Chapter 1

Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can affect other sites as well – extra-pulmonary TB. The disease is air born: germs are transmitted by inhalation of droplets containing the bacilli. The germs are expelled into the air by people who are sick with pulmonary TB, by coughing or sneezing [1]. Once people are infected, disease may ensue in some 10% of those infected [2]. Estimates for the risk of TB after infection have varied among studies between 7.3% / 100 life years [3], 2.5% in the first 5 years after infection [4] but also much higher risks (>11.5%) have been reported [5]. The extremes of age – young children and the elderly and also, the immuno-compromised, have a considerably higher risk to develop active TB [3,5].

Mortality rates of TB are high if no specific treatment is given; in studies of the natural history of the disease among sputum smear-positive and HIV-negative cases of pulmonary TB, around 70% died within 10 years; among culture-positive (but smear-negative) cases, 20% died within 10 years [6].

Currently there is no effective vaccine to prevent TB in adults, and although it provides some protection to infants and children [7,8], the TB epidemic has largely been unaffected by the current BCG vaccination programs.

Effective drug treatment was first developed in the 1940s. In 1943, the first drug that was discovered to be effective against TB was streptomycin. Albert Schatz made this discovery while working at the laboratory of Selman Waksman at Rutgers University in New Jersey [9]. Other drugs for TB, like Jörgen Lehmann's discovery of para-aminosalicylic acid (PAS) followed shortly thereafter [10]. The most effective first-line anti-TB drug, rifampicin, belongs to a group of compounds that are the natural product of *Nocardia mediterranei*. Rifampicin became available for clinical use in 1968 – 11 years after the first rifamycin derivative was isolated [11]. The currently recommended treatment for new cases of drug-susceptible TB is a six-month regimen of four first-line drugs: isoniazid, rifampicin, ethambutol and pyrazinamide [1]. Further reduction of treatment duration for drug-susceptible TB was tested in recent years by adding moxifloxacin, a recent generation fluoroquinolone, to standard treatment. The hypothesis was that by adding this potent compound to standard treatment, success rates with a four-month regimen would be non-inferior to standard care. Unfortunately, successful outcome – being 96% in the standard 6-month regimen with first-line drugs – declined to 87%, thereby rejecting the hypothesis that with addition of this potent TB drug, standard treatment duration could be shortened to 4 months only [12]. This disappointing result suggests that for the slowly replicating persister phenotype *M. tuberculosis,* later generation fluoroquinolones may not have an added benefit in combination with rifampicin. Indeed rifampicin remains the most effective drug currently available to eradicate these organisms, thereby providing relatively rapid sterilizing activity [13].

Drug resistant TB (DR-TB) was identified soon after the introduction of TB treatment. The first trial with streptomycin was conducted in 1947. It showed that streptomycin was effective against pulmonary TB, but there was also evidence of some toxicity. Patients in the streptomycin group showed an initial improvement, often however followed by their subsequent deterioration when their bacilli became drug resistant [14,15]. Soon it was notice that combined therapy of streptomycin and PAS and isoniazid prevented the emergence of resistance [16].

Although its causes are genetic and microbial, DR-TB is essentially a man-made phenomenon. From a microbiological perspective, resistance is caused by a genetic mutation that results in a drug being ineffective against the mutant bacilli. From a clinical and programmatic perspective, it is an inadequate or poorly administered treatment regimen that allows a drug-resistant strain to become the dominant strain in a patient infected with TB.

There are two principal pathways leading to the development of DR-TB [1]:

(1) Acquired (secondary) drug resistance:
Acquired drug resistance is the result of inadequate, incomplete or poor treatment quality that allows the selection of mutant resistant strains in the lesions of an individual suffering from TB. If drug-susceptible TB is treated with a regimen exclusively based on a single effective TB drug, there is a high risk that bacteria with drug-resistant mutations will be selected and multiply further during the course of treatment, eventually becoming the dominant strain. If a person infected with a strain, initially resistant to a specific drug is treated with that medicine plus one additional medicine, then there is a risk of developing resistance to that additional medicine. Step-wise additions of single drugs may eventually lead to more severe patterns of drug resistance and eventually to untreatable forms of TB.

(2) Primary drug resistance:
If a patient with drug-resistant TB is the source of infection, the secondarily infected individual acquires primary resistant TB.

Simultaneous natural mutations in the *M. tuberculosis* genome resulting in resistance to more than one TB medicine are exceedingly rare: in wild-type *M. tuberculosis* strains, typically 2.25×10^{10} cell divisions result in rifampin resistance, and 2.5×10^{8} in isoniazid resistance. Still, because of the huge numbers of bacilli present in lesions of TB patients ($10^{10} – 10^{12}$) antimicrobial pressure by only one active drug may quickly result in facilitating growth of drug resistant mutants [17].

1 WHO. Guidelines an emergency update. 2008. Geneva. http://www.who.int/tb/challenges/mdr/programmatic_guidelines_for_mdrtb/en/

With subsequent monotherapy, mono-resistance may ultimately result in multi-drug resistance. Therefore, appropriate treatment with a combination of several quality-assured TB medicines dramatically diminishes the risk of selection of resistant strains. This is the rationale for using a combination of quality-assured medicines when treating TB, while ensuring good adherence.

Definitions of DR-TB [2]

- MDR-TB: Multidrug resistance: resistance to at least both isoniazid and rifampicin (the two most powerful anti-TB drugs).
- XDR-TB: Extensive drug resistance: resistance to any fluoroquinolone and to at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin), in addition to multidrug resistance.

Treatment for MDR-TB needs to be longer than the so-called short course (6 months) that has been shown to be highly effective for drug-susceptible TB; indeed it requires more expensive and more toxic drugs. For most patients with MDR-TB, the duration of treatment regimens recommended by the WHO until 2016 were 20 months, with treatment success rates still being much lower than for drug-susceptible TB. In 2016 the WHO endorsed new guidelines allowing duration of treatment could be reduced to 9-12 months for selected cases [3]. Patients who have been previously treated with 2nd line drugs; those in whom resistance to fluoroquinolones and 2nd line injectable agents has been demonstrated or is considered highly likely should still receive 20 months of treatment. Although treatment for the subset of patients with relatively uncomplicated MDR-TB is shortened by 8-9 months, treatment still lasts long; this is due to the fact that very few 2nd line drugs have the sterilizing effect to replace rifampicin. Clofazimine is one of the few drugs with the potential to eradicate persister organisms [18]. Interestingly, relatively short-course treatment regimens with promising success rates have typically included this compound [19,20].

The global TB problem [4]

In 2012, worldwide over 8 million people developed TB and approximately 1.3 million people died from the consequences of TB in 2013 [21]. The incidence of TB is especially high in resource-limited countries. Due to immigration of migrants from high prevalence countries, TB is not only a low-income country problem, but affects countries worldwide [22].

2 WHO 2013. Definitions and reporting framework for tuberculosis. TB/2013.2). WHO/HTM/.www.who.int/iris/bitstream/10665/79199/1/9789241505345_eng.pdf

3 WHO. WHO treatment guidelines for drug-resistant tuberculosis. 2016 update. http://www.who.int/tb/areas-of-work/drug-resistant-tb/MDRTBguidelines2016.pdf?ua=1

4 WHO. Global tuberculosis report 2014. http://www.who.int/tb/publications/global_report/en/ ISBN 978 92 4 156480 9; http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf?ua=1

Of the estimated 9 million people who developed TB in 2013, more than half (56%) were in the South-East Asia and Western Pacific Regions. A further one quarter were in the African Region, which also had the highest rates of cases and deaths relative to population. India and China alone accounted for 24% and 11% of total cases, respectively. An estimated 1.1 million (13%) of the 9 million people who developed TB in 2013 were HIV-positive. The number of people dying from HIV-associated TB has been falling for almost a decade. The African Region accounts for about four out of every five HIV-co-infected TB patients and TB deaths among people who were HIV-co-infected.

In 2011, an estimated 310,000 of all newly reported TB cases had MDR-TB [1] and currently, 3.5% (95% CI: 2.2–4.7%) of new TB cases and 20.5% (95%CI: 13.6–27.5%) of previously treated cases are estimated to have MDR-TB. This translates into an estimated 480.000 people having developed MDR-TB in 2013. On average, an estimated 9.0% of patients with MDR-TB have XDR-TB. MDR-TB continues to threaten the progress made in TB control. The emergence of XDR-TB has heightened this threat. XDR-TB has been identified in all regions of the world since 2006 [23], and was announced by the World Health Organization (WHO) as a serious emerging threat to global public health, especially in countries with a high prevalence of HIV infection. In many areas such as Africa, the extent of drug resistance is unknown and in most resource-constrained countries the treatment of patients with MDR-TB is absent or inadequate. Indeed, despite efforts made to fight TB and HIV co-infection, TB incidence that had declined in the last decade seems to have plateaued in recent years [24] – and this may be explained by the emergence of TB drug resistance.

TB in the Netherlands

In the Netherlands with currently 17 million inhabitants, TB incidence started to decline long before modern TB drug treatment was introduced, while after the introduction of modern drug treatment, TB incidence has continued to decline. Over the last decade the number of TB patients in the Netherlands declined with 38%. Since 2013, the annual incidence has plateaued at around 850, which is an incidence of 5.1 per 100,000 population (NTR [5]).

In 2014, the Netherlands endorsed the World Health Organization's Global End TB Strategy, which includes the objective to reduce TB incidence with 90 % by 2035. The National Tuberculosis Control Plan 2016-2020 sets out the interventions that are needed to achieve the interim-objectives of reducing TB transmission and case numbers in the Netherlands with 25% over the next 5 years. The main new intervention to reach these targets is to screen new immigrants and asylum-seekers for latent TB infections and providing preventive treatment to those infected [25].

5 NTR: Nederlands Tuberculose Register (Netherlands Tuberculosis Register).

Today, the majority of TB patients in the Netherlands are foreign born (74%). The percentage TB patients tested for HIV increased from 28% in 2008 to 51% in 2013. The percentage of HIV-infected TB patients declined over the last decade to 2.0% in 2013 (3.9% of patients with a known HIV-status). In the last five years the number of patients with MDR-TB in the Netherlands varied between 10 and 20, 1-2% of the total number of TB patients. In 2013, 17 patients with MDR-TB were registered - all were foreign born.

Although these numbers seem trivial compared to the global figures, the open borders and subsequent in-bound travel from all over the world call for vigilance. This is particularly true for MDR- and XDR-TB.

All TB cases in the Netherlands are treated according to national TB guidelines and notified to municipal health authorities with their public health TB teams that treat uncomplicated cases, and initiate contact investigation and screening activities. With declining TB rates, training was maintained while TB departments merged. The NTR was constructed and maintained by KNCV until 2012, since when register is managed at the department of Epidemiology and Surveillance of the National Institute of Public Health and the Environment in the Netherlands. Two dedicated and well-equipped TB Centres – the TB Centre in Beatrixoord, Haren, part of the University Medical Centre Groningen, and the TB Unit in Dekkerswald, part of the Radboud University Medical Centre in Nijmegen - provide care for patients with co-morbid and complicated TB, including all cases with MDR-TB.

Outline of the thesis

In Chapter 2, we explore host-pathogen interactions, the current concepts of local and systemic immune responses to *M tuberculosis* with a focus on potentially modifiable factors (like iron and vitamin D), the BCG and newer TB vaccines; and an update is given on monitoring of disease activity. We will address the importance of differentiating between real disease activity (treatment failure) and a paradoxical response, defined as clinical worsening with increasing inflammation but with decreasing mycobacterial load. The paradoxical response in HIV+ patients is called: Immune Reconstitution Inflammatory Syndrome (IRIS). This differentiation between treatment failure and paradoxical reaction / IRIS is particularly important in HIV-patients with MDR-TB. The crucial question clinicians face is whether to change their (second-line) TB-medication or continue and even consider addition of immunosuppressive drugs, like corticosteroids. Host-pathogen reactions are based on the interplay between the pathogen and its usual host, thereby providing genetic selection pressure with impact on both *M. tuberculosis* and its human host. The chapter finishes by reflecting on possible methods of prevention.

In Chapter 3, we address treatment outcome of MDR-TB in the Netherlands. The first study describes the results of the period from 1985 until 1th September 1998 and the second study describes the results of a 10-year period of 2000-2009. The case mix of MDR-TB patients is discussed with emphasis on physical and psychiatric co-morbidities and language and cultural barriers – and we briefly discuss the financial impact of MDR-TB treatment.

We discuss different factors predicting successful outcome: drug combination; drug sensitivity testing (DST) performed in a central reference laboratory; drug treatment being adjusted to DST and pharmacokinetic (PK) measurements; team commitment; collaboration in a national TB program; and financial input. We hypothesize that all of these factors – with emphasis on PK/PD modeling – predict treatment outcome.

Chapter 4 is dedicated to the aminoglycosides amikacin and kanamycin ('the injectables'). The aminoglycosides amikacin and kanamycin are considered important and effective drugs used in the treatment of MDR-TB. However they are also notorious for their side effects: nephrotoxicity and in particular ototoxicity. Therefore one of the major challenges with the injectables is to diminish their intrinsic toxicity that is drug concentration dependent - while maintaining efficacy.

Using a retrospective survey strategy of patients with culture-confirmed MDR-TB or XDR-TB and who met the inclusion criteria, we evaluate the PK parameters of the aminoglycosides amikacin and kanamycin to detect predictors for PK parameters, efficacy and toxicity. In our study we evaluate PK/PD equations as a proxy for efficacy, as well as toxicity - notably, ototoxicity or nephrotoxicity at a lower dose (median dose of 6.5 mg/kg) than recommended in the World Health Organization (WHO) guidelines (15 mg/kg/day, with a maximum of 1000 mg daily).

To balance between efficacy and toxicity, we explore a population pharmacokinetic model to help optimize drug exposure. We hypothesize that with individualized treatment, using PK/PD modeling, toxicity can be reduced while maintaining efficacy.

In Chapter 5 we focus on ertapenem, one of the carbapenems, labeled for other bacterial infections. Ertapenem is a drug that was not listed in the group 5 WHO drugs with unknown efficacy and has also not been included in the updated 2016 recommendations. We explore the potential use of this compound in the treatment of MDR-TB (including XDR-TB).

Chapter 6 is a summary of the findings of previous chapters that ends with future perspectives and conclusions.

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