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Chapter

Introduction and scope of thesis

Tuberculosis today

In 1993, tuberculosis (TB) was for the first time named a global emergency by the World Health Organization (WHO) requiring immediate action in order to prevent expansion of a TB pandemic. At that time, 2.5 million people died from TB annually (1990) (1). Today, the global TB death rate has fallen (2). But still the TB pandemic is killing 1.3 million people according to the latest WHO Global tuberculosis report – and this staggering number excludes another 374,000 people dying from TB in the context of HIV co-infection (2017) (2). In perspective, HIV was responsible for 1.0 million deaths in 2016 (3), more than a third of whom actually died from TB (2). Therefore, even to win the battle against HIV/AIDS, we do have to fight TB (*adapted from* Nelson Mandela, International AIDS conference, Bangkok 2004).

Like HIV-infected patients, diabetes mellitus (DM) patients have a high lifetime risk of developing TB compared to non-HIV or non-DM patients, respectively, due to a weakened immune system (4). Especially, hyperglycemia is associated with a high risk of falling ill from TB as well as with a poor TB treatment response (5,6). In addition, altered pharmacokinetics in DM could contribute to inadequate drug exposure (7,8). Ageing of the world population and a rising unhealthy lifestyle are important perpetrators for the increasing number of people living with DM, especially in low- and middle-income countries (9). This new and emerging DM epidemic is a challenge for TB control as well.

Historically, TB is a poverty-related disease. Today, the incidence rates are still lower in high-income countries, e.g. 5.9 per 100,000 residents in The Netherlands versus 724/100,000 in Lesotho, Southern Africa (2). However, the downtrend of notified TB cases in the Netherlands was interrupted in 2015 and this is primarily due to the influx of asylum seekers originally from Eritrea and Ethiopia (10). New TB cases in low-incidence countries result from global migration. Therefore, global TB control ought to be a concern of high-income countries as well, as is underlined by WHO's End TB Strategy (11).

In one-out-of-five previously treated TB patients that relapse, resistance emerges against rifampicin (RIF), the most important drug in TB treatment (2). Furthermore, from 2007 onwards, the first extremely drug-resistant or " totally-drug resistant" cases were reported in Italy, Iran and India (12-14). In general, drug resistance is a result of improper use of medication regarding the dose, the combination of drugs, treatment compliance and/or the availability of drugs. Also, in some parts of the world where drug susceptibility cannot be tested, the epidemic of drug-resistant TB cannot be curbed (2).

In consecutive reports, the WHO have argued that the TB pandemic is declining, but even if the current global trend of estimated new TB cases continues to decline, additional interventions are needed to end TB by 2035, which is the target elaborated on the United Nations Sustainable Development Goals (15). Intensified efforts are needed now, as it appears that the actual TB incidence in recent years has rather plateaued than decreased (16) which is likely explained by the emergence of multi-drug resistant TB (MDR-TB) among all new TB cases. In case of MDR-TB, *M. tuberculosis* is resistant against RIF and isoniazid (INH), the two most powerful first-line agents (17). And next to TB-HIV co-infected patients and current topics as global migration and changing co-morbidity profiles (e.g. diabetes), the spread of drug resistance is therefore the biggest threat of today's TB burden (18,19).

Mycobacterium tuberculosis

TB is an air borne disease and transmission occurs via inhalation of droplets, loaded with *Mycobacterium tuberculosis*, which are dispersed by sneezing or coughing of pulmonary TB patients. Major TB outbreaks have revealed that a substantial proportion of supposedly newly infected people eradicate *M. tuberculosis* before the adaptive immune response is put in motion, whereas in others, *M. tuberculosis* settles, primarily in alveolar macrophages. The underlying cause of this distinction could be important for the development of new vaccines and therapies, including immunotherapy (20).

Once settled in the lungs, *M. tuberculosis* has a genetically determined program that can be switched on toward a low metabolic and low replication rate. This "on" mode of the dormancy survivor regulator (DosR) is driven by certain stress factors, e.g. host immune defense, drug treatment and hypoxia (21). An infected individual therefore either develops TB or only becomes 'latently' infected with dormant TB bacilli. Once the immune defense of the host weakens, the dormant mycobacteria may start replicating, e.g. in ageing individuals with immunosenescence, hosts co-infected with HIV, those that develop DM, or with declining vitamin D levels. Almost one quarter of the world's population is latently infected and therefore a pending threat for the TB burden (22).

Also, for TB patients, to ensure elimination of the slowly replicating or 'persister' population of TB bacilli, treatment lasts 6 months for drug-susceptible, and up to 20 months for advanced stages of drug-resistant TB. In 2016, based on clinical outcome data, the WHO justified the use of shorter drug regimens than 18-20 months – but still, 9-12 months - with current established 2nd line anti-TB agents, for drug resistant TB, under stringent conditions (17). Therefore, next to the dire need to develop new vaccines and immunotherapies, novel anti-TB agents are needed that directly act on the dormant mycobacterial population.

Treatment of tuberculosis and optimization of treatment

A treatment regimen has to be effective, well tolerated, but also preferably short and simple to ensure patient's compliance and good quality of life. TB treatment is assumed to be effective if the patient is successfully treated with subsequent negative cultures (cured), or at least, successfully treated without failure or relapse, without development of drug resistance and without transmission of the disease in the community (23). In this perspective, in general, even treatment for drug-susceptible TB is less than ideal, while treatment for MDR-TB is not nearly as successful as it should be.

To treat drug-susceptible TB, the WHO recommends a six-month regimen of INH, RIF, pyrazinamide (PZA) and ethambutol (23). As explained, there is at best marginal decline in new TB cases, and especially the proportion of MDR/RIF resistant (RR) cases among new and among previously treated TB cases is worrisome (2). On the opposite side of the therapeutic window, drug tolerability might be a problem. Drug-induced liver injury is a well-known side effect of INH, and especially, PZA, while RIF is the least hepatotoxic 1st line TB drug (24). In case of drug-resistance or drug-intolerability, the physician may have to switch to less studied, and thus probably less effective, more toxic, less simple (e.g. intravenous) second-line agents as part of a longer treatment regimen.

According to the WHO there are four classes of drugs for TB if RIF, the most important 1st line agent, is not suitable (Table **1**). Efficacy and tolerability of the drugs forced to the latter class (D) is less substantiated than the evidence for the core agents in class A, B and C. Bedaquiline, the first drug ever FDA-approved for MDR-TB in 2012, is one of the 'add on' drugs of class D. Class D drugs are recommended to add-on to the regimen in case not enough class A, B and/or C drugs will fit an individual MDR/RR-TB case. Nowadays, fluoroquinolones are defined as most valuable agents in case of intolerability or resistance of 1st line anti-TB agents (17). Moxifloxacin is the scope of this thesis.

Table 1. Medicines recommended for the treatment of multi-drug resistant/rifampicin-resistant TB.

			Medicine
Class A	**Fluoroquinolones**		Levofloxacin
			Moxifloxacin
			Gatifloxacin
Class B	**Second-line injectable agents**		Amikacin
			Capreomycin
			Kanamycin
			(Streptomycin)
Class C	**Other core second-line agents**		Ethionamide/ Prothionamide
			Cycloserine/ Terizidone
			Linezolid
			Clofazimine
Class D	**Add-on agents**	**D1**	Pyrazinamide
			Ethambutol
			High-dose isoniazid
		D2	Bedaquiline
			Delamanid
		D3	*p*-amino-salicylic acid
			Imipenem-cilastatin
			Meropenem
			Amoxicillin-clavulanate
			(Thioacetazone)

Adapted from the WHO treatment guidelines for drug-resistant tuberculosis. 2016 update (Table 6) via URL http://apps.who.int/iris/bitstream/10665/250125/1/9789241549639-eng.pdf?ua=1.

A TB regimen contains multiple active drugs, i.e. five or six for MDR/RR-TB (17), during a longer period of time, which is not conducive for treatment compliance and a good quality of life. At this moment, phase III trials investigate the value of shorter regimes for drug-susceptible and drug-resistant TB. New (delamanid and bedaquiline) and 'old' anti-TB agents (e.g. moxifloxacin) are part of this investigation (25). As mentioned above, for MDR/RR-TB patients with no history of second line-drug use and/or second-line drug resistance, the WHO recently for the first time justified the use of a shorter, standardized regimen (17). The global focus is on minimizing treatment duration. However, the exposure to individual drugs and the drug susceptibility of the *M. tuberculosis* isolate settled in an individual patient might be as important for treatment success, especially, when factors like the pathophysiology of other diseases or conditions (diabetes, HIV, malnutrition) or interactions with other drugs accounts for variability of exposure to the anti-TB drug, or if the clinical isolate is less susceptible against the drug (7,8, 26-30). Finally, as is an important note for TB as well, there is no established target to estimate efficacy and/or tolerability of treatment for each individual drug. In the end, improvement of the global availability of tests for drug-susceptibility, including important second-line drugs, and simple point-of-care tests for measuring drug exposure in body fluids is of utmost importance to maximize the effect of each individual drug instead of (only) shortening the treatment duration (31,32).

Scope of the thesis

The above-mentioned findings suggest that the spread of rifampicin resistance is the biggest threat of today's TB burden. Although this epidemic cannot be curbed without successful treatment, of the treatment-experienced MDR/RR-TB patients, only 54% was successfully treated (i.e. cured or treatment completed) in 2016 (2). Unfortunately, TB resistant against most 2nd line drugs is increasingly common (12-14).

From 2016 onwards, a short regimen – still 9 to 12 months – is solely reserved for MDR/RR-TB patients that fulfill a large set of criteria including full susceptibility to class A (fluoroquinolone) and class B (injectable) drugs (17). Unfortunately, not many patients qualify for this shortened regimen (32-34). Shortening of treatment duration will reduce pill burden, which is in the interest of both the health care system (costs, patients' compliance) and the patient. However, regardless of the duration of treatment, for each regimen inadequate (inefficient or toxic) dosing of individual drugs will or may have implications for treatment success. Therefore, the main objective of this thesis is to gain insight in the pharmacokinetics (PK), pharmacodynamics (PD) and safety of moxifloxacin, one of the core agents in

MDR/RR-TB treatment. The second objective is to develop strategies and methods for concentration measurement in countries or areas with and without centralized laboratories.

In **chapter 2A,** we review the literature evaluating the value of fluoroquinolones as part of drug-resistant TB treatment by using a PK and PD approach. The anti-mycobacterial or PD effect of fluoroquinolones is assumed to be at maximum when there is a perfect alignment of PK (absorption, distribution, metabolism, elimination) and drug susceptibility of *M. tuberculosis*. Co-prescribed agents can influence both PK and PD (synergistic or antagonistic effect). Integrating PK and PD properties, we aimed to select the most important (novel) fluoroquinolone for drug-resistant TB treatment. Several years thereafter, in **chapter 2B**, we summarized and discussed clinical and/or bacteriological outcome of drug-resistant and (short-course) drug-susceptible TB regimens based on the most valuable 2nd line anti-TB agents, i.e. moxifloxacin, levofloxacin and gatifloxacin.

A few years before the start of the studies described in this thesis, moxifloxacin was introduced in our TB center (Beatrixoord, UMCG), one of the two TB referral centers in the Netherlands, based on promising data of *in vitro* and *in vivo* bactericidal activity (35,36). However, treatment success, or the right dose, seems to be driven by individualized treatment as both drug exposure and drug susceptibility of *M. tuberculosis* may vary (26,27). The goal of chapter 3 and 4 was therefore to gain better insight in the PK, PD and exposure of moxifloxacin. In **chapter 3** we describe the development and validation of a simple and rapid bio-analytical method to measure the moxifloxacin exposure in plasma, plasma ultrafiltrate (i.e. the protein-unbound fraction) and cerebrospinal fluid. In **chapter 4A** we retrospectively review the medical charts of TB patients, receiving 400mg once daily, on drug-drug interaction and safety data, and determined drug exposure and drug susceptibility, in order to estimate efficacy and safety of the standard dosage, used off-label in TB treatment. In the second part of chapter 4 (**4B**), we further retrospectively explore time dependency of drug exposure during TB treatment. In this study we hypothesize that TB patients during the intensive phase of treatment differ clinically from TB patients in the continuation phase of treatment in order to explain the observed PK variability. In **chapter 4C**, in a letter to the editor, we challenge the authors to find out to what extent cumulative drug exposures of high-dose rifampicin, moxifloxacin and isoniazid predict TB meningitis outcome, using an alternative analysis.

The focus of chapter 5 and 6 is development of less-invasive strategies or analytical methods for therapeutic drug monitoring (TDM). In **chapter 5** we aim to develop and validate a limited sampling strategy based on population PK and explore the question whether such strategy

adequately predicts moxifloxacin exposure with limited samples in case it is not possible or not desired to obtain a full blood curve. However, in TB endemic areas, with a low density of laboratories, the required transport conditions might not always be met, and in addition, the analytical method to be used is often too expensive. In **chapter 6** we strive to develop a simple, affordable and non-invasive point-of-care test for TDM of moxifloxacin in oral fluid. In addition, we describe the first statistically robust analytical validation of a semi-quantitative thin-layer-chromatography method.

In **chapter 7**, we discuss the clinical impact of these findings and speculate on the role of moxifloxacin in the treatment of TB. In **chapter 8**, a summary of the findings of this thesis is presented.

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