the moment of sampling in a few cases, since it generally takes around 10 to 14 days of rifampicin treatment to reach maximal induction [40]. Furthermore, we encountered a significant, but small, difference in lag time between the MFX and MFX+RIF models and in T_{max} of the included pharmacokinetic profiles. Faster absorption of moxifloxacin in combination rifampicin was found in other studies as well; however, some reported the opposite effect. This could suggest that lag time and T_{max} was not influenced by rifampicin, but more likely by other differences between the MFX and MFX+RIF group, such as concomitantly taken TB drugs or interindividual differences in absorption due to disease state.

In addition to the population pharmacokinetic models, we developed and validated LSSs using the Bayesian approach as well as multiple linear regression for MFX and MFX+RIF. LSSs of moxifloxacin have been described before. Pranger *et al.* found a Bayesian LSS with a comparable performance (RMSE=15%, MPE=-1.5%, r^2=0.90) compared to our LSSs for MFX and MFX+RIF [21]. The LSS of Magis-Escurra *et al.*

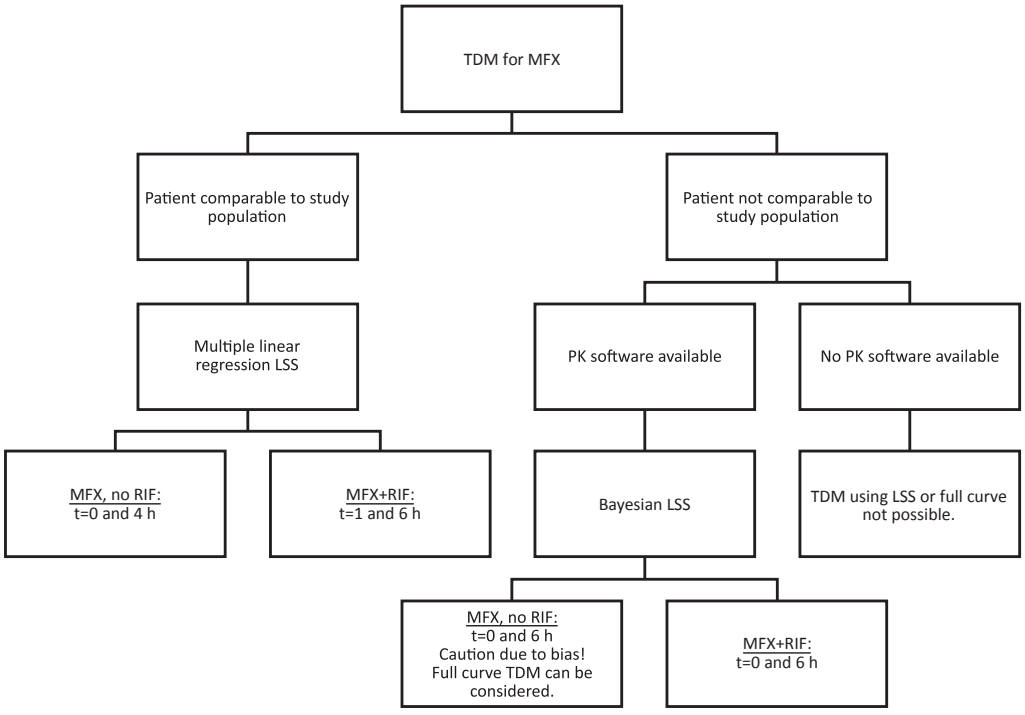
performed better (RMSE=1.45%, MPE=0.58%, r^2=0.9935) than the multiple linear regression LSSs proposed in this study [20]. However, a smaller sample size (n=12) was used to establish the equation, and this was not externally validated. Further, we provided suitable sampling strategies for multiple situations, in patients using moxifloxacin alone or together with rifampicin, and for centres that either do or do not have pharmacokinetic modelling software available. Health care professionals may select the LSS that is the most applicable to the circumstances.

The Bayesian LSS for MFX (t=0 and 6 h) showed a slight downward trend between the bias of the estimated $AUC_{0\text{-}24}$ and the mean of the estimated and actual $AUC_{0\text{-}24}$ (Figure 4). Low $AUC_{0\text{-}24}$ values were more frequently overestimated in comparison to higher $AUC_{0\text{-}24}$ values. A possible cause might be that we could not differentiate between metabolic clearance and renal clearance in both population pharmacokinetic models due to a small range of creatinine clearance in the study population. A relatively high exposure of moxifloxacin in patients with renal insufficiency could be underestimated since renal function may be overestimated and the other way around for patients with normal renal function and relatively low exposures. The pharmacokinetic modelling software will fit a curve, with the greatest likelihood of being the actual pharmacokinetic curve based on drug concentrations at 0 and 6 h, together with patient characteristics and data of the entire population. However, when the influence of creatinine clearance is not available, the software will pick a fit with average parameters, causing overestimation in low $AUC_{0\text{-}24}$ and underestimation in high $AUC_{0\text{-}24}$ ranges. We decided not to validate one of the better performing three-sample strategies from Table 5, since we focused on developing a clinically feasible LSS with a strong preference for only two samples. Furthermore, we aimed to provide a simple and well-performing alternative LSS for MFX using multiple linear regression (t=0 and 4 h). We recommend to use this LSS instead of the Bayesian LSS for MFX, particularly when low drug exposure is suspected, because overestimation of $AUC_{0\text{-}24}$ can lead to sub therapeutic dosing with treatment failure and acquired drug resistance as possible harmful consequence [26,41,42].

In this study we decided to validate one LSS for each situation (Bayesian or multiple linear regression; MFX or MFX+RIF), due to the significant influence of rifampicin on the pharmacokinetics of moxifloxacin and so there would be a suitable LSS for every patient in each health care centre. The LSSs using multiple linear regression performed rather well in our study population, but are less flexible in patients with different characteristics. A Bayesian LSS is therefore preferred for patients who are not comparable to our study populations since the population pharmacokinetic model is able to include some patient characteristics. Clinicians are guided to the best option for TDM of moxifloxacin by following the decision tree in Figure 8. For implementation

of moxifloxacin TDM using LSSs in daily practice, it would be convenient to be able to use one sampling strategy for both MFX and MFX+RIF. This study showed that it is possible to use t=0 and 6 h samples in a Bayesian LSS for both MFX as well as MFX+RIF and probably even in a multiple linear regression LSS for MFX+RIF, after successful validation. Unfortunately, a multiple linear regression strategy for MFX alone using t=0 and 6 h samples was not feasible because of inferior performance. Considering that TB patients are treated with a combination of multiple anti-TB drugs, one single LSS suitable for all drugs of interest is the ideal situation but, unfortunately, also rather challenging due to the various pharmacokinetic properties of the different drugs. Others did succeed in developing a LSS using multiple linear regression for simultaneously estimating exposure of all first-line drugs and moxifloxacin in a small population of TB patients [20]. A 2 and 6 h postdose sampling strategy is frequently used for TDM of anti-TB drugs since it is believed to be able to estimate C_{max} as well as to detect delayed absorption [38]. However, better performances were found for the LSSs proposed in this study, although the 2 and 6 h LSS performed within acceptable limits as well in the Bayesian approach and the multiple linear regression.

Figure 8. Clinical guide for choosing the best LSS for TDM of moxifloxacin alone or in combination with rifampicin.



In general, we noticed large inter-individual pharmacokinetic variation in terms of moxifloxacin concentrations (Figure 1), C_{max}, and AUC_{0-24} (Table 2) as described earlier [18], but also in K_a and CL/F (Table 4). Patients received 400, 600, or 800 mg of moxifloxacin; this obviously influenced drug concentration, C_{max}, and AUC_{0-24}, but not all variation could be explained by different dosage regimes. For MFX the AUC_{0-24} corrected to a 400-mg standard dose ranged from 10.2 to 79.1 mg*h/L, and for MFX+RIF the AUC_{0-24} corrected to a 400-mg standard dose ranged from 10.0 to 47.4 mg*h/L. This substantial interindividual variation is the reason why TDM of moxifloxacin is helpful to ensure optimal drug exposure and thus minimize the risk of treatment failure and developing acquired drug resistance [26,27]. The estimated AUC_{0-24} using one of the LSSs proposed, together with the MIC of the *M. tuberculosis* strain, will provide valuable information on the optimal moxifloxacin dose to be used in an individual patient.

A limitation to the study is the exclusion of the creatinine clearance from the population pharmacokinetic model. As discussed earlier, this could have led to the observed bias in the MFX LSS using 0 and 6 h samples since approximately 20% of moxifloxacin is eliminated unchanged in the urine. On the contrary, a well-performing LSS using multiple linear regression (t=0 and 4 h) is a suitable alternative for MFX. The lack of prospective or external validation of the population pharmacokinetic model and LSSs could be considered as another limitation. However, we were able to collect a large data set to develop the model and clinically feasible LSSs using a sufficient number of pharmacokinetic profiles. A strength of our study is that a large part of our data set consisted of drug concentrations which were collected as part of daily routine TDM. During visual check of the data we noticed several abnormal curves (both MFX and MFX+RIF) that, for instance, showed delayed absorption with T_{max} values of 4 to 6 h. These curves were not excluded from the study. The models and LSSs appeared to be able to adapt to this delayed absorption. In most cases, the subsequent decision to either increase the dose or not was similar. For these reasons, we expect the results reported here to represent the clinical practice of TDM using these LSSs very closely. The small sample size of the MFX+RIF group can be considered a limitation as well, although comparable to previously published LSS studies [21,43–46]. We consider this sample size as sufficient for exploratory objectives, since this is the first study that developed separate LSSs for moxifloxacin alone and in combination with rifampicin. Future research can build on the results described in this study.

In conclusion, we developed and validated two separate pharmacokinetic models for moxifloxacin alone and in combination with rifampicin in TB patients. We provided data to show significant differences in drug clearance and drug exposure between these groups. Furthermore, we developed and validated LSSs based on the Bayesian approach (MFX and MFX+RIF, 0 and 6 h) and multiple linear regression (MFX, 0 and 4 h; MFX+RIF, 1 and 6 h) that can be used to perform TDM on moxifloxacin in TB patients.

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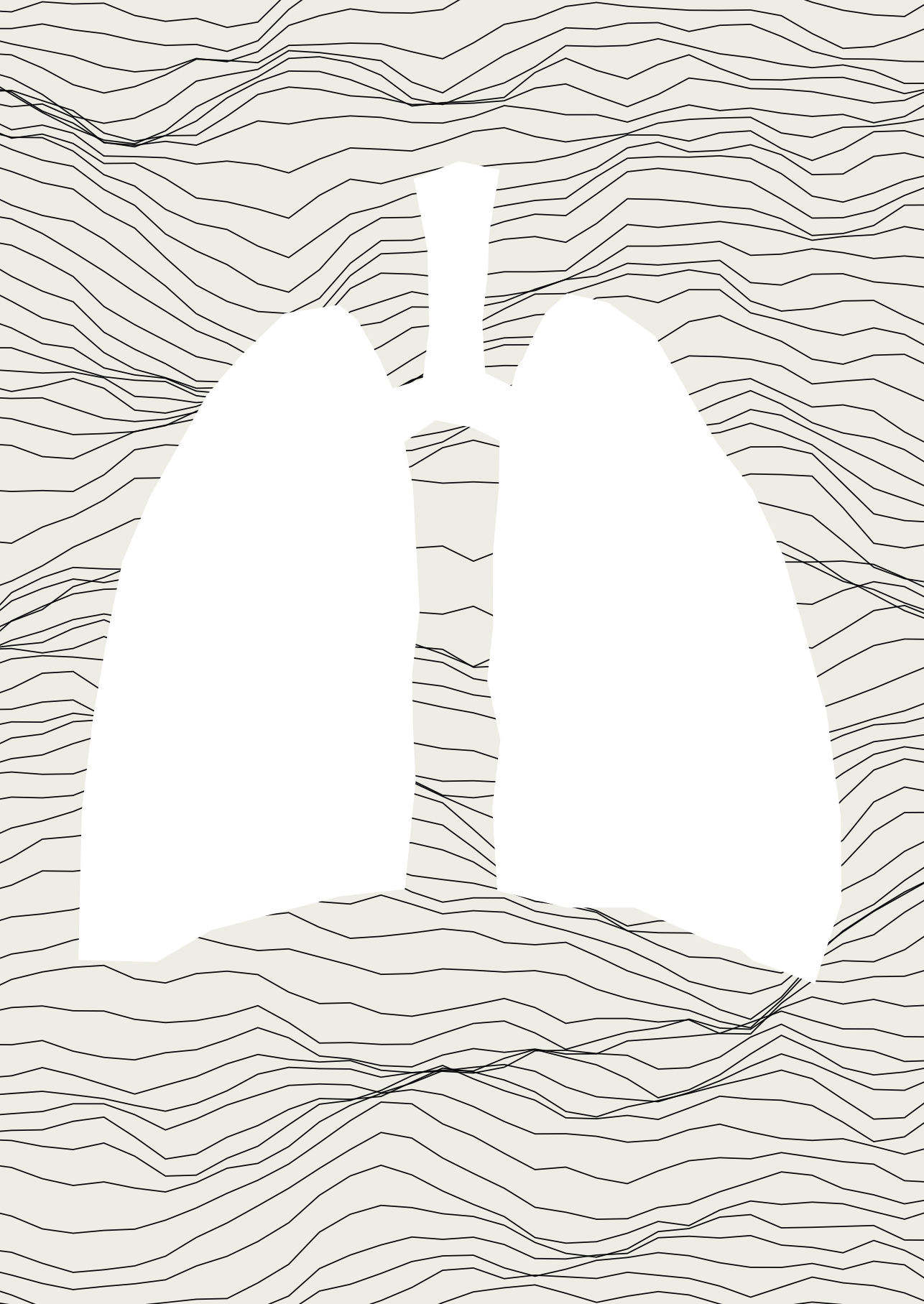

Chapter

4b

Population Pharmacokinetic Model and Limited Sampling Strategies for Personalized Dosing of Levofloxacin in Tuberculosis Patients.

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ABSTRACT

Levofloxacin is an antituberculosis drug with substantial interindividual pharmacokinetic variability; therapeutic drug monitoring (TDM) could therefore be helpful to improve treatment results. TDM would be more feasible with limited sampling strategies (LSSs), a method to estimate area under the concentration curve for the 24-h dosing interval (AUC_{0-24}) by using a limited number of samples. This study aimed to develop a population pharmacokinetic (popPK) model of levofloxacin in tuberculosis patients, along with LSSs using a Bayesian and multiple linear regression approach.

The popPK model and Bayesian LSS were developed using data of 30 patients and externally validated with 20 patients. The LSS based on multiple linear regression was internally validated using jackknife analysis. Only clinically suitable LSSs (maximum timespan, 8 h; minimum interval, 1 h; 1 to 3 samples) were tested. Performance criteria were root-mean-square error (RMSE) of <15%, mean prediction error (MPE) of <5%, and r^2 value of >0.95.

A one-compartment model with lag time best described the data while only slightly underestimating the AUC_{0-24} (mean, -7.9%; standard error [SE], 1.7%). The Bayesian LSS using 0- and 5-h postdose samples (RMSE, 8.8%; MPE, 0.42%; r^2=0.957) adequately estimated the AUC_{0-24}, with a mean underestimation of -4.4% (SE, 2.7%). The multiple linear regression LSS using 0- and 4-h postdose samples (RMSE, 7.0%; MPE, 5.5%; r^2=0.977) was internally validated, with a mean underestimation of -0.46% (SE, 2.0%).

In this study, we successfully developed a popPK model and two LSSs that could be implemented in clinical practice to assist TDM of levofloxacin.
(This study has been registered at ClinicalTrials.gov under identifier NCT01918397.)

INTRODUCTION

Tuberculosis (TB) is the leading killer from a single infectious pathogen worldwide, and poor outcomes are more frequent among patients with rifampicin-resistant (RR) and multidrug-resistant (MDR) TB. In 2016, approximately 10.4 million TB cases were identified, including 490,000 cases with MDR-TB and 600,000 with RR-TB [1]. MDR-TB and RR-TB are treated with a combination of at least five anti-TB drugs to which the *Mycobacterium tuberculosis* strain is likely to be susceptible [2]. Under programmatic conditions, the worldwide success rate of MDR-TB and RR-TB treatment is low, at 54% [1]. Recently, in fluoroquinolone (FQ)-susceptible patients, a shorter 9- to 12-month MDR-TB regimen was proposed, reducing the burden for patients and the associated costs of treatment [2]. Levofloxacin is a FQ frequently included in MDR-TB treatment because of high efficacy and a favourable safety profile [2,3]. The World Health Organization (WHO) just released a revised grouping of drugs in the treatment of MDR-TB and RR-TB that prioritises FQ together with bedaquiline and linezolid and thereby confirms the key position of FQ [4].

In general, the optimal FQ efficacy depends on the ratio of area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) to minimal inhibitory concentration (MIC) with reported target values of >100 to 125 for Gram-negative bacteria and >40 for Gram-positive bacteria [5–7]. Levofloxacin target AUC_{0-24}/MIC values for other pathogens cannot be extrapolated to *M. tuberculosis* due to its unique characteristics [8]. Recently, a hollow-fiber study indicated a levofloxacin target AUC_{0-24}/MIC in MDR-TB treatment for the first time. The target AUC_{0-24}/MIC of 146 against *M. tuberculosis* was proposed based on the concentration associated with 80% of maximum microbial kill (EC_{80}) and an AUC_{0-24}/MIC of 360 was associated with suppression of acquired drug resistance [9]. Additionally, a levofloxacin target AUC_{0-24}/MIC is being prospectively studied using linear regression of AUC_{0-24}/MIC and log-transformed time to sputum conversion in TB patients receiving various levofloxacin doses (11 to 20 mg/kg body weight) in addition to an optimized background regimen [10]. This study is expected to provide a conclusive levofloxacin target AUC_{0-24}/MIC and make a statement on the optimal levofloxacin dose to be used in TB treatment; the results of this study are expected in March 2019 (ClinicalTrials.gov, NCT01918397).

Adequate drug exposure of FQ, as key drugs in MDR-TB/RR-TB treatment, is important to prevent acquired FQ resistance, even more so in the shorter MDR-TB regimen [11]. Acquired FQ resistance can be caused by interpatient variability in pharmacokinetic parameters or *M. tuberculosis* strains with increasing resistance, leading to insufficient attainment of the pharmacokinetic/pharmacodynamic target [12–14]. Standard doses of 750 or 1000 mg levofloxacin (10 to 15 mg/kg body weight) have shown to achieve

suboptimal drug exposures and an increased risk of acquired FQ resistance [7,15]. Levofloxacin doses of 17 to 20 mg/kg body weight are suggested based on target attainment analysis, although additional data on efficacy and toxicity are still needed [15]. With the recent findings of a higher target AUC_{0-24}/MIC (146) than assumed in these studies (53 and 100), the evidence for optimal levofloxacin doses above 15 mg/kg has grown even stronger.

Therapeutic drug monitoring (TDM) of second-line anti-TB drugs, e.g. levofloxacin, is recommended by the American Thoracic Society (ATS)/Centers for Disease Control and Prevention (CDC)/Infectious Disease Society of America (IDSA) guidelines and could therefore be used to adjust individual FQ doses based on obtained pharmacokinetic data to ensure adequate drug exposure [13,16,17]. To calculate AUC_{0-24} for use in TDM, one requires a full pharmacokinetic curve with multiple blood draws throughout the 24-h dosing interval. This is not only time-consuming and expensive, but it is unacceptable to patients and therefore unfeasible in clinical practice. A limited sampling strategy (LSS) is a method that requires fewer, usually one to three, optimally timed samples to accurately estimate the AUC. LSSs can be determined using both multiple linear regression and the Bayesian approach [18]. The ease of multiple linear regression is that the resulting equation can estimate AUC with the obtained drug concentrations, although the samples should be timed exactly. The Bayesian approach is less rigid with timing of the samples and will generally result in more accurate estimates of the AUC, since it includes the population pharmacokinetic model, patient characteristics, sampling errors, and assay errors [15,18]. However, the Bayesian method requires pharmacokinetic modelling software that is not available to all clinical centres in settings endemic for MDR-TB and RR-TB. So far, only one study has described an LSS for levofloxacin. Alsultan *et al* developed an LSS based on Bayesian approach and multiple linear regression using 4-h and 6-h postdose samples to estimate AUC_{0-24} [15]. Pharmacokinetic data of only 10 TB patients were used and no external validation was performed to determine whether the population pharmacokinetic model and LSSs were suitable for other groups of patients.

The aim of this study was to develop and validate a population pharmacokinetic (popPK) model of levofloxacin in TB patients and LSSs using the Bayesian approach as well as multiple linear regression to facilitate levofloxacin TDM in daily practice.

MATERIALS AND METHODS

Study population

Three different data sets were included in this study. Data set 1 included data from a study on the pharmacokinetics of 1000 mg levofloxacin in 10 Brazilian TB patients [6,15]. Blood samples were taken at 0, 1, 2, 4, 8, 12, 18, and 24 h after the fifth dose of levofloxacin. Data set 2 consisted of levofloxacin concentrations from 20 MDR-TB patients in Kibong'oto Infectious Diseases Hospital in Tanzania. Patients received either 750-mg or 1000-mg levofloxacin doses based on body weight. Two weeks after initiating treatment, samples were taken at 1, 2, 6, and 12 h. Data set 3 included data from a pharmacokinetic study of levofloxacin in 20 MDR-TB and extensively drug-resistant TB (XDR-TB) patients in Republic Scientific and Practical Center for Pulmonology and Tuberculosis in Minsk, Belarus [7]. The data set included 750-mg and 1000-mg levofloxacin dosing regimens based on body weight. Following 7 days of levofloxacin treatment, plasma samples were drawn at 0, 1, 2, 3, 4, 7, and 12 h after drug intake.

Levofloxacin was administered to all patients under fasting conditions. As steady-state concentrations are reached on day 3, we selected data obtained at steady state [19]. Because of steady-state conditions, levofloxacin concentrations at 0 and 24 h were assumed to be equal. Informed consent was not required for this study due to the retrospective analysis of anonymous data.

Noncompartmental parameters of $AUC_{0\text{-}24,ref}$ (calculated using trapezoidal rule), dose-corrected $AUC_{0\text{-}24,ref}$ ($AUC_{0\text{-}24,ref}$ divided by levofloxacin dose in mg), C_{max}, and T_{max} were determined. C_{max} was defined as the highest observed concentration and T_{max} as the corresponding time to C_{max}.

Population pharmacokinetic model

Data sets 1 and 2 were used to develop the popPK model to ensure a proportional number of patients in model development versus external validation (30 versus 20) and because data set 2 could not be used for external validation due to a lack of 0- and 24-h data. The KinPop module of MWPharm 3.82 (Mediware, The Netherlands) was used to create a population pharmacokinetic model using an iterative two-stage Bayesian procedure. Bioavailability (F) was fixed at 1, as only oral data were available and F is known to be almost complete for levofloxacin [20]. The popPK parameters were related to this fixed F and assumed to be log normally distributed. A residual error with a concentration-dependent SD was applied (SD=0.1+0.1*C, where C is the levofloxacin concentration). Levofloxacin is mainly eliminated renally (79.6%) as unchanged drug, but it is also metabolised to desmethyl levofloxacin (1.75%) and levofloxacin-N-oxide (1.63%) in the liver [20]. Total body clearance is the composite of metabolic clearance (CL_m) and renal clearance ($Fr*CL_{cr}$, where Fr is the ratio of creatinine

clearance to renal clearance) [21]. Due to a small spectrum of creatinine clearance values in our data set, we were unable to determine the exact Fr and renal elimination. One-compartment as well as two-compartment models of levofloxacin have been described [6,15,22–24]. Firstly, a default one compartment model [15] with fixed values of CL, volume of distribution (V), and absorption rate constant (K_a) was tested, and subsequently, Bayesian estimations of V, CL, and K_a were added one by one. Additionally, a default two-compartment model [22] with fixed values of distribution rate constants (k_{12} and k_{21}), elimination rate constant (k_{10}), and central volume of distribution (V_1) was tested. K_a could not be fixed, due to an unknown population estimation of K_a because of intravenous administration in the default model. Subsequently, Bayesian estimations of the other parameters were added one by one. Finally, Bayesian estimation of lag time (T_{lag}) was added to the one- and two-compartment models and evaluated because of oral administration of levofloxacin. The final pharmacokinetic model was chosen by comparing the Akaike information criterion (AIC) values of each submodel as a measure for goodness of fit using likelihood penalization. An AIC decrease of 3 was considered significantly better [25,26].

The final model based on data sets 1 and 2 was externally validated using data set 3. The Bayesian fitted $AUC_{0\text{-}24}$ ($AUC_{0\text{-}24,fit}$) was compared with the noncompartmental $AUC_{0\text{-}24}$ calculated with the trapezoidal rule ($AUC_{0\text{-}24,ref}$). Agreement of $AUC_{0\text{-}24,fit}$ and $AUC_{0\text{-}24,ref}$ was evaluated using a Bland-Altman plot and Passing Bablok regression (Analyse-it 4.81; Analyse-it Software Ltd, Leeds, United Kingdom).

Patient characteristics and pharmacokinetic data of data set 3 used for external validation were compared with data sets 1 and 2 used to develop the pharmacokinetic model. The median (IQR) and number (%) data of the parameters were tested for significance by the Mann-Whitney U test and Fisher's exact test, respectively, using IBM SPSS Statistics 23 (IBM Corp., Armonk, NY). *P* values <0.05 were considered significant.

LSS development using Bayesian approach

Monte Carlo simulation in MWPharm was used to create 1000 virtual patients representing the data used to build the pharmacokinetic model. The reference patient for Monte Carlo simulation was chosen based on a well-fitting and representative pharmacokinetic curve in combination with representative patient characteristics (male, 50 years; BMI, 19.1 kg/m^2; serum creatinine, 80 µmol/L; dose, 16.9 mg/kg body weight). Steady-state $AUC_{0\text{-}24}$ was chosen as parameter for optimisation by the LSS. Using this method, LSSs which were able to give the best estimation of $AUC_{0\text{-}24}$, and therefore are the best choice for levofloxacin TDM, could be selected. Only LSSs using 1, 2, or 3 samples with a minimum interval of 1 h and maximum time span of 8 h postdose were tested, because of clinical suitability. The performances of the LSSs were assessed using the RMSE as a measure of precision , MPE as a measure of bias,

and adjusted r^2 (in declining order of relevance) with acceptance criteria of RMSE of <15%, MPE of <5%, and r^2 of >0.95. The LSS chosen was externally validated using data set 3 by comparing the $AUC_{0\text{-}24}$ estimated by LSS ($AUC_{0\text{-}24,est}$) with $AUC_{0\text{-}24,ref}$ using Bland-Altman plot and Passing-Bablok regression.

LSS development using multiple linear regression

Data sets 1 and 3 were used for the development of LSSs. Data set 2 had to be excluded from these analyses, since both 0- and 24-h samples were lacking, and we were unable to calculate the $AUC_{0\text{-}24,ref}$. For each LSS, pharmacokinetic curves without concentration data at the selected time points could not be included in the analysis. The levofloxacin concentrations at the sampling time points and the $AUC_{0\text{-}24,ref}$ were analysed using multiple linear regression in Microsoft Office Excel 2010. Only clinically suitable LSSs were tested (maximum timespan, 8 h; minimum interval, 1 h; 1 to 3 samples), and acceptance criteria were applied (RMSE<15%, MPE<5%, r^2>0.95). The chosen LSS was internally validated using jackknife analysis. Multiple linear regression analysis was repeated in 10 different (n-3) subanalyses, each leaving out three randomly chosen patients. All 30 patients were excluded once [27]. Each subanalysis resulted in a different equation to the estimate the $AUC_{0\text{-}24}$ values using levofloxacin concentrations at the chosen sampling times. Per subanalysis, the $AUC_{0\text{-}24}$ values of the 3 excluded curves were estimated by the corresponding equation ($AUC_{0\text{-}24,est}$). $AUC_{0\text{-}24,est}$ was compared to $AUC_{0\text{-}24,ref}$ using Bland-Altman plot and Passing-Bablok regression.

RESULTS

Study population

In total, the pharmacokinetic curves from data from 30 TB patients were used to develop the popPK model, and 20 curves of TB patients were used as external validation of the model and Bayesian LSS. Baseline characteristics of age, height, weight, body mass index (BMI), and serum creatinine levels of the patients included in the development of the model were significantly different (P<0.05) from those included in the external validation (Table 1). The $AUC_{0\text{-}24,ref}$ and dose-corrected $AUC_{0\text{-}24,ref}$ of patients in data set 1 were significantly different (P<0.05) from data set 3 as well (Table 2). An overview of the median (interquartile range [IQR]) levofloxacin concentrations of the pharmacokinetic curves is provided in Table 3.

Table 1. Patient characteristics of the study population used for development of the pharmacokinetic model versus external validation. Data are presented as median (interquartile range [IQR]) unless otherwise stated.

Parameter	Data set 1 n=10	Data set 2 n=20	Pharmacokinetic model (data sets 1 and 2) n=30	External validation (data set 3) n=20	*P* value (model versus validation)
Sex (no [%]) Male Female	 8 (80) 2 (20)	 12 (60) 8 (40)	 20 (67) 10 (33)	 12 (60) 8 (40)	0.765[a]
Age (years)	43.5 (41.5-47.0)	38.5 (31.3-48.0)	41.5 (33.5-48.0)	30.5 (25.5-34.8)	0.002[b]
Height (m)	1.69 (1.60-1.76)	1.68 (1.63-1.74)	1.69 (1.61-1.75)	1.74 (1.66-1.82)	0.038[b]
Weight (kg)	55.5 (50.1-60.8)	51.5 (43.7-59.7)	54.6 (47.9-59.9)	63.4 (53.8-78.5)	0.001[b]
Dose (mg/kg bodyweight)	18.0 (16.5-20.0)	14.6 (12.8-17.2)	15.7 (13.6-18.1)	15.8 (12.8-16.6)	0.348[b]
BMI (kg/m^2)	19.4 (18.7-21.2)	18.3 (16.1-21.4)	18.9 (17.5-21.2)	20.6 (18.9-25.6)	0.016[b]
Serum creatinine (µmol/L)	80 (67-93)	73 (67-80)	74 (68-87)	66 (59-72)	0.014[b]

[a] Fisher's exact test
[b] Mann-Whitney U test

Table 2. Noncompartmental parameters of data sets 1 and 2 versus 3. Data are presented as the median (interquartile range [IQR]). NA, not applicable.

Parameter	Data set 1 n=10	Data set 2 n=20	Pharmacokinetic model (data sets 1 and 2) n=30	External validation (data set 3) n=20	*P* value (model versus validation)
$AUC_{0\text{-}24,ref}$ (mg·h/L)	129 (118-191)	NA	129 (118-191)[a]	105 (86-128)	0.028[b]
$AUC_{0\text{-}24,ref}$ /dose (h/L)	0.129 (0.121-0.143)	NA	0.129 (0.121-0.143)[a]	0.109 (0.088-0.127)	0.035[b]
C_{max} (mg/L)	15.6 (11.8-18.5)	8.9 (7.2-12.2)	10.3 (7.9-15.4)	10.5 (7.9-13.0)	0.649[b]
T_{max} (h)	1 (1-2)	2 (2-5)	2 (1-2)	1 (1-2)	0.073[b]

[a] Only available for dataset 1 (n=10)
[b] Mann-Whitney U test

Table 3. Overview of included pharmacokinetic curves. Median (IQR) levofloxacin concentration at each sampling time point.

Time (h)	Number of samples	Levofloxacin concentration (median [IQR]) (mg/L)
0	30	1.36 (0.95-1.58)
1	50	8.36 (5.74-12.79)
2	50	9.20 (7.63-11.31)
3	20	8.35 (7.08-9.95)
4	30	8.81 (7.23-10.34)
6	19	6.47 (5.38-8.10)
7	20	6.50 (4.70-7.08)
8	10	6.67 (6.10-7.55)
12	50	4.30 (2.88-5.08)
18	10	2.54 (2.34-3.41)
24	10	1.50 (1.30-1.71)

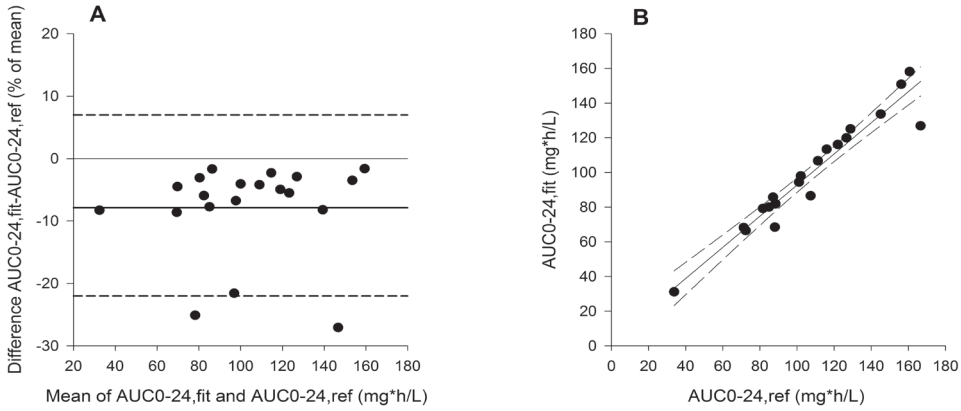
Population pharmacokinetic model

The default models resulted in an AIC value of 9950 for one-compartment and AIC of 4933 for two compartments. Based on AIC, a one-compartment pharmacokinetic model with lag time best described the data (AIC=574). A two-compartment model was not favourable (AIC=765 without lag time, AIC=592 with lag time), possibly due to too few data points during the elimination phase [25]. The popPK parameters of the final model are summarised in Table 4. External validation of the popPK model (Figure 1) showed that $AUC_{0\text{-}24}$ was slightly underestimated, with a mean of -7.9% (range, -25.1% to -1.6%; standard error [SE], 1.7%). Correlation of $AUC_{0\text{-}24,\ fit}$ and $AUC_{0\text{-}24,ref}$ with an r^2 of 0.977 was found in Passing Bablok regression.

Table 4. Pharmacokinetic parameters of the population pharmacokinetic model of levofloxacin.

Parameter	Geometric mean±SD (n=30)
CL/F (L/h)	7.1710±3.0503
V_d/F (L/kg bodyweight)	1.5148±0.2970
K_a (/h)	4.2922±5.8764
T_{lag} (h)	0.7693±0.1277

Figure 1. Bland-Altman plot (A) and Passing Bablok regression (B) of external validation of a population pharmacokinetic model of levofloxacin (n=20).



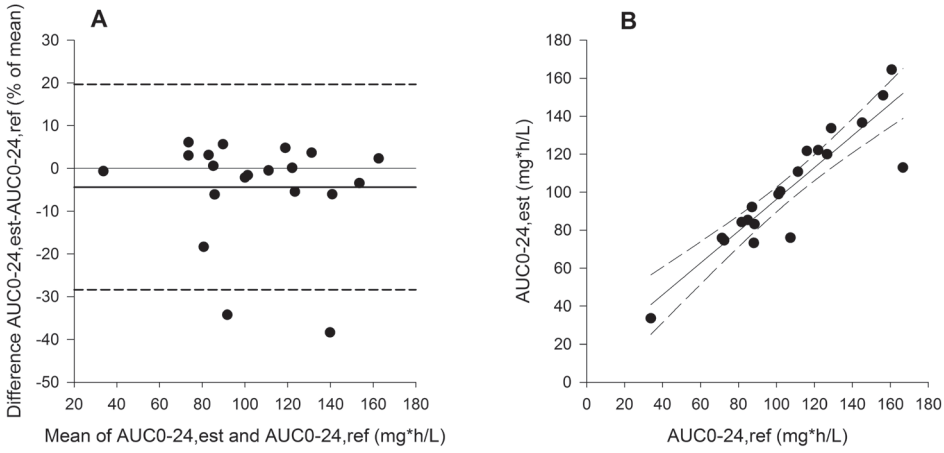
LSS development using the Bayesian approach

The three best-performing strategies are displayed in Table 5, including root-mean-square error (RMSE), mean prediction error (MPE), and r^2. All strategies using 2 and 3 samples, except at t=0 and 7 h, met the acceptance criteria (RMSE, <15%; MPE, <5%; r^2, >0.95). Overall, the LSS with samples at 0, 2, and 8 h postdose was the best-performing strategy with an RMSE of 7.1%, MPE of -0.70%, and r^2 of 0.972. However, the LSS with 0- and 5-h (RMSE, 8.8%; MPE, 0.42%; r^2, 0.957) was chosen for further evaluation because of its clinical suitability in addition to its relatively good performance. The results of the external evaluation (Figure 2) showed a mean underestimation of -4.4% (range, -38.4% to 6.1%; SE, 2.7%) and r^2 of 0.821.

Table 5. LSSs of levofloxacin using the Bayesian approach.

Sampling time point (h)			r^2	MPE (%)	RMSE (%)
6			0.847	-0.62	16.5
7			0.883	-0.29	14.4
8			0.906	0.88	12.9
0	7		0.949	0.43	9.5
0	6		0.952	0.36	9.2
0	5		0.957	0.42	8.8
0	2	7	0.970	-1.13	7.4
0	3	8	0.970	-0.93	7.3
0	2	8	0.972	-0.70	7.1

Figure 2. Bland-Altman plot (A) and Passing Bablok regression (B) of external validation of the Bayesian LSS using t=0 and t=5 h sampling (n=20).



LSS development using multiple linear regression

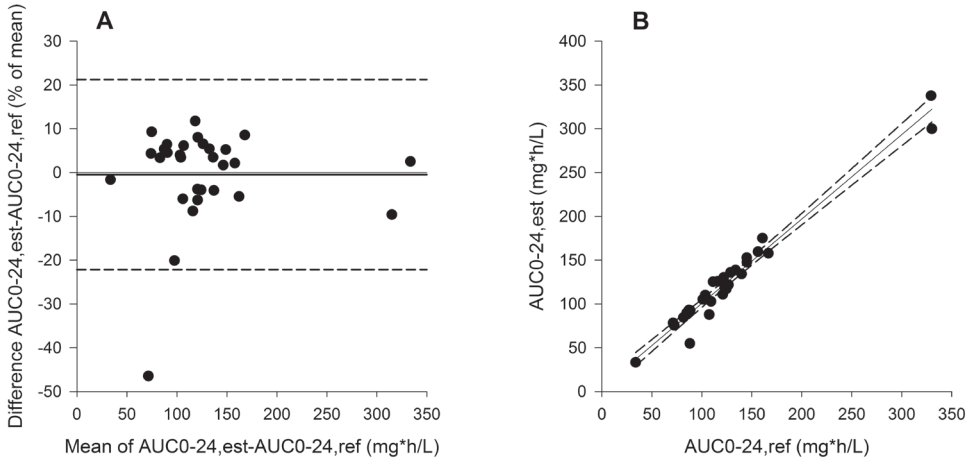
The three best-performing LSSs with and without an 8-h are displayed (Table 6), including the number of included curves (N), RMSE, MPE, and r^2. Again sampling at 0, 2 and 8-h postdose was the best-performing LSS, with an RMSE of 1.7%, MPE of 1.4%, and r^2 of 0.997. LSS of 4- and 8-h was the best-performing strategy with two time points with an RMSE of 2.5%, MPE of 2.1%, and r^2 of 0.997. The LSS using 0- and 4-h postdose samples showed a good performance as well, with an RMSE of 7.0%, MPE of 5.5%, and r^2 of 0.977. This LSS was chosen for further evaluation because of clinical suitability in addition to good performance. $AUC_{0\text{-}24}$ (mg·h/L) can be estimated using the equation $AUC_{0\text{-}24,est}$= 4.96+18.12*C0+10.04*C4, where C0 and C4 are the

levofloxacin concentrations (mg/L) at 0 and 4 h after drug intake, respectively. The results of the internal validation showed a mean underestimation of -0.46% (range, -46.5% to 11.8%; SE, 2.0%) and r^2 of 0.966 (Figure 3).

Table 6. LSSs of levofloxacin using multiple linear regression.

Max timespan (h)	Sampling time point (h)			Equation	N	r^2	MPE (%)	RMSE (%)
8	0			$AUC_{0\text{-}24,est}$= 65.71+35.59*C0	30	0.849	14.8	18.5
8	4			$AUC_{0\text{-}24,est}$= -22.43+16.51*C4	30	0.892	11.2	15.6
8	8			$AUC_{0\text{-}24,est}$= -16.40+21.93*C8	10	0.996	2.6	3.1
7	0	2		$AUC_{0\text{-}24,est}$= 27.84+23.87*C0+5.50*C2	30	0.923	9.1	12.9
7	1	7		$AUC_{0\text{-}24,est}$= -5.43+3.00*C1+13.88*C7	20	0.939	5.9	7.1
7	0	4		$AUC_{0\text{-}24,est}$= 4.96+18.12*C0+10.04*C4	30	0.977	5.5	7.0
8	2	8		$AUC_{0\text{-}24,est}$= -18.79+0.99*C2+20.60*C8	10	0.996	2.5	2.9
8	0	8		$AUC_{0\text{-}24,est}$= 0.11+6.48*C0+18.05*C8	10	0.997	2.2	2.5
8	4	8		$AUC_{0\text{-}24,est}$= -4.28-4.76*C4+26.98*C8	10	0.997	2.1	2.5
7	0	2	7	$AUC_{0\text{-}24,est}$= -3.01+10.58*C0+2.91*C2+11.31*C7	20	0.979	3.0	4.1
7	0	3	7	$AUC_{0\text{-}24,est}$= -2.98+10.69*C0+3.99*C3+10.18*C7	20	0.986	2.6	3.3
7	0	4	7	$AUC_{0\text{-}24,est}$= 3.10+11.79*C0+5.63*C4+7.12*C7	20	0.987	2.2	3.2
8	1	2	8	$AUC_{0\text{-}24,est}$= -16.34-1.33*C1+2.11*C2+21.05*C8	10	0.997	1.8	2.3
8	2	4	8	$AUC_{0\text{-}24,est}$= -6.51+1.04*C2-4.87*C4+25.70*C8	10	0.997	1.8	2.1
8	0	2	8	$AUC_{0\text{-}24,est}$= 1.18+8.35*C0+1.53*C2+14.86*C8	10	0.997	1.4	1.7

Figure 3. Bland-Altman plot (A) and Passing Bablok regression (B) of internal validation (n-3) of the multiple linear regression based LSS using t=0 and t=4 h sampling (n=30).



DISCUSSION

In this study, we successfully developed and validated a population pharmacokinetic model of levofloxacin in TB patients. Furthermore, we developed and validated an LSS based on multiple linear regression using 0- and 4-h samples and an LSS based on the Bayesian approach using 0- and 5-h samples.

The popPK model was able to estimate AUC_{0-24} of TB patients, with significant differences in age, height, weight, BMI, serum creatinine, and levofloxacin exposure in the external validation, with a mean underestimation of only -7.9% (Tables 1 and 2, Figure 1). The popPK parameters of the developed model were comparable to those of the prior one-compartment model in healthy volunteers [23].

Second, we developed two LSSs that can be used in clinical practice to estimate levofloxacin drug exposure. In this analysis, we considered an LSS clinically feasible if it required 1 to 3 samples with a maximal time span of 8 h postdosing. However, we feel that a smaller time span between the samples is more favourable in daily practice. Both LSSs, multiple linear regression LSS using the equation and Bayesian LSS using the popPK model, were able to adequately estimate the AUC_{0-24}. We expect no problems concerning 0-h concentrations below the limit of quantification of assays, since in our data sets the median levofloxacin concentration at 0 h was 1.36 mg/L (IQR, 0.95 to 1.58 mg/L) and no data were missing due to low concentrations.

We developed an LSS based on multiple linear regression, because it is a straightforward method that can be used at any clinical centre. It only requires the equation and the levofloxacin concentrations at 0 and 4 h after drug intake to estimate AUC_{0-24}. The 8-h single-sample LSS was not chosen for validation despite its remarkably good performance, due to the limited number of included curves. Moreover, this time point may be unfeasible in combination with directly observed treatment (DOT) at 0 h, and it may be challenging to obtain a precisely timed 8-h sample.

Bayesian LSSs, on the other hand, can only be used in centres that have access to pharmacokinetic modelling software. The Bayesian LSS resulted in other optimal sampling time points (0 and 5 h) than the multiple linear regression based LSS (0 and 4 h). This discrepancy is most likely caused by unlimited choice of time points, more patients being included in LSS development due to inclusion of data set 2, and the influence of the popPK model. The Bayesian strategy using 0- and 4-h samples was not among the three best-performing two sample strategies shown in Table 5 but still had a performance within acceptable limits (RMSE, 9.5%; MPE, 0.04%; r^2=0.949). Therefore, it would be possible to take 0- and 4-h samples and use both the Bayesian estimation and multiple linear regression to estimate AUC_{0-24}.

AUC_{0-24} estimated by LSS produced a slight underestimation which is acceptable and expected to be clinically irrelevant. In a comparison of $AUC_{0-24,ref}$ with $AUC_{0-24,est}$, the underestimation resulted in a different decision whether to increase the levofloxacin dose or not in only 1 out of 30 patients for the LSS based on multiple linear regression and in 1 out of 20 patients for the Bayesian LSS. Target AUC_{0-24} was set at >150 mg·h/L [9] based on an MIC of 1 mg/L [13]. In MDR-TB treatment practice, the precise AUC_{0-24} is not as important to the clinician as whether or not the TDM result triggers a dose increase. Dose increments will be based on available tablets, and these are expected to account for a dose-proportional 25% (1000 to 1250 mg) to 33% (750 to 1000 mg) increase in AUC_{0-24} [20]. Moreover, the risks of treatment failure and acquired antibiotic resistance are more relevant than the potential for relatively mild adverse drug reactions compared to other anti-TB drugs and other FQ [13]. The performance of an LSS has to be balanced against its alternatives, i.e. the collection of a full pharmacokinetic curve or not performing TDM at all. Considering the current poor MDR-TB treatment results, we realize that the added value of TDM using LSSs may be substantial.

Apparently, the popPK model and therefore also the Bayesian LSS did not correctly fit three curves of data set 3, resulting in outliers (Figures 1 and 2). Two of these outliers showed slow drug absorption ($T_{max,}$ 4 and 7 h), causing difficulties in fitting. Food likely did not play a role in this slow absorption, since all patients fasted before drug intake [28]. The third outlier had a relatively high concentration at 12 h postdose, possibly due to a measurement error, and was recognised by the model as outlier. This caused a considerable difference in $AUC_{0-24,ref}$ and $AUC_{0-24,est}$ as the 12 h sample was the last sample of the curve and for that reason had a major influence on the trapezoid of 12 to 24 h and $AUC_{0-24,ref}$.

This study had other limitations. Due to the low number of concentrations collected during the elimination phase, we were unable to develop a two-compartment model. Due to a small range of serum creatinine values, we were unable to determine the fractions renally and nonrenally cleared, as well as the influence of creatinine clearance on total body clearance using Fr, which is defined as ratio of creatinine clearance to renal clearance. It must be noted that the AUC_{0-24} of patients with impaired renal function might not be adequately estimated by our model and LSS due to this limitation, as creatinine clearance is known to be associated with levofloxacin clearance [29]. The popPK model as well as the LSSs included only data of patients without renal insufficiency. The results obtained using our model in patients with renal insufficiency should be interpreted carefully. However, moxifloxacin is preferred to levofloxacin in MDR-TB treatment in case of kidney failure, because moxifloxacin is mostly eliminated by hepatic metabolism [30]. Despite these limitations, we developed a model and LSSs

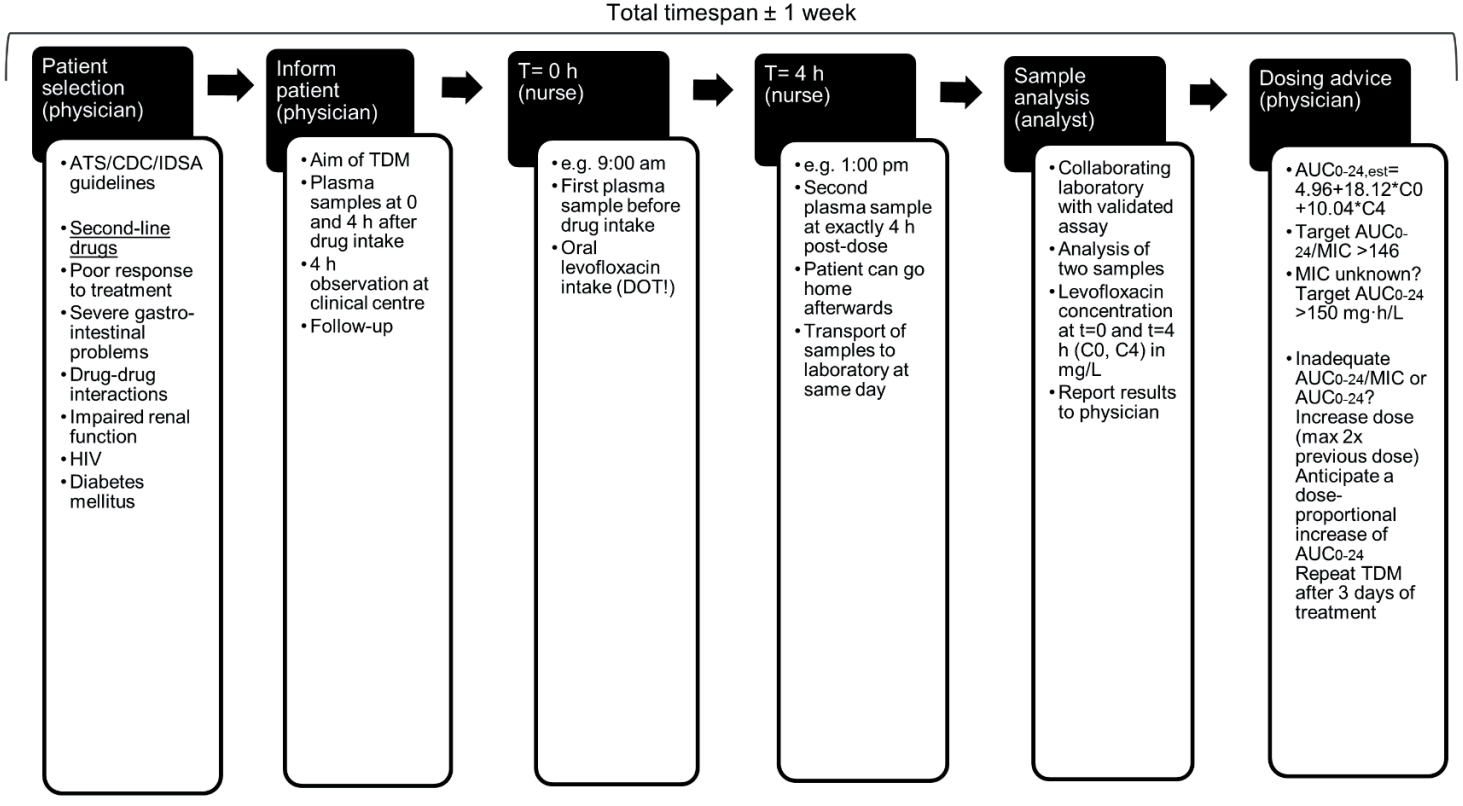
that were able to adequately predict AUC_{0-24} of a study population with significantly variable age, height, weight, BMI, and levofloxacin exposures, indicating a general suitability in a heterogeneous population of TB patients. Last, the use of retrospective data resulted in a limited number of included curves and less variability in sampling times for the LSSs using multiple linear regression. We still succeeded in developing two LSSs to adequately estimate levofloxacin drug exposure in clinical practice using just two blood samples.

The ATS/CDC/IDSA guidelines recommend TDM for patients treated with second-line anti-TB drugs, e.g. levofloxacin [16]. A validated LSS is capable of simplifying the procedure of TDM by limiting the number of required blood samples and therefore reducing the burden for patients, decreasing impact on daily schedules in the clinic, and reducing sampling costs. Using the described LSSs, it is possible to adequately predict levofloxacin exposure with only 2 plasma samples and if necessary adjust the dose based on the recently proposed target AUC_{0-24}/MIC >146 [9]. If the MIC is unknown, the target AUC_{0-24} would be approximately >150 mg·h/L, since levofloxacin MIC values of 1.0 mg/L were most frequently reported for drug-resistant *M. tuberculosis* strains [13].

By determining the individualized levofloxacin dose, treatment failure and development of antibiotic resistance may be minimized [12,13,31]. A helpful practical guideline for performing TDM of levofloxacin using the described multiple linear regression LSS is provided in Figure 4 to encourage physicians to implement TDM in their clinic [32]. We feel that TDM of anti-TB drugs should be available to most (if not all) TB patients, even in high-TB-burden areas, to support the end-TB strategy worldwide [33].

In conclusion, this study successfully developed a population pharmacokinetic model of levofloxacin in TB patients. Levofloxacin drug exposure can be adequately estimated with LSSs using 0- and 4-h postdose samples (multiple linear regression) or 0- and 5-h postdose samples (Bayesian approach).

Figure 4. Practical guideline to perform TDM of levofloxacin using an LSS based on multiple linear regression. Max, maximum.



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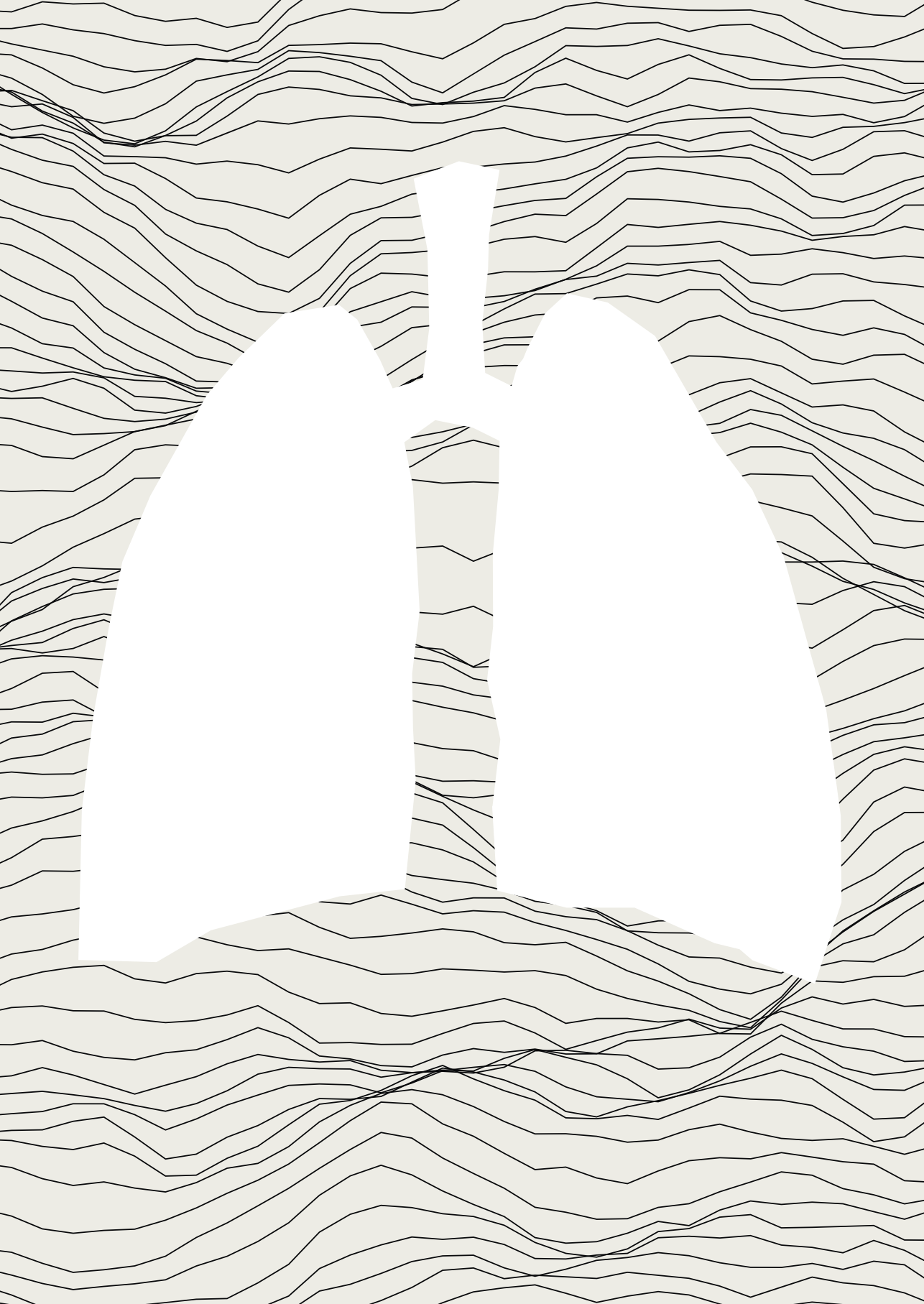

Chapter

5

Prospective Evaluation of impRoving Fluoroquinolone Exposure using Centralized TDM in patients with Tuberculosis (PERFECT) – a study protocol of a prospective multicentre cohort study.

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ABSTRACT

Introduction: Global multidrug-resistant tuberculosis (MDR-TB) treatment success rates remain suboptimal. Highly active World Health Organization (WHO) Group A drugs moxifloxacin and levofloxacin show intra- and inter-individual pharmacokinetic variability which can cause low drug exposure. Therefore, therapeutic drug monitoring (TDM) of fluoroquinolones is recommended to personalise the drug dosage, aiming to prevent development of drug resistance and optimize treatment. However, TDM is considered laborious and expensive, and the clinical benefit in MDR-TB has not been extensively studied. This observational multicentre study aims to determine the feasibility of centralized TDM and to investigate the impact of fluoroquinolone TDM on sputum conversion rates in patients with MDR-TB compared with historical controls.

Methods and analysis: Patients aged 18 years or older with sputum smear and culture positive pulmonary MDR-TB will be eligible for inclusion. Patients receiving TDM using a limited sampling strategy (t=0 and t=5 hours) will be matched to historical controls without TDM in a 1:2 ratio. Sample analysis and dosing advice will be performed in a centralized laboratory. Centralized TDM will be considered feasible if >80% of the dosing advices is returned within seven days after sampling and 100% within fourteen days. The number of patients who are sputum smear and culture negative after two months of treatment will be determined in the prospective TDM group and will be compared to the control group without TDM to determine the impact of TDM.

Ethics and dissemination: All participating centres obtained ethical clearance according to local procedures. Patients will be included after written informed consent. We aim to publish the study results in a peer-reviewed journal.

Trial registration: This study is registered at clinicaltrials.gov (NCT03409315)

INTRODUCTION

Tuberculosis (TB) is one of the major infectious diseases worldwide with an estimated number of 10.0 million new cases in 2017 [1]. In addition, multidrug-resistant TB (MDR-TB) remains a persistent problem with an estimated 458,000 new patients in 2017.[1] MDR-TB is treated from 9-20 months with a multidrug regimen [2]. The grouping of second-line anti-TB drugs was revised in 2018 by the World Health Organisation (WHO) [3]. The fluoroquinolones, specifically moxifloxacin and levofloxacin, are now considered drugs of first choice (Group A drugs), together with bedaquiline and linezolid, in the treatment of MDR-TB [2,3]. The administration of Group A medicines to patients with MDR-TB has been associated with increased treatment success and reduced mortality rates in comparison with other second-line anti-TB drugs [4]. However, the estimated prevalence of fluoroquinolone-resistance among MDR-TB cases is on the rise from 14.5% in 2011 to 22% in 2017 [5,6]. Mismanagement of MDR-TB treatment, especially the shorter regimen, could amplify the risk of drug resistance even further [7]. Importantly, antibiotic resistance can be acquired due to noncompliance but also insufficient drug exposures (e.g. inter-individual pharmacokinetic variability in patients treated with fluoroquinolones) [8–11]. Therapeutic drug monitoring (TDM) can help to prevent acquired resistance by individualising doses based on blood drug concentrations relative to the bacterial susceptibility, ideally measured as the minimal inhibitory concentration (MIC) [7,12].

Several studies described the role played by low drug concentrations on treatment outcomes [13–15]. In the light of this evidence, it can be hypothesized that TDM, which aims for adequate dosing and exposure, could improve treatment outcomes. Yet, the added value of TDM in MDR-TB treatment outcomes has not been directly studied [16,17]. One retrospective study reported the effect of TDM on the treatment results of patients with drug-susceptible TB, either with and without diabetes [18]. In the group without diabetes, TDM had a significant beneficial effect with 73% sputum culture conversion at two months amongst patients receiving TDM versus 60% in the control group. The positive effect of TDM was even larger in patients with diabetes and TB. To the best of our knowledge, such controlled studies have not yet been performed in people with MDR-TB.

The pharmacokinetic-pharmacodynamic parameter of fluoroquinolones is both time- and concentration dependent and therefore uses the ratio of area under the concentration time curve to minimal inhibitory concentration ($AUC_{0\text{-}24}$/MIC) with a target value of >146 for levofloxacin and free or unbound $fAUC_{0\text{-}24}$/MIC >53 for moxifloxacin [19,20]. However, multiple concentration measurements widely distributed over the dosing interval are required to compute the $AUC_{0\text{-}24}$. Limited

sampling strategies (LSS) could be adopted to reduce the burden of frequent sampling for both patient and personnel while providing a reliable estimation of AUC_{0-24} using only two blood samples [21,22].

Unfortunately, TDM is not always easily accessible in high TB burden areas because of practical and financial reasons. Therefore, centralized TDM could be a valuable service [23]. Large laboratories are generally well organised, have highly trained personnel with adequate performance of analytical methods leading to reliable sample analysis results [24]. In addition, centralizing the TDM procedures will engender more consistent practice from health care practitioners familiar with TDM and the provision of dosing advice for anti-tuberculosis drugs.

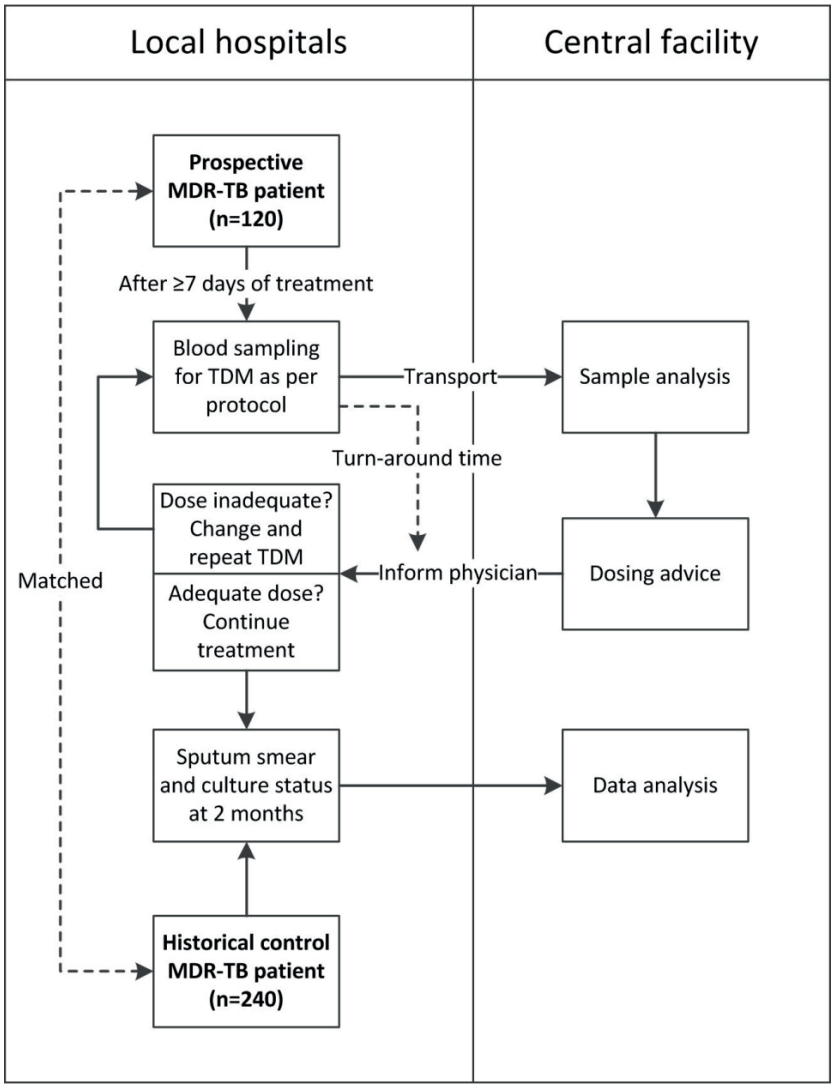
The aim of the present study is, firstly, to investigate the feasibility of centralized TDM of moxifloxacin and levofloxacin in the treatment of MDR-TB recruited in TB reference centres located in different continents. Secondly, the impact of TDM on treatment results will be assessed by comparing two month sputum smear and culture conversion rates among patients who received TDM compared with matched historical controls without TDM.

METHODS AND ANALYSIS

Study design

This observational, prospective, multicentre study aims to evaluate the feasibility of centralized TDM of moxifloxacin and levofloxacin as well as the impact of TDM on two month sputum smear and culture conversion rates of patients with MDR-TB. Study design and procedures are displayed in Figure 1. The study was registered at clinicaltrials.gov (NCT03409315) and started on 10 February 2018.

Figure 1. Workflow of study procedures in local hospitals and central laboratory facility.



Study location

University Medical Center Groningen (UMCG) in Groningen, the Netherlands, is the coordinating centre and serves as central laboratory facility for this study. The hospitals that are involved in patient recruitment are displayed in Table 1.

Table 1. List of participating hospitals and their location

Hospital	Location
University Medical Center Groningen (central lab facility)	Groningen, the Netherlands
Tuberculosis Clinic "Beatrixoord", UMCG	Haren, the Netherlands
Princess Alexandra Hospital	Brisbane, Australia
Karolinska University Hospital	Stockholm, Sweden
Instituto Nacional de Enfermedades Respiratorias	Mexico City, Mexico
Athens Chest Hospital "Sotiria"	Athens, Greece
Kibong'oto Infectious Diseases Hospital	Kilimanjaro, Tanzania
Republican Scientific and Practical Centre for Pulmonology and Tuberculosis	Minsk, Belarus
Barts Health NHS trust	London, United Kingdom
St. Orsola-Malpighi Hospital, University of Bologna	Bologna, Italy
Riga East University Hospital TB and Lung Disease Clinic	Riga, Latvia

Study population

Patients aged 18 years and older are eligible for inclusion if they are diagnosed with pulmonary MDR-TB, have positive sputum smear and culture samples at time of inclusion, are treated with either oral moxifloxacin or levofloxacin, and provide written informed consent. Pregnant or breast feeding women will be excluded. A total number of 120 patients (60 with moxifloxacin, 60 with levofloxacin) will be prospectively included and compared with 240 matched historical controls (120 with moxifloxacin, 120 with levofloxacin). Historical control patients will be matched on age, sex, *M. tuberculosis* resistance pattern of the isolate (only regimen core drugs), comorbidities (HIV, diabetes, immunosuppression), presence or absence of cavitary TB on chest radiography, and dosing of the fluoroquinolone (mg/kg body weight, ±10%) to prospectively enrolled patients in a 2:1 ratio.

Interventions

The objective of the feasibility of centralized TDM will be assessed by evaluating the process, by which a locally collected sample will be analysed in a central laboratory and subsequent dosing advice will be returned to the local physician. In brief, after at least seven days of treatment (steady state) two blood samples will be collected for TDM of moxifloxacin or levofloxacin according to a previously developed LSS [21,22]. The first sample will be collected just before drug intake (t=0

h) and the other at 5 hours after drug intake (t=5 h). Samples will be transported to the central laboratory for drug analysis and will be accompanied by a form including key patient characteristics for personalised dosing advice (i.e. sex, age, weight, height, serum creatinine, corrected QT (QTc) interval, MIC, TB presentation, start of treatment, other anti-TB drugs, and comorbidities). AUC_{0-24} will be calculated using a population pharmacokinetic model [21,22] and Bayesian dose optimisation in MWPharm++ (version 1.7.3; Mediware, Groningen, the Netherlands). Dosing is optimised based on AUC_{0-24}/MIC or AUC_{0-24} (in case MIC is unknown), taking into consideration comorbidities (HIV, diabetes, and immunosuppression) and clinical condition of the patient. The target AUC_{0-24}/MIC and AUC_{0-24} are shown in Table 1. If a dose change is necessary, TDM is to be repeated after at least seven days after the initiation of the new dose (steady state). Dose increases of moxifloxacin will not be advised in case of a prolonged QTc interval (>450 ms for males, >470 ms for females), because of safety reasons. As levofloxacin is less cardiotoxic than moxifloxacin, levofloxacin dose increases with frequent electrocardiogram monitoring are permitted in case of prolonged QTc interval. Patients with prolonged QTc interval will not be excluded from the study, since TDM can still be helpful to verify drug exposure. A closely monitored follow-up including MIC determination can be advised in case of AUC_{0-24} of 25 to 40 mg*h/L in combination with QTc interval prolongation. In case of very low moxifloxacin exposure (AUC_{0-24}<20 mg*h/L) in combination with a prolonged QTc interval, the physician will be advised to reconsider the anti-TB regimen as moxifloxacin may be less active than expected.

Laboratory methods

Drug analysis:

Measurement of moxifloxacin and levofloxacin plasma/serum concentrations will take place at the laboratory of the department of Clinical Pharmacy and Pharmacology in the UMCG, the Netherlands, and using validated liquid chromatography-mass spectrometry (LC-MS/MS) methods. The method for levofloxacin has an accuracy of 0.1-12.7%, within-run precision of 1.4-2.4%, and between-run precision of 3.6-4.1%. The calibration curve is linear over a range of 0.10 to 5.00 mg/L [25]. Accuracy of the moxifloxacin method is 2.7-7.1%, within-run precision 1.4-1.6%, and between-run precision 1.0-1.6%. The calibration curve is linear over a range of 0.05 to 5.00 mg/L [26]. Only the total moxifloxacin concentration (bound and unbound) will be measured, therefore we assume a constant protein binding of 50% [27]. Plasma and serum samples containing levofloxacin are stable for at least ten days at 50 ^{0}C and can therefore be transported to the central facility in ambient temperature, without the need of transport on dry ice [28]. The thermal stability of moxifloxacin was also tested according to the method of Ghimire *et al* and showed that moxifloxacin serum and plasma samples are stable for at least ten days at 50 ^{0}C as well (data on file).

Microbiology:
The assessment of sputum smear and culture status after two months of MDR-TB treatment will be performed according to the local procedures, but at least once a month until documented culture conversion. MIC determination is preferred but not mandatory for TDM and will be performed according to local procedures as well. To account for the differences in culture media used in drug susceptibility testing, correction factors based on the critical concentrations in the WHO-document "Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis" will be applied [29]. The target $AUC_{0\text{-}24}$/MIC values for each medium are shown in Table 2. Furthermore, second-line molecular drug susceptibility tests will be considered in case MIC data are not available.

Table 2. Target $AUC_{0\text{-}24}$/MIC and $AUC_{0\text{-}24}$ for TDM of moxifloxacin and levofloxacin in patients with multidrug-resistant tuberculosis (MDR-TB). Standard disease is defined as non-cavitary and regular disease on radiograph. Severe disease is defined as cavitary or extensive disease on radiograph.

Fluoroquinolone	Pulmonary MDR-TB	Target $AUC_{0\text{-}24}$/MIC[a]			Target $AUC_{0\text{-}24}$ (mg*h/L)
		MGIT	7H10/11	LJ	
Moxifloxacin	Standard disease	>100	>50	>25	>40
	Severe disease or comorbidities	>100	>50	>25	>60[b]
Levofloxacin	Standard disease	>150	>150[c]	>75	>150
	Severe disease or comorbidities	>150	>150[c]	>75	>200[b]

[a] Minimum inhibitory concentration (MIC) varies depending on growth media; Mycobacteria Growth Indicator Tubes (MGIT), Middlebrook 7H10/7H11, and Lowenstein Jensen (LJ) agar.
[b] Target $AUC_{0\text{-}24}$/MIC at site of cavity; therefore higher $AUC_{0\text{-}24}$ is required.
[c] Levofloxacin critical concentration of 7H11 was extrapolated to 7H10.

Data analysis plan

The primary outcome to assess the feasibility of centralized TDM will be the turn-around time, which is defined by the time between blood sampling and the peripheral centres receiving the TDM results including the dosing advice. The procedure is considered feasible if >80% of the collected samples will be reported back to the physician within seven days and 100% within two weeks. Additionally, the feasibility will be evaluated using secondary outcomes of sample quality after shipping and completeness of required information on the sample form.
Furthermore, we will evaluate the role of TDM in MDR-TB treatment by comparing the percentages of patients with sputum smear and culture conversion at two months in the enrolled groups (TDM versus control). In addition, we will evaluate the number of patients with low fluoroquinolone exposure requiring dose changes after TDM to estimate the potential gains.

Sample size calculation

As the primary endpoint was of descriptive nature and no data were available to perform a well-informed sample size calculation, it was decided to power the study on the clinical impact of TDM. The primary assumption was based on the detection of a proportional difference in sputum smear and culture positivity at two months of treatment in patients with MDR-TB undergoing TDM (35%) [30] and control patients (60%) [31]. Given an alpha error of 0.05 and statistical power of 80%, we calculated that a sample size of 60 per single group is needed (i.e. 60 prospective and 120 historical control patients for moxifloxacin and equally for levofloxacin).

ETHICS AND DISSEMINATION

This study will be performed according to the Declaration of Helsinki and Good Clinical Practice [32]. In each centre ethical clearance has been granted according to local regulations and patient recruitment has begun at most sites. Written informed consent will be obtained from all patients undergoing TDM. The need of new informed consent for historical controls was waived, because of the use of retrospective anonymous data collected for programmatic purposes or previously reported data from studies for which patients had provided informed consent.

This study includes historical patients who did not receive TDM as controls instead of prospectively randomising patients to either receive or not receive TDM for ethical reasons. The evidence that TDM actually improves MDR-TB treatment outcomes has not been confirmed in randomised controlled trials, but multiple studies have described treatment failure and risk of antibiotic resistance due to sub therapeutic drug exposure of anti-TB drugs [8,13,15,19,20]. In combination with a large between-patient pharmacokinetic variability [9,10], we hypothesize that TDM is able to improve treatment outcomes by ensuring adequate exposure in individual patients. Moreover, TDM for MDR-TB is recommended in guidelines when it is available [2,33,34]. We therefore considered it unethical to withhold TDM.

Study results will be published in a peer-reviewed journal and will be presented at an international conference.

DISCUSSION

We present an observational prospective multicentre study which aims to: a) evaluate the feasibility of centralized TDM in differently resourced settings of varying TB endemicity and geographic region and b) evaluate the role of TDM of moxifloxacin or levofloxacin on sputum smear and culture conversion rates in patients with MDR-TB after two months of treatment.

Presently, TDM is offered as an adjunctive to patients with TB in only a few hospitals worldwide and is considered to be part of the excellent clinical care [16,23,35–37]. However, general interest in TDM and MDR-TB treatment optimization has been increasing. A consensus statement on the diagnosis and treatment of MDR-TB in Europe states that TDM for second-line drugs should be used if available [34]. Moreover, the use of second-line anti-TB drugs was listed in the American Thoracic Society (ATS) guidelines as indication for TDM and TDM is also recommended in the European Union Standards for Tuberculosis Prevention and Care [33,38]. Yet, TDM is considered by some to be laborious, expensive and thus unpractical in countries with high TB incidence. Similar injurious arguments of economistic rationing of services were applied to second-line drugs for the treatment of MDR-TB in highly endemic settings and such rationing conversely led to amplification of the MDR-TB epidemic [39]. This study will focus on the feasibility of centralized TDM, which could stimulate performing TDM more often as it requires only one qualified laboratory with validated analytical methods and devices in a central location. Other options to facilitate TDM are the implementation of LSS, urine samples, dried-blood spots and saliva-screening methods [35,40–42]. Although incorporating TDM in TB treatment has shown to give high treatment success rates in low endemic countries, like the Netherlands [30], this has not yet been evaluated in well-designed randomized controlled trials [43]. This study will provide a first-ever conclusion on the value of TDM of moxifloxacin and levofloxacin on sputum smear and culture conversion of patients with MDR-TB.

It can be considered a limitation that only TDM of fluoroquinolones is performed in this study. However, moxifloxacin and levofloxacin are currently among the core drugs in the MDR-treatment regimen together with linezolid and bedaquiline [3]. Based on TDM criteria [44], we have selected moxifloxacin and levofloxacin, because they show large inter-individual pharmacokinetic variability, which emphasizes the need for personalized dosing [9,10]. Moreover, fluoroquinolone resistance is on the rise and can develop during low drug exposure [8]. TDM of fluoroquinolones aims to find the individual patients who have low drug exposure and would benefit from dose adjustment. Therefore, it is expected that TDM of fluoroquinolones will have the largest impact on MDR-TB treatment outcomes. We did not include TDM for linezolid

and bedaquiline in this study, because of unclear evidence for TDM of bedaquiline due to the novelty of the drug [45] and TDM of linezolid has focussed more on preventing toxicity [46–48].

Another limitation is that we are only evaluating interim outcomes such as sputum conversion rates at two months and will not assess outcomes at the end of treatment. However, this study is primarily designed to determine the feasibility of centralized TDM. In addition, this is the first study to evaluate the impact of fluoroquinolone TDM. We believe that reporting the results on sputum conversion rates is relevant as bacterial load and risk of acquired resistance are highest in the first months of therapy. Fast sputum culture conversion reduces the risk of transmission of *M. tuberculosis* strains which continues to sustain the MDR-TB epidemic [49]. With the results of this study we aim to design a future study to extensively evaluate TDM of all drugs in the regimen including the final treatment outcomes. However, such study would require substantial funding.

We hope that this study will show that centralized TDM is feasible and that TDM can improve the quality of treatment in terms of faster sputum conversion rates compared to historical experience. If that might be the case, the major hesitations about TDM in TB treatment can be attenuated favouring the improvement of TB management using a personalized approach [38].

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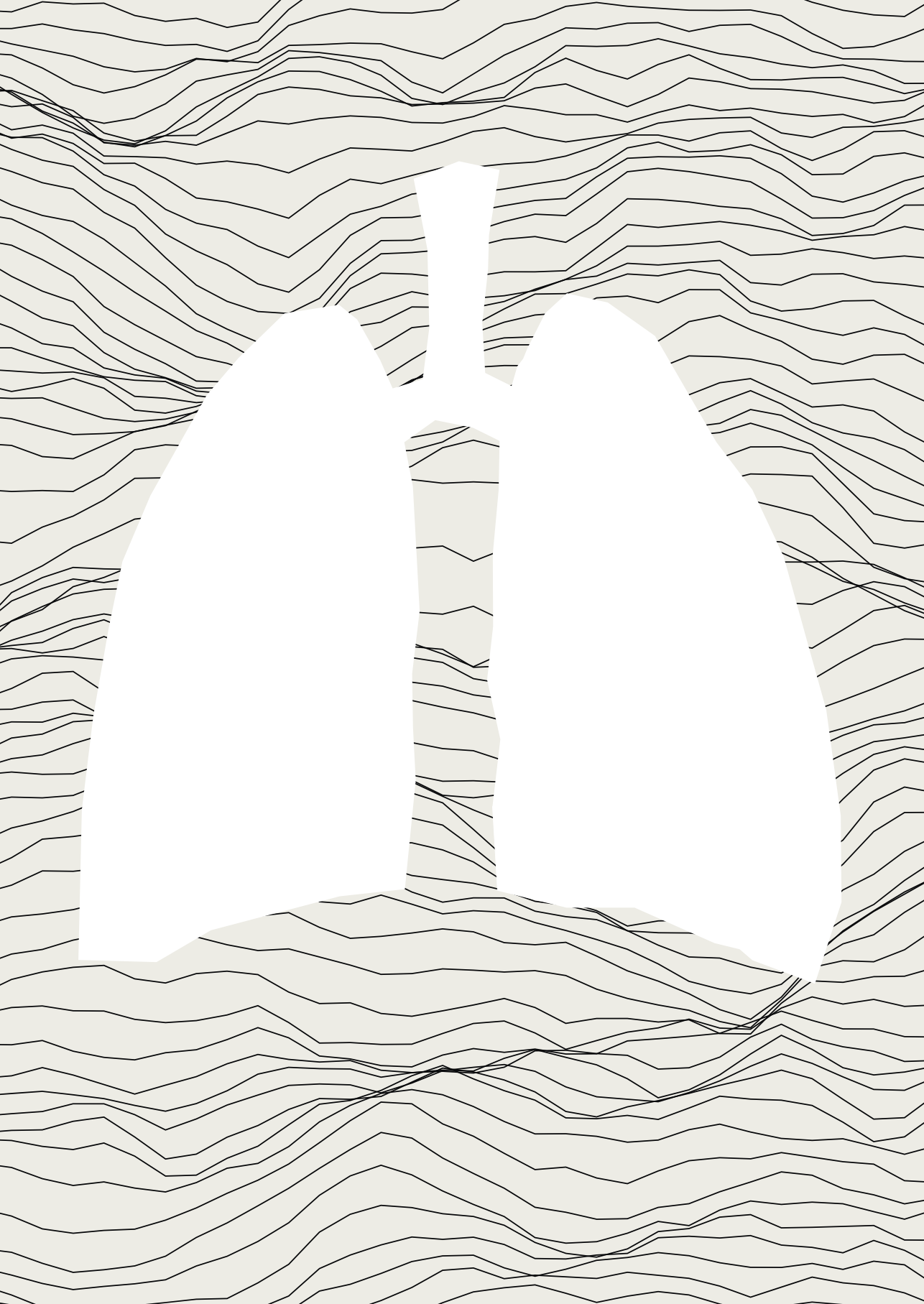

Chapter

6

General discussion and future perspectives

THERAPEUTIC DRUG MONITORING (TDM) IN TUBERCULOSIS (TB) TREATMENT

Current major issues that forestall the worldwide elimination of TB are the diagnostic gap - individuals with TB who currently go undiagnosed; poor access to bacterial susceptibility tests; poor availability of multidrug-resistant TB (MDR-TB) treatment; and finally, the substantial funding gap for health services and research [1]. TDM may play an important role to reduce the emergence of acquired drug resistance and the improvement of TB treatment outcomes by detecting and preventing inadequate drug exposure, which has been identified as one of the major causes of the MDR-TB epidemic [2–7]. TDM may also be able to reduce the transmission of TB if it is performed early in treatment, because it can increase the efficacy of anti-TB drugs and hereby accelerate sputum conversion [8]. We realize that traditional TDM might be one bridge too far for high TB burdened countries with low resources and we emphasize that in these low-resourced settings, the focus should be on TB diagnosis, availability of treatment, and bacterial susceptibility testing first. For these countries a simple point-of-care test would be very helpful to provide rapid information about individual drug exposure. Nevertheless, implementation of TDM would be beneficial in low and medium TB endemic countries with sufficient resources for national TB programs to improve treatment outcomes and proceed towards a more individualized approach. TDM is already part of standard TB care in the Netherlands and has contributed to a high MDR-TB treatment success rate of 94% when compared to a worldwide success rate of 56% [9–12].

Although it has been shown many times that suboptimal drug exposure puts patients at risk of treatment failure and acquired drug resistance [2–7], straightforward evidence that TDM actually improves treatment outcomes is still scarce [13,14]. Efficacy of TDM has been retrospectively studied in patients with drug-susceptible TB (DS-TB) and showed more sputum culture conversion after two months of treatment in the group that received TDM versus the patients that did not receive TDM [15]. Similar studies have not been performed yet in patients with MDR-TB and we hypothesise that the potential gain is even larger in this population due to the current low treatment success rates. Therefore, we designed a study to evaluate the impact of TDM on treatment results of patients with MDR-TB **(Chapter 5)**.

Presently, TB treatment is frequently started while drug susceptibility test results are lacking, due to slow mycobacterial culture test turn-around-time and unavailable rapid molecular tests or line-probe assays [8,16]. This increases the risk of inadequate treatment and stimulates development of acquired drug resistance.

Therefore, the development of an easy, rapid, and affordable method to determine bacterial susceptibility (e.g. microplate nitrate reductase assay [17]) is key. Whole genome sequencing could be the future [18], but is still difficult due to the need for sputum cultures, an incomplete database of mutations, and lack of validation [8]. Similar issues with obtaining drug susceptibility information are encountered in the implementation of TDM. TDM of antibiotics is guided by the measured drug concentrations or drug exposure in relation to the bacterial susceptibility reflected by minimal inhibitory concentration (MIC), but presently time-consuming culture methods are required to determine the MIC of the *Mycobacterium tuberculosis* strain. Performing TDM without known MIC is not recommended, since it introduces additional uncertainty due to the broad range of MIC values prevalent in *M. tuberculosis* strains [19], and therefore could decrease the effect of TDM. For instance, the MIC is assumed to be 0.25 mg/L based on the regional population MIC distribution and after analysis of the plasma samples the drug dosage is considered adequate for the individual patient. However, if the actual MIC of the involved strain is 0.5 mg/L instead of 0.25 mg/L, AUC or C_{max} needs to be twice as high to achieve the same AUC/MIC ratio or C_{max}/MIC. If the actual MIC is 1 mg/L instead of 0.25 mg/L, it even requires a four times higher AUC or C_{max}. Clearly, misassumptions like this could have a significant impact on the adequate dose for an individual patient. Another option is to use the worst case MIC, but that implies unnecessary high doses for most patients.

Plasma or serum samples are the gold standards for TDM and efficacy targets are also based on the drug concentrations and drug exposure in the central compartment. However, the antimicrobial effect is most closely related to the amount of drug present at the site of action. The plasma samples only serve as proxy for infection site concentrations because of the invasive nature of tissue sampling methods. Still, low plasma concentrations have been associated with unfavourable outcomes and can therefore be used in TDM [20,21]. Ideally, pharmacokinetic/pharmacodynamic parameters at the site of infection will be easier to determine or predict in the future, as, together with data about drug penetration into infection sites, this would increase the quality of TDM.

Despite the previously mentioned challenges that come with performing TDM, we feel that it is a suitable clinical service that is able to make a significant improvement in treatment success while reducing the emergence of drug resistance. This thesis focused on alternative methods, such as saliva sampling, LSS, and centralized TDM, that may be able to decrease the organisational and financial burden of TDM and evaluated their feasibility in TB care.

TDM USING SALIVA SAMPLES

Using saliva samples for TDM of anti-TB drugs would be interesting, because it is an easy, non-invasive, patient friendly sampling method and it has the potential for home-based sampling in remote areas [22]. Therefore, it might as well be cheaper than blood-based TDM as trained medical staff is not required to collect saliva samples [23]. However, although there are exceptions [24,25], in general salivary drug concentrations not always correlate well with plasma concentrations [26]. The major challenge of salivary TDM is that the penetration of drugs from blood into saliva is influenced by many factors. Firstly, the chemical properties of the drug play an important role and determine whether a drug is likely to passively diffuse across the membranes in the salivary gland (e.g. protein binding, pKa, molecular mass, lipid solubility) [27,28]. Physiological elements that have an influence on drug penetration into saliva are salivary flow, salivary pH, composition of saliva, involvement of drug transporters, and presence of oral cavity diseases [27–29]. Other contributing factors are drug stability in saliva, sample storage conditions, sampling methods, used materials, and assay variation. All these aspects contribute to differences in saliva-blood concentration ratios between drugs, between studies, between patients, and even within one patient.

Several studies on salivary versus blood concentrations of anti-TB drugs have been performed already and showed a substantial variation of saliva-plasma or saliva-serum ratios between these studies **(Chapter 2)**. Numerous dissimilarities, for instance in sampling procedure or study population, were observed between the studies and these could partially explain the wide range of saliva-blood ratios found in the systematic review. Only a small number of studies included patients with TB and even fewer evaluated the feasibility of salivary TDM in TB treatment. Therefore, a prospective observational study in TB patients was designed and set up to fill this knowledge gap **(Chapters 3a, 3b, 3c)**. A strength of this study is that the patients already received TDM using blood samples as part of standard care and only non-invasive saliva samples had to be additionally collected. Moreover, all TB drugs being part of the individualized treatment regimens were studied and therefore data was mainly collected for frequently used preferential anti-TB drugs (rifampicin, isoniazid, moxifloxacin, linezolid). In preparation for this study, a safe sampling method was used to process saliva samples of sputum culture positive patients without infection hazard of TB bacteria present in their oral cavity **(Chapter 3d)**. This sampling method utilizes membrane filtration to successfully sterilize saliva, but on the other hand is expected to introduce additional costs and more variability due to different sampling methods.

The results of this study were mostly in line with the theoretical background and chemical properties of the drug. Rifampicin is known to have a high protein binding of 80-90% [30] and demonstrated very low saliva-serum ratios in our study, while isoniazid saliva-serum ratios were significantly higher due to a low protein binding of 10-15% [31]. Amikacin did not penetrate into saliva at all, likely due to ionization and polarity of the molecule. Saliva-plasma ratios of moxifloxacin were very high and this corresponds with a large volume of distribution [32–34]. Interestingly, in some patients moxifloxacin salivary concentrations were greater than the simultaneously collected plasma concentrations, but the underlying mechanism remains unknown. Theoretically, a drug can be unionized in plasma, but becomes ionized after it transfers to saliva due to differences in pH between these two matrices. Because ionized molecules cannot easily diffuse across membranes, the drug could get trapped in saliva and this results in saliva-blood ratios above 1. However, we did not detect any association between salivary pH value and the saliva-plasma or saliva-serum ratio. Therefore other mechanisms that could cause a high salivary concentration are more likely, such as the involvement of active transporters in addition to passive diffusion [29].

The main conclusion of the study was that salivary TDM is not an equal alternative to traditional blood-based TDM, since it is not feasible for all TB drugs nor is it as precise as TDM using blood samples **(Chapters 3a, 3b, 3c)**. In the light of the practical advantages of salivary TDM, we feel that a larger variability can be accepted for saliva screening methods to identify patients with low drug exposure, to monitor adherence, to determine isoniazid acetylator phenotype or to select other individuals who could benefit from blood-based TDM. Future studies could particularly focus on the development of convenient semi-quantitative methods using saliva samples [22]. A clinically relevant example is a screening method for low levofloxacin drug exposure using salivary trough concentrations [25]. Furthermore, a proof of concept study on salivary TDM of new anti-TB drugs (e.g. bedaquiline) could be valuable once more efficacy data and PK/PD targets are available for these drugs [35].

Nevertheless, only a saliva-blood ratio established and validated in clinical research is not sufficient for implementation of salivary TDM in daily TB care. Firstly, new analytical methods need to be developed for drug analysis in saliva or current methods need to be cross validated in saliva. LC-MS/MS was used to analyse the patient samples in our study, but we realize this technique is expensive and not always available in high TB burdened countries with limited resources. Therefore, it would be helpful to develop other analytical methods (e.g. using HPLC-UV) that are able to analyse anti-TB drugs in saliva or centralize drug analysis in reference laboratories [22]. Other elementary lab experiments that have to be performed beforehand are recovery testing of the

sampling materials as well as determination of drug stability in saliva (especially in case of home-based sampling). Last but not least, logistics as well as training of personnel and patients should be organized. Clearly, whereas collecting saliva samples is straightforward, the overall concept of salivary TDM is not.

LIMITED SAMPLING STRATEGIES (LSS)

This thesis also focused on using LSS as method to decrease the burden of TDM for patients as well as health care personnel and to reduce costs. A LSS is able to estimate individual drug exposure using a small number, usually one to three, of appropriately timed plasma or serum samples [36–38]. After analysis of the blood samples, the individual AUC can be assessed using the drug concentration results together with either a population pharmacokinetic model or equation established by multiple linear regression [39]. Each approach has its own advantages and disadvantages. Multiple linear regression is simple and readily available, yet timing of samples is rigid and it can only be used in a patient with comparable characteristics to the population included in the development dataset. In contrast, a population pharmacokinetic model is more flexible in terms of timing of samples and patient characteristics, but requires modeling software. A strength of the LSS developed in this thesis (**Chapters 4a, 4b**) is that both approaches were used to develop separate LSS. One of the validated LSS can be chosen based on availability of modeling software, patient characteristics, and the preferences of the clinician.

By minimizing the number of samples, the accuracy of the AUC estimation will also be reduced. LSS are all about finding the minimal number and optimal timing of samples that is required for acceptable estimation of drug exposure. Slight deviations between estimated and actual AUC are accepted (RMSE<15%, MPE<5%, r^2>0.95) [36,37,40]. For target AUC/MIC of anti-TB drugs, there usually is a cut-off value instead of a narrow range of target exposure [41–45]. Therefore it is unlikely that minor bias or a slightly decreased precision will have a significant effect on dosing decisions after TDM with LSS. For instance, our multiple linear regression LSS for levofloxacin using t=0 h and t=4 h samples **(Chapter 4b)** would have resulted in a different dosing decision in only 1 of 30 patients when compared with regular TDM, which is considered acceptable.

Appropriate validation is key to evaluate the performance of the proposed LSS before it can be safely used in clinical practice. Preferably, external validation is performed in a separate dataset to ensure that the LSS is able to adequately estimate drug exposure in a new patient [46]. If possible, the dataset for external validation should be collected in a significantly different population to test the robustness of the model. In case there is no separate dataset available for the targeted patient population, internal validation

should be performed instead [47]. A LSS that is only internally validated should be used with caution in patients who differ from the study population as the performance of the LSS remains unknown. An internally validated LSS can always be externally validated later on, once a suitable dataset becomes available, to test whether it is suitable for the aimed population as well. We consider this more efficient than developing a new and comparable LSS in every patient population. Besides, this will maintain a clear overview of available LSS and its applications.

Frequently, LSS are developed for only one drug at the time as we did in this thesis. However, this results in many different LSS which are not easily merged into one general LSS for the entire drug regimen of a patient with TB. For example, we developed a LSS for moxifloxacin using t=0 h and 4 h samples **(Chapter 4a)**. Previously, a LSS for linezolid was developed using t=0 h and t=2 h samples [36]. Simultaneous TDM of the group A drugs moxifloxacin and linezolid is preferred from the programmatic treatment point of view. However, using these two LSS it would already require three samples (0, 2, and 4 h), but likely even more if yet another drug needs to be monitored. So far, two studies have been published that developed a LSS for all first-line TB drugs at once, one additionally included moxifloxacin [48,49]. It would be very helpful if there also was a LSS available for a combination of commonly prescribed drugs in MDR-TB treatment (e.g. group A drugs). After all, TDM is particularly recommended for MDR-TB patients because of suboptimal treatment outcomes and toxicity of the second line drugs [50].

We feel that LSS are promising to be implemented in TB treatment, because they are satisfactorily precise and can make use of already existing analytical methods and procedures. On the other hand, LSS still require venipuncture in a health facility and do not have the advantage of home-based sampling unless dried-blood spots or other suitable home sampling methods are developed and validated. Yet, we do see great potential in LSS together with dried-blood spot sampling [51], because of the already available methods for dried blood spot analysis of anti-TB drugs, high sample stability, and home-sampling possibilities [52–57].

CENTRALIZED TDM

As was proposed before, centralizing drug analysis in core laboratories may be the way to go to increase the use of TDM in TB treatment [22]. It likely reduces the costs of TDM, because expensive analytical equipment has to be available in only few locations and is efficiently used for multiple health care facilities. Additionally, the quality of TDM is more likely to be improved due to extensive experience, highly trained personnel, sophisticated equipment, and participation in proficiency programs using quality

control rounds with reference laboratories [58]. On the other hand, centralizing TDM will introduce logistic challenges due to the numerous transports of samples from local health facilities to the central laboratory. Therefore, we aimed to evaluate the feasibility of centralized TDM primarily using the turn-around-time between sampling and sharing dosing advice **(Chapter 5)**. The strength of this prospective multicenter study is that it uses LSS from **Chapter 4a** and **Chapter 4b** to reduce the burden of TDM. Fortunately, levofloxacin and moxifloxacin are rather stable in plasma and serum samples and can therefore be transported at room temperature conditions **(Chapter 5)**. However, this might be different for other TB drugs that are less stable and would require more expensive transport using for example dry ice. For these drugs, dried-blood spots might be a solution as sample stability usually is prolonged [52,59]. In the future, we ideally see centralized TDM joining forces with LSS and perhaps also dried-blood spots to increase the use of TDM in TB treatment.

CONCLUSION

This thesis focused on strategies to decrease the burden of TDM and hereby stimulating performing TDM in TB treatment. Based on the studies compiled in this thesis, we can conclude that salivary TDM cannot be seen as equal alternative for blood-based TDM but can be useful as semi-quantitative screening method at location for some anti-TB drugs. LSS are accurate in estimating drug exposure if properly developed and are valuable to decrease the burden of TDM by minimizing the number of required samples. Developing accurate and clinically feasible LSS for relevant drug combinations will be the next step towards more frequent practice of TDM. Furthermore, centralizing TDM in a central laboratory is expected to reduce the financial burden, while increasing the quality of TDM. However, centralized TDM might be logistically challenging and its feasibility remains to be determined.

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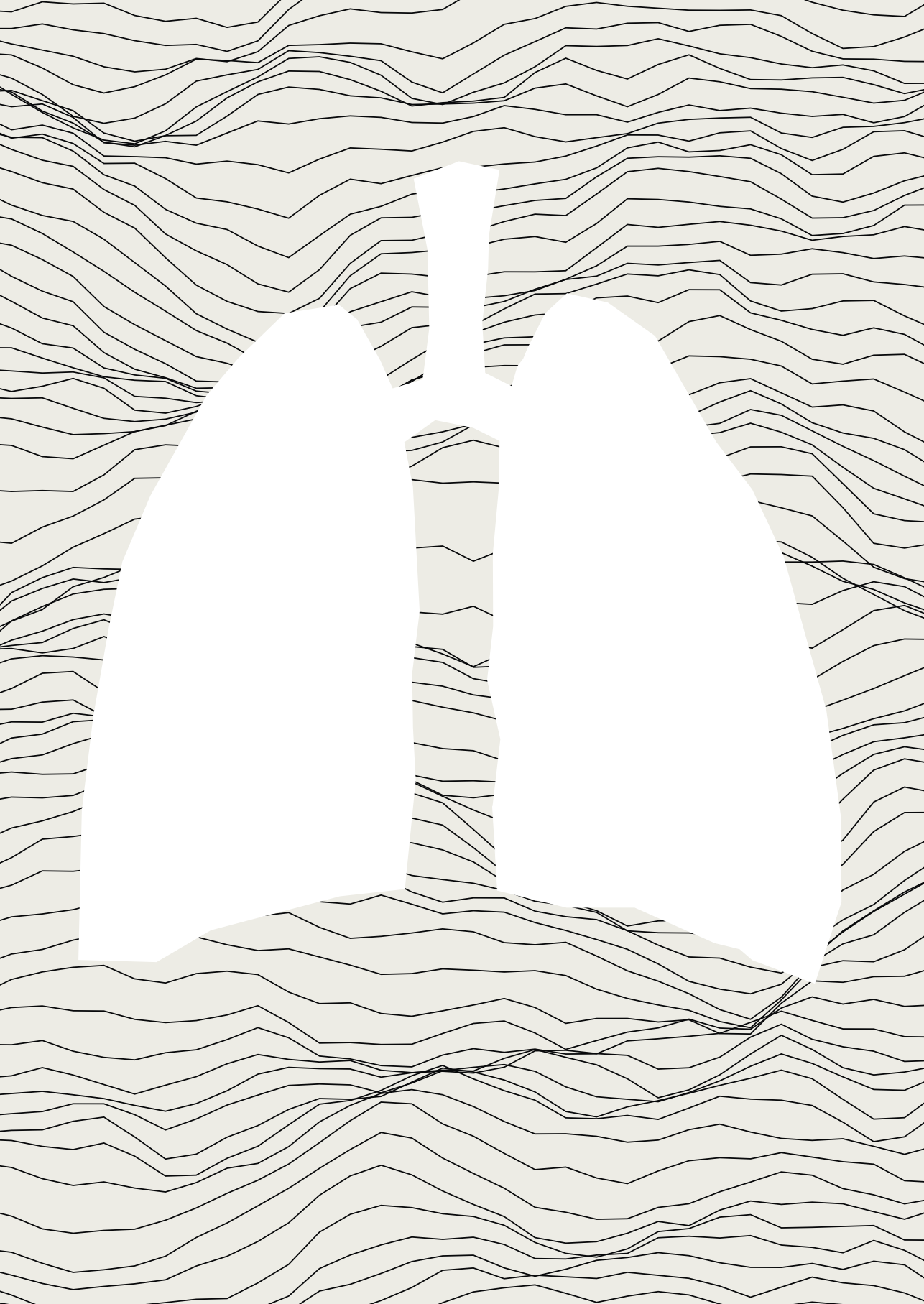


Chapter 7

Summary

The ambition to curb the persistent worldwide tuberculosis (TB) epidemic is challenged by two important problems; the development of acquired drug resistance and the low treatment success rates for multi-drug resistant tuberculosis (MDR-TB). An underlying cause is the combination of large inter-individual variability in drug exposure and decreasing bacterial susceptibility. Individualised dosing of anti-TB drugs using therapeutic drug monitoring (TDM) may be an important step to improve MDR-TB treatment outcomes and minimize the development of acquired drug resistance. However, TDM is considered laborious, expensive, and consequently has not been widely implemented yet. This thesis aimed to study the feasibility of salivary TDM, limited sampling strategies (LSS), and centralized TDM as alternative methods to reduce the burden of TDM as well as to stimulate the programmatic implementation of TDM.

In **Chapter 2**, we reviewed the literature and identified pharmacokinetic studies which reported anti-TB drug concentrations in both saliva and blood of humans. The aim of this study was to provide an overview of the available data on saliva-blood ratios of anti-TB drugs and to detect knowledge gaps to be filled in by future studies. In total, we included 42 studies with data on rifampicin, isoniazid, moxifloxacin, ofloxacin, gatifloxacin, amikacin, linezolid, amoxicillin/clavulanate, doripenem, and clarithromycin. Large variation in study population, sampling procedure, and saliva-plasma or saliva-serum ratios was observed between studies. The conclusion of this chapter was that, based on the available literature, salivary TDM likely is not possible for all anti-TB drugs due to highly variable saliva-plasma or saliva-serum ratios, but it is worthwhile to further investigate salivary TDM for each individual TB drug especially those that have not been studied yet.

Because many studies included in our review (Chapter 2) did not include patients with TB nor did they evaluate the feasibility of salivary TDM, we decided to perform a prospective observational cohort study in patients with TB (**Chapters 3a, 3b, 3c**). This study included all TB drugs being part of the treatment regimen. Patients consecutively enrolled in this observational study received traditional blood-based TDM as part of standard of care. Saliva was simultaneously sampled with blood and the measured paired drug concentrations were used to calculate saliva-plasma or saliva-serum concentration ratios. Additionally, non-compartmental $AUC_{0\text{-}24}$ saliva-plasma or saliva-serum ratios were assessed. To minimize the infection hazard of processing saliva samples of sputum culture positive patients, we developed and tested a secure sampling method (**Chapter 3d**). Culture fluids containing at least 10^5 to 10^6 CFU/mL of different *Mycobacterium tuberculosis* strains were successfully sterilized using membrane filtration with a pore size of 0.22 μm. This experiment provided evidence for the conclusion that membrane filtration is suitable for safe collection of saliva samples.

Chapter 3a studied the feasibility of salivary TDM of the first-line TB drugs rifampicin and isoniazid. Rifampicin showed very low saliva-serum ratios which can be explained by a high protein binding. We concluded that rifampicin serum AUC_{0-24} could be adequately estimated by applying a correction factor of 6.5 to the AUC_{0-24} in saliva. In contrast, isoniazid saliva-serum ratios were highly variable, especially between patients, but the exact cause remains unclear. For that reason, isoniazid TDM using saliva samples was assumed to be unfeasible using the described methods. It might still be interesting to explore the option of determining acetylator phenotype using salivary isoniazid concentrations.

Chapter 3b focused on moxifloxacin and linezolid; both are preferred (group A) drugs for the treatment of MDR-TB. We felt that salivary TDM of linezolid has great potential due to constant saliva-serum ratios. To adequately predict the serum AUC_{0-24} of linezolid, a correction factor of 1.2 must be applied to the AUC_{0-24} determined in saliva. Moxifloxacin saliva-plasma ratios were noticeably high, but very variable and therefore unpredictable. Based on this data, we concluded that saliva is not a suitable alternative matrix for TDM of moxifloxacin.

Chapter 3c aimed to determine the potential for C_{max}/MIC guided TDM of amikacin using saliva samples. However, amikacin could not be quantified in any of the saliva samples, not even in the saliva samples collected at serum T_{max} which were expected to represent salivary C_{max}. This low penetration into saliva could be explained by the fact that amikacin is a highly polar compound and as a result does not easily pass through membranes. As the salivary C_{max} could not be determined in any of the patients, we concluded that salivary TDM using C_{max}/MIC is unfeasible for amikacin.

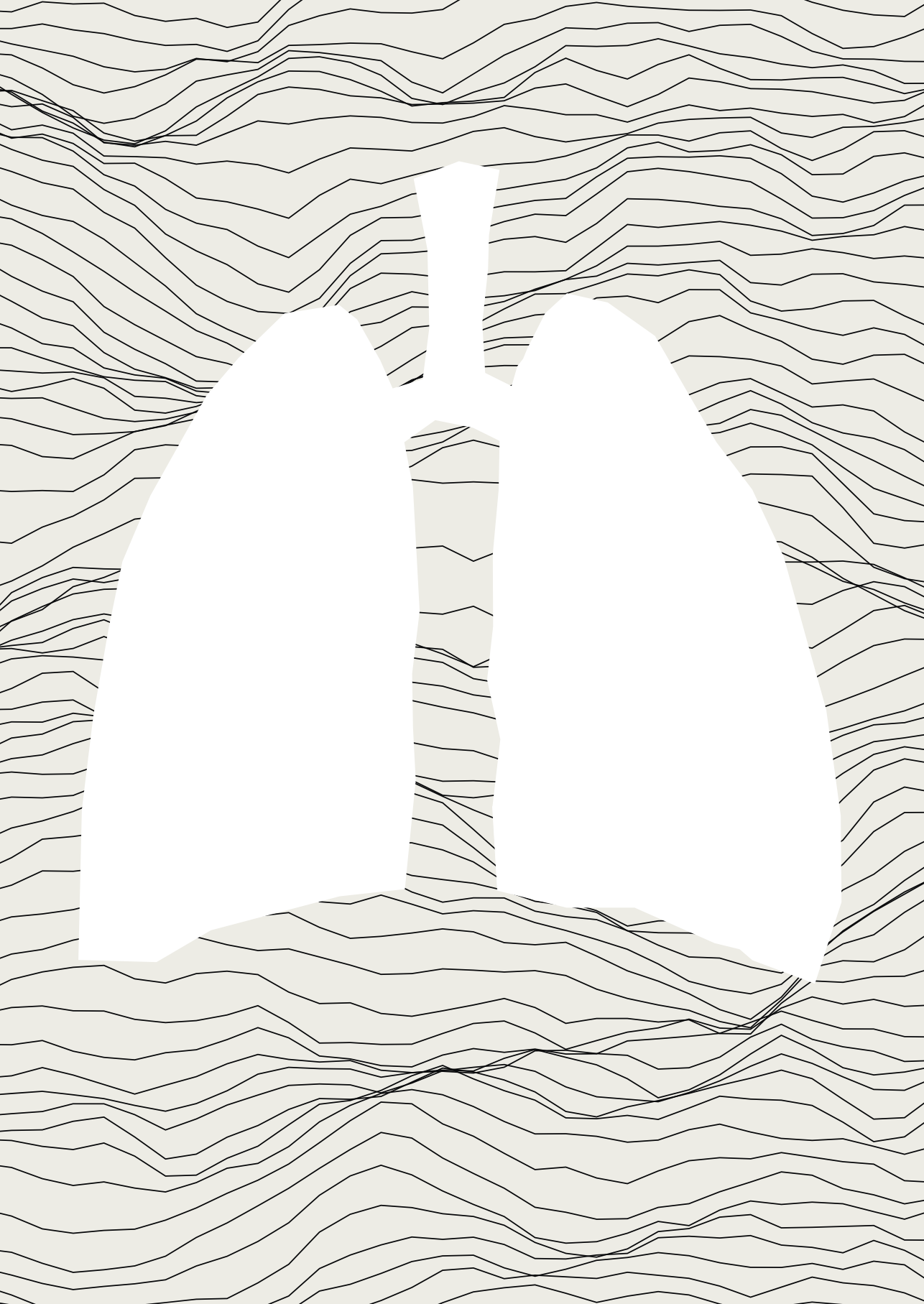
Another alternative sampling method studied in this thesis is the use of a limited sampling strategy (LSS) that requires only a small number of ideally timed samples to estimate AUC_{0-24}. In **Chapters 4a and 4b,** we aimed to develop and validate population pharmacokinetic (popPK) models as well as LSS using the Bayesian approach and multiple linear regression.

Chapter 4a included moxifloxacin pharmacokinetic data of 101 patients with TB. Separate popPK models and LSS were created for moxifloxacin alone (77 patients) and in combination with rifampicin (24 patients), because rifampicin is known to significantly increase moxifloxacin clearance and subsequently decrease moxifloxacin exposure. One-compartment popPK models with lag time were developed as they best described the data. Well-performing Bayesian LSS for both moxifloxacin alone and in combination with rifampicin included two samples; one collected before drug intake (t=0 h) and a second sample 6 h after drug intake. Using multiple linear regression, AUC_{0-24} can be adequately estimated with t=0 h and t=4 h samples for moxifloxacin alone, whereas with t=1 h and t=6 h samples when in combination with rifampicin. The popPK model and LSS were all successfully validated using jackknife analysis.

Chapter 4b focused on another important fluoroquinolone in MDR-TB treatment: levofloxacin. Pharmacokinetic data of 30 patients with MDR-TB was used to develop the popPK model and corresponding LSS. The model and Bayesian LSS were externally validated in 20 other MDR-TB patients. The multiple linear regression LSS was internally validated using jackknife analysis. The data was best described by a one-compartment model with lag time. We found that levofloxacin $AUC_{0\text{-}24}$ could be adequately estimated using the Bayesian approach using a sample collected before drug intake (t=0 h) and one collected 5 h thereafter (t=5 h). The LSS using multiple linear regression required t=0 h and t=4 h post dose samples to provide a reliable estimation of levofloxacin $AUC_{0\text{-}24}$. The LSS for moxifloxacin and levofloxacin described in Chapters 4a and 4b are ready for implementation in clinical practice and were already applied in one of our studies (Chapter 5).

We proposed a study design for a prospective multicentre study in **Chapter 5** with the primary aim to evaluate the feasibility of centralized TDM of moxifloxacin and levofloxacin. Sample analysis and clinical dose decisions are performed in a central facility to reduce the costs and to increase the quality of TDM. The turn-around-time between moment of sampling at the local hospital and the clinician receiving dosing advice was chosen as parameter for feasibility. Secondary, this study intended to determine the impact of TDM by comparing sputum culture conversion after two months of treatment between patients who received TDM and historical controls without TDM. A strength of this study is that the burden of TDM for patients was decreased by using LSS. Moreover, this is the first prospective study to investigate the effect of TDM on treatment results of patients with MDR-TB.

All chapters were discussed and put into perspective in **Chapter 6**. The place for TDM in TB treatment and its efficacy was debated. The need for drug susceptibility testing at start of TB treatment as well as MIC determination for TDM was highlighted as we feel this is an important issue that must be solved by developing rapid and cost-effective tests. We discussed numerous causes for the observed high variability in saliva-blood ratios in general. Based on the results in Chapters 2, 3a, 3b, and 3c, we concluded that TDM using saliva samples can be an attractive alternative for some anti-TB drugs such as linezolid and rifampicin, but is not feasible for others (e.g. moxifloxacin, isoniazid, amikacin), and therefore is not equivalent to regular TDM. We proposed to use saliva in semi-quantitative screening methods to identify the patients who could benefit from blood-based TDM. Furthermore, this chapter included the advantages and limitations of the different types of LSS and the need for proper validation of the LSS. Moreover, we discussed the additional value of developing LSS for a combination of second-line anti-TB drugs, since it was not available yet and would significantly reduce the burden of TDM. Additionally, we briefly reviewed the value of centralized TDM and the necessity for determination of its feasibility. Lastly, we provided our overall conclusion of this thesis on the feasibility of salivary TDM, LSS, and centralized TDM.



Epilogue

Samenvatting

Tuberculose (TB) is een infectieziekte die veroorzaakt wordt door de tuberkelbacil, ook wel *Mycobacterium tuberculosis* genoemd. Een TB infectie wordt in de meeste gevallen verspreid door het inademen van kleine druppeltjes in de lucht met daarin de *M. tuberculosis* bacteriën. Deze druppels kunnen gevormd worden wanneer iemand met een besmettelijke vorm van een TB infectie in de longen (pulmonale TB) hoest of niest. Bekende symptomen van een actieve pulmonale TB infectie zijn een aanhoudende hoest, nachtelijk zweten, vermoeidheid en gewichtsverlies.

Tegenwoordig komt TB in Nederland weinig voor, maar wanneer gekeken wordt naar de wereldwijde aantallen staat TB in de top 10 meest voorkomende doodsoorzaken. In 2018 ontwikkelden wereldwijd ongeveer 10 miljoen mensen een actieve TB infectie en in datzelfde jaar zijn 1,45 miljoen patiënten door TB overleden. Hoewel TB in de meeste gevallen goed te behandelen is met antibiotica, lukt het dus maar niet om de TB epidemie te verslaan.

Een actieve TB infectie wordt, wanneer de bacterie normaal gevoelig is, behandeld met een combinatie van geneesmiddelen (rifampicine, isoniazide, pyrazinamide, ethambutol). Er wordt gedurende twee maanden met deze vier middelen behandeld, gevolgd door vier maanden behandeling met alleen rifampicine en isoniazide. Echter, wanneer de TB bacterie resistent is voor de twee belangrijkste geneesmiddelen (rifampicine en isoniazide), dan wordt dit multidrug-resistente TB (MDR-TB) genoemd. De behandeling van MDR-TB duurt wel 9 tot 20 maanden en is minder succesvol dan de behandeling van normaal gevoelige TB. Daarnaast is er een grotere kans op bijwerkingen door het gebruik van niet goed verdraagbare geneesmiddelen. Er zijn veel verschillende antibiotica die gebruikt worden in de MDR-TB behandeling en deze zijn op voorkeur ingedeeld in verschillende groepen. Bij start van de behandeling kiest de arts hieruit 4 tot 5 middelen die naar verwachting het meest effectief zijn voor de patiënt op basis van het resistentiepatroon van de TB bacterie.

GEÏNDIVIDUALISEERDE TB BEHANDELING MET THERAPEUTIC DRUG MONITORING

Een actueel en belangrijk probleem is de ontwikkeling van resistentie tegen antibiotica die gebruikt worden in de TB behandeling. De TB bacterie kan resistent worden door inefficiënte therapie, bijvoorbeeld bij een te lage blootstelling aan het geneesmiddel. Een te lage blootstelling kan worden veroorzaakt door farmacokinetische variatie tussen individuen. Dit houdt in dat wanneer dezelfde dosering aan verschillende

personen wordt gegeven, er erg wisselende geneesmiddelconcentraties in het bloed gevonden worden. Dit geeft dus ook een grote variatie in effectiviteit tussen deze personen en daarom is er dus niet één dosis die voor iedereen geschikt is. Voor meerdere TB geneesmiddelen is het al bekend dat er grote farmacokinetische variatie bestaat. Daarom wordt er in verschillende richtlijnen geadviseerd om therapeutic drug monitoring (TDM) toe te passen. TDM is een methode waarbij er bloedmonsters wordt afgenomen bij de patiënt om daarin de geneesmiddelconcentraties te laten meten door een laboratorium. Met behulp van deze informatie en andere patiëntkarakteristieken, zoals geslacht, leeftijd, gewicht en nierfunctie, wordt er bepaald of de huidige dosering voor deze patiënt adequaat is, of dat er een dosisaanpassing nodig is. Zo wordt er voor iedere individuele patiënt een dosering op maat gevonden. TDM kan hierdoor het slagingspercentage van de behandeling vergroten en het risico op ontwikkeling van resistentie verlagen. Daarnaast kan het ook toegepast worden om de bijwerkingen van de TB geneesmiddelen te verminderen door te zoeken naar de laagst mogelijke effectieve dosering.

Bij TB geneesmiddelen is niet alleen de blootstelling aan het geneesmiddel van belang, maar ook de gevoeligheid van bacterie voor het geneesmiddel. Deze gevoeligheid kan namelijk per geneesmiddel sterk variëren tussen verschillende stammen van de tuberkelbacil. De gevoeligheid van de bacterie kan getest worden in een microbiologisch laboratorium en wordt uitgedrukt in de minimale groei remmende concentratie (MIC). De MIC is gedefinieerd als de laagste concentratie van het geneesmiddel waarbij de TB bacterie niet meer groeit. De verhouding tussen de blootstelling en de gevoeligheid van de bacterie bepaalt de effectiviteit van het middel en daarom wordt deze verhouding uitgerekend als onderdeel van de TDM. De gemeten geneesmiddelconcentraties worden uitgezet tegen de tijd van afname en deze grafiek vormt dan een zogenaamde concentratie-tijd curve. Voor de meeste TB middelen is de oppervlakte onder deze concentratie-tijd curve (AUC) in relatie tot de gevoeligheid de beste maat voor de effectiviteit en daarom wordt voor deze middelen de AUC/MIC ratio berekend om te bepalen of de dosering adequaat is.

In Nederland is TDM al een standaard onderdeel van de MDR-TB behandeling, maar dat is lang niet overal zo georganiseerd. In ontwikkelingslanden met hogere aantallen TB patiënten wordt TDM nog gezien als extra service, omdat men het tijdsintensief, organisatorisch lastig en duur vindt. Er zijn diverse methodes om de financiële en organisatorische lasten van TDM te verlagen, waarvan er enkele worden beschreven in dit proefschrift.
Van oudsher wordt TDM uitgevoerd met bloedmonsters, maar speeksel wordt gezien als interessant alternatief, omdat de speekselconcentratie een goede afspiegeling kan zijn van de concentratie in bloed. Het verzamelen van een speekselmonster

is eenvoudig, patiëntvriendelijk en mogelijk ook goedkoper dan bloedafnames. Vooral voor kinderen en mensen die een hekel hebben aan bloedprikken is het een prettige methode. Daarnaast kunnen patiënten getraind worden om zelf thuis de speekselmonsters te verzamelen waardoor het aantrekkelijk is voor gebieden met lange reistijden naar de ziekenhuizen.
Om de AUC nauwkeurig te bepalen zijn meerdere, soms wel 6 tot 8, bloedmonsters nodig. Daarom kan de uitvoering van TDM ook vereenvoudigd worden door het toepassen van limited sampling strategieën (LSS). Deze LSS vereisen minder monsters dan bij reguliere tijdschema's, omdat deze afnamemomenten ideaal gepland zijn. Hierdoor is er met minder monsters, meestal maar 1 tot 3, alsnog voldoende informatie beschikbaar om een betrouwbare inschatting te geven van de AUC.
Een derde methode om de drempel voor de toepassing van TDM te verlagen, is het centraliseren van TDM in een hoofdlaboratorium. De patiënt laat dan bloed afnemen bij een gezondheidscentrum of ziekenhuis in de buurt, waarna de monsters worden verstuurd naar het centrale laboratorium. In het laboratorium worden de metingen uitgevoerd en het doseeradvies opgesteld. Doordat dit alleen plaatsvindt in een gespecialiseerd laboratorium met veel expertise, zullen dat de analyseresultaten en doseeradviezen van betere kwaliteit zijn. Bovendien verbetert het de kosteneffectiviteit van TDM, omdat de schaarse en dure analyseapparatuur optimaal wordt gebruikt.

Het doel van dit proefschrift was om de mogelijkheden van TDM in speeksel, LSS en gecentraliseerde TDM te onderzoeken om de organisatorische en financiële last van de uitvoering van TDM te verlagen en zo de toepassing van TDM in de behandeling van TB wereldwijd te bevorderen.

TDM IN SPEEKSEL

In **hoofdstuk 2** hebben we in de literatuur gezocht naar studies die concentraties van TB middelen in zowel bloed als speeksel rapporteren. We hebben 42 studies geïncludeerd met data over rifampicine, isoniazide, moxifloxacine, ofloxacine, gatifloxacine, amikacine, linezolid, amoxicilline/clavulaanzuur, doripenem en claritromycine. Er zaten grote verschillen tussen de studies, vooral wat betreft de studiepopulatie, de methode van speekselafname en de gevonden speeksel-bloed concentratieratio's. De conclusie van dit hoofdstuk was dat TDM in speeksel waarschijnlijk niet geschikt is voor alle TB geneesmiddelen door grote variatie in speeksel-bloed ratio's tussen individuen, maar dat het wel de moeite waard is om extra onderzoek te doen naar de mogelijkheden van TDM in speeksel.
Omdat veel studies in hoofdstuk 2 geen patiënten met TB hebben geïncludeerd en de praktische uitvoerbaarheid van TDM in speeksel niet hebben onderzocht, hebben we een prospectieve studie opgestart om de missende informatie aan te vullen

(**hoofdstuk 3a, 3b, 3c**). In deze studie hebben we gelijktijdig bloed en speeksel afgenomen bij TB patiënten om zo de geneesmiddelconcentraties in beide vloeistoffen te kunnen vergelijken en de speeksel-bloed ratio's te kunnen berekenen. Wanneer de speeksel-bloed ratio's relatief constant zijn, kunnen deze als omrekeningsfactor gebruikt worden om in toekomstige patiënten de concentratie in bloed te voorspellen met behulp van de gemeten speekselconcentratie.
Het speeksel van besmettelijke patiënten kan TB bacteriën bevatten, dus het verwerken van deze monsters brengt een infectierisico met zich mee indien er geen voorzorgsmaatregelen getroffen worden. Daarom hebben we in **hoofdstuk 3d** een methode ontwikkeld om veilig speeksel te kunnen verzamelen bij deze patiëntengroep. Door het speeksel te filtreren door een membraanfilter met hele kleine poriën (<0,22 µm) werden alle TB bacteriën verwijderd. Deze methode is succesvol gebruikt in onze studie.
In **hoofdstuk 3a** is speeksel TDM voor de eerstelijns geneesmiddelen rifampicine en isoniazide onderzocht. De conclusie was dat de AUC van rifampicine in bloed nauwkeurig kon worden voorspeld door de AUC in speeksel te vermenigvuldigen met een factor 6,5. Voor isoniazide bleek echter dat TDM in speeksel niet goed bruikbaar was door de grote variatie in speeksel-bloed ratio's tussen de patiënten, maar de precieze oorzaak hiervan bleef onbekend.
In **hoofdstuk 3b** richtten wij ons op twee voorkeursmiddelen in de behandeling van MDR-TB, namelijk moxifloxacine en linezolid. We concludeerden dat de AUC van linezolid in bloed nauwkeurig kon worden berekend door het vermenigvuldigen van de AUC in speeksel met een factor 1,2. Voor moxifloxacine werden hoge, maar ook zeer wisselende speeksel-bloed ratio's gevonden, waardoor het niet haalbaar leek om TDM van moxifloxacine in speeksel uit te voeren.
In **hoofdstuk 3c** onderzochten wij de mogelijkheid om amikacine TDM in speeksel uit te voeren. Amikacine was in geen enkel speekselmonster van de zes geïncludeerde patiënten terug te vinden. Omdat amikacine dus blijkbaar onvoldoende doordringt in speeksel om het te kunnen meten, hebben we geconcludeerd dat speeksel TDM niet mogelijk is voor amikacine.

LIMITED SAMPLING STRATEGIEËN (LSS)

Voor het ontwikkelen van LSS zijn eerst farmacokinetische modellen nodig. Dit zijn modellen die het verloop van de geneesmiddelconcentraties in de tijd voor een individu kunnen voorspellen met behulp van een grote dataset met informatie uit een eerdere patiëntenpopulatie. In **hoofdstuk 4a en 4b** zijn vervolgens twee verschillende soorten LSS ontwikkeld. De eerste maakt gebruik van het farmacokinetische model en houdt rekening met veel factoren, maar is daardoor ook relatief complex. De tweede is gebaseerd op lineaire regressie, waarbij alleen de geneesmiddelconcentraties

worden gebruikt om de AUC te berekenen met behulp van een simpele formule. Beide manieren hebben dus voordelen en nadelen. Daarom hebben we beide soorten LSS ontwikkeld, zodat er per situatie besloten kan worden welke LSS het meest geschikt is. Voor **hoofdstuk 4a** zijn gegevens verzameld van 101 TB patiënten die onder andere met moxifloxacine behandeld werden. Er zijn afzonderlijke modellen en LSS gemaakt voor de patiënten met moxifloxacine zonder rifampicine (77 patiënten) en moxifloxacine in combinatie met rifampicine (24 patiënten), omdat bekend is dat rifampicine de klaring van moxifloxacine uit het lichaam beïnvloedt en dus ook de AUC. Wij concludeerden dat de AUC van moxifloxacine goed kan worden voorspeld (zowel met als zonder rifampicine) met behulp van het model en twee bloedafnames. Het eerste monster werd verzameld net voor de inname van het geneesmiddel (t=0 uur) en de tweede op 6 uur na inname (t=6 uur). Voor de lineaire regressie LSS waren afnamemomenten op t=0 uur en t=4 uur geschikt voor moxifloxacine zonder rifampicine en op t= 1 uur en t=6 uur voor moxifloxacine in combinatie met rifampicine.
In **hoofdstuk 4b** richtten wij ons op LSS voor levofloxacine wat, naast moxifloxacine, een ander eerste keus geneesmiddel voor de behandeling van MDR-TB is. Er zijn gegevens van 30 MDR-TB patiënten gebruikt voor het ontwikkelen van een model en LSS. Een aparte dataset met 20 andere MDR-TB patiënten werd gebruikt om naderhand de prestaties van het model en LSS te testen. Het afnemen van monsters net voor geneesmiddelinname (t=0 uur) en 5 uur daarna (t=5 uur) was geschikt voor het schatten van de levofloxacine AUC met behulp van het model. Voor lineaire regressie waren bloedafnames op t=0 uur en t=4 uur vereist voor het nauwkeurig berekenen van de levofloxacine AUC.
We concludeerden dat de LSS beschreven in hoofdstuk 4a en 4b per direct gebruikt kunnen worden voor TDM van moxifloxacine en levofloxacine in de dagelijkse praktijk.

CENTRALISEREN VAN TDM

We hebben in **hoofdstuk 5** een protocol beschreven voor een toekomstige studie die de praktische uitvoerbaarheid van gecentraliseerde TDM zal evalueren. De voordelen van gecentraliseerde TDM liggen vooral in de kostenbesparing en kwaliteitsverbetering, maar het zal naar verwachting ook logistieke uitdagingen geven, zoals bij het transport van de monsters. Daarom is ervoor gekozen om de tijd tussen de bloedafname bij de patiënt en het moment dat de arts het doseringsadvies ontvangt (turn-around-time) te bepalen als maat voor de praktische uitvoerbaarheid. Een turn-around-time van 7 dagen wordt gezien als acceptabel. Daarnaast zal de impact van TDM op de behandelresultaten onderzocht worden, omdat verwacht wordt dat TDM de effectiviteit van de behandeling verbetert. Hiervoor worden de behandelresultaten van de patiënten in deze studie vergeleken met de uitkomsten van controlepatiënten die geen TDM hebben gekregen. Een voordeel van deze studie is dat het gebruikt maakt van de in hoofdstuk 4a en 4b

ontwikkelde LSS voor moxifloxacine en levofloxacine, waardoor er per patiënt maar twee monsters nodig zijn om de TDM uit te voeren. Daarnaast is het de eerste studie die rechtstreeks het effect van TDM op de behandelresultaten van MDR-TB zal onderzoeken.

CONCLUSIE

Gebaseerd op de studies in dit proefschrift hebben we geconcludeerd dat TDM in speeksel geen gelijkwaardig alternatief is voor reguliere TDM met bloedmonsters, omdat het niet geschikt is voor alle TB geneesmiddelen doordat de concentraties in speeksel erg variabel zijn of te laag om te kunnen meten. Er zijn LSS ontwikkeld die in staat zijn om nauwkeurig de AUC van moxifloxacine en levofloxacine in te schatten met behulp van slechts twee monsters. Wij zien de LSS als waardevolle methode om de belasting door TDM te verlagen voor patiënt en zorgverlener en moedigen daarom het gebruik van LSS aan. Centraliseren van TDM in gespecialiseerde laboratoria is naar verwachting een goed idee om de kosten van TDM te verlagen en de kwaliteit van TDM te verbeteren, maar er is nog implementatieonderzoek nodig om de praktische uitvoerbaarheid te bepalen.

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About the author

Simone Hildegarde Johanna van den Elsen was born on April 27th, 1994 in Etten-Leur, the Netherlands. She attended high school (VWO) at Dr. Knippenberg College in Helmond and graduated in 2011. Thereafter, she moved to Groningen to study Pharmacy at the University of Groningen, where she received a bachelor's degree in 2014.

Simone's interest in research was excited during the master's program (2014-2018). She conducted a research project on therapeutic drug monitoring of anti-tuberculosis drugs in saliva at the University Medical Center Groningen (UMCG, department of Clinical Pharmacy and Pharmacology) under the supervision of prof. dr. J.W.C. Alffenaar and dr. M.S. Bolhuis. Her enthusiasm for this project led to the initiation of a PhD trajectory that officially started in September 2017. The first steps of her PhD research were taken during the remaining part of her master's studies.

In June 2018, Simone earned her master's degree of Pharmacy (PharmD). One month later, she started her first job as compounding pharmacist at the department of Clinical Pharmacy and Pharmacology of the UMCG and combined this with the finalisation of this PhD thesis. At the moment, she aspires to a career in hospital pharmacy and aims to continue research on PK/PD of anti-infective drugs.

Simone has developed a passion for coffee as a result of her experience as barista at an espresso bar. Additionally, she enjoys the beauty of nature and photography, particularly when both are combined.

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