Tuberculosis (TB) is caused by infection with *Mycobacterium tuberculosis*. It is one of the infectious diseases with the highest morbidity and mortality in the world. In 2014, an estimated 9.6 million people were infected with TB and 1.5 million people died due to TB [1].

Patients with active pulmonary TB are the main source of transmission of *M. tuberculosis*. The disease is spread in the air when people, who are sick with pulmonary TB, expel bacteria, for example by coughing. A relatively small proportion of people infected with *M. tuberculosis* will develop active TB disease. In more than 90% of persons infected with *M. tuberculosis*, the pathogen is contained as asymptomatic latent infection [2]. Worldwide, one third of the population, approximately two billion people, have latent infection and are at risk for reactivation [2].

TB typically affects the lungs (pulmonary TB), but can affect other sites as well (extrapulmonary TB). Symptoms of active disease are a productive cough often with other respiratory symptoms like shortness of breath, chest pain or haemoptysis and fever with night sweats, fatigue and loss of appetite and body weight [3]. Extrapulmonary TB can affect virtually any organ in the body, with a variety of clinical manifestations and therefore it requires a high index of clinical suspicion [2].

The most common method for diagnosing TB worldwide is sputum smear microscopy. The use of rapid molecular tests to diagnose TB and drug-resistant TB is increasing. In countries with more developed laboratory facilities, cases of TB are also diagnosed via culture methods, which is currently the reference standard [1].

Without adequate treatment, TB mortality rates are high [1]. Drug-susceptible TB is treated with the first-line anti-TB drugs, isoniazid, rifampicin (rifampin), pyrazinamide and ethambutol, during the first two months, continued with isoniazid and rifampicin for another four months [3][4]. In general, treatment success rate continues to be high among new TB cases [1]. However, healthcare providers are still confronted with treatment failure on a regular basis. In multidrug-resistant TB (MDR-TB), *M. tuberculosis* is resistant to isoniazid and rifampicin. In 2014, an estimated 480,000 people developed MDR-TB [1]. MDR-TB is difficult and costly to treat, as first-line treatment is not effective. Besides, treatment takes much longer than six months in drug-susceptible TB, generally it takes 20 months.

### 1.1 First-line anti-TB treatment

Isoniazid and rifampicin are still the cornerstones of treatment in drug-susceptible TB. Both drugs are cheap and readily available worldwide. Rifampicin is bactericidal, by inhibition of the \(\beta\)-subunit of RNA polymerase of *M. tuberculosis*. It is highly effective and extremely valuable in TB treatment, as rifampicin is capable of
Isoniazid is a prodrug that is activated by the mycobacterial enzyme KatG. It exerts its activity by inhibiting mycolic acid synthesis, which is essential for mycobacterial cell wall integrity. If *M. tuberculosis* lacks the katG enzyme, it shows resistance to isoniazid. Isoniazid is mostly active in the first few days of therapy as it exhibits the most potent early bactericidal activity (EBA), the decrease in number of bacilli in sputum in the first two treatment days, among the anti-TB drugs.

Pyrazinamide is a prodrug that is bio-activated by the pyrazinamidase enzyme inside *M. tuberculosis*. The mechanism of action for pyrazinamide is not clearly understood. It has been suggested to act by inhibiting fatty acid synthase I. But also inhibition of protein translation or depletion of cellular adenosine triphosphate have been proposed. Pyrazinamide is an essential component in the treatment of *M. tuberculosis* and is supposed to play an important role in new multidrug regimens which focus on shortening treatment duration.

Ethambutol acts by inhibiting the synthesis of arabinogalactan, which is part of the mycobacterial cell wall. It is used to protect rifampicin from acquired drug resistance and is the least potent of the four first-line anti-TB drugs.

### 1.2 Pharmacology and therapeutic drug monitoring

Pharmacokinetics describes the behaviour of a drug in the patient’s body, including absorption, distribution, metabolism and excretion, whereas pharmacodynamics describe the biochemical or pharmacological effect of a drug at the site of action in the patient’s body. Together, both parameters determine the pharmacological profile of the drug.

In infectious diseases, the pharmacological effect of the drug is directed against the pathogen. The minimum inhibitory concentration (MIC), a measure of potency of the drug for the microorganism, is the lowest concentration at which the drug inhibits growth of *M. tuberculosis*. In case of first-line anti-TB drugs, actual MICs are often unknown. Critical concentrations or breakpoints have been defined as the MIC above which maximum tolerated doses fail to effectively kill *M. tuberculosis*. Conventionally, these critical concentrations were 1.0 mg/L for rifampicin and 0.2 mg/L and 1.0 mg/L for low-level and high-level resistance for isoniazid. However, Gumbo et al. advocated the use of much lower critical concentrations, 0.0625 mg/L for rifampicin and 0.0312 and 0.125 mg/L for isoniazid, suggesting that *M. tuberculosis* is resistant at a much lower level.

The efficacy of anti-infective drugs is not only dependent on the pathogens related MIC, but also on the exposure of the drug in the patient. The area under
the concentration-time curve (AUC) over 24 h in steady state (AUC$_{0-24}$) represents this exposure. Studies in both hollow fiber infection models and murine models showed that the AUC divided by the MIC (AUC/MIC ratio) is the best predictive pharmacokinetic/pharmacodynamic parameter for determination of the efficacy of first-line anti-TB drugs [9–15]. More importantly, these data were recently confirmed in TB patients, as poor long-term outcome was predicted by low AUC values of pyrazinamide, rifampicin and isoniazid [7].

For a long time, it was suggested that poor compliance was the cause of acquired drug resistance of *M. tuberculosis*. Now it has become clear that pharmacokinetic variability is much more likely to be the driving force of drug resistance [16]. In the study by Pasipanodya et al., low maximum concentrations (C$_{\text{max}}$) or peak levels of rifampicin or isoniazid preceded all (three) cases of acquired drug resistance in 142 TB patients [7]. Therefore, both AUC and C$_{\text{max}}$ are important parameters to investigate. This can be done using therapeutic drug monitoring (TDM).

The International Association of Therapeutic Drug Monitoring and Clinical Toxicology defined TDM "as a multi-disciplinary clinical specialty aimed at improving patient care by individually adjusting the dose of drugs for which clinical experience or clinical trials have shown it improved outcome in the general or special populations. It can be based on an a priori pharmacogenetic, demographic and clinical information and/or on the a posteriori measurement of blood concentrations of drugs (pharmacokinetic monitoring) and/or biomarkers (pharmacodynamic monitoring)."

TDM has been used when it is impossible to measure the pharmacodynamic effect of the drug faster or in a more direct way. For antibiotics this is often true, as it is both difficult and time-consuming to observe whether the infection is being treated adequately. If the infection is not treated adequately, it may be too late to turn the tide. However, in the case of anti-TB drugs, a therapeutic range or target has not been established. Until recently, the available data was based mostly on measurements in TB patients, rather than target values based on outcome. It has long been thought that exposure was sufficient and TDM was not indicated. Hence, TDM of first-line anti-TB drugs has not been performed on a regular basis.

Moreover, obtaining a full concentration-time curve to calculate the AUC values is a laborious and expensive procedure and it is therefore not feasible in clinical practice. Alternative strategies to easily evaluate drug exposure are urgently needed. An optimal sampling procedure based on a population pharmacokinetic model may help to overcome these problems. This method implies that a limited number of appropriately timed blood samples are needed to adequately predict the AUC as a measure for drug exposure [17–19].
However, there are more hurdles to take. Not many laboratories are capable of performing bioanalysis, as no commercially available assays exist and thus all methods have to be developed in-house. As a consequence, TDM has long been thought to be too costly and too difficult. Finally, once one has decided on performing TDM of first-line anti-TB drugs, the logistics of the blood samples is complicated by both the instability of the drugs in blood and the contagiousness of the material.

Treatment with the first-line anti-TB drugs, though usually successful, is increasingly challenged by the emergence of drug resistance, toxicity, relapse and non-response. It is believed that for both rifampicin and isoniazid higher doses might well be indicated and tolerated [5, 20–22].

We hypothesize that it is necessary to move away from the ‘one size fits all’ approach [23]. The aim of this thesis is to develop methods to facilitate TDM of first-line anti-TB drugs and to optimize treatment in drug-susceptible TB patients.

### 1.3 Outline of the thesis

In Chapter 2, a review is presented on the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) in TDM of anti-infective drugs. Pharmacokinetic and pharmacodynamic parameters are discussed. We explore aspects of new matrices such as saliva and new sampling techniques like dried blood spot (DBS) and their analysis.

The objective of Chapter 3 was to develop a fast, simple and reliable LC-MS/MS method for the simultaneous determination of isoniazid, pyrazinamide and ethambutol in human serum for TDM and pharmacokinetic studies.

In Chapter 4, we describe a recently initiated international interlaboratory quality control (QC) or proficiency testing programme for the measurement of anti-TB drugs. In the first round of this programme, the four first-line anti-TB drugs and the second-line TB drugs moxifloxacin and linezolid were included. The programme addresses the utility of an ongoing QC programme in this area of bioanalysis.

The objective of Chapter 5a was to develop an optimal sampling procedure based on population pharmacokinetics to predict $AUC_{0–24}$ of rifampicin. In Chapter 5b, we discuss the need of well-designed pharmacokinetic studies to produce reliable data and relevant outcome.

In Chapter 6, we performed a retrospective study in TB patients in which we determined the correlation of clinical variables with exposure of isoniazid and rifampicin. The aim was to discuss the maximum dose of isoniazid and rifampicin, which is guided by the World Health Organization [3].
Chapter 1. Introduction

Concomitant food is known to influence pharmacokinetics of first-line anti-TB drugs in healthy volunteers. However in treatment-naive TB patients who are starting with drug treatment, data on the influence of food intake on the pharmacokinetics are absent. In Chapter 7 we performed a prospective randomized crossover pharmacokinetic study that aimed to quantify the influence of food on the pharmacokinetics of isoniazid, rifampicin, ethambutol and pyrazinamide in TB patients, starting anti-TB treatment.

In Chapter 8 the outcome of the research in this thesis is discussed and future perspectives are presented. This chapter is based on a letter to the editor in which we stress the importance of well-designed randomized controlled trials to investigate the effectiveness of TDM on outcome.

References


