Multi-Drug Tuberculosis in the Netherlands

Personalised treatment and outcome

Richard van Altena

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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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Chapter 2

Immunology

a | Immunology in Tuberculosis: Challenges in Monitoring of Disease Activity and Identifying Correlates of Protection

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ABSTRACT: Humans have always lived with tubercle bacilli. Host susceptibility – both inherited and acquired – determines whether an individual infected with *Mycobacterium tuberculosis* will eventually fall ill and develop tuberculosis (TB). After infection with *M. tuberculosis*, a latent TB infection may ensue reflected by immune recognition of specific antigenic epitopes expressed by *M. tuberculosis* – the Region of Difference 1 proteins ESAT-6 and CFP-10 leading to interferon gamma release in vitro. Multi-Drug-Resistant TB has emerged as an enormous infectious threat in certain regions in the world, and the Acquired immunodeficiency by co-infection with HIV has accelerated the TB epidemic even further. A paradoxical response – or Immune Response Inflammatory Syndrome in the context of treatment of HIV co-infection - is an increased inflammatory reaction during effective reduction in the bacterial load. This should be differentiated from treatment failure. A huge challenge is to develop novel markers that can differentiate paradoxical responses from treatment failure.
We discuss the role of protection of vaccines – especially BCG, iron metabolism and the role of vitamin D.

KEYWORDS: *Mycobacterium tuberculosis*, immune response, interferon gamma, BCG, vitamin D, iron, immune response inflammatory syndrome.

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INTRODUCTION

Humans have struggled with tuberculosis (TB) for thousands of years. *Mycobacterium tuberculosis*–specific gene insertions (including insertion segment 6110) have been retrieved from bone lesions in mummies in Egypt dating back as far as 2000 BC [1]. Several studies around the world support the concept that lineages of *M. tuberculosis* closest to its ancestral origin are *M. africanum* (I, II) hail from the African continent [2]. Within the *M. tuberculosis* complex, several other pathogens may affect humans. *M. bovis* should be considered a zoonosis. This micro-organism shares a common ancestor with *M. tuberculosis* and is an important pathogen in several different other mammals than man [3].

In this paper we briefly review host-pathogen interactions, give a summary of the current concepts of local and systemic immune response to *M. tuberculosis*, and focus on potentially modifiable factors (i.e., the micro-nutrients iron, and vitamin D); we discuss BCG and newer TB vaccines currently in development; and provide an update on monitoring of disease activity. Clinicians are increasingly challenged by patients who do not respond favourably to antituberculosis-treatment. Failure to respond to treatment may be clinically indistinguishable from a paradoxical response. We define the term 'paradoxical response' here as clinical worsening with increasing inflammation, but with decreasing mycobacterial load suggesting a beneficial response at the bacteriological level but paradoxically, a clinical deterioration. In the context of immune recovery as a result of treatment for HIV co-infection as well as TB treatment, the pro-inflammatory response is referred to as Immune Response Inflammatory Syndrome or IRIS. We discuss current assays and tools to differentiate paradoxical response from treatment failure, and discuss inherited as well as environmental and nutritional factors that confound this response.

TUBERCULOSIS: HOST-PATHOGEN INTERACTIONS

The concept that in the *M. tuberculosis* complex, *M. bovis* should be considered a subset most closely related to the ancestor of modern *M. tuberculosis* strains has been abandoned [4, 3]. Although *M. bovis* infection is indeed a zoonosis, TB is not. Humans are indeed the almost exclusive reservoir of *M. tuberculosis*. The interplay between the pathogen and its usual host has provided genetic selection pressure that has both impacted on *M. tuberculosis* and on its human host [5]. Selection pressure by the human host appeared to reduce the variability of genes of *M. tuberculosis* that are essential for survival and duplication but also the epitopic repertoire of antigens that are recognized by human T-cells [6]. An important feature of *M. tuberculosis* is its capacity - under environmental pressure such as low oxygen pressure and immune surveillance by the human host – to go into a dormant phase by expression of special genes [7, 8]. This unique feature allows *M. tuberculosis* to latently infect one third of the world's human population. Only in the last 60 years, the battle with *M. tuberculosis* could change in favor of the human host, with the

introduction of effective antimycobacterial treatment [9]. Not immunity alone, but highly effective drugs could now fight TB [10]. This was of course a historical revolution, and when Selman Waksman was honored with the Nobel Prize in Physiology and Medicine in 1952 for his discovery of streptomycin, the first drug shown to be effective against TB, he believed that TB control could be expected. In his Banquet Speech he said: 'The Great White Plague, which only 10 years ago was thought to be immune to drug therapy, is gradually being eliminated. Even persons afflicted with those forms of tuberculosis, such as meningitis and miliary, which were nearly always fatal, now have a better than even chance of recovery. Streptomycin pointed a way. Later supplemented with PAS and more recently with isoniazid, it has brought the control of this disease within sight'. With the introduction of rifampicin and pyrazinamide, two decades later, short course therapy had become available [11] and the world's attention for TB waned. These successes were however followed by the emergence of HIV/AIDS that weakened the immune defense of individuals latently infected with *M. tuberculosis* (LTBI) [12, 13, 14, 15, 16]; and the development of multi-drug resistant TB (MDR-TB) and extensively-drug resistant TB (XDR-TB) [17]. TB has remained and even re-emerged as a major deadly disease with an evolving burden of drug resistance. In 2008 alone, 9.3 million new cases world-wide, and 1.3 million people killed 2 and among these, 440,000 individuals with MDR-TB [18]. The concept that drug resistant strains would represent strains that are less transmissible or less 'fit' or virulent has largely been abandoned [19]. Indeed, MDR-TB and XDR-TB (Table 1) appear to be both transmissible and virulent [14]. Although previous TB treatment has been identified as an independent risk factor for MDR-TB, most patients presenting with MDR-TB have no treatment history and appear newly infected [20]. One of the clade 1 strains, the Beijing strain [21], has been shown to be highly transmissible, replacing other lineages and strains in certain parts of the world. The Beijing strain has also appeared more virulent than comparator strains, and it is associated with MDR-TB [22]. One important aspect is that in areas in the world where the epidemics of TB and HIV/AIDS overlap, such as in certain townships in the Cape Town area in South Africa, TB has emerged unprecedentedly [23]. For instance, prevalence data reported from the Kayelisha Township, an area with a high HIV/AIDS burden, were as high as 1,000 bacteriologically confirmed TB cases per 100,000 population [24] which is currently around the highest prevalence figures reported around the world.

TABLE 1. Antituberculosis drugs - definitions of MDR- and XDR-TB

First-line oral agents	Injectables	Fluoroquinolones	Other second line agents	Agents with unclear efficacy
Group 1	Group 2	Group 3	Group 4	Group 5
Isoniazid (H)	Kanamycin (Km)	Moxifloxacin (Mfx)	Etionamide (Eto)	Clofazimin (Cfz)
Rifampicin (R)	Amikacin (Am)	Levofloxacin (Lfx)	Protionamide (Pto)	Linezolid (Lzd)
Pyrazinamide (Z)	Capreomycin (Cm)	Ofloxacin (Ofx)	Cycloserin (Cs)	Amoxiclav, Imipenem
Ethambutol (E)			PAS	Clarithromycin (Clr)

NB: The use of the fluoroquinolone ciprofloxacin is no longer encouraged

Multi-Drug Resistant TB (MDR-TB) denotes resistance to the two most powerful TB drugs currently in use: isoniazid (H) and rifampicin (R). Extensively Drug Resistant TB (XDR-TB) denotes MDR-TB plus resistance to one group 2, and one group 3-drug. With the advent of MDR-TB and XDR-TB, and especially in HIV co-infected patients, clinicians need novel tools to discriminate increase in mycobacterial load with failing treatment from ongoing or increasing inflammation despite effective bacterial killing, a condition referred to as paradoxical response [25, 26, 27, 28, 29]. Interestingly, certain anti-tuberculosis drugs notably PAS have an immuno-modulatory effect, apart from their anti-mycobacterial action [30].

As explained earlier, the context of immune reconstitution in patients with HIV co-infection, treated with combined Anti-Retroviral Therapy (cART), the phenomenon of paradoxical increase in inflammation is referred to as IRIS [31, 32]. Here we will refer to IRIS also in case of 'unmasking' TB co-infection in HIV patients started on cART [33, 34]. TB accounts for 20% of the reported IRIS cases and is common in resource limited settings [35].

LOCAL AND SYSTEMIC IMMUNE RESPONSES TO *M. TUBERCULOSIS*

Most *M. tuberculosis* infections do not result in clinical TB. *M. tuberculosis* bacilli persist in mononuclear phagocytes and Dendritic Cells (DCs) where they may survive [7]. The macrophage is the primary host cell for mycobacterial infection and is also the source for the cytokines. These cytokines play an important role in recruiting other immune cells for the formation of the granuloma. The *M. tuberculosis* bacilli, during their residence in the phagosome secrete proteins, which after degradation are presented by MHC class II molecules to CD4+ T cells. The MHC class I molecules present to the CD8+ T cells. CD4+ T cells are polarized into Th1 and Th17 cells, which perform effector functions. CD8+ T cells contribute to protection by cytolytic mechanisms and IFN-γ production. Upon activation,

macrophages also express the cytokines IL-10 and IL-12. These molecules counteract each other as IL-12 helps in the expression of IFN-γ from lymphocytes and IL-10 down regulates macrophage activation. The MHC class I-restricted CD8+ T cells also contribute to protective immunity against TB. These CD8+ T cells secrete perforin and granulysin, which lyse host cells and attack *M. tuberculosis* directly. T regulatory cells (Treg) are CD4+ T cells involved in regulation of self-tolerance, autoimmunity and suppression of immune responses during infections [36]. These Treg cells secrete regulatory cytokines, notably interleukin-10 (IL-10) and Transforming Growth Factor-β (TGF- β), which suppress Th1 immune responses [37] (see Fig. 1).

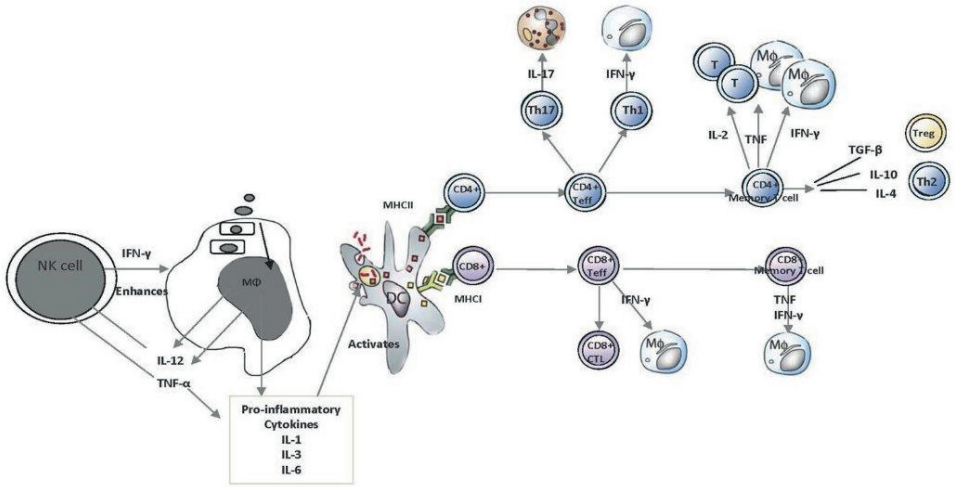

FIG. (1). Cross-talk between cytokines.

Post infection of tuberculosis in the lung and the macrophages and DC's stimulate specific CD4+ and CD8+ T cells. The initial interaction between the TB bacilli and macrophage results in the expression of cytokines the IL-12 and TNF- α which act upon the other cells of the immune system such as the NK cells and DC cells. Infected macrophages respond in an autocrine manner to the cytokines derived from the antigen specific CD4 cells. CD4+ cells are differentiated into Th1 and Th17 cells to perform effector functions. Memory T cells develop, with co-expression of multiple cytokines, notably IL-2. TNF, and IFN- γ and co-expression of cytolytic molecules (38). Treg cells produce transforming growth factor-beta (TGF- β) and IL-10, which tries to suppress the function of Th1 cells (36, 37).

Abbreviations: NK - natural killer cells. Teff - effector T cells; Th- T helper cell; Mφ - macrophage; CTL - cytolytic T lymphocyte; TNF - Tumor necrosis factor; IFN- γ - interferon-gamma; TGF-β - transforming growth factor - beta; IL - interleukin; MHC - major histocompatibility complex; PNG - polymorphonuclear granulocyte.

When *M. tuberculosis* bacilli are engulfed by macrophages, intra-cellular killing may result if the vacuole fuses with the lysosome to allow acidification to occur [38]. Although certain mutants of *M. tuberculosis* are unable to block fusion due to altered expression in acyltrehalose glycolipids [39], most wild-type *M. tuberculosis* are able to arrest this process resulting in persistence of *M. tuberculosis* in macrophages and latent infection. Protective cellular immune responses are elicited by antigen processing to Thelper 1 cells (Th1) orchestrating the immune response by inflammatory mediators, cytokines and chemokines. Interferon-gamma (IFN-γ) is the dominant cytokine reflecting Th1 protective immune response. Generally, Th1-type pro-inflammatory mediators are in turn set off by counteracting regulatory T-cells producing IL-10 and TGF-β [37]. A localized infectious focus may result that is contained in a granulomatous inflammatory response. A granuloma is a complex host response pattern with macrophages providing a secured niche with a necrotic centre in which live but dormant *M. tuberculosis* may persist. Tumor Necrosis Factor-alpha (TNF-α) is an essential cytokine for granuloma formation and maintenance, and interference with TNF-α (fusion, receptor blockade) jeopardizes the host granulomatous immune response. Interleukin 12 (IL-12) is an important cytokine in the Th1 response too. The granulomas reflecting contained TB infection are known as the Ghon focus, usually situated in the lung [31, 40]. Recently a CD4 cell associated, non IFN-γ driven pathway has been shown to be important too [41].

Latent Tuberculosis Infection (LTBI) reflects the situation in which the infection is contained and controlled without elimination of live *M. tuberculosis*. LTBI may either persist indefinitely [42], or change to complete eradication of live *M. tuberculosis*; or fail, with progression to overt TB. Clinical data suggest that during LTBI hardly any replication seems to occur, as evidenced by the fact that strains within one cluster originally isolated and subsequently emerging after LTBI appear genetically identical [43]. Recent evidence from whole *M. tuberculosis* genome sequence comparisons in a macaque model however has emerged that in fact replication continues during latency at a rate at least as fast as during fast replication [44]. The authors have explained this by assuming DNA change as a result of oxidative stress and they produce some evidence for this hypothesis in their paper. Most of the knowledge on the local immune response with granuloma formation has been derived from murine animal models [45]. Few studies have used non-human primate animal models in exploring the complex process of granuloma formation in TB [46]. The concept that granuloma formation merely provides protection has been challenged [47, 48].

Although most of the evidence was derived from a zebrafish model of *M. marinum* infection, the authors found that the presence of the critical virulence region ESX1 - that contains the Region of Difference-1 (RD-1) genes, are essential to induce host macrophage cell death by the apoptotic pathway. The tuberculous granulomas have long been

considered host-protective structures formed to contain infection. However, work in zebrafish infected with *M. marinum* suggests that granulomas contribute to early bacterial growth [47, 48]. Early Secretory Antigenic Target – 6 (ESAT-6) appears to induce matrix metalloproteinase-9 (MMP-9) in epithelial cells neighboring infected macrophages. MMP-9 enhanced recruitment of macrophages, which contributed to nascent granuloma maturation and bacterial growth. Disruption of MMP-9 function attenuated granuloma formation and bacterial growth [49]. IFN-γ activated macrophages are able to kill *M. tuberculosis* by inducing NO-dependent apoptosis [50]. Apoptosis allows the host to fight *M. tuberculosis* effectively by containing the micro-organism [51]. On the other hand, apoptotic host cells contain viable *M. tuberculosis* bacilli, that are able to escape host IFN-γ immune defense by reduced expression of Ag85B [52]. When these micro-organisms in turn are engulfed by newly attracted host macrophages, this process might allow *M. tuberculosis* to replicate and survive in the host. Although this may occur in human disease, and may explain some of the epidemiology and the intricate host-pathogen interaction, failed granuloma formation as is seen in immuno-compromised hosts is a risk factor for multiplication of the organisms and the subsequent spread of disease as well. Apart from apoptosis, *M. tuberculosis* also significantly induces host cell death by necrosis, by causing plasma membrane micro-disruptions. Resealing of these lesions, a process crucial for preventing necrosis and promoting apoptosis, requires translocation of vesicles derived from the lysosomal and Golgi apparatus to the plasma membrane. Plasma membrane repair depends on prostaglandin E2, which regulates synaptotagmin 7, the calcium sensor involved in the lysosome- mediated repair mechanism. By inducing production of lipoxin A4, which blocks prostaglandin E2 biosynthesis, virulent *M. tuberculosis* prevents membrane repair and induces necrosis [53, 54]. Apart from MMP-9, MMP-1 is also an important inflammatory mediator that is upregulated during *M. tuberculosis* infection. As mentioned earlier, certain drugs – PAS being the most clear example – may have immune-modulatory effects. PAS being chemically closely related to acetosal, appears to modulate the immune response in *M. tuberculosis* – infected isolated human macrophages. MMP-1 (and not MMP-7) appears to be stimulated through the p38 mitogen activated protein kinase (MAPK) signal transduction pathway, and the signaling by p38 MAPK is inhibited by PAS [30]. Necrosis resulting in the release of many live *M. tuberculosis* bacilli escaping the host immune surveillance, with unlimited spread and multiplication presents the greater risk for the host. In summary, a granuloma in the context of LTBI is no longer considered a passive quiescent lesion with low cell turnover and low metabolic activity, but rather a dynamic, immunologically active battlefield of immune cells and replicating bacilli. An ever expanding array of newly detected pro-inflammatory cytokines and chemokines appears involved in this struggle between the host and the pathogen, in a delicate balance with apoptotic cell signalling and necrosis.

IMPORTANCE OF RD-1 GENES

Over the last decade, the RD-1 genes have been shown to be highly specific for *M. tuberculosis* [55, 56]. The RD-1 genes coding for TB-specific proteins - ESAT-6 and Culture Filtrate Protein-10 (CFP-10) are not present in *M. bovis* BCG, nor in most of the environmental mycobacteria. The only non-tuberculosis mycobacteria currently known to contain RD-1 genes are *M. szulgai*, *M. marinum* and *M. kansasii*, making tests based on detection of RD-1 proteins potentially useful in clinical practice. Three commercial tests have been developed for use in detection of LTBI – with one also marketed for active TB. The tests are known as IFN-γ Release Assays (IGRA) and either measure the number of IFN-γ positive cells (TSPOT-TB; Oxford Immunotec, UK), or the concentration of IFN-γ after stimulation with Mtb-specific antigens (Quantiferon TB Gold; and Quantiferon TB Gold-in-Tube; both manufactured by Cellestis, Carnegie, Australia). IGRA provide a sensitivity approaching 90% for TB diagnosis using culture of *M. tuberculosis* as the reference test [31, 57, 58, 59, 60]. The problem with IGRA is that these tests do not differentiate between TB and LTBI; and their use as a marker of success or failure in TB has yielded controversial and generally disappointing results (reviewed in [57]); [61, 62]. Genetic expression patterns derived from whole genome analysis studies in two different cohorts of TB patients appear to reflect a highly specific pattern for active TB, differentiating it from LTBI, and healed TB, after completion of treatment [63]. The sophistication of this study is high, and although the study provides much detailed information, a general pattern that emerges from this study is that it largely confirms the importance of upregulation of many genes in the IFN-γ post-signal transduction pathways (JAK-2, STAT-1).

Besides, also IFN-γ/δ pathways appear upregulated, including their post-signalling downstream pathways (STAT-2). This powerful approach might lead to detecting novel useful markers for disease activity and treatment response in the future.

ACQUIRED IMMUNOLOGICAL SUSCEPTIBILITY – ROLE OF HIV

The interplay of *M. tuberculosis* and HIV has been reviewed in detail [16, 15, 9, 14, 31]. Here, we summarize the most important research findings. HIV replication is enhanced at sites with active TB. Reactivation of TB in granulomas where viable dormant *M. tuberculosis* bacilli persist, can at least partly be explained by failure of CD4 cells to secrete IFN-γ and TNFβ. TNFα is essential for formation and maintenance of granuloma formation, and lack of TNFα results in decay and resolution of granulomas. Weakened macrophage killing of intracellular *M. tuberculosis* bacilli and impaired specific anti- *M. tuberculosis* CD4 cell function reflected by impaired secretion of IFN-γ, TNFα and IL2 to *M. tuberculosis* specific stimuli all contribute to the devastating effects of HIV coinfection in TB patients. During TB treatment, paradoxically increased inflammatory responses may mimic failure to respond to treatment and this paradoxical reaction is even worse following cART in which

case the immune phenomenon is referred to as IRIS. Until recently the treatment of HIV infection was postponed at least until the initial intensive phase of TB treatment had been completed, this policy has recently been challenged. In a retrospective analysis, patients in whom cART was postponed until at least two months after start of TB treatment had a significantly decreased chance of survival compared to individuals who were started on cART earlier on [64]. Recently a randomized controlled trial was reported that confirmed that early start of cART in HIV co-infected TB patients is beneficial [65], and also further evidence has emerged in favour of starting cART early on during treatment of TB in HIV-coinfected TB patients [66].

IMMUNE GENETIC SUSCEPTIBILITY

Several genetic studies in different founder populations have shown that immunological defense against development of TB is genetically driven [67, 68]. Many different genetic polymorphisms are associated with development of disease or LTBI, but different genes emerged from different genetic founder populations [69, 70, 71, 72, 73, 74]. Genetic polymorphisms in the SLC11A gene that is believed to regulate macrophage bactericidal function by metal (iron) transport have been shown to be associated with susceptibility to TB [75]. Not all genetic polymorphisms that have been shown to be associated with TB have a clear biological explanation yet. In a recent genome-wide association analysis from Ghana and The Gambia, 11,425 individuals were studied. In this combined study, rs4331426, located in a gene-poor region on chromosome 18q11.2, was associated with disease (combined P = 6.8 x 10-9, odds ratio =1.19, 95% CI = 1.13–1.27) [76].

VACCINATION; THE ROLE OF BCG

BCG has not been shown to provide protection against the development of pulmonary TB [77]. In the 1970s, the Indian government ordered a trial to identify the possible protective role of BCG. This largest study ever was conducted in Chingleput, India, with nearly 270,000 participants receiving either a locally manufactured ('Madras strain') BCG, a French product of BCG, or placebo vaccination, with a follow-up of 15 years. After 7.5 years of follow-up, the actively vaccinated groups had similar rates of pulmonary TB proven by sputum smear microscopy and culture [78, 79]. International WHO teams confirmed that the study was meticulously conducted. At 15 years follow-up, BCG still failed to show any protection against bacillary-proven TB [80].

A large BCG study conducted later in Malawi likewise failed to show any protection against TB [81]. A post-hoc analysis of the Chingleput trial revealed a low level of overall protection against TB meningitis and other lethal forms of TB in infants and young children (27%, 95% CI - 8 to 50%). In a more recent meta-analysis the protective effect of BCG for infants and young children to develop severe forms of TB is estimated to be at around

50% [82]. In the analysis of an outbreak of TB among infants and children in a nursery in London resulting from a nursery teacher who had had symptomatic pulmonary TB for several months, BCG-vaccinated children appeared protected against LTBI by 66% using IGRA [83] but in this small-sized study the confidence intervals overlaps the much smaller effects reported in earlier studies [84].

The WHO recommends BCG vaccination in the newborn [85] but in newborns and infants with HIV co-infection BCG should not be given [86] as there is considerable risk for severe and potentially lethal disseminated BCG infection [87]. In a recent study from Tanzania, an international research team found evidence that TB patients with previous BCG vaccination as evidenced by a scar had better sputum conversion rates at two months after start of treatment than those without previous BCG vaccination [88]. One problem in the interpretation of this finding is that scar formation following BCG intra-dermal vaccination is highly variable, and does not necessarily correlate with immune protection against TB [84].

BCG vaccination in infants results in complex patterns of cytokine responses, with clearly IFN-γ being the dominant response [89]. Although BCG induces a large array of cellular immune responses in humans and experimental animal models, good correlates of protection have not been identified [84, 90, 91].

Obviously there is a dire need for new, better vaccines, and some 12 novel components are now considered for clinical trials. Most of these moieties aim at boosting a primary vaccination with BCG [91, 92, 93]. An important challenge is to develop correlates of protection so that the response to novel vaccines can effectively be monitored. Potential candidates to be explored as possible surrogate markers for beneficial response should best be identified during follow-up of individuals enrolled in prophylactic vaccine trials, but surrogate parameters might also be identified in patient cohorts during treatment.

THE ROLE OF IRON

Iron is known to play an important role in host-pathogen interactions. The withholding of intracellular iron has been a host defense strategy against intracellular pathogens such as mycobacteria [94]. Iron is essential as a cofactor of enzymes involved in vital cellular functions ranging from respiration to DNA replication. It is not freely available to the intracellular organisms for in the presence of oxygen and at physiological pH, it exists mainly in insoluble ferric complexes. In higher organisms iron is available in bound forms such as transferrin, lactoferrin and ferritin, thereby limiting the levels of free iron required for bacterial survival [95, 96]. The concentration and the availability of iron are important in determining the outcome of the infection with pathogenic mycobacteria [97, 98]. Supplementing iron to patients suffering from various infections may enhance infections by viruses, other bacteria and various parasitic organisms [99]. Excess iron

(Fe), from dietary iron, causes individuals to be more susceptible to TB. Iron overload of both macrophages and hepatocytes is common in sub-Saharan African populations. A plausible reason is drinking of a traditional beverage of high iron content that is brewed in non-galvanized steel containers. Increased iron supply will result in *M. tuberculosis* growth, and iron overload is a known risk factor for infections, as this may worsen the course of disease [100]. Mild iron deficiency causes a significant impairment in the immune status and reduces the capacity to control infections [101]. More severe iron deficiency due to chronic peptic ulceration and blood loss may on the other hand protect against TB. The high prevalence of Helicobacter pylori in populations where TB and other lethal infections remain endemic suggests the host-pathogen interplay of these infections has coevolved. *H. pylori* infection appears to be associated with protection against TB [102].

Mycobacteria acquire iron by secreting high-affinity iron chelators called siderophores that sequester ferric iron. The ferric–siderophore complexes are transported into the bacteria and iron is released in the cytoplasm, probably by reduction. Mycobacteria produce two classes of siderophores, the intra-cellular mycobactins and the extra-cellular exochelins or the carboxymycobactins (also called exomycobactins). Mycobactin is the major intracellular hydrophobic siderophore of most mycobacteria. By virtue of its hydrophobic nature mycobactin acts as a repository for holding iron within the cell envelope before its release into and through the cytoplasmic membrane [103]. A ten-gene cluster designated mbt A-J, contains the core components necessary for mycobactin biogenesis. Mutant *M. tuberculosis* lacking a gene from mycobactin biosynthesis has decreased ability to grow in human macrophages thereby establishing that iron acquisition from the host iron sources is an essential pre-requisite for mycobacteria to be pathogenic [104]. Enzymes involved in mycobactin biosynthesis are important targets for the design of specific inhibitors for TB treatment [105]. One of the oldest anti-TB drugs is p-aminosalicylic acid (PAS) that is believed to exert its effect by the inhibition of mycobactin biosynthesis [94, 105]. *M. tuberculosis* is physically deprived of iron when it is within macrophages [106]. Unless the bacterium is able to acquire iron by synthesizing mycobactin and carboxymycobactin, its virulence is greatly diminished. Ferric-siderophore complexes are recognized by specific surface receptors and translocated through the plasma membrane by ABC-type transporters that are energy dependent [107]. Inactivation of *M. tuberculosis* irtA (Rv1348) or irtB (Rv1349) genes, which encode membrane proteins of the ABC transporter family, results in decreased ability of *M. tuberculosis* to replicate in low-iron medium and to utilize ferric-exomycobactin as the sole iron source [108]. Probably, IrtA-NTD functions as a flavin/ferric reductase that reduces iron in the imported Fe3+-exomycobactin complex for its assimilation [109]. Iron regulates not only the iron acquisition machinery but also the expression of virulence factors / toxins in several other bacterial systems [95, 110].

M. tuberculosis scavenges iron from the host-cell through the transferrin - iron

acquisition pathway. IFN-γ, important for the protection against TB, also influences cellular iron status. IFN-γ activation of human monocytes down-regulates transferrin receptor numbers on the cell surface and the rate of macrophage iron acquisition from holotransferrin thereby decreasing iron availability to intracellular microorganisms that utilize transferrin iron [98].

The siderophore-dependent iron acquisition pathways in *M. tuberculosis* were well established as discussed above. Recent studies [111, 112] demonstrate a newly characterized pathway, whereby *M. tuberculosis* can use free heme and heme from hemoglobin as an iron source. The genomic region, Rv0202c-Rv0207c was identified to be responsible for the passage of heme iron across the mycobacterial membrane. The discovery of a unique mycobacterial heme acquisition pathway could open new avenues for drug targets.

In summary, the role of iron in TB is complex, with nutritional factors as well as genetic polymorphisms in iron-handling transport systems (e.g. SLC11A1, formerly called: NRAMP-1) playing a complex role [100, 97].

THE ROLE OF VITAMIN D

Vitamin D (vit D) is known to play an important role in host immune defense against *M. tuberculosis*, and vit D deficiency is closely associated with active TB [113]. 1-α 25(OH)2 vit D, the active form, targets various immune cells modulating both innate and adaptive immune responses. The enzyme 25-OH- vit D 1-α-hydroxylase converts the 25-OH- vit D to the active 1-α 25(OH)2vit D. IFN-γ potentiates this effect by up-regulating 1α – hydroxylase [114, 115]. Several mechanisms of anti-mycobacterial activity for 1-α 25 (OH)2 vit D have been described. Exogenous 1-α 25 (OH)2 vit D induces a superoxide burst and enhances phagolysosome fusion in *M. tuberculosis* -infected macrophages [116]. TLR activation of human macrophages, by mycobacterial antigens, results in the up-regulation of expression of vit D receptor (VDR) and the vit D -1 α -hydroxylase genes. This leads to induction of the antimicrobial peptide cathelicidin and killing of *M. tuberculosis* [117]. The cathelicidin antimicrobial peptide is cleaved to LL-37, which restricts the growth of *M. tuberculosis*. Interestingly, several genes classically regarded as being essential for protection against TB are actually down-regulated by 1-α 25 (OH)2 vit D, including IL-12, tumor necrosis factor, and IFN-γ [118]. This is indicative that vit D therapy could both switch on beneficial microbicidal mechanisms, and at the same time decrease the expression of inflammatory mediators that may be contributing to pathology [119, 120].

Serum concentrations of vit D in TB patients are generally but not invariably lower than in healthy controls. In a genetically homogeneous immigrant population of Gujarati Asians in the London area with high rates of TB there was an association between TB and vit D deficiency [121]. In a double-blinded randomized controlled trial, a single dose of 100,000 U (2.5 mg) of vit D3 enhanced anti-mycobacterial immunity in healthy tuberculin

skin test-positive donors [122]. VDR polymorphisms determine the activity of the receptor and might represent potential markers of host susceptibility to TB. The association between vit D physiology and infectious disease is also supported by genetic studies implicating polymorphisms in the gene encoding the VDR in disease susceptibility [123]. There are numerous VDR polymorphisms, including a common Fok1 restriction fragment length polymorphism (RFLP) that shifts translational initiation to an ATG three codons downstream. The Taq1 and Bsm1 RFLPs are present in the 3' untranslated region. Genetic studies have linked VDR polymorphisms with susceptibility to *M. tuberculosis* infection and treatment outcome. In the past few years, more studies addressing the impact of VDR polymorphisms on TB susceptibility were conducted in different populations. In a case control study of 2,015 African subjects, homozygotes for Taq1 polymorphism (genotype tt) were significantly underrepresented in TB patients [124]. In the study on Gujarati Asians [121], the ff genotype of the Fok1 RFLP was associated with the extent of pulmonary TB in vit D deficient patients. The same study suggested a synergistic association between the T allele and vit D deficiency. The results show that 23% of healthy contacts were of the genotype non-tt and vit D deficient as compared with 46% of TB patients and increasing to 52% of individuals with severe disease. Sunlight exposure modulates skin production of vit D, and studies with vit D supplementation conducted in Africa may therefore not necessarily apply to pigmented individuals with TB in moderate climate zones with less sunlight exposure [125, 126]. In moderate climates, 25-OH-D levels among TB patients are typically low [127] and supplementation of vit D in TB patients may be advantageous; in a small subset of subjects with tt Taql VDR genotype it was associated with enhanced sputum culture conversion rates [128]. Interestingly, vit D may be enhanced by BCG vaccination as has been suggested in a study in infants with and without previous BCG vaccination [129].

In summary, elucidation of the mechanisms by which antimicrobial peptides restrict the growth of *M. tuberculosis* could lead to novel pharmacologic approaches. The interaction between vit D, VDR, and effector molecules such as IFN-γ is complex and further studies are warranted to understand metabolic, nutritional and immunological factors in the pathogenesis of TB.

MONITORING DURING TB TREATMENT

Diagnostic and disease monitoring assays including the detection of drug-resistant strains of *M. tuberculosis* have been reviewed recently [57, 130]. The adherence to treatment is supported by directly observed therapy (DOT) with witnessed drug ingestion [131]. The response to treatment is monitored clinically and by cultures, side effects are mainly monitored by liver tests. Liver test abnormalities although relatively uncommon are the most frequent reason for treatment interruption [132, 133, 134, 135]. In the WHO guidelines

[131], the suggestion is made to monitor sputum microscopy after the intensive phase – and with smear-positive sputum, a repeat microscopy after three months should be made, with culture and drug sensitivity testing if sputum smear microscopy is still positive after three months. Drug intolerance and liver toxicity are important but relatively infrequent events during follow-up [31]. MDR-TB is a rapidly emerging epidemic. In 2008, 440,000 new cases were detected with an alarming death toll of 150,000 [18, 9], and treatment outcome and treatment duration in XDR-TB are increasingly challenging the health care system [136, 137, 17]. At the level of individual health care, clinicians are faced with only few tools to monitor treatment.

In pulmonary TB (PTB), serial sputum culture may conceivably help to detect treatment failure. Simply testing for sputum microscopy and even culture at any one time point after start of treatment [138] was neither sensitive nor specific enough to guide therapy, but it also failed as a continuous monitoring tool [139] and the technique is not helpful in extra-pulmonary TB (EPTB) [57]. Sputum microscopy is insensitive to differentiate live bacilli from dead bacilli, and PCR-based techniques are similarly insensitive. Real-time quantitative PCR has the potential to measure the presence of *M. tuberculosis* DNA semi-quantitatively by the number of cycle times required for a positive signal. Electronic nose technology has not yet been developed to a level that makes it a promising tool in the near future [140, 141]. Lipo-arabinomannan (LAM) is a cell wall constituent of mycobacteria. Although not entirely specific for *M. tuberculosis*, serial urinary secretion of LAM might provide a tool for monitoring especially when the antigen load is high, such as in EPTB in HIV-co-infected individuals with low <200 cells/μL) [142]. IGRA has been proposed as a monitoring tool but it has largely failed to live up to expectations in this respect as the test remained positive in individuals responding to therapy [57, 61]. Neopterin is a metabolic product of guanosine-triphosphate and is released by macrophages following stimulation by IFN-γ. It has been considered a marker of cellular immune system activation.

Neopterin has been suggested as a marker for diagnosis of TB [143, 144] either alone or in a panel of markers [145, 144].

FUTURE DIRECTIONS

The number of individuals infected with MDR-TB and XDR-TB is increasing. In the absence of novel powerful drugs [146, 9, 147], immune enhancing therapies like therapeutic vaccines, and modalities of treatment interfering with the iron and vitamin D should be addressed and controlled for in future studies. Nutritional intake as well as ultraviolet-induced skin production of vit D are important to consider. Immune-based monitoring will need to be further developed before it can be introduced in clinical practice [63]. Rapid detection of MDR-TB with molecular tools has been possible since almost two decades [148] but an elegant, rapid, robust, and easy-to-use point-of-care system has

only recently been reported [149, 150]. Differentiating failing treatment caused by MDR-TB from paradoxical responses, notably, IRIS in HIV-co-infected patients is critically important [32, 151] especially as evidence is now emerging that corticosteroids may be beneficial in IRIS [152]. New insights into the pathogenesis of IRIS help identify biomarkers that could be useful in predicting or diagnosing IRIS. Studies on immunopathogenesis of IRIS have shown a significant activation of both innate and adaptive immune responses with elevation of plasma or serum chemokines and cytokines. Markers of inflammation such as C-reactive protein, interferon-inducible protein 10 or IFN-γ may be helpful as predictors of IRIS events. In addition, TB-associated IRIS is associated with a prominent Th1 response that can be heightened even prior to ART initiation in cases of unmasking TB, and may assist in early diagnosis. Future studies are needed to elucidate the diagnostic value of IRIS biomarkers [153, 154].

ABBREVIATIONS

M. tuberculosis	=	*Mycobacterium tuberculosis*
TB	=	Tuberculosis
PTB	=	Pulmonary TB
EPTB	=	Extra- pulmonary TB
LTBI	=	Latent Tuberculosis Infection (immune recognition of - and assumed infection with *M. tuberculosis* without clinical disease)
MDR-TB	=	Multi-drug resistant TB (i.e., drug resistant to at least isoniazid and rifampicin)
XDR-TB	=	MDR-TB, with additional resistance to: one of the injectable agents amikacin, kanamycin, or capreomycin - and to one of the fluorquinolones
IGRA	=	Interferon gamma release assay - reflecting immune recognition of region of difference-1 coded proteins of *M tuberculosis*: ESAT-6 and CFP-10
TNF- α	=	Tumor necrosis factor alpha
TGF- β	=	Transforming Growth-Factor β
IFN- γ	=	Interferon-gamma
IL-10, IL-12	=	Interleukin 10, and 12
HIV	=	Human immunodeficiency virus
IRIS	=	Immune response inflammatory syndrome (denotes paradoxical response following cART in HIV-co-infected TB patients)
cART	=	Combined anti-retroviral therapy (HIV-treatment)
Vit D	=	Vitamin D
VDR	=	Vitamin D receptor
BCG	=	Live-attenuated *M. bovis* Bacille Calmette Guerin.
ESAT-6	=	Early Secretory Antigenic Target-6
CFP-10	=	Culture Filtrate protein-10
RD-1	=	Region of Difference-1 (region in the ESX-1 gene coding for ESAT-6 and CFP-10)
ESX-1	=	Genetic region in *M tuberculosis* genome interrupting fusion of phagolysosome
MMP-1, -7, 9	=	Matrix metalloproteases 1,7 and 9
P38 MAPK	=	P38 mitogen activated protein kinase (several classes have been described)
LAM	=	Lipo-arabinomannan
TGF-β	=	Transforming growth factor - β
DOT	=	Directly Observed Therapy

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Chapter 3

Individualised MDR-TB treatment

a | Multidrug-resistant tuberculosis: long-term treatment outcome in the Netherlands.

Geerligs WA, Van Altena R, De Lange WCM, Van Soolingen D, Van Der Werf TS.
Int J Tuberc Lung Dis. 2000 Aug;4(8):758-64. PubMed PMID: 10949328.

SETTING: Tuberculosis units (Beatrixoord, Haren; and Dekkerswald, Groesbeek) in the Netherlands.
OBJECTIVE: To study the long-term treatment outcome of patients with multidrug-resistant tuberculosis (MD-RTB).
DESIGN: Descriptive analysis of all consecutively admitted patients with MDR-TB between 1 January 1985 and 1 September 1998, with follow-up until 1 August 1999.
RESULTS: Of 44 patients (31 male) enrolled in the study, 33 were foreign born and none were human immunodeficiency virus positive. At diagnosis 38 patients had sputum-smear positive pulmonary TB, and converted culture negative after a mean of 6 weeks, while six converted to negative later (mean 69 weeks). Most patients had micro-organisms resistant to several antimycobacterial drugs (mean _ median: 5), including resistance to isoniazid and rifampin. In-patient treatment lasted a mean of 164 days (range 31–481), and patients were treated with six drugs on average. Side effects were common.
Treatment lasted for a mean of 608 days (range 268– 1626); five patients are still on treatment. Four patients were operated for TB, and two others were operated for post-TB sequelae. During the follow-up period six patients died, of whom three had active TB; 33 (75%) were considered cured.
CONCLUSION: Mortality was only 14% after a mean follow-up period of 53 months. MDR-TB can be successfully treated, but requires much effort from both patients and carers, and the costs may be higher than is affordable in resource-poor countries.

KEY WORDS: multidrug-resistant tuberculosis; tertiary care centre; outcome; survival

MULTIDRUG-RESISTANT tuberculosis (MDR-TB) is defined as a form of tuberculosis (TB) caused by strains of *Mycobacterium tuberculosis* that are resistant to at least isoniazid (INH) and rifampin (RMP). Drug resistance results from mutations in the genetic material of these mycobacteria.[1] One in every 10^6 mycobacteria is naturally resistant to INH because of spontaneous mutations in the katG gene,[2–5] which encodes for the katalase-peroxidase reaction, or the inhA gene.[3,4] One in every 10^8 –10^{10} mycobacterial divisions, mutations of the genes which encode for the RNA polymerase subunit B (rpoB) occur, resulting in RMP resistance.[6,7] The overall incidence of RMP-resistant mutants is estimated at one in 10^7 .

MDR-TB can emerge in several ways.[8–10] The chance of spontaneous mutations occurring, resulting in simultaneous resistance to INH and RMP, is exceedingly small. The chance of developing primary multiple drug resistance can be calculated by multiplying the mutation rates for the individual drugs, and is estimated to occur in one in 10^{13} mycobacteria.

Even in patients with high bacterial loads, i.e., in smear-positive pulmonary tuberculosis with an estimated mycobacterial load of 10^8 –10^9 micro-organisms, the chance of harbouring simultaneous INH and RMP-resistant tubercle bacilli resulting from spontaneous mutation is negligible, and would occur in only one in 10^4 –10^5 patients. Drug resistance is therefore believed to result from inadvertent monotherapy. Once resistance mutation has occurred, these resistant mutants soon replace the drug-sensitive bacterial population, and if monotherapy is in effect given to a patient with a high bacterial load, resistant mutants will again replace the microbial population, resulting in acquired drug resistance. Combination anti-tuberculosis treatment is therefore only effective in overcoming the problem of drug resistance if the mycobacterial population remains sensitive to at least two compounds.[11] Inadequate treatment may result from several sources: inaccurate prescription by a physician, inaccurate delivery of medication by the pharmacy, poor intrinsic quality or poor bioavailability of the drugs delivered by the pharmacy, bowel absorption disorders, and patient non-compliance. In a personal communication by Vegter, quoted by Lambregts-van Weezenbeek,[10] it was noted that 56% of all major errors in TB treatment observed during home visits were due to mistakes made by physicians and pharmacists. Another way of acquiring MDR-TB is by transmission of multiresistant mycobacteria. In the Netherlands, most cases of MDR-TB are imported from other countries, due to fugitive migration and international travel of the local and immigrant populations. In a 1993–1994 cohort study in the Netherlands, the majority of MDR-TB patients appeared to be foreign-born.[12] MDR-TB is notoriously difficult to treat for a number of reasons. Because of resistance to the most powerful bactericidal agent available, INH, sputum conversion is delayed in patients with MDR-TB compared to those infected with normally sensitive tubercle bacilli. Resistance to RMP is attributed to the fact that MDR-TB patients have a greater risk of relapse because this agent has not only bactericidal properties, but also a sterilising effect. As the first-line drugs RMP and INH cannot be used, patients with MD-RTB have to be treated with the so-called second-line agents. These drugs are generally less effective, i.e., their sterilising capacity is limited, and they are therefore not suitable for short-course chemotherapy (SCC). These drugs also have considerably more side-effects. The need to treat patients with MDR-TB over considerably longer periods of time than 6 months, with drug schedules that have a considerably higher toxicity profile, reduces the chance of patients complying with the prescribed treatment. Prolonged time for sputum conversion carries yet another risk—a prolonged period of transmission of the disease. It has been shown that MDR-TB patients

are equally likely to infect persons in their direct environment—as evidenced by a positive tuberculin skin test—as patients with drug-susceptible tuberculosis.[13] Directly observed therapy, short course (DOTS) has proved successful in many TB programmes throughout the world, both in poor-resource countries [14–17] and in affluent societies.[18,19] MDR-TB can be seen as a failure to supervise drug compliance in TB treatment, and therefore DOTS has become a standard approach for combating drug-susceptible TB.[20,21] Supervised treatment of MDR-TB is even more critical as reserve drugs are being used as a last resort.[22,23] In this paper we describe the results of intensive, predominantly in-patient, fully supervised treatment for MDR-TB patients in the Netherlands.

METHODS

Sites

Beatrixoord and Dekkerswald are the two specialized referral centres for tuberculosis in the Netherlands. Because of the complicated treatment that MDR patients require, and the need to prevent nosocomial transmission of MDR-TB, many physicians in the Netherlands refer MDR patients to one of these two centres for treatment.

Data retrieval

Retrospective data were collected of all patients who were admitted and treated for MDR-TB during the period 1 January 1985 to 1 September 1998. Most patients had received treatment prior to admission to Beatrixoord or Dekkerswald, but it was impossible to obtain valid data about this period. Data were collected on demographics, hospitalisation and treatment, the number of drugs used, duration of treatment and serious side-effects, disease localisation, symptoms and results of sputum smears and cultures with drug susceptibility test results. Risk factors for MD-RTB were assessed as follows: a history of TB treatment, recent immigration, close contact with patients with known MDR-TB, and non-compliance with anti-tuberculosis treatment. In vitro sensitivity test results were retrieved from the WHO/IUATLD-recognised National Reference Laboratory for Tuberculosis, National Institute of Public Health and the Environment, Bilthoven, the Netherlands. The modified absolute concentration method with 99% growth inhibition using Middlebrook 7H10 culture medium was used to determine sensitivity (NCCLS document M24-pV, 110:10. Antimycobacterial susceptibility testing). Breakpoints used for in vitro susceptibility are given in Table 1.

TABLE 1 Breakpoints for in vitro sensitivity and resistance

	Upper limit	Lower limit
Agent	for sensitivity	for resistance
Isoniazid	< 0–2 mg/L	>1 mg/L
Rifampin	< 1 mg/L	>1 mg/L
Pyrazinamide	< 50 mg/L	>100 mg/L
Ethambutol	< 5 mg/L	>10 mg/L
Streptomycin/kanamycin/ amikacin	< 5 mg/L	>10 mg/L
Ciprofloxacin/ofloxacin	< 2 mg/L	>2 mg/L
Rifabutin	< 2 mg/L	>2 mg/L
Prothionamide/ethionamide	< 5 mg/L	>5 mg/L
Cycloserine	< 50 mg/L	>50 mg/L
Thioacetazone	< 2 mg/L	>2 mg/L
Clofazimine	< 2 mg/L	>2 mg/L
Clarithromycin	< 16 mg/L	>16 mg/L

Side-effects were considered present if they were serious enough to discontinue medication or adjust the dosage. The time to sputum conversion was defined as the time from admission to the time of collection of the first in a series of two or more consecutive negative culture results, at least 2 weeks apart.[24] Treatment failure was defined as a positive culture after 5 months of adequate therapy;[25] and adequate therapy was defined as a regimen of drugs containing at least two drugs with in vitro susceptibility against the isolate;[24] such therapy was invariably instituted within 2 weeks after admission. Patients were considered compliant with therapy if they reported on a regular basis to the out-patient clinic and if they took their medications regularly according to their physician, family or specialised TB nurses.

Patients were followed until 1 August 1999. When these patients were no longer followed up by our tuberculosis unit out-patient departments, their general practitioners were contacted by telephone about their current state of health. Patients were considered cured if they had no clinical or microbiological signs of TB at the moment of follow-up. The costs of drug treatment, admission fees, and out-patient treatment were estimated based on 1999 prices in US$.

RESULTS

Patient characteristics

During the study period, 44 patients (31 males and 13 females) were treated for MDR-TB in the two centres. Eleven were indigenous to the Netherlands, two came from other European countries, 13 from Africa, nine from the Middle East and eight from the Far East. We were unable to trace the native country of one patient. The mean age at diagnosis was 33 years (range 10–82). Twenty-five patients were proven human immunodeficiency virus (HIV) negative; the remaining 19 were not tested as they did not belong to any of the known risk groups for HIV infection.

Disease characteristics

Most (38) of the patients had pulmonary tuberculosis, four had extra-pulmonary tuberculosis, and the remaining two had both pulmonary and extra-pulmonary disease. Only four were asymptomatic at hospitalisation, while the others had obvious symptoms.

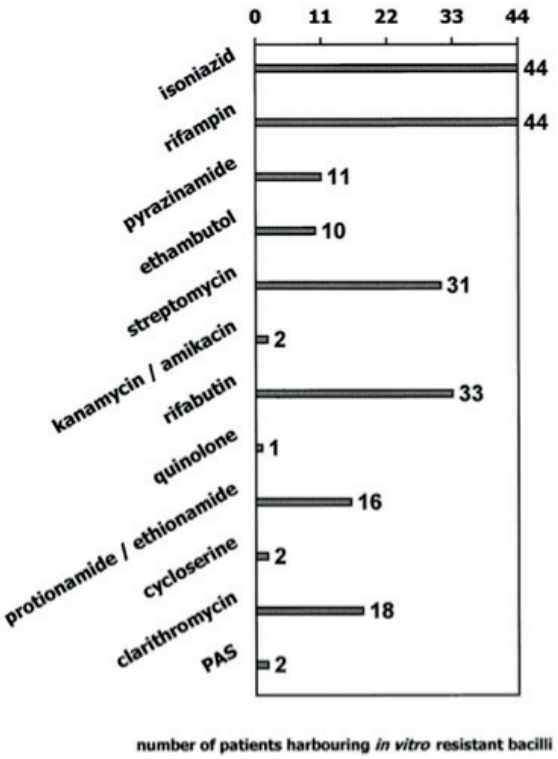

FIGURE 1 In vitro resistant tubercle bacilli recovered from 44 MDR-TB patients.

Twelve patients had fever, 34 had pulmonary complaints and 35 had constitutional symptoms. Most patients had organisms that were resistant to more agents than only INH and RMP, with resistance to a median and mean number of five drugs. Data on in vitro resistance to the separate agents are listed in the Figure.

Twenty-four of the patients had immigrated to the Netherlands less than two years before diagnosis; 34% had a history of previous treatment for TB and 36% had had close contact with a known TB patient.

Treatment and costs

At the start of treatment all patients were admitted to one of the units (39 to Beatrixoord, and five to Dekkerswald), and were discharged home after a mean admission episode of 164 days (range 31–481). All patients received an individually tailored combination of anti-tuberculosis drugs, depending on the drug susceptibility reports, and based on previously prescribed anti-tuberculosis drugs, when known. In the latter period of the study, the general policy was to start with a regimen of an aminoglycoside, a quinolone and two to three additional drugs, but for patients treated in the 1980s the drug prescribing policy was less specific. The number of different compounds for each individual patient was six on average (range 4–9).

Drug therapy was continued for a mean period of 608 (268–1626) days. Five patients had not completed treatment at the time of writing, but were responding well. Their drug therapy was scheduled to be continued for a mean of 124 days. Twenty-four patients suffered significant side effects. An overview of all drugs prescribed, all experienced side effects and the duration of drug treatment is given in Table 2.

In Table 3, the number and range of antimycobacterial agents are given for each pattern of in vitro test results against five first-line antimycobacterial agents. Despite the recorded drug resistance, most patients were still treated with INH because it was assumed that if the in vitro catalase reaction proved positive, a subset of the micro-organisms in the clinical isolate were still INH-susceptible. Three patients underwent a pneumonectomy because of treatment failure; shortly after the operation, their cultures became negative.

One patient underwent an elective pleuropneumonectomy in order to reduce the risk of relapse from a destroyed lung. Two other patients underwent a lobectomy after they were considered cured of their TB, because of an aspergilloma in an old cavernous lesion. Forty-one patients had sputum examination on a regular basis.

TABLE 2 Anti-tuberculosis drug treatment in 44 patients with MDR-TB; duration of treatment and reported side effects

Agents	Patients (N)	Duration in days (range)	Total side effects (n)	Side effect
Isoniazid	36	471 (20-1684)	3	N, S, L
Rifampin	5	341 (85-534)		
Pyrazinamide	38	461 (18-1001)	5	L, J
Ethambutol	42	526 (44-1474)	4	I, K, V
Aminoglycoside	40	108 (20-383)	6	H, I, N, K
Quinolone	38	504 (31-898)	4	L, I, J, K
Rifabutin	9	276 (14-1474)	1	S
Prothionamide	16	244 (6-617)	6	P, L, I
Thioacetazone	4	511 (415-651)		
Cycloserine	5	193 (16-478)	2	N, P
Gamma interferon	1	21		
Clofazimine	39	533 (75-1474)	2	S, K
Clarithromycin	2	520 (443-596)		
Co-amoxiclav	1	61		

N = neurological disorders; S = itching; L= liver-test abnormalities; J = joint complaints; I = gastro-intestinal complaints; K = renal dysfunction; V = visual disorder; H = acoustic symptoms; P = mental disturbances.

At the time of admission, 29 patients were sputum culture positive, and 22 of these also had a positive direct sputum smear. After a mean period of 6 weeks (range 1–20), 23 patients converted to sputum culture negative. Therapy failed initially in six patients, but after a period of 69 weeks on average (range 24–181), their sputum also converted. The total costs per patient for treatment amounted to US$60,000.

Follow up

The mean follow-up period from the first day of hospitalization was 1610 days (range 268 4288)— about 53 months. The mean survival from the day of diagnosis was 1904

days (range 421–5382)—about 62 months. Six patients (14%) died during the follow-up period. In only one patient was the cause of death directly related to TB: he died of a relapse. Two other patients dying during treatment for active TB died from other causes, one from *Staphylococcus aureus* sepsis and the other from complications of pancytopenia in concomitant Gaucher's disease. The remaining three patients died of progression of pulmonary carcinoma and other unrelated conditions after completion of their TB treatment, without any evidence of TB relapse.

Five patients had not yet completed therapy, but had no clinical or microbiological signs of active TB. The remaining 33 patients (75%) are considered cured of their MDR-TB. Only one patient had a relapse, 2 years after completion of treatment; this was the only patient to die as a result of active TB.

TABLE 3 Drug resistance patterns to first-line anti-tuberculosis agents and subsequent treatment decisions

		Number of patients for whom each of the listed drugs was prescribed										
Patterns of multiple drug resistance	**No. of patients with each resistance pattern**	**Isoniazid**	**Rifampin**	**Pyrazinamide**	**Ethambutol**	**Aminoglycoside***	**Quinolones†**	**Rifabutin**	**Prothionamide**	**Cycloserine**	**Thioacetazone**	**Clofazimine**
INH/RMP	7	6		7	7	6	5	2	1	1	1	7
INH/RMP/SM	16	13	2	16	16	14	14	3	3	2	1	11
INH/RMP/EMB	4	2		4	4	4	4		1	1	1	4
INH/RMP/PZA	2	2	1	2	2	1	2		2			2
INH/RMP/SM/EMB	6	5	1	6	5	6	5	2	2		1	6
INH/RMP/PZA/SM	6	5		3	6	6	6	1	5			6
INH/RMP/SM/EMB/PZA	3	3	1		2	3	2	1	2	1		3
All patterns	44	36	5	38	42	40	38	9	16	5	4	39

* amikacin, kanamycin or streptomycin.

† Ciprofloxacin or ofloxacin.

INH = isoniazid resistance; RMP = rifampin resistance; SM = streptomycin resistance; EMB = ethambutol resistance; PZA= pyrazinamide resistance.

DISCUSSION

This study shows that MDR-TB can be cured in the vast majority of cases in the setting described. MDR-TB has been referred to as the 'new tuberculosis' which is more often than not a deadly scourge for mankind.[26] This view is largely based on several earlier reports on long-term treatment outcome in MDR-TB.

One large study in 171 patients showed poor outcome after a mean follow-up period of 5 years, in which only 56% of patients responded, and 20% of mortality was attributed to TB.[27] Another report by Park et al. showed that HIV-positive patients have a particularly poor prognosis: 77% of HIV-positive MDR-TB patients died, whereas HIV-negative patients had a mortality of 20%.[28] Another important factor influencing prognosis in this study was the institution of inadequate treatment. One South African study showed that only 27% of the patients were considered cured after a follow-up period of 5 years.

Treatment outcome of MDR-TB was therefore comparable with the treatment outcome of (drug-sensitive) TB in the period before antimycobacterial drug treatment was available.[29]

Not all of our pulmonary TB patients (29 of 40 patients with pulmonary involvement) were sputum culture or smear positive. This may reflect the possibility that in 11 of these patients the treatment started by their referring doctors had converted them to negative, and that the pre-hospital treatment period should be added to estimate the total time required for their treatment.

Not all the patients were tested for HIV co-infection, notably in the initial years of the study. We have assumed that these patients were HIV-negative, both because they did not belong to any known risk group for HIV infection, and because they remained in good health after completion of TB treatment in the absence of retroviral therapy. We have no data to suggest any measurable benefit from some of the medications prescribed such as clofazimine, clarithromycin and co-amoxiclav (Tables 2 and 3, Figure), and we are well aware of the controversy about their effectiveness. The data presented merely suggest that the care provided appeared effective for most of the patients. Although the costs involved for treatment compared favourably with a report from the United States by Mahmoudi, who calculated an average of US$180000 (in 1993) per patient,[30] it is clear that such costs cannot be afforded in most resource-poor countries. We believe, however, that not all reports of poor treatment outcome of MDR-TB are disappointing due to financial restraints.[31,32]

Our results confirm the hypothesis that MDR-TB can be cured, provided that patients are treated with an adequate drug combination, by a committed team who apply the principles of close supervision.[22,23] Mortality after a median follow-up of 53 months was 14%, with half of the patients dying during TB treatment, and only one death (2–3%) directly caused by active TB. Because patients are treated with drugs that are known to be potentially toxic and have significant side-effects, and because of the need for

prolonged use of the drugs, it is even more important to motivate patients to comply with treatment than in normally drug-sensitive TB.[33,34] Despite the serious side-effects in 22 patients, only nine appeared to be non-compliant with therapy at any one time during the treatment period. We believe that this is an important aspect of the treatment of MDR-TB.[35] Our practice has been to emphasise education and psychosocial support of patients. The DOTS-plus strategy implies close treatment supervision by our nurses, a practice that is continued after discharge from the unit by a specialised nurse from the Municipal Health Service. Special care is taken so that patients feel free to call or contact health staff informally whenever they experience any problems.

A limitation of the study is that our patients agreed to be admitted—some patients might not have agreed to be referred to one of the units in the first place, and might have absconded. Compulsory treatment is not possible under Dutch law, and some MDR-TB patients may have experienced difficulties due to cultural or language barriers. Dutch immigration authorities have agreed to allow illegal immigrants who subsequently appear to suffer from disease to complete their medical treatment before any court sentence to expel them from the Netherlands is implemented. In the earlier quoted national Dutch cohort survey, six of 19 patients defaulted, four were transferred outside the Netherlands, and only nine were considered cured.[12]

In the Netherlands, the number of TB cases has been low. The 1997 incidence was 9.5/100 000 population (Tuberculosis Index 1997, Royal Netherlands Tuberculosis Association, The Hague, 1999), and this figure has changed little over recent years. The problem of resistance is also limited—12% of TB patients have bacilli with resistance to one or more agents, and only 1% have multiresistant bacilli.[10] Although MDR-TB may not be a major health problem in the Netherlands, it certainly is a large problem world-wide. Pablos-Mendez et al. showed that few countries participating in the global study on the prevalence of drug-resistant TB were free from the problem of MDR-TB, [25] with prevalence rates ranging from nearly 0 to 22% (mean 2.2%). The highest prevalence of MDR-TB was found in Asia, the former Soviet Union, the Dominican Republic and Argentina. The higher prevalence rates for MDR-TB appeared to correlate with the lack of a well organised national anti-tuberculosis programme, with implementation of the WHO-TB control strategy. The best way to combat MDR-TB is to prevent it.[22] Migration and international travel have rendered regional and national efforts futile. To fight MDR-TB and TB in general requires both a global and a holistic approach.[36]

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b | Antituberculosis-Drug Resistance

N Engl J Med 1998; 339:1079-1080October 8, 1998DOI: 10.1056/
NEJM199810083391511

To the Editor:

The report by Pablos-Méndez et al. pointing out the alarming worldwide threat imposed by multidrug-resistant tuberculosis prompts us to comment on the clinical, social, and financial impact of this problem.

We studied the outcome of treatment in 30 consecutive patients (19 male and 11 female) with multidrug-resistant tuberculosis who were referred to our tertiary care tuberculosis unit between 1985 and 1996. The mean age was 34 years (range, 15 to 82); none of the patients were HIV-seropositive; and 23 were foreign-born. The majority (27 patients) had pulmonary tuberculosis, typically with apparently active disease. All patients were culture-positive on admission, with strains that were resistant to isoniazid and rifampin (by definition), but streptomycin resistance was present in strains from 20 patients, and resistance to both pyrazinamide and ethambutol was present in strains from 5 patients. In 17 patients, previous tuberculosis treatment was documented, and 10 patients were known to have had no prior treatment. Twenty-seven patients with pulmonary tuberculosis had smear-positive sputum samples. Smears of sputum samples from most of these patients converted to negative within eight weeks, but four converted to negative only after a median period of two years. Inpatient treatment lasted a mean of 173 days (range, 31 to 481).

Patients were treated with seven drugs on average. Side effects were common, as were language and cultural barriers between patients and care providers. Yet compliance was high, with directly observed chemotherapy during in-patient treatment. Tuberculosis treatment was continued until six months after the conversion of sputum cultures; one patient received additional surgical treatment for the control of tuberculosis, and two others underwent surgery for aspergilloma after successful tuberculosis treatment.

The estimated cost of treatment (including the cost of drugs and the admission fees) was $60,000 per patient. The mean length of follow-up was 2053 days (range, 497 to 3892); none of the patients were lost to follow-up. Patients were considered cured if the clinical response was favorable and if cultures and directly obtained smears remained negative after the completion of treatment. Six patients died, three with active tuberculosis and three of unrelated conditions. Our results compare favorably with the results of Goble et al.[1] but are similar to those for patients without HIV in the studies by Park et al.[2] and Telzak et al.[3]

The costs of treating multidrug-resistant tuberculosis are tremendous, both economically and in terms of human suffering, although in affluent societies the mortality rate may have been overestimated in the past. Pablos-Méndez et al. rightly call for increased global efforts to combat the effects of poor tuberculosis-control programs, especially in developing countries.[4]

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c | Multidrug resistant tuberculosis

Letters to the Editor

We read with interest the report by White and Moore-Gillon[1] on the resource implications of multidrug resistant tuberculosis (MDR-TB) in the UK. We studied the outcome of 44 HIV negative patients with MDR-TB admitted to two tertiary care tuberculosis units in the Netherlands between 1985 and 1998.[2 3] Most (38 patients) had pulmonary tuberculosis. The mean admission period was 164 days and all patients received an individually tailored combination of antituberculosis drugs for a mean period of 608 days. We estimated the cost of treatment per patient to be US$60 000 which included admission fee, costs for outpatient visits, and the costs of drug provision. Although we did not include cost of toxicity monitoring and additional procedures, our costs compare favourably with those of White *et al*[1] (mean £60 000) and Mahmoudi[4] (mean US$180 000).

In the Netherlands the number of patients with tuberculosis resistant to any antituberculous drugs is limited to 11%, and only 0.6% of the bacilli are MDR. Between 1993 and 1997 only 43 cases of MDR-TB were identified, of which 28% had received previous treatment for tuberculosis (Index Tuberculosis 1998, Royal Netherlands Tuberculosis Association, The Hague, 2000). This suggests that transmission of MDR bacilli rather than inadequate treatment contributes to the resistance problem in the Netherlands. In poor resource countries, directly observed short course chemotherapy of tuberculosis is generally recommended to prevent the occurrence of MDR-TB. We feel that new rapid molecular methods for detecting resistance should be developed to limit the period of potential (nosocomial and community) transmission of MDR bacilli and thus prevent the emergence of MDR-TB. Such tests should then be made available to poor resource countries at an affordable price.

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Comment on: Resource implications of patients with multidrug resistant tuberculosis. [Thorax. 2000]

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d | Highly successful treatment outcome of multidrug-resistant tuberculosis in the Netherlands, 2000-2009

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Summary

SETTING: Simultaneous resistance to the two key anti-TB drugs isoniazid (INH) and rifampin (RMP) characterizes multidrug-resistant tuberculosis (MDR-TB). MDR-TB is a scourge requiring toxic, prolonged treatment and is associated with poor outcome. The Netherlands is a country with a long-standing intertwined well-resourced TB service where all patients have a culture-confirmed diagnosis in a central reference laboratory.
OBJECTIVES: To assess treatment outcomes of MDR-TB patients over a period of ten years in The Netherlands.
DESIGN: Demographic, clinical and microbiological features of all patients with MDR-TB that started treatment in 2000-2009 in the Netherlands were analyzed, using national registry and patient records.
RESULTS: Characteristics of the 113 patients with MDR-TB were: M/F ratio 1.57; 96% foreign born; median age 29 yrs; 96 (85%) pulmonary TB, 56 (50%) smear-positive sputum; 14 (12%) HIV co-infected. Of the 104 (92%) that started MDR-TB treatment, 86% had a successful outcome using a median of 6 active drugs; 8 had pulmonary surgery. HIV negative status was associated with successful outcome (adjusted OR 2.1; 1.1-3.8).
CONCLUSION: High success rates in MDR-TB were achieved with close collaboration of all

stakeholders, reaching targets set for drug-susceptible TB. HIV remained an independent risk factor for unsuccessful treatment outcome.

KEY WORDS: Tuberculosis, Multidrug-Resistant, HIV, public health, microbial sensitivity test, therapeutic drug monitoring, outcome

INTRODUCTION

Multi-Drug Resistant Tuberculosis (MDR-TB) is an emerging epidemic, with 480,000 incident cases estimated annually, most from Eastern Europe, China and India [1-2]. The magnitude of the problem may be much larger, because in many highly TB-burdened areas, drug susceptibility testing is unavailable. The WHO European Region (WER), that includes former Soviet Union states, has the highest MDR-TB burden. Outcome of MDR-TB is generally poor; only 34% of the 2010 MDR-TB cohort in WER completed treatment; and only 48% of MDR-TB cases who started treatment globally in 2010 had a favourable outcome [3]. Studies reporting treatment success of 60-70% [4] therefore do not reflect service conditions [5].

Reports on maximally achievable favourable outcome from affluent countries are scant [6-9]. Follow-up of patients is limited in some studies [8], and selective reporting may result from failing registration systems.

In the Netherlands, with 17 million inhabitants, all TB cases are notified to Municipal Health Authorities with their Public Health TB teams (MHTB) that treat uncomplicated cases, and initiate contact investigation and screening activities. A national Tuberculosis Register (NTR) was maintained by KNCV; in 2012, the National Institute for Public Health and the Environment (RIVM) took over. Two dedicated TB centres provide care for patients with co-morbid and complicated TB, and all patients with MDR-TB are admitted to these units in accordance with the national TB guideline. We report treatment outcomes of MDR-TB in the Netherlands in 2000-2009.

METHODS

Data collection

Retrospective data were collected of all patients diagnosed with MDR-TB between January 2000 and December 2009. Patients diagnosed before, but starting therapy during the study period, were also included; patients diagnosed in 2009, but starting therapy in 2010, were excluded. MHTB physicians and pulmonologists were approached to follow-up MDR-TB patients and ascertain that they were either well, without symptoms suggesting absence of relapse; or had relapsed, had defaulted or deceased; and if so, from TB or any other cause. Data on all TB patients with *Mycobacterium tuberculosis* complex

culture confirmation were obtained from the NTR. Follow-up after treatment completion was either by the TB centres, the attending physician or the municipal health authorities.

Bacteriology including drug susceptibility testing (DST)

Demographic and clinical data on previous TB treatment, microbiology, hospitalization, drugs used, and outcome were retrieved from the two TB centres and the MHTB.
All *M. tuberculosis* isolates were submitted to the RIVM for identification and DST; the absolute concentration method was used for most second-line TB drugs; for moxifloxacin and linezolid three different concentrations were tested to assess the minimal inhibitory concentration (MIC).

MDR-TB treatment and monitoring of adverse effects (AE)

TB drug combinations were individually tailored. Treatment history, age, co-morbidities and co-infections (hepatitis B and C, HIV) were recorded. DST results and previous TB treatment were considered in designing treatment regimens. As a rule, treatment was continued for 18 months – and at least 12 months after (sputum) culture converted. Sputum conversion was defined as > 2 consecutive negative cultures performed at least 4 weeks apart. In the framework of ongoing studies, several patients had pharmacokinetic (PK) measurements and dosages were adjusted according to PK and MIC results [10-11].

Nursing staff of the two TB centres directly supervised treatment; specialised nurses from the MHTB continued Directly Observed Therapy (DOT) after discharge if necessary, or less stringent forms of adherence support if feasible. To monitor AE, regular laboratory tests for renal and liver injury, and monthly audiometry and ophthalmological assessments were made. AE were scored if medication was interrupted, stopped or the dosing adjusted.

The time to sputum conversion during MDR-TB treatment was defined as the time from the start of MDR-TB treatment to the time of collection of the first in a series of two or more consecutive negative culture results, at least 4 weeks apart.

Definitions

MDR-TB was defined as TB caused by *M. tuberculosis* complex isolates that are resistant to at least INH and RMP [1, 12, 13]. XDR-TB is defined by *M. tuberculosis* isolates resistant to INH, RMP, and to any of the tested fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin or moxifloxacin) and to at least one of three injectable second-line drugs (amikacin, capreomycin or kanamycin). INH, RMP, ethambutol, pyrazinamide and streptomycin were considered 1st line drugs.

The WHO standard definitions were also used to define treatment outcome: completion, cure, death during treatment, failure, default and transferred out [12-15].

Patients were categorized as either previously treated or treatment-naïve. Previous treatment was defined as a history of TB treatment for > 4 weeks. Delay was defined as the number of days between the start of 1st line TB treatment assuming drug-susceptible TB and start of MDR-TB treatment, an indirect measure for a cumulative delay caused by the time to *M. tuberculosis* culture positivity, for the time to report DST results, and for health care providers to start appropriate therapy.

Ethics

As this study was a chart review, no ethical approval was needed under Dutch law (WMO).

Statistical Analysis

For comparison of categorical variables, we used X^2 test with continuity correction or 2-sided Fisher exact test as appropriate. For comparison of the mean diagnostic delays, we used the Mann-Whitney U test. Bivariate logistic regression was performed to assess characteristics associated with MDR-TB among all culture-confirmed TB patients. Variables with a p-value <0.25 were considered for inclusion in multivariable modelling. By backward elimination, the most parsimonious model was selected through -2 log likelihood testing. A P value < 0.05 was considered statistically significant. For all statistical analysis we used SPSS version 20 (SPSS Inc., Chicago, Ill, USA).

RESULTS

Demographic and clinical characteristics

Of the 113 MDR-TB patients diagnosed during the study period, 5 (4.4%) patients were Dutch, 12 (10.6%) came from other European countries, 49 (43.4%) from Africa, 42 (37.2%) from Asia and five patients (4.4%) from elsewhere (Table 1); 66 (58.4%) of the patients had immigrated to the Netherlands < two years before diagnosis. Median age at diagnosis was 29 (IQR: 24-37) years; 37 (32.7%) were detected by active case finding (contact investigation or screening). Pulmonary TB was detected in 96 (85.0%) of the patients, 56 (58.9%) were sputum smear microscopy positive and another two tested positive in broncho-alveolar lavage (BAL) microscopy; 17 patients (15.0%) had extra-pulmonary TB only; 35 (31%) had previous TB treatment; 14 (12.4%) patients were co-infected with HIV.

Table 1 provides details of culture-confirmed MDR and non-MDR-TB cases. MDR-TB cases were typically in age group 15-29 years, foreign-born, and < 2 years resident in the Netherlands. MDR-TB cases were more often identified by active case finding than non-MDR-TB cases. MDR-TB cases had more often pulmonary disease, a history of TB treatment and were more often HIV co-infected. In multivariate analysis, MDR-TB cases were less often > 45 years, more often were born in other European countries, Asia or Africa, had a

duration of stay in the Netherlands < 2 years, had pulmonary disease, a history of previous TB treatment, and HIV co-infected.

Of the 113 patients (M:F: 69 : 44), 104 were treated for MDR-TB - nine patients never started MDR-TB treatment: two were asylum seekers who had to leave the country before Immigration Authorities were notified about their disease status. One asylum seeker could not be traced, two immigrant MDR-TB cases had already returned to their home country when DST results became available. In a 9-year old child with TB lymphadenitis clinicians decided not to start MDR-TB treatment, because the lymph node almost completely regressed after three months standard TB treatment by the time DST results became available; the child was closely monitored thereafter. Three patients were only diagnosed with MDR-TB post-mortem.

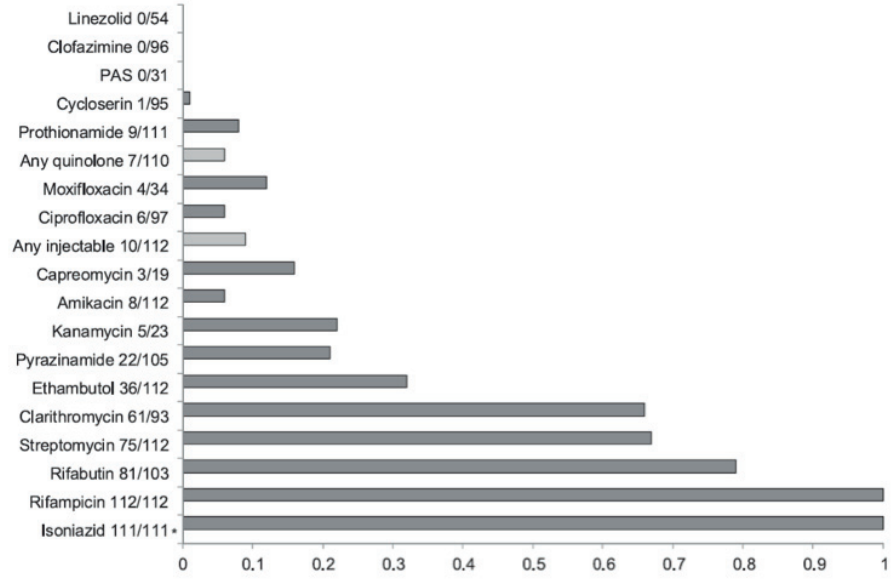

FIGURE 1 In vitro drug resistance of *M. tuberculosis* isolates of patients with MDR-TB in the Netherlands, 2000–2009.* One isolate of a rifampicin-resistant case had an inhA mutation; however, drug susceptibility against isoniazid could not be determined and the case was considered as MDR-TB. PAS¼para-aminosalicylic acid; MDR-TB¼multidrug-resistant tuberculosis.

Drug resistance patterns

Figure A shows the drug susceptibility test results for 112 of the 113 MDR-TB patients. The remaining case was clinically and epidemiologically diagnosed with MDR-TB based on documented exposure to a relative with MDR-TB, but without culture confirmation. *M. tuberculosis* isolates were resistant to median 5 drugs (IQR: 4-5, maximum 10); see Figure 1.

In the first four years of the study period, isoniazid was prescribed in 15 patients because of a positive in vitro catalase reaction, suggesting that some of the organisms were still isoniazid-susceptible; later this practice was abandoned; no high-dose isoniazid was prescribed. Fifteen (14.3%) of 105 isolates tested for all first-line agents - from the total pool of 112 MDR-TB cases - were resistant to all first-line TB drugs. Of 103 MDR isolates, 22 (21.4%) tested susceptible to rifabutin; the rifabutin-susceptible strains were predominantly identified in the first few years of the study period. Ten of 112 MDR isolates (8.9%) were resistant to at least one of the aminoglycosides and 7 of 110 (6.4%) isolates for at least one of the fluoroquinolones. Four (3.6%) fulfilled the criteria for XDR-TB.

TABLE 1 Demographic and disease-related factors of MDR-TB and culture-confirmed non-MDR-TB cases in the Netherlands, 2000–2009

	MDR-TB (n = 113)* n (%)	Culture-confirmed non-MDR-TB (n = 8915) n (%)	OR (95%CI)	aOR (95%CI)†
Demographic factors				
Sex				
Female	44 (38.9)	3605 (40.4)	1	
Male	69 (61.1)	5310 (59.6)	1.1 (0.73–1.6)	
Age, years				
0–14	2 (1.8)	233 (2.6)	0.39 (0.10–1.6)	0.99 (0.24–4.2)
15–29	64 (56.6)	2937 (32.9)	1	1
30–44	36 (31.9)	2639 (29.6)	0.63 (0.42–0.95)	0.71 (0.46–1.1)
45–59	8 (7.1)	1393 (15.6)	0.26 (0.13–0.55)	0.41 (0.19–0.89)
≥ 60	3 (2.7)	1713 (19.2)	0.08 (0.03–0.26)	0.19 (0.06–0.64)
Country or region of birth				
The Netherlands	5 (4.4)	2588 (29.0)	1	1
Rest of Europe	12 (10.6)	468 (5.2)	13.7 (4.6–37.8)	4.1 (1.3–12.2)
Asia	42 (37.2)	1967 (22.1)	11.1 (4.4–28.0)	4.8 (1.8–13.0)

	MDR-TB (n = 113)* n (%)	Culture-confirmed non-MDR-TB (n = 8915) n (%)	OR (95%CI)	aOR (95%CI)†
Africa	49 (43.4)	3165 (35.5)	8.0 (3.2–20.1)	3.0 (1.1–7.9)
Americas and Oceania	5 (4.4)	727 (8.2)	3.6 (1.0–12.3)	1.2 (0.28–5.2)
Arrived in the Netherlands <2 years				
No	44 (38.9)	7114 (79.8)	1	1
Yes	66 (58.4)	1718 (19.3)	6.2 (4.3–9.1)	2.9 (1.9–4.5)
Unknown	3 (2.7)	83 (0.9)	5.8 (1.8–19.2)	14.6 (2.9–72.6)
Disease-related factors				
Active case finding				
Yes (contact investigation/ screening)	37 (32.7)	1471 (16.5)	2.5 (1.7–3.7)	
No	76 (67.3)	7444 (83.5)	1	
Site of tuberculosis				
Extra-pulmonary TB	17 (15.0)	2875 (32.2)	1	1
PTB	96 (85.0)	6040 (67.8)	2.7 (1.6–4.5)	2.4 (1.4–4.1)
PTB sputum smear-positive	56 (58.9)	3465 (48.6)		
PTB BAL microscopy-positive	2 (2.1)	530 (10.8)		
PTB sputum/BAL microscopy-negative	38 (40.0)	2575 (42.6)		
Previous history of anti-tuberculosis treatment				
Yes	35 (31.0)	361 (4.0)	10.6 (7.0–16.1)	9.5 (6.1–14.9)
No or unknown	78 (69.0)	8554 (96.0)	1	1
HIV co-infection				
Yes	14 (12.4)	451 (5.1)	2.7 (1.5–4.7)	2.1 (1.1–3.8)
No or unknown‡	99 (87.6)	8464 (94.9)	1	1

* Including one MDR-TB case without culture-confirmation (see text). All MDR-TB isolates were *Mycobacterium tuberculosis*, except for one isolate identified as *M. bovis*.
† Including adjustment for year of diagnosis (not shown).
‡ 9 MDR-TB and 7646 non-MDR-TB cases had unknown HIV status.
MDR-TB = multidrug-resistant TB; OR = odds ratio; CI = confidence interval; aOR = adjusted OR; TB = tuberculosis; PTB = pulmonary TB; BAL = bronchoalveolar lavage; HIV = human immunodeficiency virus.

Treatment

MDR-TB patients received on average 44 days (median 35 days) standard TB treatment prior to MDR treatment; in the period 2000-2004 the median delay in initiating MDR-TB treatment was 45 days (IQR: 28-79), in the period 2005-2009, the delay was reduced to 26 days (IQR: 13-50; P<0.01; data not shown).

Most (95) patients started in-patient MDR-TB treatment in one of the TB centres, according to protocol. Patients were hospitalized for a median of 92 (IQR: 61-154; maximum 512) days.

MDR-TB patients were treated with median six active drugs (IQR: 5-6; range: 3-10), and the regimen almost invariably included an aminoglycoside, initially given daily, but if discharged with this drug, to be continued typically 5d/wk; based on PK and MIC, we typically prescribed 7.5 mg/kg of kanamycin or amikacin bodyweight daily for 6 months) and a fluoroquinolone – usually, moxifloxacin 400mg (Table 2). If susceptible, ethambutol and pyrazinamide were added. Second-line drugs often prescribed were prothionamide/ethionamide, clofazimine 100mg 5-7 d/wk, typically started after cessation of injectables; and linezolid (from 2003 onward; typically 300 mg bid or less as assessed by PK/PD). Prothionamide was the drug most often discontinued due to side effects. Of the 104 patients that started MDR treatment, 43 experienced adverse effects (Table 2).

Eight patients had thoracic surgery (lobectomy or pneumonectomy); three patients underwent pneumonectomy because of persistently positive sputum smears with culture conversion soon after surgery. One patient needed pleuro-pneumonectomy of a destroyed lung. Two other patients with aspergillomas had a lobectomy after therapy completion; the two others had limited procedures.

Sputum smear microscopy and culture conversion

Sputum smear and cultures were performed weekly during admission. Figure 2A shows time to smear and Figure 2B time to culture conversion; 24 of 55 sputum smear-positive MDR-TB cases (one sputum smear-positive patient died before MDR-TB treatment) had already negative smears at initiation of MDR-TB treatment. In the other 31 cases sputum smear conversion occurred after median 49 days (IQR 25-131 days). Four still had positive smears after 180 days, and converted after 208, 273, 307 and 426 days. Forty-five of 89 culture-positive pulmonary MDR-TB cases had one or more sputum samples culture-positive during treatment, with culture conversion after median 46 (IQR 20-76) days; one patient only converted 280 days after start of treatment.

TABLE 2 Anti-tuberculosis drug treatment and discontinuation of drugs due to side effects for 104 multidrug-resistant cases treated in the Netherlands, 2000–2009

Anti-tuberculosis drugs used	Patients n	Treatment duration, days Mean (min–max)	Median [IQR]	Discontinuation n	Side effects
Isoniazid	15	400 (37–723)	378 [274–548]		
Rifampicin	0				
Ethambutol	68	405 (6–730)	456 [270–548]	2	Visual
Pyrazinamide	55	297 (6–730)	209 [62–546]	6	Gastro-intestinal, joint, liver
Rifabutin	14	394 (263–617)	364 [328–468]		
Amikacin	64	165 (6–549)	165 [89–193]	3	Hearing
Kanamycin	23	147 (47–394)	113 [92–197]	2	Hearing, renal
Capreomycin	3	394 (243–691)	249 [243–243]		
Any injectable	88*	172 (6–691)	160 [92–209]		
Ciprofloxacin	4	218 (56–550)	133 [56–465]	1	Gastro-intestinal
Levofloxacin	43	448 (6–730)	508 [365–549]	1	Gastro-intestinal
Moxifloxacin	57	400 (37–611)	442 [277–548]	2	Neurological, tendon
Any fluoroquinolone	101*	425 (6–730)	485 [364–549]		
Prothionamide	72	323 (6–638)	348 [146–528]	16	Gastro-intestinal, liver, psychiatric
Cycloserine	14	317 (7–598)	360 [129–417]	2	Psychiatric
PAS	3	354 (12–659)	12 [394–394]	1	Gastro-intestinal
Clofazimine	74	343 (7–706)	374 [91–547]	2	Itching, gastro-intestinal
Clarithromycin	8	398 (61–579)	491 [219–529]		
Linezolid	53	99 (12–706)	56 [26–91]	5	Renal dysfunction, hearing
Cotrimoxazole	4	341 (90–502)	386 [155–482]		
Thiacetazone	7	153 (7–357)	116 [8–314]		
Doxycyclin	2	27 (26–27)	27 [26–26]		

* Two patients switched between injectables, three patients switched between fluoroquinolones.
IQR = interquartile range; PAS = para-aminosalicylic acid.

TABLE 3 Treatment outcome of all MDR-TB cases diagnosed and of those who started drug treatment, The Netherlands, 2000–2009

	All MDR-TB cases (n = 113)* n (%)	Cases who started MDR-TB treatment in the Netherlands (n = 104) n (%)
Favourable outcome	89 (78.8)	89 (85.6)
Cured	47 (41.6)	47 (45.2)
Completed	42 (37.2)	42 (40.4)
Unfavourable outcome	24 (21.2)	15 (14.4)
Died	9 (8.0)*	6 (5.8)
Defaulted/stopped	8 (7.1)	8 (7.7)
Transferred out	1 (0.9)	1 (1.0)
Unknown or no treatment	6 (8.0)*	

* Nine MDR TB patients did not start MDR-TB treatment (see text).
MDR-TB = multidrug-resistant tuberculosis.

Treatment outcome

Of the 104 MDR-TB patients starting MDR-TB treatment, 85.6% had favourable outcome (Table 3); of the total of 113 patients diagnosed with MDR-TB, 78.8% had successful treatment outcome. Only 7 out of 14 (50%) HIV-infected MDR-TB patients completed treatment (5 died during treatment and 2 defaulted/stopped treatment; 91.1% of MDR-TB patients with negative or unknown HIV status completed treatment ($p < 0.01$).

The median duration of treatment was 445 days. For those completing treatment, treatment lasted median 18.2 months or 546 (IQR: 424-549; range 183-730) days.

Only 28 of 98 patients were consistently followed up for at least 24 months; 28 patients had zero follow-up days after treatment discontinuation or completion, mainly because they left the country. One patient died during the follow-up period. No relapses were observed.

After completion, one patient still had arthritis due to pyrazinamide, one other had visual impairment (ethambutol), and two had symptomatic bronchiectasis.

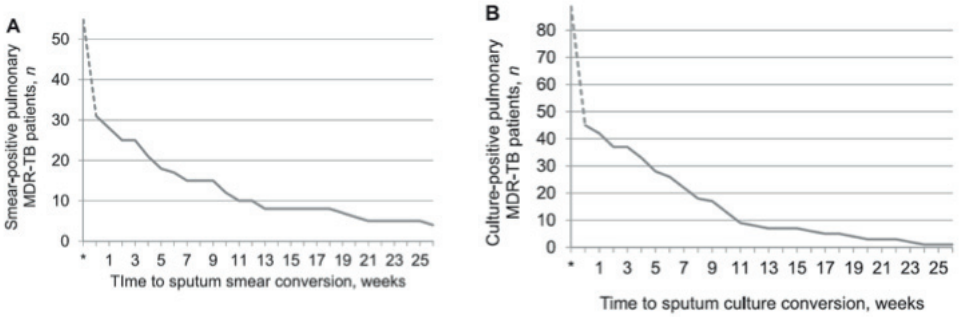

FIGURE 2 A) Time to smear microscopy conversion during MDR-TB treatment in the Netherlands, 2000–2009. The survival curve shows the time to smear conversion of 31 patients with a positive smear at the start of MDR-TB treatment. An additional 24 patients had one or more positive smears at the start of drug-susceptible anti-tuberculosis treatment, but negative smears at the start and throughout MDR-TB treatment (shown with dashed line).

FIGURE 2 B) Time to culture conversion during MDR-TB in the Netherlands, 2000–2009. The survival curve shows the time to culture conversion of 45 patients with culture-positive smear at the start of MDR-TB treatment. An additional 44 patients had 71 positive cultures at the start of drug-susceptible anti-tuberculosis treatment but negative cultures at the start and throughout MDR-TB treatment (shown with dashed line). * Before MDR-TB treatment. MDR-TB = multidrug-resistant tuberculosis.

DISCUSSION

Here, we show that MDR-TB can be cured in the vast majority of cases treated in The Netherlands. This high success rate is the compound result of many different factors. With our data we are unable to determine the relative contribution of each individual component: adequate drug combination; DST performed in a well-coordinated fashion in a central reference laboratory; drug treatment tailored to DST and PK; TB centres and MHTB workers that apply DOT; a well-coordinated TB program; and a well-resourced setting.

Using all of these components, we show that outcome in the 104 MDR-TB patients (including four patients with XDR-TB) were equal to targets set for drug-susceptible TB: 85.6 % of the patients that started treatment achieved a favourable outcome (i.e., cured or completed treatment). Outcome was similar in the two decades preceding the current study period [16]. A recently published meta-analysis provides evidence that drug susceptibility-targeted therapy provides added value for survival [17]. These results were achieved in patients with significant physical and psychiatric co-morbidities and language and cultural barriers, and with considerable input of human and financial resources.

Treatment duration is the major challenge for case holding. The updated 2011 WHO guidelines [1, 14] suggest extending the minimum duration of treatment by two more months, as improved treatment success has been associated with the longer treatment duration of MDR-TB 18. Intensive phase of treatment should therefore last at least 8 months, and total duration of treatment should be extended to 20 months. The duration may be adjusted for some patients based on their clinical and bacteriologic response. Studies from Bangladesh 19 and Niger 20 suggest that fluoroquinolone-based treatment of MDR-TB might be shortened under specific circumstances to 9-12 months, well below the target currently set for MDR-TB at 20 months [1, 15]. Although treatment was typically individually tailored and based on DST results for second line drugs, many patients received linezolid [10,21] which is now considered a powerful WHO group 5 drug; and clofazimine, which has been associated with improved outcome [22-23], possibly because of its activity against *M. tuberculosis* persisters [24-25]. The added value of rifabutin prescribed to a minority of our patients, and the regular dose-INH to those with strains showing catalase activity, remains questionable; we largely abandoned these in recent years without apparent loss of efficacy.

Our results contrast with most of reported series from well-resourced settings [6-9] and even more to reports reflecting service conditions [1, 5]. Collaboration of all stakeholders may be the key to this success. Public-private collaborations are important to improve TB outcome [1,15,18].

Current guidelines for MDR-TB treatment have low level of evidence, and controversies remain [18], e.g., on the number of anti-TB drugs required, duration of (parenteral) drug administration, standardized versus individualised regimens, and the role of surgery [26]. Indeed, MDR-TB management is predominantly based on observational studies and expert opinion [27]. Our results were obtained without adding any of the new drugs - bedaquiline [28, 29], delamanid [30], sutezolid [28, 31, 32] and pretomanid [28,33].

CONCLUSIONS

With close collaboration of all stakeholders, MDR-TB outcome equalled that of the target for drug-susceptible TB in The Netherlands. Absence of HIV co-infection favoured successful outcome.

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e | Shorter treatment for multidrug-resistant tuberculosis: the good, the bad and the ugly.

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Abstract: WHO shortened MDR-TB treatment to 9-12 months for some patients - fitting 85 of 172 Dutch patients, 2000-2015

To the Editor:

We welcome the initiative by the Guideline Development Group (GDG) members to issue the 2016 update of World Health Organization (WHO) treatment guidelines for drug-resistant tuberculosis (TB) [1]. With one in two patients currently failing on treatment for multidrug-resistant (MDR)-TB, primarily as a result of the difficulties presented by cumulative drug toxicity, logistics, costs and subsequent poor adherence to therapy [2], a shorter regimen for selected patients would be a tremendous asset, even though the GDG argues that the recommendation is conditional, and the scientific evidence for the recommendation is low.

Since the first reports on the efficacy of a regimen of only 9 months for MDR-TB [3, 4], two more studies have been published to support the concept of shorter regimens [5, 6] while the STREAM (Evaluation of a Standard Treatment Regimen of Anti-tuberculosis Drugs for Patients with MDR-TB) study is still enrolling [7]. However, shortening therapy would only apply for selected patients without prior use of or proven resistance to fluoroquinolones (group A) or second-line injectable agents (group B). At least five active drugs should be available for the intensive phase (4–6 months). Further exclusions are extrapulmonary TB, additional resistance to pyrazinamide (PZA) and pregnancy. Clofazimine [8] and linezolid [9] were regrouped as core agents in group C with ethionamide or prothionamide, while para-aminosalicylic acid was deferred to group D.

The GDG noted the lack of evidence for shortening treatment for extrapulmonary MDR-TB and did not recommend doing so, even though drug-susceptible extrapulmonary and pulmonary TB are treated with similar schedules and durations of treatment. MDR-TB treatment requires bacteriological monitoring, which is more feasible in sputum samples from pulmonary MDR-TB patients than samples from extrapulmonary MDR-TB cases.

Excluding PZA resistance is problematic. PZA susceptibility testing is difficult and not widely available, especially in low-resource settings. However, recent studies have shown excellent concordance of molecular and phenotypic susceptibility [10, 11], and testing molecular susceptibility to this important drug may become an important asset, even in low-resource settings.

We retrospectively analysed how many MDR-TB patients in the Netherlands would potentially have benefitted from the new guidelines; we therefore studied the data of all 172 consecutive patients that had started treatment since 2000. Data on patients treated between 2000 and 2009 have been published previously [8].

Five of these 172 patients had earlier MDR-TB treatment, four were pregnant, 30 had extrapulmonary MDR-TB, 28 had additional resistance to group A and/or group B drugs and 52 had PZA-resistant organisms. Four had extensively drug-resistant TB (these patients were included in our earlier report [8]), and all of these cases had a favourable outcome. As multiple exclusion criteria clustered in some patients (table 1), nearly half (85 (49.4%) out of 172) of our patients would meet the criteria for the new shorter regimen. Interestingly, 58% of the 2000–2009 cohort, but only 36% (25 out of 69) of the 2010–2015 cohort would be eligible for shortened treatment. Of the 54 patients starting treatment between 2010 and 2013, 46 (85.2%) completed treatment successfully, one died from an unrelated cause, four interrupted their treatment and three left the Netherlands to complete their treatment elsewhere. Therefore, the high success rate at ~85% reported earlier [8], without failure or relapse was maintained.

The evidentiary table on page 20 in the guidelines [1] is based on data from 1205 patients;

89 of those were lost to follow-up. Outcome in those without resistance to quinolones, injectables or PZA was successful in 121 (96.8%) out of 125, which is obviously excellent, but the lower limit of the confidence interval (77.3–99.6%) calls for caution.

MDR-TB treatment outcome in the Netherlands with conventional treatment of 20 months duration [8, 12] has been highly successful with very limited toxicity, and no failure or relapse. We benefitted from an extraordinary collaboration between tertiary treatment centres and public health physicians, with hardly any loss to follow-up. We have treated patients in whom treatment was extremely long, considering the fact that their lesions and their bacterial load were limited. Whether treatment duration is important in cavitary lesions and in patients with a large initial bacterial load needs to be investigated in future studies.

TABLE 1 Reasons for ineligibility of 172 multidrug-resistant tuberculosis (MDR-TB) patients in the Netherlands to receive treatment of a shortened duration (9–12 months) than regular care (18–20 months), 2000–2015

	Previous MDR-TB drugs	Pregnancy	Extra pulmonary TB	Resistance to FLQ# or second-line injectable drugs¶	PZA resistance	Total
Previous MDR-TB drugs	5	0	0	4	3	
Pregnancy		4	2	0	0	
Extrapulmonary TB			28	2	9	
Resistance to FLQ# or second-line injectable drugs¶				22	12	
PZA resistance					28	
Patients meeting exclusion criterion	5	4	30	28	52	87

Data are presented as n.
FLQ: fluoroquinolone;
PZA: pyrazinamide.
#: group A;
¶: group B.

We believe that part of our success is due to an approach using tailored pharmacokinetic/pharmacodynamic (PK/PD) modelling. Drug susceptibility testing in MDR-TB is important [13], but merely reporting a test result below or above the European Committee on Antimicrobial Susceptibility Testing breakpoint may not provide sufficient precision. Clearly, the contribution of each individual drug depends on drug exposure relative to the drug susceptibility of the organism [14]. Molecular tests such as the MTBDRplus (version 2.0; Hain Lifescience, Nehren, Germany) to predict susceptibility to second-line drugs currently do not provide a minimum inhibitory concentration (MIC) value to allow for PK/PD calculations, while MIC values for second-line drugs have gradually changed over time. All of these considerations imply that vigilance is warranted with shortened treatment. Modern diagnostic tools should enable physicians to act promptly if treatment is stalling [15]. We believe that drug concentration monitoring has added value to fast molecular tests and drug susceptibility testing to support the shortened regimen by preventing slow response due to low drug exposure [16]. Synergy between second-line TB drugs may further benefit the short course of treatment [17].

We welcome shortened treatment and we agree that the good news of shortened treatment duration should be carefully monitored. Only four of our eligible patients were still sputum smear microscopy positive after 4 months of treatment; 81 (95%) would therefore have continued treatment for 9 months only, according to the new guideline; for these 81 patients, 9 months of treatment would remain good news during the planned 9 months of treatment, while only four would have had to extend their treatment to 12 months according to the new guidelines. However, if many more patients among the 85 starting the 9- (or 12-) month regimen would relapse because of failure to sterilise persister organisms, their treatment would become a negative experience, with a treatment duration of 9 months plus an additional 20 months.

Both for low-resource settings and for programmes that currently have excellent treatment results, monitoring is therefore essential to avoid the bad and ugly effects of what is intended to be simply good.

Chapter 4

Aminoglycosides

a | Hearing loss and nephrotoxicity in long-term aminoglycoside treatment in patients with tuberculosis.

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SUMMARY

OBJECTIVE: To investigate the ototoxic and nephrotoxic effects of long-term use of aminoglycosides.

DESIGN: Patients treated for tuberculosis with aminoglycosides were evaluated for hearing loss and nephrotoxicity for a minimum of 14 days.

RESULTS: Hearing loss of 15 decibels (dB) at two or more frequencies, or at least 20 dB hearing loss at at least one frequency, was found in 18% of our total population treated with aminoglycosides (amikacin, kanamycin and/or streptomycin). In the group treated with kanamycin this percentage was 15.6. None of the factors sex, age, treatment duration, total aminoglycoside doses or first serum creatinine concentration, was found to be associated with hearing loss. Nephrotoxicity percentages at the end of treatment with aminoglycoside or kanamycin are 7.5% (1.9%) and 4.5% (2.3%) respectively, using the definition increase of serum creatinine ≥27 µmol/l (≥44 µmol/l). Patients developing nephrotoxicity had a longer duration of treatment and received larger total doses.

CONCLUSIONS: Patients developing nephrotoxicity had a significantly longer duration of treatment with aminoglycosides, and received a larger total dose. We did not find any factor significantly associated with the development of hearing loss. In the long-term treatment of tuberculosis with aminoglycosides, ototoxicity seems to be a greater problem than nephrotoxicity.

KEY WORDS: hearing loss; nephrotoxicity; aminoglycosides; kanamycin; tuberculosis

INTRODUCTION

Both ototoxicity and nephrotoxicity are known to be major possible side-effects of treatment with aminoglycosides. In our Tuberculosis Centre patients are treated, often for many months, with aminoglycosides. Very little is known about the side-effects of long-term treatment with aminoglycosides.

The aim of this retrospective study was to investigate the overall auditory and nephrotoxic effects of aminoglycosides after long-term use. Aminoglycosides are known to have some degree of toxicity to the eighth cranial nerve; both vestibular and auditory divisions may become affected. In the case of cochlear damage, hearing loss occurs as a result of degeneration of the hair cells of the cochlea, beginning at the basal coil and progressing to the apex. High frequency hearing loss is followed by loss of lower frequencies. In the early stages of ototoxicity, damage is limited to the higher frequencies and does not usually affect frequencies utilised in conversational hearing. Vestibular disturbance is found predominantly in the vestibular sensory cells from the crista ampullaris, and causes ataxia and nystagmus. Neither cochlear nor ampullar cells can regenerate once they have been destroyed.[1–6]

Ototoxicity does not generally appear until 5 days after the start of aminoglycoside treatment.[3] It has been shown experimentally that toxicity in the cochlear and the vestibular portions may vary between different aminoglycosides. Streptomycin is predominantly vestibulotoxic, while amikacin appears to be exclusively cochleotoxic.[7] Amikacin and kanamycin were found to be similarly ototoxic in cats.[8]

Aminoglycoside antibiotics are nephrotoxic by inducing necrosis of the proximal tubules ranging from focal to diffuse lesions. Aminoglycosides are not metabolised, and they are excreted almost exclusively by glomerular filtration. The onset of glomerular dysfunction does not start until 5–7 days after initiation of treatment. Increase in serum creatinine is usually noted during the second week of treatment. The clinical threshold of aminoglycoside nephrotoxicity is determined by the rate of necrosis and the rate of regeneration of proximal tubular cells.[9–11]

Although many comparative studies of aminoglycoside nephrotoxicity in humans are available, there is no consensus about the differences in nephrotoxicity of the aminoglycosides.[7,11,12] Streptomycin was found to be the least nephrotoxic in a study comparing the effects of aminoglycosides on renal function and structure in the rat, while amikacin was found to be as toxic as kanamycin. The relevance of these findings to the situation in humans remains unclear.[13] The number of free amino groups seems to correlate with the relative nephrotoxicity of these compounds. Both amikacin and kanamycin have four free amino groups, while streptomycin has three, and therefore streptomycin is considered to be the least toxic.[14]

MATERIALS AND METHODS

Patients and aminoglycosides

All patients hospitalised in the Tuberculosis Centre Beatrixoord in the period January 1995 to July 2000 and treated for a minimum period of 14 days with streptomycin, amikacin or kanamycin, were included in this retrospective chart study. Diagnoses were multidrug-resistant tuberculosis, tuberculosis with intolerance or resistance to isoniazid or rifampicin, or infections with environmental mycobacteria. Hearing loss and nephrotoxicity were determined for all patients receiving these aminoglycosides.

Consecutive audiograms were evaluated for hearing loss for each patient. The first audiogram of each patient was considered as the baseline audiogram. All audiograms were obtained in a sound-proof auditory test chamber with an audiometer at 250, 500, 1000, 2000, 4000 and 8000 Hz. If necessary, translators were available.

An audiogram is considered to be normal with a maximum of one value between 20 and 30 decibels (dB), not involving conversational hearing ability, and the rest between 0 and 20 dB. Hearing loss was defined as a loss of 15 dB at two or more frequencies, or a minimum of 20 dB hearing loss of at least one frequency between 0.25 and 8.0 kHz. The time between the audiograms was variable. In our retrospective study, not enough data were available to be able to reach conclusions regarding signs or symptoms of vestibular dysfunction.

Serum creatinine levels were determined at the start of aminoglycoside treatment, with normal values between 60 and 110 µmol/l, and were evaluated throughout the whole period of treatment. Aminoglycoside-related nephrotoxicity was defined as a rise in the serum creatinine concentration of 27 µmol/l (0.3 mg/ml) and 44 µmol/l (0.5 mg/ml) at any time during treatment. The reversibility was determined by the creatinine level at the end of treatment. Serum creatinine tests were obtained very frequently, almost daily at the start of treatment. When the patient's situation improved, the tests were performed less frequently.

Age, sex, total duration of aminoglycoside treatment, total aminoglycoside doses and serum creatinine level were determined before treatment commenced.

For continuous variables, differences between groups were compared using the Mann-Whitney rank sum test. The X^2 test was used for analysing categorical data. A P value of less than 0.05 was accepted as indicating statistical significance.

RESULTS

One hundred and ten patients treated with kanamycin, amikacin, streptomycin, or a combination of these, were included in this study. There were 81 (73.6%) men and 29 (26.4%) women, with a mean age of 35.7 years (range 10–83, standard deviation [SD] 16.0). The total number of weeks for which patients received aminoglycosides was 11.8 (SD 8.1), during which they received 53.2 g of any aminoglycoside (range 8–191 g, SD 32.2).

At the start of treatment, the aminoglycosides were given daily because of the high bacterial load; after a certain period a switch was made to a dosage schedule of five or three times a week, in order to be able to treat for a longer period. The hypothetical reasons for this were to minimise the toxic effects and to achieve optimal results. In general the average dose varied between 750 and 1000 mg by intravenous infusion, depending on serum drug and creatinine levels and body mass. The choice between the three different aminoglycosides was based on sensitivity tests and cost. The preferred choice was kanamycin. No patient received more than one aminoglycoside at a time.

Ninety patients were treated with kanamycin (K), seven with streptomycin (S), and two with amikacin (A). Two aminoglycosides were used in 10 patients (5 KS, 4 KA, 1 AS) and one patient received all three aminoglycosides during the period of treatment.
Treatment was changed for reasons of cost, availability of drugs, and problems of drug resistance.

Of the 90 patients treated with kanamycin only, there were 67 (74.4%) men and 23 (25.6%) women, with a mean age of 36.9 years (range 14–83, SD 16,5). They received 46.3 g of kanamycin (SD 24.6), over 10.2 weeks (SD 6.2).

TABLE 1 Hearing loss observed during and at the end of treatment

Aminoglycoside	Patients *n*	Hearing loss during treatment *n* (%)	Hearing loss last audiogram *n* (%)
Kanamycin (K)	45	9 (20.0)	7 (15.6)
Streptomycin (S)	5	3 (60.0)	3 (60.0)
Amikacin (A)	2	1 (50.0)	1 (50.0)
K + S	3	0	0
K + A	4	0	0
S + A	1	0	0
K + S + A	1	0	0
Total	61	13 (21.3)	11 (18.0)

Audiograms

Only one audiogram was available for 49 patients. The audiograms of 18 of these were considered normal. Of the 31 abnormal audiograms, 12 did not interfere with conversational hearing ability, and 19 did. One patient complained of loss of conversational hearing ability after 5 weeks of treatment. The audiogram confirmed this result, but unfortunately there was no earlier audiogram.

Two or more audiograms were available for the remaining 61 patients, who received 62.9 g aminoglycosides (range 15–181, SD 34.8), over 11.9 weeks (range 3–46, SD 8.0). Hearing loss was observed in 13 (21.3%) patients; it was bilateral in seven (53.9%) and unilateral in six (46.1%). Recovery of hearing loss occurred in three patients. At the last audiogram, four (6.6%) showed bilateral and seven (11.5%) showed unilateral hearing loss (Table 1). In five patients only the high frequencies (4000 and 8000 Hz) were involved, and in one patient only the low frequencies (250, 500, 1000 Hz) were involved. In seven patients there was a loss of conversational hearing ability: in five cases there was already conversational hearing loss at the start of treatment, while in the other two patients only the highest frequency of conversational hearing ability (4000 Hz) was involved.

Improvement of hearing of 15 dB or more during treatment was found in at least 22 of all patients

(36.1%), and an improvement of 20 dB or more in 10 patients (16.4%). In the group of patients with hearing loss an improvement of 15 dB was found in four (30.8%), and one of 20 dB or more in three (23.1%) patients.

In the group of patients receiving kanamycin, two or more audiograms were available from 45 patients. They received 42.6 g aminoglycosides (range 14–97, SD 22.9) over 9.4 weeks (range 3–25, SD 5.0). Hearing loss was shown in nine (20.0%) of the patients, three (6.7%) bilateral and six (13.3%) unilateral. Recovery of hearing loss developed in two patients. Unilateral hearing loss was observed at the last audiogram in five patients (11.1%) and bilateral in two (4.4%) (Table 1). We did not find any factor significantly associated with the development of hearing loss (Table 2).

TABLE 2 Univariate associations of independent factors related to hearing loss at any time during treatment

Factors	Hearing loss	Without hearing loss	*P* value
Aminoglycoside treatment			
Sex (%)			
Male	83.3	73.5	
Female	16.7	26.5	0.477
Age (years)	41.5 ± 23.5	33.2 ± 13.0	0.591
Duration of AG			
treatment (weeks)	10.1 ± 5.8	12.4 ± 8.4	0.437
Total dose of AG (g)	75.4 ± 40.7	59.5 ± 32.7	0.252
T0 creatinine level (µmol/l)	70.4 ± 9.5	68.9 ± 16.4	0.721
Kanamycin treatment			
Sex (%)			
Male	88.9	77.1	
Female	11.1	22.9	0.436
Age (years)	41.9 ± 24.2	35.5 ± 14.0	0.771
Duration of AG			
treatment (weeks)	7.9 ± 3.4	9.9 ± 5.3	0.405
Total dose of AG (g)	66.4 ± 36.4	49.0 ± 22.1	0.211
T0 creatinine level (µmol/l)	70.7 ± 11.2	71.4 ± 13.8	0.940

AG = aminoglycoside; T0 = time zero.

Creatinine serum concentrations

There was no initial serum concentration available for three patients; none of these patients had any serum creatinine concentration suggesting nephrotoxicity.

The initial and subsequent serum creatinine concentrations were available for the remaining 107 patients, 33 of whom had a serum creatinine concentration of 59 µmol/l or lower, and none of whom had a higher concentration than 110 µmol/l. They received 53.5 g aminoglycosides (range 8–191, SD 32.5), over 11.9 weeks (range 2–46, SD 8.1). An increase in serum creatinine concentration of at least 27 µmol/l (0.3 mg/ml), and 44 µmol/l (0.5 mg/ml) throughout treatment was observed in respectively 18 (16.8%) and 10 (9.3%) of the

patients. In respectively 10 and eight cases, the increased serum creatinine concentration returned to normal values, and the increased serum creatinine concentration was still detectable in respectively eight (7.5%) and two (1.9 %) patients at the end of treatment (Table 3). Only one patient had a serum creatinine concentration (140 µmol/l) higher than the normal values (60–110 µmol/l).

All serum creatinine concentrations were available for the 88 patients receiving kanamycin. They received 46.6 g aminoglycosides (range 8–143, SD 24.8) over 10.2 weeks (range 2–44, SD 6.3). Nephrotoxicity during treatment, with increases of at least 27 µmol/l and 44 µmol/l, was observed in respectively 13 (14.8%) and six (6.8%) of these patients. In respectively nine and four cases the serum creatinine concentration returned to normal values, and an increase in serum creatinine concentration was still detectable in respectively four (4.5%) and two (2.3%) patients at the end of kanamycin treatment (Table 3).

In the univariate analysis, patients developing nephrotoxicity had a significantly longer duration of treatment with aminoglycosides and received a larger total dose (Table 4).

TABLE 3 Nephrotoxicity observed during and at the end of treatment

Aminoglycoside	Patients *n*	Increase ≥27 µmol/l during treatment n (%)	Increase ≥27 µmol/l end of treatment n (%)	Increase ≥44 µmol/l during treatment n (%)	Increase ≥44 µmol/l end of treatment n (%)
Kanamycin (K)	88	13 (14.8)	4 (4.5)	6 (6.8)	2 (2.3)
Streptomycin (S)	7	2 (28.6)	1 (14.3)	2 (28.6)	0
Amikacin (A)	2	1 (100)	1 (100)	1 (100)	0
K + S	4	1 (25)	1 (25)	0	0
K + A	4	0	0	0	0
S + A	1	0	0	0	0
K + S + A	1	1 (100)	1 (100)	1 (100)	0
Total	107	18 (16.8)	8 (7.5)	10 (9.3)	2 (1.9)

TABLE 4 Univariate associations of independent factors related to the development of nephrotoxicity

Factors	Nephrotoxicity	Without nephrotoxicity	*P* value
Aminoglycoside treatment			
Sex (%)			
Male	72.2	73.5	
Female	27.8	27.0	0.944
Age (years)	34.7 ± 18.3	36.2 ± 15.8	0.342
Duration of AG			
treatment (weeks)	19.9 ± 11.7	10.3 ± 6.1	0.000*
Total dose of AG (g)	86.1 ± 44.2	46.8 ± 25.1	0.000*
T0 creatinine level (µmol/l)	67.7 ± 14.9	70.0 ± 16.4	0.588
Kanamycin treatment			
Sex (%)			
Male	69.2	74.7	
Female	30.8	25.3	0.680
Age (years)	37.1 ± 20.2	37.3 ± 16.0	0.609
Duration of AG			
treatment (weeks)	17.6 ± 10.2	9.0 ± 4.2	0.001*
Total dose of AG (g)	76.6 ± 30.6	41.4 ± 19.6	0.000*
T0 creatinine level (µmol/l)	70.3 ± 15.4	70.8 ± 15.8	0.888

* Statistical significance.
AG = aminoglycoside; T0 = time zero.

DISCUSSION

In this retrospective study, we examined the toxic effects of long-term intravenous aminoglycoside treatment. In most studies, the length of aminoglycoside treatment had a maximum of 2–3 weeks. In our study, patients had a minimum treatment of 2 weeks, with a mean of 11.8 (SD 8.1) and a maximum of 46 weeks. They received an average of 53.2 g of aminoglycosides (range 8–191 g, SD 32.2).

In our centre, 21.3% developed hearing loss during treatment with aminoglycosides, 20.0% with kanamycin, 18.0% at the last audiogram of patients treated with aminoglycosides, and 15.6% at the last audiogram of patients treated with kanamycin

(Table 1). These incidences are higher than the average percentages of hearing loss for amikacin (13.9%) found by Kahlmeter and Dahlager in clinical studies published between 1975 and 1982.15 More recent studies about the toxicity of kanamycin are not available. In the literature, there is a discrepancy between on the one hand the clinical observations that very few patients receiving aminoglycosides complain of developing hearing loss, and on the other hand the reported incidence of hearing loss of up to 41% in studies in which auditory thresholds were obtained. Reasons for this discrepancy could be diverse: patients are unlikely to complain of hearing loss until considerable damage has been done, and there is no universally agreed upon standard for the definition of drug-induced hearing loss.[16]

Hearing loss does not generally appear until at least 5 days after the start of aminoglycoside treatment. Not all cases of hearing loss may be due to the ototoxic effects of aminoglycosides: patients receiving no ototoxic drugs can have auditory changes considered to represent the established criteria of ototoxicity.[16]

Brummett found test-retest differences suggestive of 20–30% hearing loss in a group of healthy volunteers who were not taking any known ototoxic drugs.[17] We found an improvement in hearing of 15 dB or more in 22 patients (36.1%) and an improvement of 20 dB or more in 10 patients (16.4%). In the group with hearing loss, at least one improvement of 15 dB was seen in four patients (30.8%), and an improvement of 20 dB in three (23.1%) patients. There may be more than one explanation for this: inaccurately obtained audiograms, fluctuating conductive hearing losses (allergies, middle ear and atmospheric pressure changes, collapsed external auditory canals, the common cold, and other sources), use of other ototoxic drugs, attentiveness, learning effects and motivation of the test subjects.[17] Due to the lack of many baseline audiograms, we are unable to say anything about hearing loss over the total period of treatment. The reasons for missing audiograms made at the start of treatment include the fact that critically ill patients very frequently cannot undergo baseline auditory testing before receiving aminoglycosides, and some patients started their aminoglycoside treatment in other hospitals where such tests were not being performed.

In many cases we could not determine which abnormalities in baseline audiograms were pre-existent, and which were the result of cochlear damage induced by aminoglycosides. Pre-existing high frequency hearing loss may be related to advancing age, congenital defects, previous ear infections or noise exposure.

Risk factors in the development of auditory toxicity are still a matter of discussion. Moore et al. identified risk factors that included duration of aminoglycoside use, bacteraemia, fever, liver dysfunction and hypovolaemia. Age did not reach statistical significance.[18] Conversely, Gatell et al. found that only age was retained as an independent factor in the development of auditory toxicity.[19] In our study, age, sex, total duration of

aminoglycoside treatment, total aminoglycoside dose and serum creatinine level before the start of treatment were not significantly associated with the development of hearing loss (Table 2). Because of the lack of data about vestibular dysfunction, we can not draw any conclusions about vestibular toxicity.

The incidence of nephrotoxicity of aminoglycosides or kanamycin only was 16.8% or 14.8%, using the increase of serum creatinine levels ≥27 µmol/l over the total period of treatment as the definition.

Incidences at the end of treatment were respectively 7.5% and 4.5%. Using the definition of serum creatinine increase ≥44 mol/l, the incidence decreases to 1.9% overall, and to 2.3% for kanamycin, at the end of treatment (Table 3). Incidences in the literature vary between 2% and 50%. Kahlmeter and Dahlager, in a review of aminoglycoside toxicity of clinical studies published between 1975 and 1982, found a 9.4% average incidence of nephrotoxicity for amikacin.[12,15] As far as we know, no investigations have been done recently on the nephrotoxicity of kanamycin.

One reason for the relatively low rate of nephrotoxicity found in our centre could be the understanding that a once-daily aminoglycoside regimen reduces the potential for toxicity.[20] Second, many different definitions of nephrotoxicity are used. Third, the study of different patient populations may produce very different incidences of nephrotoxicity. A complex combination of drug- and patient-related factors, such as dose duration, dose regimen, prior aminoglycoside treatment, choice of drug, associated drugs, patient age, prior renal or hepatic insufficiency, patient illness and hypovolaemia are involved in the development of nephrotoxicity. It has been noted before that the highest incidence of renal damage has occurred in severely ill populations.[7,12,14,18,21] In our study we did not include data on other medications that patients received, patient illness, volume depletion or prior hepatic insufficiency, so it is not possible to determine whether there were any interactions.

It is of note that only one of our patients had a serum creatinine value at the end of treatment that was higher than the normal values. Increased creatinine values can also be the result of increasing muscle mass. At the start of treatment, many patients are severely ill and often improve physically during treatment.

We found that patients who developed nephrotoxicity had a significantly longer duration of treatment with aminoglycosides, and received larger total doses. None of the patients had a creatinine level above the normal values suggestive of pre-existing renal failure (Table 4).

As there was no follow-up of most patients after the end of treatment, we cannot say anything about the long-term reversibility of damage to the kidneys.
Both hearing loss and nephrotoxicity (increase of serum creatinine ≥27 µmol/l) developed in five patients and was seen at the end of treatment in two patients.

There are some limitations to this retrospective study: the lack of many baseline audiograms, the variability in the frequency of obtaining audiograms and serum creatinine tests, the lack of data about vestibular dysfunction, other medications the patients received, the patients' illnesses, volume depletion and prior hepatic insufficiency, and the different aminoglycosides and schedules used. A prospective study based on the frequency of serum creatinine levels and of obtaining audiograms may clarify the frequency of evaluations, while a prospective study based on dosing schedules and serum aminoglycoside levels may clarify the differences in toxicity.

CONCLUSIONS

In our study, age, sex, total duration of aminoglycoside treatment, total aminoglycoside dose and serum creatinine level before the start of treatment were not significantly associated with the development of hearing loss. Patients developing nephrotoxicity had a significantly longer duration of treatment with aminoglycosides and received larger total doses, a significance we did not find in hearing loss. In the long-term treatment of tuberculosis with aminoglycosides, hearing loss seems to be a greater problem than nephrotoxicity.

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b | Reduced chance of hearing loss associated with Therapeutic Drug Monitoring of Aminoglycosides in the treatment of Multidrug Resistant Tuberculosis

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Submitted

Keywords: pharmacokinetics, pharmacodynamics, amikacin, TDM, tuberculosis
Key points: the occurrence of nephrotoxicity and ototoxicity was not significantly correlated with therapeutic parameters in the treatment with aminoglycosides, yet the extent of ototoxicity was correlated with the dose per kg bodyweight.

ABSTRACT

Hearing loss and nephrotoxicity are associated with prolonged treatment duration and higher dosage of amikacin and kanamycin. In our Tuberculosis Center, we have employed therapeutic drug monitoring (TDM) targeting pre-set pharmacokinetic/pharmacodynamic (PK/PD) surrogate endpoints in an attempt to maintain efficacy while preventing (oto-)toxicity. To evaluate this strategy, we retrospectively evaluated medical charts of TB patients treated with amikacin or kanamycin in the period 2000 - 2012.

Patients with culture-confirmed multi- or extensively drug resistant tuberculosis (MDR/XDR-TB) receiving amikacin or kanamycin as part of their TB treatment for at least 3 days were eligible for inclusion in this retrospective study. Clinical data, including C_{max}, C_{min} and audiometry data were extracted from the patients' medical charts.

80 patients met the inclusion criteria. The mean weighted C_{max}/MIC ratio obtained from 57 patients was 31.2 for amikacin and 12.3 for kanamycin. The extent of hearing loss was limited and correlated with the cumulative drug dose per kg body weight during daily administration. At follow-up, 35 (67.3%) of all patients had successful outcome; there were no relapses.

At a median dose of 6.5 mg/kg a correlation was found between the dose per kg bodyweight during daily dosing and the extent of hearing loss in dB at 8000 Hz. This study suggests that the efficacy at this lower dosage is maintained with limited toxicity. A randomized controlled trial should provide final proof of the safety and efficacy of TDM-guided use of aminoglycosides in MDR-TB treatment.

BACKGROUND

Amikacin and kanamycin are almost similar aminoglycosides and are both are considered very useful as second line injectable drugs for the treatment of multidrug resistant tuberculosis (MDR-TB)(1). MDR-TB is caused by *Mycobacterium tuberculosis* resistant to at least isoniazid and rifampicin. Although *in vitro* activity of amikacin and kanamycin appeared high against *M. tuberculosis* (2, 3), early bactericidal activity was low (4). In addition, extremely resistant TB (XDR-TB) is resistant to at least one aminoglycoside and any fluoroquinolone.

According to World Health Organization (WHO) guidelines, aminoglycosides are administered in a dose of 15 mg/kg/day with a maximum of 1000 mg daily in the treatment of patients with MDR-TB (5). Although cross resistance between amikacin and kanamycin is thought to be nearly complete (6-7) isolates resistant to one may still be susceptible to the other aminoglycoside and in vitro susceptibility should therefore be evaluated for each drug (8). Toxicity of aminoglycosides is profound and permanent, and hearing loss and nephrotoxicity have been observed in 8-37% of the patients receiving these drugs for any period of time (9-11). These adverse effects may aggravate with prolonged treatment and higher dosage (1). In a study based on data of 28 TB patients in Botswana treated with 15-25 mg/kg amikacin daily, 7 patients developed hearing loss. The cumulative area under the curve (AUC) and duration of amikacin treatment were predictors of hearing loss (12).

Aminoglycosides are not metabolised – renal excretion is the only elimination pathway. Patients with increased serum creatinine values and/or nephrotoxic co-medication run a higher risk for encountering nephrotoxicity (1). Because of these serious adverse events monitoring is advised and should consist of a baseline evaluation (audiogram, vestibular testing, Romberg testing and serum creatinine measurement) and a monthly evaluation during treatment (questionnaire regarding auditory or vestibular symptoms and serum creatinine) (1). Aminoglycoside-related ototoxicity generally manifests first at high frequencies, sometimes without the patients noticing their hearing loss (13). Regular monitoring gives the opportunity to alter the provided therapy in order to prevent more extensive hearing loss.

Pharmacokinetic (PK) and pharmacodynamic (PD) parameters have increasingly gained attention in the development of drugs and treatment of TB in recent years (14). Data regarding PK and PD parameters in TB are however scarce. For other bacterial infections, predominantly Gram-negative infections, e.g., caused by *Pseudomonas aeruginosa*, the maximum concentration (C_{max}) to mean inhibitory concentration (MIC) ratio is the most relevant PK/PD parameter to assess the efficacy of aminoglycosides (15-16). Additionally, it was shown that PK parameters of aminoglycosides may vary and the patients may benefit from individualized treatment (17-21). In our TB Center, we have used PK/PD parameters

targeting a surrogate endpoint of a C_{max}/MIC ratio >20, in an attempt to maintain efficacy while preventing (oto-)toxicity Therefore we performed a retrospective survey to evaluate the PK parameters of amikacin and kanamycin to detect predictors for PK parameters, as well as efficacy and toxicity.

PATIENTS, MATERIALS AND METHODS

In this retrospective study we evaluated all patients with culture-confirmed MDR-TB or XDR-TB, either pulmonary or extrapulmonary, receiving amikacin or kanamycin as part of their TB treatment for at least three days (steady state) who were hospitalized at the Tuberculosis Centre Beatrixoord between 1st of August 2000 and 16st of May 2012. Only patients older than 17 years were included. As retrospective data were collected the Institutional Review Board of the University Medical Center Groningen waived the requirement for research subjects to give informed consent (METc 2013/492).

Data collection

Medical history, age, sex, weight, length, ethnicity, co-morbidity, type of diagnosis, localisation of TB, MIC of amikacin and kanamycin, resistance pattern, dose and duration of TB treatment, creatinine levels at baseline and adverse events (hearing loss and renal dysfunction) were collected from the patients' medical records. Parameters such as the cumulative dose and the dose per kg bodyweight were calculated based on the gathered data. Serum levels of routine TDM of amikacin and kanamycin and the MIC of the sputum isolates were also retrieved from the patients' records. Adverse events were monitored using audiometric monitoring and the determination of the serum creatinine as described below.

Serum level measurements

C_{max} samples, obtained 30 min after a one-h infusion, and C_{min} samples obtained immediately before infusion were collected. Amikacin concentrations were determined by fluorescence polarization immunoassay (TDx or Architect, Abbott laboratories, Illinois, USA) with a lower limit of quantitation (LOQ) of 1.5 mg/L. Kanamycin concentrations were determined using a validated analytical method using liquid chromatography with coupled tandem mass spectrometry (TSQ Quantum, Thermo Fisher, San Jose, CA, USA) with a LOQ of 0.1 mg/L (22).

Drug susceptibility testing

The sputum isolates were subjected to drug susceptibility testing for amikacin and kanamycin at the Dutch National Mycobacteria Reference Laboratory (National institute

for Public Health and the Environment; RIVM). The Middlebrook 7H10 agar dilution method was applied for drug susceptibility testing of the isolate(s) (23). Drug susceptibility testing was not repeated during the treatment, except when the physicians expected the development of drug resistance based on clinical non-improvement. Sputum samples for microscopy (fluorescent staining) and culture were collected weekly and were sent to the national reference laboratory for analysis.

PK/PD analysis

The C_{max}/MIC ratio and time to sputum and culture conversion was calculated and considered to be a proxy parameter for efficacy. The aminoglycoside dose was adjusted based on the amikacin and kanamycin concentration and MIC.

Based on the peak and through levels, the $AUC_{0\text{-}24h}$ was estimated with the use of a validated population pharmacokinetic model using MW/Pharm 3.81 (Mediware, The Netherlands) (24). The C_{max}/MIC was consecutively calculated by dividing the C_{max} by the median MIC of 1 mg/L (amikacin) and 2.5 mg/L (kanamycin). A weighted C_{max}/MIC was calculated for each patient by the following formula:

$$weighted\ \frac{C_{max}}{MIC} = \frac{\sum days\ of\ treatment\ with\ dose\ X * \frac{C_{max}\ attained\ using\ dose\ X}{MIC}}{total\ treatment\ duration\ (days)}$$

Adverse events and clinical outcome

Adverse events of the aminoglycosides were assessed by evaluation of ototoxicity and renal function at baseline and during treatment. Audiometry was performed monthly at 250, 500, 1000, 2000, 4000 and 8000 Hz. Hearing loss was defined as 20 dB reduction in hearing threshold from baseline irrespective of side (right or left ear) or frequency (25). Audiometry was usually performed every 3 to 4 weeks during aminoglycoside treatment. Renal function was evaluated at least once a week by measuring creatinine in serum. Renal toxicity was defined as more than 50% increase in the baseline serum creatinine concentration at any moment during the treatment, in accordance with the common toxicity criteria (CTC) (26). Treatment outcome was evaluated two years after completion of treatment using common WHO criteria (27).

Statistics

All statistics were performed using SPSS 20 (SPSS, Virginia, IL). *M. tuberculosis* isolates showing no growth at <1 mg/L were statistically analysed as 1 mg/L. Differences in gender and type of aminoglycoside were assessed using Mann-Whitney U-tests. Determinants in nephrotoxicity and ototoxicity were also assessed using Mann-Whitney U-tests, except

for the gender (Chi-squared test), and the use of other co-medication (Fisher's Exact Test). Correlations between the extent of nephrotoxicity and ototoxicity and continuous or categorical factors were calculated using Spearman's coefficient. The correlation between clearance and distribution volume and the occurrence of side effects was assessed using Spearman's coefficient. The relation between the nephrotoxicity, classified by the CTC criteria as binary or categorical and demographic data was determined by Spearman's rank-order correlation test. Relations between the weighted C_{max}/MIC and time to sputum and culture conversion was assessed using simple linear regression and CART (CHAID) tree classification analysis. All P-values below 0.05 were considered significant.

RESULTS

Patient characteristics

Eighty patients with a median age of 30.5 (IQR; 25.0 – 39.0) years met the inclusion criteria; 37 (46.3%) patients were female and 43 (53.8%) were male. Patient characteristics at baseline are presented in table 1. Drug susceptibility testing was performed for all patients. All except three patients had a favorable outcome. One patient stopped due to drug addiction related problems and two patients were transferred to other hospitals without follow-up. Blood levels of 57 patients (71%) were retrievable from the patient files.

TABLE 1: Patient characteristics at baseline (total n = 80)

	N (%) or median (IQR)	
Common parameters	**Amikacin**	**Kanamycin***
Male (%)	26 (48.1)	17 (65.4)
Female (%)	28 (51.9)	9 (34.6)
Age (yr)	30 (25 – 39)	31 (25 – 40)
Weight (kg)	61.4 (55.2 – 68.4)	57.2 (50.0 – 68.2)
BMI (kg/m^2)	21.2 (19.4 – 23.6)	20.5 (18.5 – 22.4)
Ethnicity (%)		
-European	7 (13.0)	2 (7.7)
-Asian	17 (31.5)	4 (15.4)
-African	14 (25.9)	12 (46.2)
-Other	14 (25.9)	7 (26.9)
-Unknown	2 (3.7)	1 (3.8)

	N (%) or median (IQR)	
Tuberculosis		
Localisation		
Pulmonary (%)	42 (77.8)	19 (73.1)
Extra-pulmonary (%)	6 (11.1)	3 (11.5)
Both pulmonary and extra-pulmonary (%)	6 (11.1)	4 (15.4)
Drug Susceptibility		
MDR (%)	52 (96.3)	26 (100)
XDR (%)	2 (3.7)	0
Comorbidity		
Diabetes Mellitus type 1 (%)	3 (5.6)	1 (3.8)
Diabetes Mellitus type 2 (%)	3 (5.6)	1 (3.8)
HIV co-infection (%)	4 (7.4)	4 (15.4)
Creatinine level at baseline	64.0 (50.8 – 77.3)	69.5 (51.3 – 77.3)

Results are presented as median with interquartile range between brackets or as number of patients (n) with the percentage between brackets (%). BMI = body mass index; MDR = Multi Drug Resistant; XDR = extensively drug resistant; HIV = human immunodeficiency virus.

Pharmacokinetic and pharmacodynamics

All patients but one started with a daily dosing regimen with a median dose of 400.0 (IQR; 400.0 – 568.2) mg with a median duration of 85 (IQR; 60 – 111) days. From these patients, 36 patients continued their aminoglycoside treatment – after the initial daily treatment – in a 5 times-weekly regimen with a median dose of 400.0 (IQR; 387.5 – 500.0) mg and a median duration of 61 (IQR; 56 - 78) days. One patient did not receive the first daily dosing schedule and was treated with the 5 times-weekly regimen from start. After this 5 times-weekly regimen, 27 patients received a 3 times-weekly regimen with a median dose of 400.0 (IQR; 350.0 – 500.0) mg with a median duration of 61 (IQR; 54 - 82) days. Four patients immediately received the three times weekly regimen after the daily regimen. Co-medication used is displayed in table 2.

TABLE 2: Anti-tuberculosis medication (total n = 80)

Fluoroquinolones	**N (%)**
Levofloxacin	21 (26.3%)
Moxifloxacin	57 (71.3%)
Second line injectable agents	
Amikacin	54 (67.5%)
Kanamycin	25 (31.3%)
Both amikacin and kanamycin	1 (1.3%)
Capreomycin	2 (2.5%)
Other core second-line agents	
Linezolid	62 (77.5%)
Protionamide	52 (65.0%)
Clofazimine	64 (80.0%)
Cycloserine	8 (10.0%)
Add-on agents (D1)	
Pyrazinamide	32 (40.0%)
Ethambutol	58 (72.5%)
Add-on agents (D3)	
Thioacetazone	7 (8.8%)
Others / not classified	
Rifabutin	11 (13.8%)
Clarithromycin	11 (13.8%)
Azithromycin	3 (3.8%)
Co-trimoxazole	7 (8.8%)
Ciprofloxacin	4 (5.0%)
Ertapenem	7 (8.8%)

Treatment details are displayed in table 3. All C_{max} levels and AUCs are displayed in figure 1a and 1b. The trough level was below 3 mg/L in all patients. The C_{max} and AUC correlated both with the dose per kg bodyweight ($r = 0.53$ and 0.25, $p < 0.05$). The C_{max} and AUC were both not significantly different between both aminoglycosides ($P = 0.86$ and 0.61). The median dose per kg bodyweight was slightly, yet significantly, higher in male (6.7 mg/kg) in comparison to female patients (6.0 mg/kg; $P = 0.025$) for both aminoglycosides.

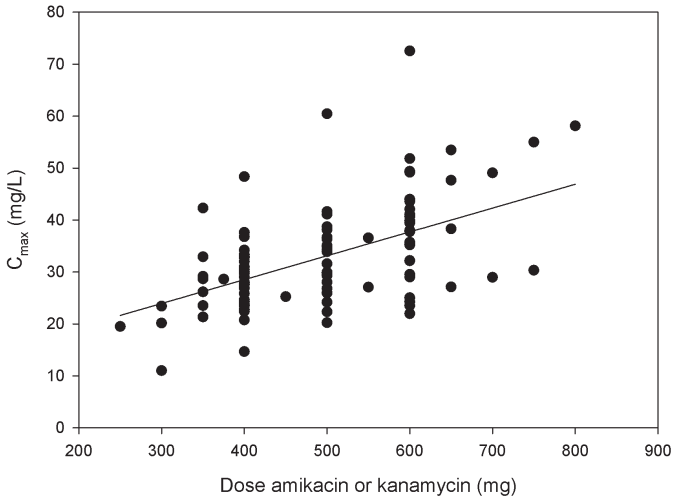

FIGURE 1A

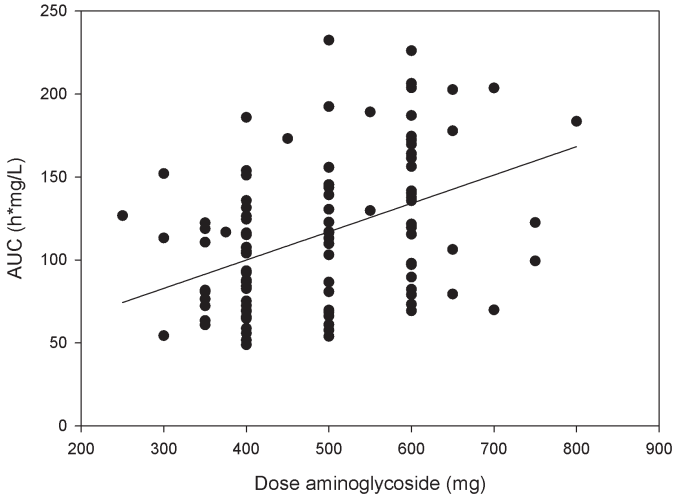

FIGURE 1B

The median treatment duration with amikacin was 166 days (IQR; 78 - 202 days) with a median cumulative dose of 791.0 (IQR; 522.0 – 1,281.6) mg/kg. With kanamycin, the median treatment duration was 124 (IQR 82 – 193) days with a median cumulative dose of 860.7 (IQR; 569.2 – 1,337.5) mg/kg. Treatment duration and cumulative dose were not significantly different between both aminoglycosides (P = 0.650 and P = 0.945) or between genders (P = 0.813 and P = 0.265).

TABLE 3: Treatment details and side effects

	n (%) / median (IQR)	
	Amikacin	**Kanamycin***
Common parameters		
Duration of hospital stay (days)	92.5 (67.3 – 162.3)	110.0 (90.5 – 186.5)
Duration of treatment with aminoglycosides (days)	138.0 (69.8 – 187.0)	104.0 (82.0 – 179.8)
Creatinine (µmol/L) after 90 days of treatment	80.0 (66.0 – 93.0)	77.0 (62.0 – 100.5)
Creatinine (µmol/L) l after 180 days of treatment	82.0 (70.0 – 95.0)	83.5 (67.8 – 101.5)
Observed side effects		
Nephrotoxicity *1	11 (22.9)	9 (34.6)
Ototoxicity *2	4 (9.1)	5 (21.7)

*1 Nephrotoxicity is defined as a serum creatinine of more than 1.5 times the baseline serum creatinine at any time during treatment

*2 Ototoxicity defined as reduced hearing at any frequency >20dB by audiometry, any time during treatment compared to baseline.

*The patient is included in the kanamycin results, as this aminoglycoside represented the largest treatment period.

The median MIC value for amikacin and kanamycin was, with and without resistant cases (MIC > 5 mg/L) 1.0 mg/L (range 1 – 20 mg/L, n = 67) and 2.5 mg/L (range 1 – 20 mg/L, n = 12), respectively. The achieved mean weighted C_{max}/MIC was 25.0 for both aminoglycosides. With amikacin, the mean weighted C_{max}/MIC was 31.2, while a mean weighted C_{max}/MIC of 12.3 was obtained using kanamycin. The mean cumulative $AUC_{0\text{-}24h}$ was 15,205 mg/L*h*days for amikacin and 15,518 mg/L*h*days for kanamycin.

Adverse events and clinical outcome

Serum creatinine levels of 20 patients (25.0%) were considered elevated as displayed in table 3. All except six patients were classified as a grade 1 toxicity, five patients as grade 2 toxicity and 1 patient as grade 3 toxicity according to the CTC (26).

The total dose (P = 0.230), duration (P = 0.301), weighted C_{max} (P = 0.824), cumulative AUC (P = 0.970), age (P = 0.404), body weight at the start of the treatment (P = 0.121) and BMI were all non-significantly related to the occurrence of nephrotoxicity. All co-administrated drugs were also non-significantly related to nephrotoxicity (P > 0.05, Fischer's exact test), except for the drug co-trimoxazole (P = 0.01, n = 7), ethambutol (P = 0.034, n = 58) and levofloxacin (P = 0.044, n = 21). Cycloserine was also correlated with the occurrence of

nephrotoxicity (P = 0.02, Fishers' exact test). Five patients on cycloserine developed some extent of nephrotoxicity. Nephrotoxicity occurred already before the start of cycloserine.

Regression analysis on the different grades of nephrotoxicity and the factors mentioned did not reveal independent predictors for toxicity; see table 4. Furthermore, no significant increase of the incidence of nephrotoxicity was observed with diabetes mellitus type 2 (Mann-Whitney U-test p = 0.404). The relation between diabetes mellitus type 1 and nephrotoxicity showed a non-significant trend (P = 0.079). In addition, we performed several probit models in order to establish possible factors associated with the occurrence and extent of nephrotoxicity. However, the cumulative AUC, weighted trough and treatment duration did not correlate with the occurrence and extent of nephrotoxicity.

TABLE 4: Spearman correlations of different factors predicting nephrotoxicity

Classification		Total dose	Total duration	Dose mg/kg	Baseline serum creatinine
CTC > 50% binary*1	P =	0.226	0.313	0.159	0.000*
CTC >50% regression*2	P =	0.200	0.321	0.220	0.001*

* Significant at 95% significance level
*1 Serum creatinine above 50% of the baseline at any moment during treatment as defined by the common toxicity criteria [26]

Audiometry results were available in 70 patients (87.5%), generally at the start of the aminoglycoside treatment and thereafter every 3 – 4 weeks. The results of the audiometry showed hearing loss in 9 patients (11.3%, table 3), predominantly at higher frequencies (4000 and 8000 Hz). The mean hearing loss was 37.5 dB (range 25.0 – 50.0) at 4000 Hz and 46.1 dB (range 25.0 – 70.0) at 8000 Hz. Cumulative dose (P = 0.421), dose per kg bodyweight (P = 0.741), duration (P = 0.644), bodyweight (P = 0.978), gender (P = 0.386), age (P = 0.155) and BMI (P = 0.432) did not correlate with the occurrence of ototoxicity.

The $AUC_{0\text{-}24h}$, weighted C_{max} and duration of therapy did not relate to the occurrence or extent of ototoxicity using Probit models. Also, the weighted C_{max} was not related to the occurrence and extent of hearing loss (P > 0.05). Furthermore, none of all co-administrated drugs correlated with ototoxicity (P > 0.132). The administration of cycloserine was also not correlated with the occurrence of ototoxicity (P = 0.66, Fishers' exact test). In total, eight patients used cycloserine, of which one patient experienced hearing loss.

Regression analysis was performed on the extent of hearing loss at 8000 Hz in decibels (dB) of all patients with hearing loss (n = 9). The dose received during the daily regimen was correlated with hearing loss in dB at 8000 Hz (P = 0.004, R = 0.851).

Data on clinical outcome were available of 52 patients. Of all patients, 35 (67.3%) had successful outcome, fifteen patients were lost to follow-up (28.8%) and two patients (3.8%) died within the follow-up period of 2 years. None of the patients had a documented treatment failure or relapse. Simple linear regression between the weighted C_{max}/MIC and time to sputum and culture conversion did not reveal any linear relationship (P = 0.44 and 0.64, respectively). In addition, we performed a CART (CHAID) tree classification analysis to establish any links between Cmax/MIC, cumulative dosage and time to sputum and culture conversion. However, this did not yield any significant results.

DISCUSSION

This study showed a low level of hearing loss in the investigated cohort, predominately in high frequencies as expected. Treatment outcome in patients receiving aminoglycosides given in a lower TDM guided dose, was good. This may be explained by the fact that C_{max} was related to the MIC in individual patients. Although of retrospective nature these findings are important as amikacin and kanamycin form the cornerstone of today's MDR-TB treatment.

A recent prospective study using classification and regression tree (CART) analysis showed that a cumulative AUC of amikacin above 87,232 mg/L*h*days significantly increases the probability of ototoxicity to 10% (12). This study in 28 patients, 10 of whom had earlier aminoglycoside exposure, found audiometry-confirmed hearing loss in 7 (25%) of the patients studied. The peak and trough concentration of amikacin did not correlate with the occurrence of ototoxicity. By using blood concentration guided dosing, our mean cumulative AUC was well below this threshold of 87,232 mg/L*h*days, which could explain the relatively low incidence of ototoxicity in our population. This should be an argument for minimizing the cumulative AUC during aminoglycoside treatment.

The occurrence of ototoxicity varies amongst different studies. According to the study of Peloquin *et al*. (28), the incidence of hearing loss after treatment with aminoglycosides was 37%. De Jager *et al*. found an incidence of 21.3% during treatment (9). This is higher than in our study, with an incidence of hearing loss in 11.3% of all patients. No difference in demographics was found between the group with and without ototoxicity. Therapeutic parameters, particularly dose, cumulative dose, duration and C_{max}, were all non-significantly correlated with ototoxicity; making ototoxicity prediction with these parameters not possible. The lack of relationship between C_{max} and the daily dose is consistent with a previous study (28). It has, however, previously been shown that the duration of treatment and the cumulative dose are associated with the occurrence of ototoxicity. However, the cumulative $AUC_{0\text{-}24h}$ in our population did not reach the threshold value of 87,232 mg/L*h*days (12), which could explain this difference.

Based on the above, regular audiometry should be common practice (29). This regular audiometry could be difficult in programmatic settings due to logistical problems or lack of equipment and trained personnel. However, it has been shown that audiological monitoring using a smartphone connected to headphones, preferable with passive noise cancelling, correlates well with professional audiometry (30, 31). This could be a viable option in developing countries. When there is evidence of ototoxicity, a possible solution could be to administer the aminoglycosides five times or even three times a week, according to WHO guidelines (32, 33). The effect of this dosing regimen on the clinical efficacy has however not been established. When reducing the dose, recommendations on the C_{max}/MIC ratio need to be taken into account to avoid loss of efficacy (34, 35).

The prevalence of nephrotoxicity in our study was comparable with an earlier report from our Center (16.8%) (9) and with the report by Peloquin *et al*. (11.6%) (28). No significant influence of different factors on either the occurrence or the extent of nephrotoxicity was found. This finding is in line with the earlier study of Peloquin *et al* (36). The results of the current cohort are in contrast with an earlier study from January 1995 to July 2000 performed in our center (9). In the earlier cohort, the total dose and duration of the aminoglycoside therapy were significantly correlated with nephrotoxicity. Applied doses in the earlier study were, however, more than a two-fold higher than the dose used in our study (750 – 1000 mg vs. 400 mg). It is, however, questionable whether the serum creatinine is the right tool to measure nephrotoxicity. A raise in serum creatinine could also be related with increased muscle mass and weight gain, which is often seen during successful TB treatment.

The use of co-trimoxazole was correlated with the occurrence of nephrotoxicity. Co-trimoxazole, a combination of trimethoprim and sulfamethoxazole, is known to increase the serum creatinine, since trimethoprim decreases the tubular secretion of creatinine (37, 38). This finding is supported by the fact that a clear time relationship between the co-trimoxazole administration and the elevation in serum creatinine was found in 5 out of 6 patients. The serum creatinine value has, however, limited predictive value during treatment with co-trimoxazole due to the specific inhibition of clearance of the creatinine molecule.

The dosage applied in our study is a two-fold lower than the 15 mg/kg recommended by the WHO, (5) yet outcome was favourable in the vast majority of patients, and in those with unfavourable outcome, aminoglycoside dosage was not a predictor of poor outcome. All but 3 patients completed their treatment and were well when discharged after a median of 150.5 days of treatment. This showed that the therapy provided was effective. This is

supported by the finding that of all patients with follow-up data, 35 (67.3%) did not have a relapse after 2 years. We therefore hypothesize that the dose of aminoglycosides can be decreased, taking into consideration that the C_{max}/MIC recommendations are met, when co-administrated with other highly active medication, such as linezolid, clofazimine and moxifloxacin, without apparent loss of efficacy.

Dosing based on the C_{max}/MIC of aminoglycosides should be used rather than dosing based on body weight in order to improve treatment outcomes, as the C_{max}/MIC is correlated with clinical outcome. This means that analytical techniques in order to analyse amikacin or kanamycin in serum with high throughput rates should be made available in all TB programmes to deliver fast and accurate results. In addition, simple drug susceptibility testing in order to establish a precise MIC value should also be available (39). Both PK and PD analysis requires trained and experienced personnel with equipment. However, it would be feasible to centralize these facilities in order to concentrate knowledge and reduce costs.

With accurate dosing based on the C_{max}/MIC, the cumulative AUC can be minimized in order to reduce ototoxicity. It should be noted that the cumulative AUC threshold value of 87,232 mg/L*h*days was established in a prospective study with only 28 patients (12) and its validity needs to be tested in larger cohorts. With our proposed limited sampling strategy, the $AUC_{0\text{-}24h}$ can be predicted with only 2 serum samples (24), which can be analysed in a centralized laboratory in order to estimate the $AUC_{0\text{-}24h}$. Treating physicians should be aware of the patients' cumulative $AUC_{0\text{-}24h}$ in order to reduce or possibly avoid hearing loss. It should be noted that the trough level of aminoglycosides should not be used to change the dose and to assess the risk of ototoxicity. In addition, there is a large variation in C_{max} and $AUC_{0\text{-}24h}$ (and thus efficacy and toxicity) as shown in figure 1a and 1b, which cannot be explained by the administered dose alone. This is an additional reason to use PK/PD guided dosing.

One limitation of this study was the rather imprecise method to determine the MIC. We analysed the MIC of amikacin <1 mg/L as 1 mg/L in our statistical analysis, however, it has been shown that many isolates have MICs below 1 mg/L for amikacin (40). Therefore, the weighted $C_{max/}$MIC could be higher for amikacin than reported, increasing its efficacy.

After more than 30 years of medical practice prescribing aminoglycosides in a dose of 15 mg/kg, we believe that a formal study is warranted between standard of care, and an individualised approach based on drug susceptibility and drug concentrations. With the dosage of 6.5 mg/kg used in this study and the old breakpoint MIC of 2 mg/L for amikacin

and 5 mg/L for kanamycin determined using the Middlebrook 7H10 agar method (41), the C_{max}/MIC ratio would be 12.5 and 5. However, the median MIC found in this study is lower than the breakpoint MIC found and sufficient C_{max}/MIC ratios were reached. In vitro testing using a hollow fiber infection model should be performed to detect the optimal C_{max}/MIC ratio as has already been done for other anti-TB drugs (42-45). Combining amikacin or kanamycin with other drugs in this setup seems rational since the treatment of MDR-TB is based on a treatment regimen with a combination of anti-TB drugs. Additional effect of single drugs in a multidrug regimen can therefore be evaluated. Based on these data a new MDR-TB dosing strategy can be designed to improve efficacy while toxicity may be reduced.

In conclusion, a lower, TDM-guided dosage of aminoglycosides resulted in an acceptable treatment outcome with relatively low percentages of hearing loss. However, this approach should be validated in a prospective randomized trial.

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c | Limited sampling strategies for Therapeutic Drug Monitoring of amikacin and kanamycin in Patients with Multidrug-Resistant Tuberculosis

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ABSTRACT

Amikacin and kanamycin are considered important and effective drugs used in the treatment of multidrug resistant tuberculosis. Unfortunately, the incidence of toxicity is high and related to high drug exposure. To balance between efficacy and toxicity a population pharmacokinetic model may help to optimize drug exposure. MDR-TB patients who had received amikacin or kanamycin as part of their treatment and had routinely received therapeutic drug monitoring were evaluated. A population pharmacokinetic model was developed and subsequently validated. Using this model a limited sampling model was developed. Eleven patients receiving amikacin and nine patients receiving kanamycin were included in this study. Median observed $AUC_{0\text{-}24h}$ was 77.2 (IQR; 64.7– 96.2) mg*h/l for amikacin and 64.1 (IQR; 55.6 – 92.1) mg*h/L for kanamycin. The pharmacokinetic model was developed and validated using n-1 cross-validation. An limited sampling model was developed based on two samples obtained at 1 and 4 hours after administration with an R2 of >0.99 and a bias and Root Mean Squared Error of -0.04% and 2.5%, respectively. We developed a robust population model that is suitable for predicting the $AUC_{0\text{-}24h}$ of amikacin and kanamycin. This model in combination with the limited sampling strategy developed can be used in daily routine to guide dosing but also to assess $AUC_{0\text{-}24h}$ in phase III studies.

KEYWORDS: amikacin, kanamycin, tuberculosis, pharmacokinetics, pharmacokinetic model, limited sampling

1. INTRODUCTION

Tuberculosis is a life-threatening disease. Around 1.4 million people die as consequence of this disease every year [1]. Multidrug resistant tuberculosis (MDR-TB) is caused by strains of *Mycobacterium tuberculosis* resistant to at least rifampin and isoniazid. In 2011, an estimated 310,000 of all newly reported TB cases had MDR-TB [1]; and in the most recent WHO report on TB, the incidence of MDR-TB is estimated at around 480,000 [2]. Treatment success is associated with prolonged duration of therapy of a minimum of 18 months with second line drugs [3].

Amikacin and kanamycin are classified as group 2 - injectable agents - for the treatment of MDR-TB [4]. Recommended dosages are 15 – 20 mg/kg with a maximum of 1000 mg daily for both amikacin and kanamycin [4]. The reported minimal inhibitory concentration (MIC) of amikacin and kanamycin is 0.5-1 mg/L and 1-2 mg/L, respectively [5].

The pharmacodynamic index of aminoglycosides is usually quantified in the maximal blood concentration (Cmax) divided by the MIC. Aminoglycoside dosing regimens with multiple doses per day were designed to reach certain Cmax levels, while minimizing Cmin levels was required to avoid toxicity. However, in order to detect inter- and intra-individual differences in clearance or distribution volume, the area under the curve (AUC) might be a more sensitive pharmacokinetic parameter in comparison with the Cmax or Cmin [6].

Inter-individual variation in pharmacokinetics may contribute to toxicity and effectiveness. Zhu et al. claimed that the AUC of streptomycin in 19 patients differed from 124 – 680 µg·hr/ml while the Cmax differed from 9 – 107 µg/ml [7]. Inter-individual variation in Cmax was also observed for amikacin (median 46 mg/L, range 26-54 and kanamycin (median 44 mg/L, range 33-65) [8]. This urges the need for a pharmacokinetic model to assess inter-individual variability.

Side effects of aminoglycosides are ototoxicity and nephrotoxicity. The prevalence of ototoxicity varies from 18% [9] to 37% [8] and nephrotoxicity varies from 7.5% [9] to 15% [8]. Treatment duration and the cumulative dose were correlated with these side effects, and not the dose, or the dosing frequency [8-10]. In addition to the cumulative dose, the cumulative $AUC_{0\text{-}24h}$ is also related to both nephrotoxicity and ototoxicity [11-13]. A retrospective evaluation of a Dutch cohort showed that an MDR-TB treatment regimen including aminoglycoside drug concentration guided dosing resulted in high effectiveness with excellent treatment outcome, without severe adverse drug reactions [14]. During the study period, we observed no treatment failures, nor any documented relapses using this relatively low dose of aminoglycosides in an analysis of all MDR-TB patients diagnosed and treated in the Netherlands [14]. A population pharmacokinetic model makes it possible to prospectively acquire pharmacokinetic data of aminoglycosides in the treatment of TB in order to design new optimized regimens in the treatment of MDR-TB.

As collecting full blood plasma curves of amikacin or kanamycin to estimate the $AUC_{0\text{-}24h}$ and clearance (CL) is expensive and burdensome for patients, a limited sampling strategy to perform TDM will help to improve pharmacotherapy and reduce costs [15]. The objective of this study is to develop a population pharmacokinetic model of amikacin and kanamycin to assess both the $AUC_{0\text{-}24h}$ and Cmax based on retrospective data. This model could be used in a prospective study to evaluate both toxicity and efficacy. Furthermore, a limited sampling strategy will be designed using this pharmacokinetic model.

2. MATERIALS AND METHODS

2.1. Study population

All patients at the Tuberculosis Center Beatrixoord (University Medical Center Groningen (UMCG), University of Groningen, Haren, the Netherlands) who were diagnosed with MDR-TB after January 1st 2000 and met the inclusion criteria were included in this retrospective study. Inclusion criteria included age (≥ 18 years), treatment with amikacin or kanamycin longer than 2 days, availability of at least 3 plasma concentrations from one dose at the same day. Medical and demographic data were collected from the medical records. Demographic data included age, length and body weight at start of treatment. Medical data included the aminoglycoside used, the administered dose and serum creatinine at baseline. This study was evaluated by the local ethics committee (IRB 2013-492) and was according to the Dutch law allowed due to its retrospective nature. Drug susceptibility was determined using the Mycobacteria Growth Indicator Tube (MGIT) method by the Tuberculosis Reference Laboratory of the National Institute for Public Health and the Environment (RIVM, The Netherlands).

2.2. Pharmacokinetics

Data on the plasma concentration of the patients included were retrieved from the laboratory information system. Blood analyses were performed with a validated liquid chromatography mass spectrometry (LC-MS/MS) (amikacin and kanamycin) [16] or with a validated Axsym (amikacin) (Abott, Chicago, IL) method. Both methods were validated on precision and accuracy according to the FDA guidelines [17]. All pharmacokinetic calculations were performed using MW\Pharm 3.81 (Mediware, Groningen, the Netherlands) [18]. Individual pharmacokinetic parameters, including AUC, half-life, clearance, distribution volume and the elimination rate constant were calculated using the KinFit module of MW\Pharm using one-compartment analysis.

For amikacin and kanamycin, a model was developed separately using MW\Pharm using a one-compartment model as described earlier [19]. We were not able to evaluate the performance of a two-compartment model, since there the number of samples at

the elimination phase of the curve was insufficient. Differences in pharmacokinetic parameters between both aminoglycosides were analysed using Mann-Whitney U-tests.

Furthermore, a final model was developed with the amikacin and kanamycin curves combined. The distribution of the parameters of the final model developed was assessed by histograms generated by MW\Pharm. Furthermore, the predicted concentrations were compared with the observed concentrations using residual plots. The influence of the covariates age, weight, height, gender, body surface area, lean body mass and creatinine clearance on the renal elimination constant and distribution volume were tested for significance using MW\Pharm. The population parameters of the final model and their 95% confidence intervals were calculated using a bootstrap method (n = 1000).

The elimination constant was calculated by the following formula: Kel = Kelm (metabolic elimination rate constant) (fixed to 0) + Kelr (renal elimination rate constant) * CLcr (creatinine clearance in ml/min/1.73m2). The free fraction was estimated at 0.04 – 0.08. The fat distribution was estimated at 0.4. Assay errors were set to 0.1 + 0.035*[measured concentration], which captured the variation of both methods.

2.3. Limited sampling strategies

A pharmacokinetic population model was developed using the KinPop module of MW\Pharm. This module uses an iterative two-stage Bayesian population procedure [20]. The pharmacokinetic parameters were assumed to be log-normally distributed. The Kelr and distribution volume V1 used to calculate the limited sampling strategies was calculated by the pharmacokinetic model (shown in table 3).

Using Monte Carlo simulations, plasma concentrations at 8 points in 8 hours were calculated for 1,000 virtual patients. Only models to optimize AUC were developed. Only practical sampling strategies were evaluated with a minimum time span between two sampling points of 1 hour with a maximum of 8 hours after administration. Only strategies with an Root Mean Squared Error (RMSE) <10% were considered. The ability of the limited sampling model to predict the Cmax was assessed by entering both the T=1 and T=4 concentrations combined into the model. The difference between the model-predicted Cmax and the limited sampling predicted Cmax was calculated.

2.4. Statistics

All statistics were performed using SPSS 22 (SPSS, Virginia, IL). Validation of the pharmacokinetic model developed was performed by calculating new pharmacokinetic models based on experimental data of subsequently n – 1 patients, which was previously used successfully [21,22]. With this 'n-1' pharmacokinetic model, $AUC_{0\text{-}24h}$ of the excluded patient was calculated. The $AUC_{0\text{-}24h}$ calculated with the model was compared with the n-1 validation AUC with a Bland-Altman plot. Furthermore, all pharmacokinetic

parameters of the n-1 model, including the $AUC_{0\text{-}24h}$, were compared with the population pharmacokinetic model using Wilcoxon Signed Rank tests. Differences in pharmacokinetic parameters between amikacin and kanamycin were assessed using Mann-Whitney U tests. In addition, correlations between demographic and pharmacokinetic data were tested for significance with Spearman correlations or in the case of categorical data with Mann-Whitney U-tests.

3. RESULTS

In total, 30 plasma concentration curves were retrieved from the medical dossiers of 20 patients. Sample times of the individual curves varied between individuals and curves, with a maximum time span of 24 hours. Eleven patients had received amikacin 400 mg once daily, which resulted in 16 plasma concentration curves. In addition, 14 curves were retrieved from nine patients who had received kanamycin 400 mg once daily. The median BMI was 20.3 kg/m2 (IQR 18.8 – 22.0), with a median dose per kg body weight of 6.9 mg/kg (IQR 6.3 – 7.8). Demographic data is shown in table 1.

TABLE 1. Patients characteristics.

	Amikacin group (n = 11)		Kanamycin group (n = 9)	P-value
Sex [n (%)]				
Male	5	(45.5)	4 (44.4)	0.66[b]
Female	6	(54.5)	5 (55.6)	
Height (m)	1.75	(1.68–1.85)	1.62 (1.55–1.69)	0.02[c]
Weight (kg)	60.0	(57.0–70.4)	51.0 (46.3–58.4)	0.02[c]
Age (years)	26	(24–43)	31 (24.5–36.5)	0.75[c]
Dose/kg body weight (mg/kg)	6.67	(5.68–7.02)	7.85 (6.86–8.64)	0.02[c]
BMI (kg/m2)	20.2	(19.6–21.4)	20.5 (16.7–23.6)	0.82[c]
SCr (µmol/L)	64.0	(52.0–68.0)	59.5 (46.5–70.5)	0.88[c]

BMI, body mass index; SCr, serum creatinine.
[a] Data are median (interquartile range) except for sex.
[b] Fisher's exact test.
[c] Mann–Whitney U-test.

The median $AUC_{0\text{-}24hv}$ of amikacin (400 mg) was 77.2 (IQR; 64.7 – 96.2) h*mg/L. The median $AUC_{0\text{-}24h}$ of kanamycin (400 mg) was slightly lower: 64.1 (55.6 – 92.1) h*mg/L). The coefficient of variation of the $AUC_{0\text{-}24h}$ was 33%, indicating that the number of patients included in the model is sufficient to achieve a power level of >80% [23].

TABLE 2. Pharmacokinetic parameters of the population model.

	Median (IQR)		Median (IQR)	
	Amikacin model (n = 16)	**Kanamycin model (n = 14)**	**P-value[a]**	**Overall model (n = 30)**
CL (L/h)	4.62 (4.05–5.35)	5.30 (4.64–5.85)	0.270	5.07 (4.27–5.85)
V_d (L)	12.0 (9.14–15.3)	11.4 (8.50–13.5)	0.423	11.9 (8.70–13.9)
AUC_{0-24h} (h mg/L)	86.7 (75.1–99.0)	75.6 (68.4–86.5)	0.257	79.1 (68.5–93.9)
C_{max} (mg/L)	26.2 (22.7–34.4)	26.6 (24.0–32.7)	0.766	26.6 (23.5–35.9)

IQR, interquartile range; CL, clearance; V_d, volume of distribution, AUC_{0-24h}, 24-h area under the concentration–time curve; C_{max}, maximum serum concentration.
[a] Two-tailed exact Mann–Whitney U-test.

Population models of all amikacin and kanamycin curves at 400 mg were first built separately. The pharmacokinetic parameters of these models are displayed in table 2. All parameters were compared using Mann-Whitney U-tests, however, none of the parameter was significantly different between both models.

Therefore, we decided to pool the amikacin and kanamycin curves and to develop a new 'combined' model for both amikacin and kanamycin to include more variability in the model in order to increase the robustness of the model. In figure 1, a plot of the amikacin and kanamycin concentration time curves is shown. The population pharmacokinetic parameters and corresponding 95% confidence intervals are shown in table 3. The estimated $AUC_{0\text{-}24h}$ was 79.1 (IQR; 68.5 – 93.9) h*mg/L with a Cmax of 26.6 (IQR; 23.5 – 35.9) h*mg/L. This model was cross-validated using the proposed n-1 methodology. The RMSE in predicting the $AUC_{0\text{-}24h}$, T1/2, Vd, CL and Cmax was 0.36 h*mg/L, 0.004 h, 0.04 L, 0.004 L/h and 0.03 mg/L, respectively. A Bland-Altman plot concerning the $AUC_{0\text{-}24h}$ prediction is displayed in figure 2. One outlier was observed, with a deviation of ca. 2 h*mg/L in the $AUC_{0\text{-}24h}$.

TABLE 3. Population pharmacokinetic parameters.

Parameter	Mean (95% CI)	S.D. (95% CI)
k_{elr}	**0.00384 (0.00341–0.00432)**	**0.00143 (0.00113–0.00167)**
[h^{-1} (mL/min/1.73 m2)$^{-1}$]		
V_d corrected for lean body mass (L/kg)	0.2073 (0.1878–0.2284)	0.0664 (0.0456–0.0858)

CI, confidence interval; S.D., standard deviation; k_{elr}, renal elimination constant; V_d, volume of distribution.

The influence on the covariates age, weight, height, gender, body surface area, lean body mass and creatinine clearance on the renal elimination constant and distribution volume was tested for significance with MW\Pharm. The height (P = 0.0046) and creatinine clearance (P = 0.009) correlated with the renal elimination constant. In addition, gender (P = 0.037) correlated with the distribution volume.

TABLE 4. Limited sampling strategies. [a]

Time point(s) of sampling post-dose	r	Prediction bias (%)	RMSE (%)
2 h	0.984	−3.02	8.6
3 h	0.975	−0.96	10.2
4 h	0.944	0.63	14.9
1 h and 4 h	0.998	−0.04	2.5
1 h and 5 h	0.997	0.03	3.2
1 h and 3 h	0.997	−0.4	3.3
2 h and 4 h	0.996	−0.24	3.8
1 h and 6 h	0.996	0.27	4.1
1, 4 h and 5 h	0.999	−0.09	1.7
1, 4 h and 6 h	0.999	−0.09	1.8
1, 3 h and 5 h	0.999	−0.19	1.8
1, 3 h and 6 h	0.999	−0.19	1.8
1, 2 h and 5 h	0.999	−0.26	1.8

RMSE, root-mean-squared error.

[a] Strategies are sorted by RMSE. Only the top five limited sampling strategies with two and three sampling points are shown.

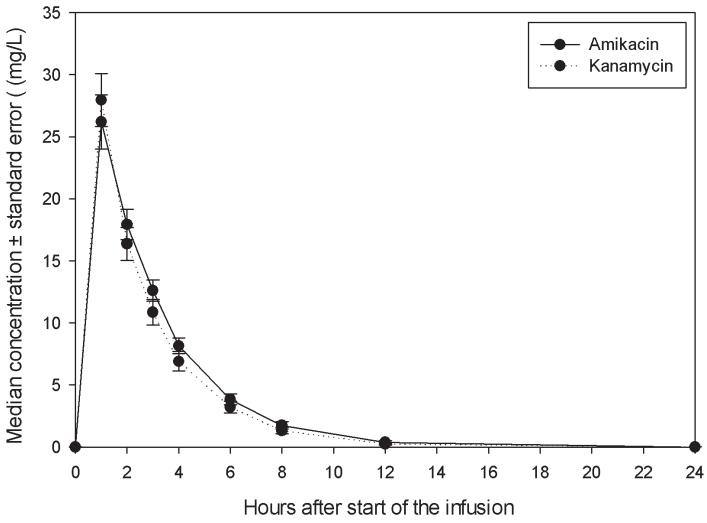

FIGURE 1.

The $AUC_{0\text{-}24h}$, CL, t1/2, Cmax, Tmax and distribution volume resulting from the n – 1 validation were compared with all curves fitted to the population pharmacokinetic model; all parameters showed no difference (AUC: p = 0.363, CL: p = 0.414, t1/2: p = 0.317, Cmax: p = 0.490, Tmax: p = 1.000, Vd: p = 0.472).

The volume of distribution per kg body weight was higher in men than in women (median: 0.24 vs. 0.19 L/kg, P = 0.022, Mann-Whitney U-test). The Cmax was higher in women (median: 29.2 in women vs. 23.3 mg/L in men, P = 0.012, Mann-Whitney U-test); however, the $AUC_{0\text{-}24h}$ was not significantly different (median: 78.3 (95% CI: 63.3 – 89.0) in men vs. 86.7 (95% CI: 71.4 – 111.7) h*mg/L in women, P = 0.285, Mann-Whitney U-test). Furthermore, volume of distribution, T1/2 and the Cmax correlated with the patients' body weight and height (Spearman correlations, two-tailed test of significance). The $AUC_{0\text{-}24h}$ was correlated with the Cmax computed by the model: $AUC_{0\text{-}24h}$ = 1.636*Cmax + 36.190 with a correlation coefficient (r) of 0.61 using simple linear regression.

Based on the 'combined' population kinetic model, we developed a limited sampling strategy based on a patient with an average weight (59.9 kg), height (1.68 m) and serum creatinine (63 mmol/L) and 35 years of age. Different limited sampling strategies were evaluated and subsequently the RMSE, bias and correlation coefficient of the AUC were calculated. These different limited sampling strategies are displayed in table 4. The RMSE is the most important parameter, since this indicates the precision in the prediction of the $AUC_{0\text{-}24h}$. Sampling at 1 and 4 after start of the infusion resulted in an RMSE of 2.5% with a

prediction bias of -0.04%, respectively. The Cmax calculated by the model was compared with the Cmax calculated by the model based only on the concentrations at T=1 and T=4. The median difference was -0.04% (IQR; -0.28 – 0.38%).

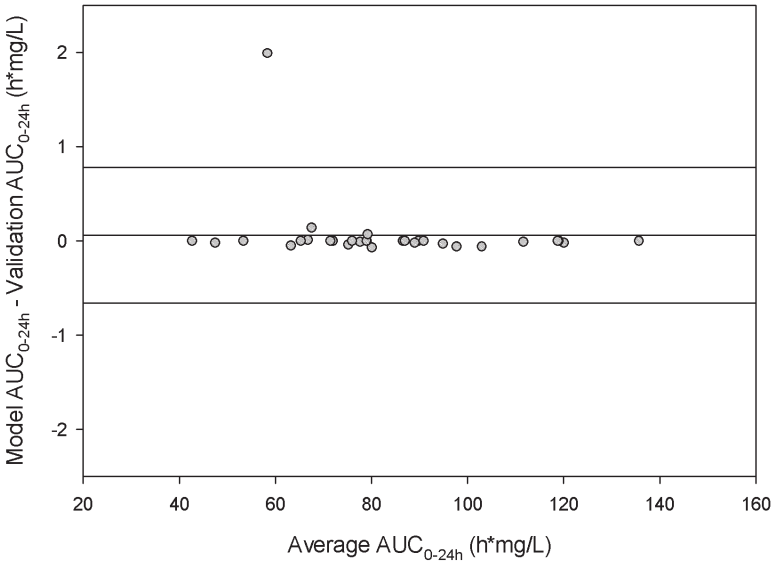

FIGURE 2.

4. DISCUSSION

We developed the first limited sampling strategy of amikacin and kanamycin in patients with tuberculosis. The RMSE found in predicting the $AUC_{0\text{-}24h}$ from samples at 1 and 5 hours is very low (2.9%). The model was successfully validated using the proposed n-1 cross-validation methodology. Since none of the relevant pharmacokinetic parameters showed a significant difference between amikacin and kanamycin, the final pharmacokinetic model was identical for both drugs. This model was considered appropriate for the assessment of individual pharmacokinetics during daily patient care. Furthermore, this limited sampling model could be used to assess drug exposure in randomized controlled trials evaluating efficacy of new regimens in the treatment of TB.

The pharmacokinetic parameters of the population model are higher than those in neutropenic patients (CL 5.07 vs. 4.43 L/h, Vd 11.9 vs. 8.92 L). This could be due to the use of a two-compartment model, while we used a one-compartment model. The authors found that the one-compartment model was unable to fit peaks and 12-24h trough levels. However, our model did not seem to have this disadvantage. [24]. We evaluated

2-compartment models which provided a slightly better fit to our data, however, these models provided unrealistic curves between 12 and 24 hours post-dose. A 1-compartment model did not seem to have this disadvantage.

The distribution volume per kg body weight of critically ill patients is higher (0.39 – 0.45 L/kg vs. 0.20 L/kg in this study) [25]. However, these critically ill patients were experiencing sepsis or a septic shock, and gained volume during the first hours of resuscitation explaining the higher distribution volume. A study with healthy volunteers showed that the pharmacokinetic parameters of amikacin are comparable with our population, except for the clearance, which is slightly lower in our population (V1 11.0-11.15 vs. 11.9 L, CL 6.8-7.6 vs. 5.07 L/h, depending on the amikacin dose of 7.5 or 15.0 mg/kg) [26]. This difference in clearance might be caused by the nephrotoxic potential of these aminoglycosides during an extended period of time or the simultaneous administration of other antibiotics in the treatment of TB. Due to the differences in population pharmacokinetics, it may be necessary to re-evaluate the proposed limited sampling strategy in other populations.
The distribution volume and Cmax appeared to be significantly different between genders. As women have commonly a higher percentage body fat in comparison to men, and aminoglycosides are very hydrophilic, this is an understandable correlation. In addition, the height and weight of women is generally lower than in men, which also affects the Cmax and Vd. When targeting a certain Cmax level, this would result in lower dosages for women, while the $AUC_{0\text{-}24h}$ was not significantly different between both genders.

Using the AUC/MIC ratio instead of the Cmax/MIC-ratio to monitor efficacy needs to be validated in an in vitro model for infection [27] and subsequently tested in a prospective clinical trial. Nevertheless, evidence in animal models suggests that the $AUC_{0\text{-}24h}$/MIC ratio predicts the efficacy of the aminoglycoside therapy [28], and we speculate that this ratio can also be applied to humans [29]. But this needs to be confirmed in a hollow fiber model as has already been done for moxifloxacin [27].

In our TB center, drug concentration-guided dosing of aminoglycosides is daily routine. The average dose given is 6.7 mg/kg, which is lower than the dose recommended by the World Health Organisation of 15 – 20 mg/kg [4]. Within our center, aminoglycoside dose is based on individualised treatment based on the Cmax/MIC ratio [30,31]. A retrospective study was performed to evaluate the treatment outcome with a treatment regimen incorporating this lower TDM-guided dosing and showed favourable results [14]. It should however be noted that an additional prospective study is necessary to confirm the efficacy of this relatively low dosage.

Although common practice, estimating the $AUC_{0\text{-}24h}$ with only a peak-level measurement (Cmax) appears to be unreliable with a correlation coefficient of only 0.61. The addition of a trough level 24 hours post-dose did not improve this estimation. However, measuring at 1 and 5 hours post-dose resulted in a high correlation of >0.99 and a low RMSE and bias. In

addition, a fair estimation of the $AUC_{0\text{-}24h}$ could be based on a one-point estimate 3 hours post-dose.

Oral drugs used in the treatment of MDR-TB show strong correlations between the $AUC_{0\text{-}24h}$ and the serum concentration 6 hours post dose [32]. The $AUC_{0\text{-}24h}$ of aminoglycosides can be easily predicted with the sample times used to assess the exposure of oral drugs. With this strategy, the estimation of the $AUC_{0\text{-}24h}$ of several anti-TB drugs with only two or three samples is possible.

Fluoroquinolones and aminoglycosides are the cornerstone of MDR-TB treatment, however, resistance development and toxicity are causes for concern. Treatment with fluoroquinolones, such as moxifloxacin, can be optimized using PK/PD modelling [22]. With this work we have shown that the assessment of the aminoglycoside exposure using a limited sampling strategy is accurate. This limited sampling strategy provides a good estimation of the $AUC_{0\text{-}24h}$ and is therefore suitable for use in outpatient clinics, but also during TDM in prospective clinical trials.

5. CONCLUSIONS

This study showed that the $AUC_{0\text{-}24h}$ of amikacin and kanamycin can be predicted using a limited sampling strategy in combination with the developed population pharmacokinetic model. This strategy can be used to optimize TB treatment by reducing toxicity while maintaining efficacy but may also be included in phase III studies to collect data on drug exposure.

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Chapter 5

Ertapenem

a | Pharmacokinetics of ertapenem in patients with multidrug-resistant tuberculosis

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KEYWORDS: Ertapenem, multidrug-resistant tuberculosis, pharmacodynamics, pharmacokinetics, safety.

Abstract

Treatment of multidrug resistant (MDR) and extensively drug resistant (XDR) tuberculosis (TB) is becoming more challenging because of increased level of drug resistance against second line tuberculosis drugs. One promising group of antimicrobial drugs are carbapenems. Ertapenem is an attractive carbapenem for the treatment of MDR and XDR-TB because its relative long half-life enables once daily dosing.

A retrospective study was performed for all MDR-TB suspected patients at the Tuberculosis Center Beatrixoord of University Medical Center Groningen (Haren, The Netherlands) who received ertapenem as part of their treatment regimen between the first of December 2010 and the first of March 2013. Safety and pharmacokinetics were evaluated.

Eighteen patients were treated with 1000 mg ertapenem for a mean of 77 days (range 5-210). Sputum smear and culture were converted in all patients. Drug exposure was evaluated in 12 patients The mean AUC_{0-24} was 544,9 (range 309 – 1130) mg*h/L. The mean Cmax was 127.5 (73.9 – 277.9) mg/L.

In general ertapenem treatment was well tolerated during MDR-TB treatment and showed a favourable PK/PD profile in MDR-TB patients. We conclude that ertapenem is a highly promising drug for the treatment of MDR-TB that warrants further investigation.

INTRODUCTION

Treatment of multidrug resistant (MDR) and extensively drug resistant (XDR) tuberculosis (TB) is becoming more challenging because of increased level of drug resistance against second line tuberculosis drugs. New drugs are being evaluated in clinical trials, but only bedaquiline and delamanid have entered the market to date. Therefore, antimicrobial drugs, which have been developed and labeled for other bacterial infections may be of potential use in the treatment of MDR-TB.

One promising group of antimicrobial drugs are carbapenems [1, 2]. An early in vitro experiment showed that imipenem and meropenem were active against *J* [3]. Chambers and co-workers showed that imipenem has anti-mycobacterial activity in mice and humans [4]. Imipenem and meropenem are currently listed as group 5 drugs for the treatment of MDR-TB [5]. More recently, clinical experience of carbapenems in MDR-TB patients showed promising results [6, 7].

Carbapenems are poor substrates for beta lactamase C (BLaC) due to rapid acylation and slow deacylation. Therefore, unlike beta-lactams, they are not rapidly hydrolyzed by BLaC and therefore maintain their potential activity against *M. tuberculosis* [8]. The binding of carbapenems to the LD transpeptidases results in inhibition of the peptidoglycan polymerization of the cell wall [9]. Combined with a beta lactamase inhibitor, such as clavulanate, activity against *M. tuberculosis* is higher [10].

Efficacy of carbapenems is correlated with the percentage of time the free plasma drug concentration transcends the MIC (Tfree>MIC). Maximal bactericidal activity is reached if the time above MIC is at least 40% of dosing interval [11, 12]. To reach this target for gram positive, gram negative and anaerobic bacterial infections ertapenem is given intravenously in a dose of 1000 mg once daily [13]. Ertapenem has the advantage over other carbapenems because of a long half-life of 4 h enabling once daily dosing [12], which is attractive for MDR-TB treatment. Another advantage is that ertapenem is not affected by drug-drug interactions as it is neither metabolized by cytochrome P450 nor a substrate for P-glycoprotein [14].

To include ertapenem among the other carbapenems as a group 5 drug for the treatment of MDR-TB additional pharmacokinetic and safety data are urgently needed [15]. Therefore the objective of this study was to evaluate pharmacokinetics and safety in patients that received ertapenem as part of their treatment MDR-TB regimen.

PATIENTS AND METHODS

Patients

All patients suspected to MDR-TB at the Tuberculosis Center Beatrixoord of the University Medical Center Groningen (Haren, The Netherlands) who received ertapenem as part of their treatment regimen between first of December 2010 and the first of March 2013 were included in this retrospective study. The study was evaluated by the Medical Ethical Review Board of the University Medical Center Groningen (metc 2013-492). The need for written informed consent was waived for the retrospective collection and analysis of anonymous data because it was not required under Dutch Law (WMO). For each MDR-TB suspects, age, gender, weight, length, ethnicity, drug susceptibility pattern, localization of tuberculosis, antiretroviral therapy, sputum conversion, adverse effects induced by ertapenem, dose, total exposure to ertapenem, and duration of treatment were collected.

Drug susceptibility to Ertapenem

Drug susceptibility testing (DST) of ertapenem was performed with and without clavulanic acid using the Middlebrook 7H10 agar dilution method at the Dutch National Tuberculosis Reference Laboratory (National Institute for Public Health and the Environment RIVM), Bilthoven, The Netherlands) [16].

Pharmacokinetics and pharmacodynamics

All patients received ertapenem in a dosage of 1000 mg once daily, given as intravenous infusion in 30 min. In all MDR-TB patients routine plasma concentrations were collected at steady state to assess drug exposure to enable individualized dosing. For plasma sampling a peripheral intravenous catheter was inserted. Patency of the peripheral catheter was maintained by a saline drip. Before a blood sample was taken, the drip was stopped and the first 4 mls of blood were discarded. The samples were collected before administration and at t = 1, 2, 3, 4, 5, 6, 8, 12, hrs post-dosage and stored at –80 °C until analysis. Plasma concentrations were assessed and validated using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) in the laboratory of Clinical toxicology and Drugs Analysis of the department of Clinical Pharmacy and Pharmacology at the University Medical Center Groningen [17]. Population pharmacokinetic parameters were calculated using the KinPOP module. Both KinFIT and KinPOP were part of the software package MWPharm 3.82 (Mediware, The Netherlands). The Tfree>MIC was calculated as this has been proposed as the best pharmacokinetic/pharmacodynamic parameter to predict in vivo efficacy of carbapenems [10]. Free drug concentration was assumed to be 5% [12, 18]. Eucast minimal inhibitory concentrations for ertapenem (non-species related) of 0.5 and 1.0 mg/L were used to calculate Tfree>MIC.

Safety and tolerability

Reported adverse effects (AE) in medical charts were used to evaluate the safety of ertapenem. Specific attention was paid to AE's mentioned in earlier studies: i.e. diarrhea and vomiting. The Naranjo algorithm was used to evaluate for causality between adverse effects that occurred and ertapenem [19].

Statistics and pharmacokinetic evaluation

SPSS 20 was used as statistical software (SPSS, Virgina, IL). Correlation between pharmacokinetic parameters and patient characteristics were analyzed using the Spearman correlation coefficient. MIC data were statistical analyzed using a methodology for censored MIC data [20].

RESULTS

Patients

Eighteen patients treated with ertapenem, mean age 29 (range 13 - 66 years), were retrieved. Ertapenem was part of the treatment regimen because of suspected extensive drug resistance, intolerance to second line drugs or combination of both. Based on the results of the drug susceptibility test ertapenem was discontinued in three patients who appeared to have drug susceptible TB. Gender was unequally distributed between patients as 8 were male (44.4%) and 10 patients were female (55.5%). The mean body mass index was 21.3 (range 13.7-32.6) kg/m2. Patients originated predominantly from Africa (11/18) and Europe (5/18). Patients were primarily diagnosed with pulmonary TB (13/18), extra pulmonary sites were involved in 7 patients.

Prescribed dosage of ertapenem was 1000 mg once daily in all patients. Mean total treatment duration of ertapenem was 77 days (range 5-210 days). Drug resistance pattern of the patients to anti-tuberculosis agents are shown in table 1. Most prescribed anti-TB drugs were: moxifloxacin (17/18), injectable (16/18), linezolid (15/18), clofazimine (8/18), clarithromycin (6/18) and pyrazinamide (5/18).

In total 15 patients completed treatment and were cured. Three patients were to lost to follow up. All patients with positive sputum-smear converted within a mean period of 17 days (range 0-97 days). Cultures remained negative after culture conversion and no relapse of MDR-TB was observed.

TABLE 1 Drug resistance patterns of the 18 study patients

Drugs by group #	Resistant	Sensitive
Group 1: first-line oral drugs	17 (94.4)	1 (5.55)
Isoniazid	15 (83.3)	3 (16.6)
Rifampicin	8 (44.4)	8 (44.4)
Pyrazinamide	11 (61.1)	6 (33.3)
Ethambutol	13 (72.2)	4 (22.2)
Rifabutin		
Group 2: injectable agents		
Streptomycin	14 (77.7)	4 (22.2)
Amikacin	3 (16.6)	14 (77.7)
Kanamycin	3 (16.6)	14 (77.7)
Capreomycin	5 (27.7)	12 (66.6)
Group 3: fluoroquinolones		
Moxifloxacin	2 (11.1)	15 (83.3)
Group 4: oral bacteriostatic second-line agents		
Protionamide	7 (38.8)	10 (55.5)
Group 5: agents with unclear role in treatment of drug-resistant TB		
Linezolid		17 (94.4)
Clarithromycin	3 (16.6)	7 (38.8)
Clofamizine		8 (44.4)
Other		
Ertapenem		18 (100)

Data are presented as n (%). TB: tuberculosis. #: groups defined by the World Health Organization.

Drug susceptibility of M. tuberculosis to ertapenem

All the *M. tuberculosis* strains appeared susceptible to ertapenem. However, actual determination of the MIC was complicated by the fact that ertapenem itself appeared an instable compound at 37°C [17]. This was confirmed by the fact that after 7 days MIC values were lower than after 14 days. In addition, freshly prepared plates showed lower MIC's compared to plates stored at 4°C. The refrigerator-stored plates showed lower MIC's than plates stored at room temperature. If ertapenem was combined with clavulanic acid all MIC's were even lower.

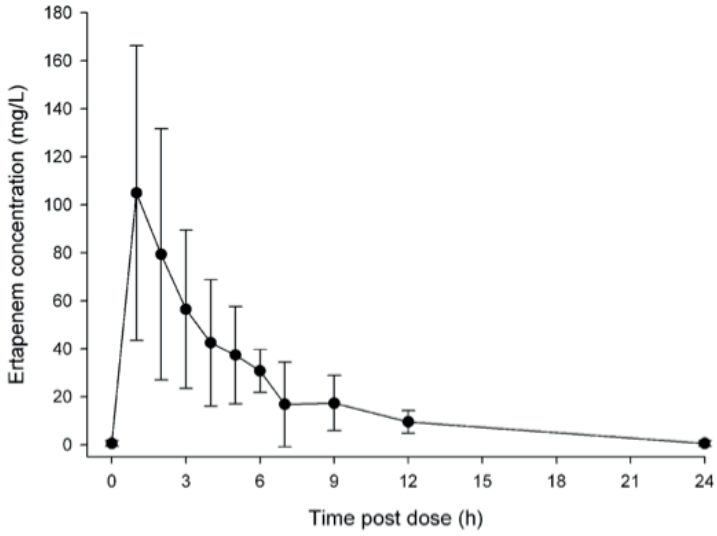

FIGURE 1: Ertapenem plasma concentration–time curves in 12 patients.

Pharmacokinetics and pharmacodynamics

The plasma concentration time curves were obtained in 12 patients with MDR-TB. In the remaining 6 patients routine plasma concentrations were collected at a time point at which they did not yet receive ertapenem or this drug was no longer administered. Three patients had multiple plasma concentration time curves and these were consistent. The mean curve is shown in figure 1. The mean $AUC_{0\text{-}24}$ was 544,9 (range 309 – 1130) mg*h/L. The steady state pharmacokinetic parameters are shown in table 2. Based on a MIC of 0.25 mg/L, 11 out of 12 patients exceeded a minimum of 40% time above MIC. In 9 patients the MDR-TB remained susceptible with a MIC of 0.5 mg/L. Except for 2 patients, none exceeded a minimum of 40% time interval with a MIC of 1 mg/L. The pharmacokinetic population model (KinPOP) of ertapenem showed a clearance of 2.26 (range 0.86-3,19) L/h/1.73m^2 and a volume of distribution of 8.79 (range 4.76-13,57) L.

Safety and tolerability

In general ertapenem was tolerated very well. In three patients treatment with ertapenem was stopped. One of these patients suffered from Crohn's disease and developed MDR-TB after multiple dosages of infliximab, a TNF alpha-blocker [21-22]. This patient experienced allergic fever, shortly after administration of ertapenem and ethambutol. After reintroduction this happened again (Naranjo score= 4). Both adverse events subsided after withdrawal of the offending drug. In the second patient ertapenem was stopped

after an increase in liver enzymes (ASAT: 109 / ALAT: 255) after 13 days of treatment with ertapenem. However, after two months, while this patient was still on treatment without ertapenem, liver enzymes remained elevated (Naranjo score = 1). In one patient kanamycin, linezolid and ertapenem were stopped due to line sepsis. This was considered not-to-be related to ertapenem. After removal of the venous access port, the patient recovered. Ertapenem and a new venous access port were not reintroduced, since it was not indicated anymore, due to low bacillary load at that time and these IV drugs could be substituted with oral antimycobacterial drugs. None of the patients experienced diarrhea, vomiting or dizziness.

TABLE 2 Pharmacokinetic parameters of ertapenem

Study	AUC_{0-24} h·mg·L^{-1}	Cmax mg·L^{-1}	Half-life h	Volume of distribution L	Clearance L·h^{-1}
1 g i.v. in MDR-TB	544.9	127.5	2.4	7.3	2.1
patients	(309–1130)	(73.9–277.9)	(2.047–3.528)	(2.612–11.1)	(0.0884–3.231)
1 g i.v. in healthy	572.1	154.9	4	8.2	1.8
volunteers [18]	(572–672)	(145–175)	(3.8–4.4)		

Data are presented as mean (range) and were calculated using KinFIT. AUC_{0-24}: area under the concentration–time curve up to 24 h; Cmax: maximum observed plasma concentration; MDR-TB: multidrug-resistant tuberculosis.

DISCUSSION

This is the first study, following a clinical report of ertapenem [6], presenting pharmacokinetic and safety data in patients with MDR-TB. In comparison with healthy volunteers, MDR-TB patients showed lower AUC_{0-24} values. Mean values of volume of distribution and clearance of MDR-TB patients were higher compared to healthy volunteers. Our observation is consistent with other studies that showed a lower drug exposure of ertapenem in patients with infectious diseases [23, 25]. More surprising was the inter-variability in AUC between patients with MDR-TB. Other antimycobacterial drugs also show highly variability and lower drug exposure in TB patients as well [2, 26, 27]. It is not yet completely clear why drug exposure is lower in TB patients. Apparently, stage of disease and altered body composition may potentially help to explain this observation.

Since ertapenem belongs to the class of beta-lactams, ertapenem has a time-dependant bactericidal activity. The Tfree>MIC is therefore important to evaluate the efficacy of ertapenem against *M. tuberculosis*. Nicolau and colleagues indicated that in case meropenem showed 40 %T>MIC bactericidal activity is observed whereas 20 %T>MIC appeared to have bacteriostatic activity. Other studies have mentioned this T>MIC as well

[11, 12, 14]. Protein binding of ertapenem was assumed to be 5%, since ertapenem shows concentration-dependent plasma protein binding. Healthy volunteers, whom average a peak plasma concentration of 150 mg/L after the end of infusion of 1 g of ertapenem, have a percentage of 8 % unbound protein. When total drug plasma concentration declines below 50 mg/L peak plasma concentration, the percentage of unbound ertapenem is circa 5% [18]. It is very promising to notice that the non-species related breakpoint of ertapenem of 0.5 mg/L was exceeded for more than 40% of the day in the majority of patients assuming that patients have a protein binding of 5%. At a higher MIC value of 1 mg/L bacteriostatic activity could be expected. Therefore ertapenem seems a very attractive drug for MDR-TB treatment. It seems warranted that doses of ertapenem higher than 1g/day should be used in the treatment of MDR-TB. However, in vitro experiments evaluating PK/PD targets for ertapenem against *M. tuberculosis* have yet to be performed. The hollow fiber infection model is suitable for PK/PD experiments and has already been used successfully before [28].

Besides pharmacokinetics of ertapenem in patients with MDR-TB, additional safety data are described for the first time as well. Only one patient, with Crohn's disease, experienced AE, which might be potentially related to ertapenem. One can speculate this may be related to an infusion related AE, due to a developed immune disorder and eventually a consequence of an infliximab treatment. Drug induced fever is a common AE of infliximab in the treatment of Crohn's Disease [29]. AE's of other carbapenems, such as diarrhoea, nausea, vomiting, headache and rash are well documented and found to be mild [1, 30]. According to the product leaflet, ertapenem is given for a maximum of two consecutive weeks. Previous studies explored the safety and tolerability of ertapenem for this period of time and concluded that adverse side effects were mild to moderate [13]. In our study we showed that AE did not increase during prolonged treatment.

The measurement of actual MIC values was complicated by the fact that ertapenem is an instable compound at 37°C. As drug susceptibility testing for *M. tuberculosis* takes at least two weeks at 37°C, it is highly likely that the initial drug concentration decreases rapidly in time. Unfortunately, with the current drug susceptibility systems, e.g. MGIT or plate, this problem cannot be overcome, as the drug concentration in the medium cannot be corrected for a decrease in concentration due to degradation of the drug. Recently the hollow fiber infection model solved this problem as drug concentrations can be increased to correct for degradation [31]. As systems are expensive and difficult to manage its not likely that routine DST will be performed using hollow fiber systems. Another alternative may be the use of E-tests [32]. This is much cheaper and easier to employ but it is unclear if it can help to overcome the instability of ertapenem.

The most important limitation of our study is the retrospective character and its limited sample size, and absence of control group, thereby preventing a meaningful conclusion on efficacy of ertapenem. Secondly the inability to define MIC for ertapenem in clinical isolates is another limitation. Nevertheless all patients were cured and no relapse was noticed after being treated with combination regimen including ertapenem. Likely the combination of drugs contributed to sputum culture conversion and favorable treatment outcome. This is in line with recently published data on tolerability and outcomes in 5 patients receiving ertapenem [6].

A recent editorial proposed a new classification of antituberculosis drugs. It marked the potential of carbapenems as group 5 drugs, however carbapenems are still in need of proper evaluation and clinical evidence [33]. Before ertapenem can be labeled as a group 5 drug and used as part of MDR-TB treatment, a valid procedure to test drug susceptibility has to be made available. Ideally, the use of ertapenem would be supported by the results of a clinical trial.

In conclusion this study provides new knowledge on the use of ertapenem in patients with MDR-TB, presenting pharmacokinetic and additional safety data.

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b | Pharmacokinetic Modeling and Limited Sampling Strategies based on healthy volunteers for Monitoring of Ertapenem in MDR-TB patients

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Submitted

Abstract

Background: Ertapenem is a widely used broad-spectrum antibiotic and can be administered once daily. Ertapenem is one of the newer carbapenems that is explored against *Mycobacterium tuberculosis*. The most important pharmacokinetic/pharmacodynamics (PK/PD) parameter for ertapenem seems to be the free 40 percent of the time above the minimal inhibitory concentration (*f* 40%T>MIC). To be able to calculate the *f* 40%T>MIC, a good indication of the plasma concentration profile is mandatory. To assess 40%T>MIC in MDR-TB patients, a limited sampling strategy was developed using a population pharmacokinetic model based on healthy volunteers.

Methods: A population two-compartment model was based on data from forty-eight healthy volunteers using a two-stage Bayesian procedure. Bland-Altman analysis was performed to evaluate the correlation between the area under the plasma concentration-time curve from 0-24h ($AUC_{0\text{-}24h}$) calculated and the $AUC_{0\text{-}24h}$ estimated with the pharmacokinetic model. Plasma concentrations of 12 patients with MDR-TB were fitted into the model. A Monte Carlo simulation (n=1000) was used to calculate limited sampling strategies. Additionally, the *f* 40%T>MIC for MDR-TB patients was estimated with the population pharmacokinetic model.

Results: The pharmacokinetic parameters determined with bootstrap analysis were CL_m 1.06 L/h, V1 0.08 L, f_r 0.13, V_2 0.05 L and CL_{i2} 2.56 L/h. This pharmacokinetic model was shown to estimate the $AUC_{0\text{-}24h}$ in MDR-TB patients with an overestimation of 6.8 (range: -17.2 – 30.7)%. The best performing limited sampling strategy was found to be sampling at 1 and 5h (R^2 = 0.78, mean predictive error = -0.33% and a %root mean square error = 5.5). Drug exposure was overestimated by a mean percentage of 4.2 (-15.2 – 23.6)%. Considering a free fraction of 5% and the MIC set at 0.5 mg/L, 10 out of 12 patients would have exceeded a minimum of *f*40% T>MIC.

Conclusions: A pharmacokinetic model and limited sampling strategy, developed using data from healthy volunteers, showed to be adequate to predict drug exposure in MDR-TB patients.

KEYWORDS: Ertapenem, Tuberculosis, Pharmacokinetics, Pharmacokinetic model, Limited sampling.

INTRODUCTION

Ertapenem is a broad spectrum carbapenem antibiotic, used against a range of infectious diseases (1). Like for all other beta-lactam antimicrobial products, the efficacy of ertapenem is characterized by time-dependent killing. Carbapenems have anti-bacterial activity when the plasma concentration exceeds the minimal inhibitory concentration at least 40% of the time (40%T>MIC) (1,2). Although not yet studied in tuberculosis (TB) patients, *free* 40%T>MIC is expected to be an important pharmacodynamic parameter (3). Carbapenems in combination with clavulanic acid has created interest since activity was shown in a murine model of TB (3). Additionally, a recent study showed that carbapenems efficiently inactivated peptidoglycan cross-linking in *M. tuberculosis* (3,4). Recently a new susceptibility testing method to estimate the MIC of ertapenem has been introduced (5) showing that ertapenem might be more potent *in vitro* than previously thought because its chemical degradation had never been taken into account (6). To date only a limited number of MDR-TB patients have been treated with ertapenem as part of a multidrug regimen. Based on this data, the drug appeared well tolerated during prolonged treatment (7,8). However, ertapenem is not yet added to the World Health Organization (WHO) list of anti-TB drugs, in contrast to imipenem and meropenem.

Pharmacokinetics of ertapenem have typically been studied in healthy volunteers (9), people with obesity (10,11), patients with renal failure (12-14) and critically ill patients with various pathologies (15-17). Lower drug exposure was observed in obese individuals (11), and an increase in dose interval was needed in patients with renal insufficiency with an estimated glomerular filtration rate (eGFR) below 30 mL/min/1,73m^2 (13), suggesting that the optimal dose of ertapenem is different for various health conditions. A recent study on pharmacokinetics of MDR-TB patients suggested that there was substantial pharmacokinetic variability in these patients (7).

For studies exploring the use of ertapenem against *M. tuberculosis* it would be valuable to assess *f* 40% T>MIC in patients. To be able to calculate the *f* 40% T>MIC, a good indication of the plasma concentration profile is mandatory. However, measurement of the plasma concentration over the entire 24h dosing interval is time consuming, expensive and burdening to patients. A limited sampling strategy can be used to predict this plasma concentration profile as has been done for other anti-TB drugs (18-20).

The aim of this study was to develop a population pharmacokinetic model and a limited sampling strategy, in order to estimate drug exposure of ertapenem in MDR-TB patients.

MATERIALS AND METHODS

This study was based on two data sets. The first set was comprised of forty-eight healthy volunteers from five clinical studies receiving 0.25 to 3 g i.v. doses of ertapenem (9). The second set was comprised of a retrospective dataset of patients with MDR-TB, receiving 1g ertapenem administered once daily via a 30 minute infusion at the Tuberculosis Center Beatrixoord, University Medical Center Groningen, The Netherlands between December 1, 2010 and March 1, 2013 (7). Plasma concentrations of ertapenem were collected at steady state before administration and at 1, 2, 3, 4, 5, 6, 8 and 12 hours after administration. Ertapenem plasma concentrations were analyzed by a validated liquid chromatography-tandem mass-spectrometry (LC-MS/MS) method (21). Both data sets included demographic and medical data, such as age at start of treatment, height and body weight at the time of pharmacokinetic assessment and serum creatinine at the time of pharmacokinetic assessment.

Population pharmacokinetic model

All pharmacokinetic calculations were performed using MW\Pharm 3.82 (Mediware, Groningen, The Netherlands). The drug plasma-concentrations of the forty-two healthy volunteers were used to develop a two-compartmental population pharmacokinetic model using the KinPOP module of MW\Pharm. The clearance was calculated with CL = CL_m (metabolic clearance (L/h)) * BSA (body surface area (m^2)) /1.85 + f_r (drug clearance – creatinine clearance ratio) * CL_{cr} (creatinine clearance (L/h)). The area under the plasma concentration-time curve from 0 up to 24 hours ($AUC_{0\text{-}24h}$) was calculated using the log trapezoidal rule. Ertapenem plasma concentrations were analysed using an iterative two-stage Bayesian (ITSB) procedure. Distribution pharmacokinetic parameters were assumed to be log-normally distributed and the residual error was assumed to be normally distributed with SD = 0.1 + 0.1*C, in which C is the observed plasma concentration of ertapenem. The parameters of population model were assumed to be log-normally distributed and calculated using bootstrap analysis (n = 1000).

Internal validation was performed by leaving three healthy volunteers out of the pharmacokinetic model, creating n-3 sub models. N-3 sub models were obtained by randomization using Microsoft Excel 2010. The $AUC_{0\text{-}24h}$, estimated was obtained by fitting the curve of the three left out volunteers in the corresponding n-3 sub models. Agreement between $AUC_{0\text{-}24h}$, and estimated and $AUC_{0\text{-}24h}$ was assessed by Bland-Altman (BA) analysis and Passing and Bablok regression and subsequent residual plot. Fitting the individual data of MDR-TB patients in the two-compartmental pharmacokinetic model externally validated the population pharmacokinetic model. BA analysis was also used to assess the agreement between the $AUC_{0\text{-}24h}$ of MDR-TB patients resulting from the external validation and the $AUC_{0\text{-}24h}$ of MDR-TB patients calculated with the KINFIT module of MW\Pharm 3.82.

Healthy volunteers received multiple or single doses of ertapenem. Multiple doses were given on day one and day eight and were treated as single doses on day one to prevent duplication.

Limited sampling strategies

A Monte Carlo simulation was used to calculate limited sampling strategies. This simulation consisted of 1000 random patients drawn from the population pharmacokinetic model. For each patient limited sampling strategies were calculated at 1 to 4 sampling time points by Bayesian MAP procedure. We only considered evaluation of sampling time points from 0-6 h for limited sampling strategies, since this would be suitable for application in clinical practice.

Performance was considered suitable for application in prospective studies if the adjusted r squared (R^2) was > 0.95, the root mean squared error (RMSE) was < 15%, and the mean prediction error (MPE) was <5%.

Predication of free 40%T>MIC

The ertapenem concentration-time curve of each patient was used to establish if the *f* 40%T>MIC was reached. For this cause the time that the concentration-time curve was above the MIC was assessed in MW/Pharm. The percentage protein unbound ertapenem used for the assessment was 5-10% (4,7). European Committee on Antimicrobial Susceptibility Testing (EUCAST) MIC values for ertapenem (non-species related) of 0.5 and 1.0 $mg \cdot L^{-1}$ were used to calculate *f* 40%T>MIC. Exposure was considered adequate if the concentration was 40% of time above MIC which corresponds with 9.6 h of each 24 h interval.

Statistics

Differences between the population characteristics and pharmacokinetic parameters of healthy volunteers and TB patients were calculated using the Mann Whitney U test. All statistics were calculated with Analyse-it™ for Microsoft Excel (version 2.30).

TABLE 1: Baseline characteristics of healthy volunteers versus multidrug-resistant tuberculosis patients

	Healthy volunteers n=42	MDR-TB patients n=12	p-value
Sex [n (%)]			
Male	36 (86%)	5 (42%)	0.0083[a]
Female	6 (14%)	7 (58%)	
Age (years; median, IQR)	31 (23-38)	25 (18-30)	0.064[b]
Weight (kg; median, IQR)	78.9 (72.2-83.8)	55.5 (47.3-70.3)	0.000[b]
Height (cm; median, IQR)	178 (172-183)	167 (164-174)	0.004[b]
Body mass Index (kg/m2; median, IQR)	24.5 (23.6-26.2)	19.2 (17.9-23.7)	0.002[b]
Ethnicity			
Black (%)	5 (12%)	7 (58%)	0.000[c]
Caucasian (%)	36 (86%)	3 (25%)	
Asian (%)	1 (2%)	1 (8%)	
Other (%)	0 (0%)	1 (8%)	
Serum Creatinine *	0.9 (0.8-1.1) **	0.5 (0.4-0.7)	0.000[b]
Dose(mg)/weight(kg)	18.0 (14.2-21.1) **	12.9 (6.0-20.0)	0.044[b]
Samples/patient	28 (16-48)	7 (6-8)	

*On the day the plasma concentrations were determined

** Some patients received more than one dose with subsequent serum creatinine

a Fisher exact test

b Mann-Whitney U test

c Pearson Chi-Squared test

RESULTS

Data set

Data of forty-two healthy volunteers was used to develop the population pharmacokinetic model. The baseline characteristics of healthy volunteers and MDR-TB patients were shown to differ significantly ($P<0.05$), except for age (table 1). The median age of the subjects was 31 (23 - 38) years and body mass index was 24.5 (23.6 – 26.2) kg/ m^2. Mean

$AUC_{0\text{-}24h}$ value for 1g ertapenem once daily was 540.57 (491.85 – 551.36 mg*h/L). The steady state pharmacokinetic parameters are based on a two-compartmental model, and are displayed in table 2.

TABLE 2: Two-compartmental steady-state pharmacokinetic parameters of ertapenem in healthy volunteers

	Healthy volunteers (n=42)[a]
AUC [t=∞] (h.mg/l)	529.31 (263.48 – 775.05)
AUC [t=24h] (h.mg/l)	519.81 (257.53 – 747.25)
CL (l/h)	1.87 (1.72 – 2.03)
CL_{12} (l/h)	3.27 (1.98 – 3.41)
V_1(l)	5.86 (5.09 – 6.53)
V_2 (l)	3.97 (3.34 – 4.42)
V_{ss} (l)	9.83 (9.12 - 10,49)
V (l)	11,46 (10,19 - 11,98)
k_{10} (/h)	0,33 (0,28 - 0,36)
k_{12} (/h)	0,62 (0,32 - 0,60)
k_{21} (/h)	0,76 (0,55 - 0,81)
$t_{1/2}$ 1 (h)	0,62 (0,44 - 0,69)
$t_{1/2}$ 2 (h)	4,31 (3,93 - 4,55)
MRT (h)	5,35 (4,83 - 5,64)

Data are presented as geometric mean (range)

AUC = Area under the concentration-time curve; CL = clearance; V_{ss} = Volume of distribution at steady state; V_1 = Central compartment volume; V_2= Peripheral compartment volume; V = Volume of distribution; CL_{12} = inter-compartmental clearance; MRT = mean residence time; k_{12} and k_{21} = first-order inter-compartmental transfer rate constants between the central and peripheral compartments; k10 =elimination rate constant ; $t_{1/2}$ 1 and 2 = half-life of ertapenem in the central (1) and peripheral (2) compartment

TABLE 3: Final population pharmacokinetic model parameters at a 1g dose

	Healthy volunteers (n=18)	Tuberculosis patients (n=12)	p-value*
Cl_m (l/h)	1.08 (1.05 – 1.10)	0.98 (0.92 – 1.03)	0.0030
f_r	0.153 (0.139 – 0.159)	0.108 (0.087 – 0.136)	0.4460
V_1 (l/kg)	0.084 (0.078 – 0.089)	0.087 (0.079 – 0.104)	0.0130
CL_{12} (l/h)	2.546 (2.536 – 2.553)	2.560 (2.545 – 2.574)	0.0470
V_2 (lkg)	0.0542 (0.0540 – 0.0543)	0.0539 (0.0538 – 0.0544)	0.4290
AUC_{0-24h} (h.mg/l)	540.57 (491.85 – 551.36)	573.07 (435.62 – 603.89)	0.5534
C_{max} (mg/l)	154.29 (143.21 – 167.15)	175.78 (156.72 – 184.41)	0.0904

* Mann-Whitney U test

Cl_m = apparent clearance of the metabolite; V_1 = volume of distribution of the central compartment; f_r =ertapenem clearance/creatinine clearance ratio; V_2 = volume of distribution in the peripheral compartment; CL_{12} = inter-compartmental clearance; AUC_{0-24h} = area under the curve from 0 to 24 h; C_{max} = maximal concentration of ertapenem.

Pharmacokinetic model

Results of population pharmacokinetic parameters are shown in table 3. It appeared that the CL_m as well as the inter-compartmental clearance (CL_{12}) was significantly higher ($P<0.05$) in healthy volunteers than in MDR-TB patients. AUC_{0-24h} values observed from the population pharmacokinetic model were underestimated by mean 0.3% (range: -8.1 – 7.6), when compared with the AUC_{0-24h} values calculated with KINFIT (MW\Pharm 3.82) - shown in Figure 1.

The observed and model calculated ertapenem AUC_{0-24h} were assessed for agreement, using Passing and Bablok regression in figure 2a. From the residual blot in figure 2b can be seen that the data looks heteroscedastic, meaning that at a higher AUC_{0-24h} the AUC_{0-24h} cannot be predicted as well with the model as with a lower AUC_{0-24h} value. However, the spread is equal on both sides of the regression line and the distance is minimal as can be seen in figure 2a.

AUC_{0-24h} values of the MDR-TB patient data in the population-pharmacokinetic model were underestimated by mean 6.8% (range: -17.2 – 30.7), when compared with the calculated AUC_{0-24h}. The estimated AUC_{0-24h} correlated with the calculated $AUC_{0-24h,}$ as determined with a Bland-Altman analysis - shown in Figure 3.

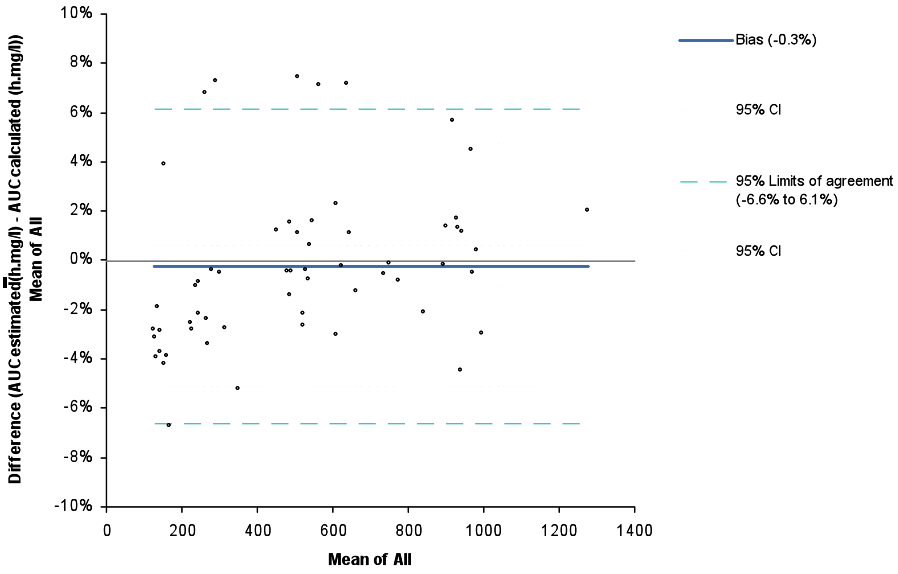

FIGURE 1: Internal validation of the population pharmacokinetic model. Bland-Altman plot showing the agreement between the area under the concentration-time curve for 24h ($AUC_{0\text{-}24h}$) of healthy volunteers estimated with the population pharmacokinetic model and the $AUC_{0\text{-}24h}$ calculated with KINFIT (MW\Pharm 3.81).

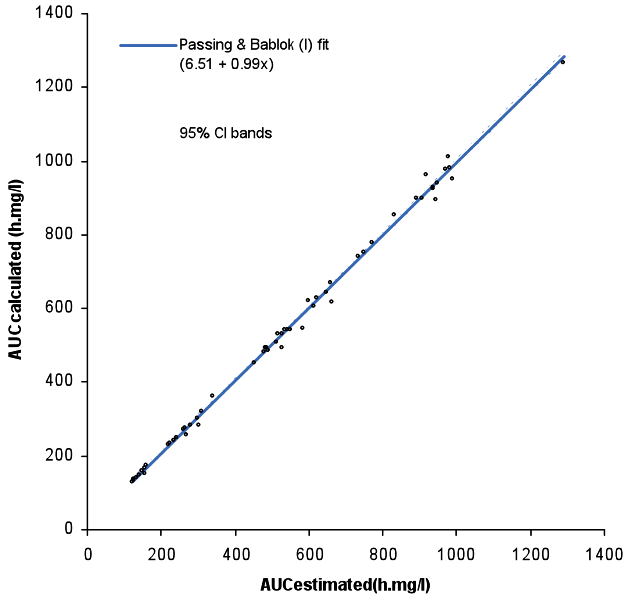

FIGURE 2A: Passing and Bablok regression. Showing the agreement between the area under the concentration-time curve for 24h ($AUC_{0\text{-}24h}$ calculated with KINFIT (MW\Pharm 3.81) and the $AUC_{0\text{-}24h}$ estimated with the population pharmacokinetic model (dotted lines: 95% confidence interval (CI)).

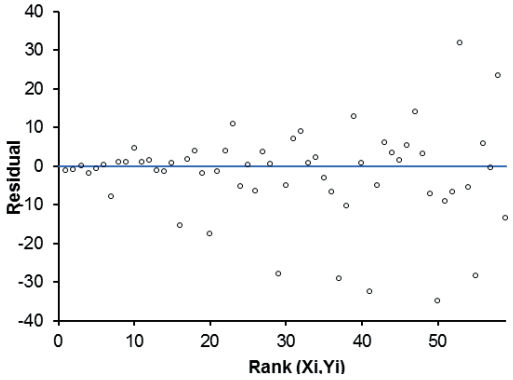

FIGURE 2B: Residual plot. Residuals translated from the Passing and Bablok regression line. The blue line is the regression line in figure 2a.

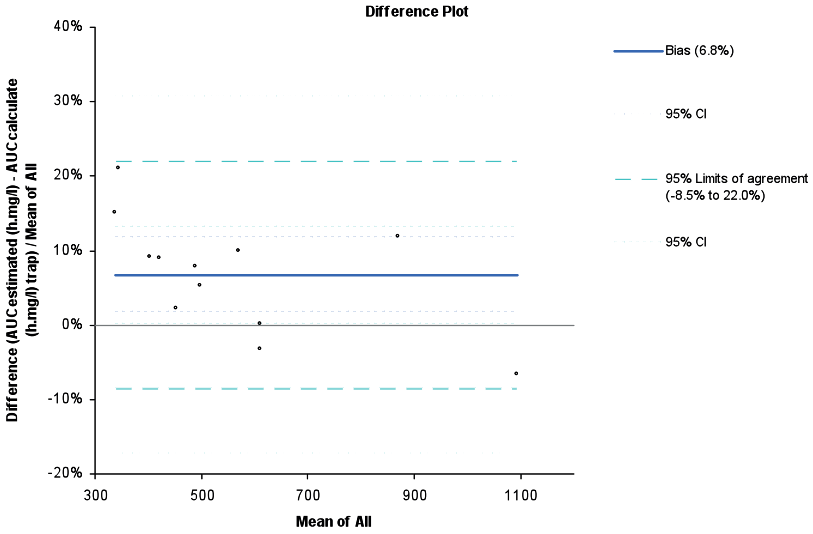

FIGURE 3: External validation of the population pharmacokinetic model. Bland Altman plot showing the agreement of the area under the concentration- time curve ($AUC_{0\text{-}24h}$) of multidrug-resistant tuberculosis patients calculated with KINFIT and the $AUC_{0\text{-}24h}$ estimated with the population pharmacokinetic model.

The pharmacokinetic parameters were determined with bootstrap analysis and are shown in table 4.

TABLE 4: Pharmacokinetic parameters of the population model (Bootstrap analysis)

Parameter	Mean (95% CI)	± st. dev. (95% CI)
CL_m (L/h)	1.06 (0.54 – 1.34)	0.16 (0.09 – 0.23)
V_1 (L/Kg)	0.08 (0.08 – 0.08)	0.01 (0.01 – 0.01)
f_r	0.13 (0.07 – 0.24)	0.04 (0.02 – 0.06)
V_2 (L/Kg)	0.05 (0.05 – 0.06)	0.00 (0.00– 0.00)
CL_{12} (L/h)	2.56 (2.34 – 2.85)	0.14 (0.11 – 0.27)

Cl_m = apparent clearance of the metabolite; V_1 = volume of distribution of the central compartment; f_r =ertapenem clearance/creatinine clearance ratio; V_2 = volume of distribution in the peripheral compartment; CL_{12} = inter-compartmental clearance

Limited sampling strategies

Using the population pharmacokinetic model, limited sampling strategies were evaluated and the R^2, bias and RMSE were subsequently determined. Limited sampling strategies with 1 to 4 sampling time-points are shown in Table 5. None of the limited sampling strategies reached the predetermined threshold of $R^2 > 0.95$. Four sampling time points, at 1, 2, 3 and 5h, enabled the best prediction of ertapenem exposure reflected by $AUC_{0-24h,}$ considering bias, RMSE and R^2 ($R^2 = 0.87$, mean predictive error = -0.33% and a %RMSE = 4.4). Two sampling time-points also reached favourable %RMSE and mean predictive error, of 5.5 and -0.33% respectively.

The AUC_{0-24h} estimated by applying this two sampling time-point limited sampling strategy was compared with the AUC_{0-24h} calculated with KINFIT (MW\Pharm 3.81) using Bland-Altman analysis. This strategy overestimated the AUC_{0-24h} by 4.2% (-15.2 – 23.6) (figure 4).

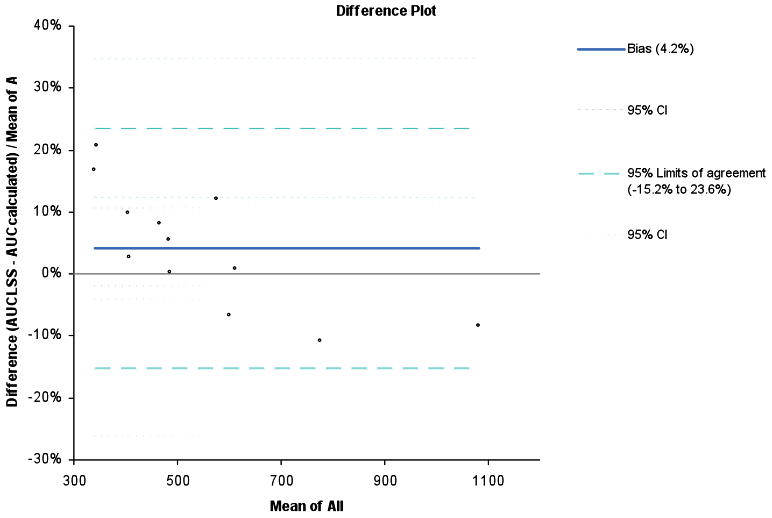

FIGURE 4: Validation of the limited sampling strategy. The Bland-Altman plot shows the agreement between the area under the concentration-time curve for 24h ($AUC_{0\text{-}24h}$) from multidrug-resistant tuberculosis patients obtained from the population pharmacokinetic model applying the limited sampling strategy of 1 and 5h and the $AUC_{0\text{-}24h}$ calculated with KINFIT (MW\Pharm 3.81).

TABLE 5: Best-performing limited sampling strategies

First sampling time point (h)	Second sampling time point (h)	Third sampling time point (h)	Fourth sampling time point (h)	Coefficient of determination (r^2)	Mean predictive error (%bias)	% RMSE[a]
6				0.52	-0.35	7.8
4				0.54	-0.22	7.6
5				0.57	-0.09	7.4
1	6			0.73	-0.59	6.0
1	4			0.77	-0.45	5.8
1	5			0.78	-0.33	5.5
1	3	4		0.83	-0.49	5.0
1	2	5		0.83	-0.42	4.9
1	3	5		0.83	-0.39	4.9
1	2	4	5	0.86	-0.44	4.5
1	2	3	4	0.88	-0.53	4.4
1	2	3	5	0.87	-0.33	4.4

a Relative mean standard error

%T>MIC

Considering a free fraction of 5% and the MIC set at 0.5 mg/L, 10 out of 12 patients would have exceeded a minimum of 40% *f* T>MIC (range 8.68h – 24h) thereby having sufficient therapeutic effect in MDR-TB Patients.; with the MIC set at 1 mg/L, 9 of 12 patients would have had therapeutic concentrations in excess of 40% of time reflecting a therapeutic benefit from the ertapenem administration (range 6.72h – 19.6h).

DISCUSSION

This is the first study showing that a population pharmacokinetic model of ertapenem based on data of healthy volunteers can predict pharmacokinetics of ertapenem in patients with MDR-TB, even though the baseline characteristics of both healthy volunteers and MDR-TB patients differed significantly (table 1). We showed that the $AUC_{0\text{-}24h}$ of MDR-TB patients could be estimated with this population pharmacokinetic model with a mean overestimation of 6.8% (± 2.8).

The robustness of this pharmacokinetic model was validated using a n-3 cross-validation, showing an underestimation of 0.3%. The limited sampling strategy that we present here can be used to assess individual drug exposure of ertapenem in TB patients with limited treatment options. Moreover, the model and limited sampling strategy can be used to evaluate drug exposure of ertapenem in phase II studies studying early bactericidal activity of ertapenem in TB patients. Such a study is urgently needed to provide data on efficacy of the potential attractive TB drug.

In the population pharmacokinetic model, multiple doses were treated as single doses on day one to avoid duplication, as an earlier study had found that there was no accumulation of ertapenem following dosing over eight days and mean plasma concentrations were found to be very similar on day one as well as on day eight (9). We did not include the subjects receiving a 3g dose, since they had a longer infusion time which complicated the model.

Pharmacokinetic modeling of ertapenem has been performed in previous studies, but it has never been performed for application in MDR-TB treatment. Based on previous reports and recent pharmacokinetics studies on ertapenem (10-17, 22-24), we evaluated concentration time curves in both a one-compartmental and a two-compartmental model. A two-compartmental model was found to be the best fit. We found that MDR-TB patients have a higher drug exposure than healthy volunteers (table 3), this is in contrast to a previous study looking at the exposure of ertapenem in MDR-TB patients in comparison with the exposure in healthy volunteers. However, a higher exposure for MDR-TB patients could be explained with the significantly lower clearance, metabolic as well as inter-compartmental for MDR-TB patients (table 3). Several studies looking at the exposure of

ertapenem in patients compared with healthy volunteers have shown that the exposure in patients varies greatly (14,15). Additionally, there is a large pharmacokinetic variability in the MDR-TB patients (table 3), making the range of $AUC_{0\text{-}24h}$ rather large. More research is needed to find an explanation for this high variability.

Using a limited strategy with sampling time points at 1 and 5 hours, we found a low %RMSE (5.5) and low mean predictive error (-0.33%). The adjusted R^2 did not reach >0.95, as none of the limited sampling strategies did. A Bland-Altman analysis was performed for the limited sampling strategy, showing an estimated $AUC_{0\text{-}24h}$ of 4.2% (-15.2 – 23.6) bias. Additionally, a limited sampling strategy using two sampling time points would give the least burden to patients as it is minimally invasive and least time-consuming. The blood samples were collected for another cause, therefore no data was available between 5 and 8 hours.

As there are limited options for treating MDR-TB and resistance of antibiotics is an emerging problem, we think it is time to start assessing efficacy of ertapenem in MDR-TB patients in Phase II clinical trial testing the early bactericidal activity. The developed limited sampling strategy can be used to evaluate drug exposure and thereby reduce costs and burden for the study subjects.

CONCLUSION

A pharmacokinetic model and limited sampling strategy bases on data from healthy volunteers was able to predict the $AUC_{0\text{-}24h}$ and *f 40*%T>MIC in MDR-TB patients. This model can be used in phase II studies.

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Chapter 6

Discussion & Future Perspectives

In this thesis, we describe the global and Dutch MDR-TB problem and we discuss treatment strategies to combat MDR-TB with a special focus on aminoglycosides and ertapenem, using pharmacokinetic / pharmacodynamics modeling to optimise outcome while minimizing dose-dependent toxicity and adverse effects.

Current WHO strategies to combat the TB epidemic by identifying and treating individuals with the most infectious forms of TB using sputum microscopy for case detection, followed by the DOTs strategy [1] have failed to curb the emergence of DR-TB, in particular MDR- and XDR-TB. In this thesis we do not focus on detection but describe several methods and approaches that we have used in The Netherlands to combat and even to prevent DR-TB, and conclude with some recommendations for low-resource, high-burden TB countries.

In **Chapter 2**, we discuss immunological aspects of TB. Improved knowledge of the biology of *M. tuberculosis* might help in the discovery of novel molecules to target *M. tuberculosis*. We argue that a better understanding of the host immune response may help in distinguishing between paradoxical reactions / IRIS on the one hand, and on-going active TB as a result of failed treatment on the other hand. We also discuss immunotherapeutic and immuno-prophylactic strategies, by designing improved vaccines, to replace or improve the currently only vaccine available – BCG. BCG has been around since almost a century and currently, it is globally the most widely used vaccine. BCG vaccination is however still controversial. It is particularly tragic that, despite much progress in recent years, the use of scarce resources to administer BCG must still be based mainly on blind faith [2].

Paradoxical reactions, IRIS or active TB

During treatment for TB, either for drug-susceptible or drug resistant TB, differentiation between a paradoxical reaction / IRIS and on-going TB disease reflecting treatment failure – is extremely important. Obviously, the two entities need a dramatically different approach. Only follow-up can distinguish the two entities with certainty, and biomarkers or surrogate parameters have not been identified to differentiate these entities, perhaps with the exception of sputum culture status at month 2-3 after start of treatment [3]. Symptoms can be completely overlapping and paradoxical reactions or IRIS, even when the mycobacteria are no longer viable, can also cause significant morbidity and mortality due to an exacerbated immune response. During treatment for drug-susceptible TB, a return of symptoms may either reflect a paradoxical reaction / IRIS but if treatment failure is the cause of recurrent symptoms, acquired drug resistant TB has to be taken into account, while treatment failure in MDR-TB might be caused by acquisition of additional resistance. Excluding active disease requires culture and drug susceptibility testing that

is not only time consuming, but also necessitates considerable infrastructure and incurs huge costs. Even currently available DNA-based molecular testing platforms may not differentiate between the presence of DNA of killed and viable bacilli.

Novel vaccination and immune modulating strategies for both active and latent TB

Boosting or immunomodulation of the immune system may have important future implications in eradicating TB. It may help to prevent reactivation of latent TB and also to combat active TB [4].

Pre-exposure vaccines are designed to prevent TB infection while post-exposure vaccines are either meant to enhance immunity following exposure to viable TB bacilli by boosting immunity, to prevent subsequent TB, or to curb/combat on-going latent TB infection by enhancing lysis of dormant bacilli in macrophages. Therapeutic vaccination is designed to help treating active TB, in combination with antibiotic drug treatment.
Although BCG is impressive with respect to its low cost and its high safety [5], the present regimen of BCG (pre-exposure) vaccination soon after birth provides limited protection in children against disseminated and meningeal TB and has had no impact on adult pulmonary TB, and hence, no impact on the epidemiology of TB in the population. This finding probably reflects a lack of effectiveness in adults, especially in the context of early exposure to environmental mycobacteria [6].

To reduce the global burden of disease, new vaccination strategies against TB need to induce a far better immunity than that achieved with BCG vaccine, not only in infants but also in adolescents and adults. In infants, an optimal vaccine would fully prevent initial infection. Present vaccine candidates are intended to only reduce the initial bacterial burden with containment of remaining *M. tuberculosis* organisms and will therefore neither eradicate the pathogen, nor prevent stable infection [7].

Immunologically contained *M tuberculosis* is thought to transform from a metabolically active replicating form into a dormant form with minimal metabolism and replication that can lead to disease reactivation at a later stage [1,8,9,10]. To prevent this reactivation, appropriate post-exposure or therapeutic vaccines are needed to target dormant and persistent *M. tuberculosis*. Post-exposure vaccines should also prevent reinfection of individuals with latent infection, notably in regions with high TB prevalence [11]. The rationale behind post-exposure vaccine strategies, as part of TB treatment is by enhancing cellular immune response eventually to enhance bacterial killing, thereby shortening treatment duration and preventing TB relapse [12]. These strategies have recently been listed among host-directed therapies [13,14,15].

Latent infection with *M. tuberculosis* is actively controlled by the immune response and once immunity fails or wanes, TB may reactivate. Better understanding of immunological mechanisms can form the basis for rational design of new vaccination strategies against TB [16,17]. Immunotherapy (such as DNA vaccines) may reduce the duration of chemotherapy (being an adjunct to chemotherapy) and may also reduce reactivation TB [18]. One problem in designing novel vaccines is that current immunogenicity markers have differed among different research groups [19]. Most researchers believe that enhancing certain cytokines e.g., interferon-gamma responses and TNF-alpha by CD4+ and CD8+ T cells reflects enhanced autophagy in macrophages translating into enhanced protection against TB or TB infection [20]. This paradigm has recently been challenged in experimental animals studies [21,22,23] as well as in human vaccine trials [24,25]. Immunotherapy though it may contribute to TB treatment, should first of all be safe [26]. Although a large multicenter trial of *M. indicus-pranii* – one of five candidate therapeutic vaccines [27] – has not shown efficacy for the end point chosen, safety appeared excellent [28].

Conclusions and recommendations: we advocate that new vaccines are urgently needed to reach the WHO Sustainable Development Goal of substantially reducing and eliminating TB as a public health problem by 2030 [29,30,31]. Long-term vaccination strategies need to target these more ambitious goals. Even though vaccine development will have a price, the return of investment will greatly exceed original costs [32].

In **Chapter 3** we describe the treatment results of two observational studies of all patients in the Netherlands diagnosed with drug resistant TB (DR-TB). We demonstrated a high success rate: 86% of those started on treatment had a favourable outcome thereby reaching targets set for DR-TB.

The optimal composition and duration of currently available MDR-TB treatment regimens are still uncertain [33,34]. In a large individual patient data meta-analysis of 9,153 patients, overall treatment results were less favourable than the Dutch data —treatment success was achieved in around 60% of all patients. Treatment success was significantly associated with the specific durations, the number of likely effective drugs for the initial intensive and continuation phases of therapy, and with the use of later generation quinolones. However, because of important limitations in the included studies, cautious interpretation of these results is needed [35]. The evidence of efficacy of the former WHO class 5 drugs is still limited [36] and especially drug resistance emerging during treatment is associated with poor outcome [37]. Our strategy to prevent emergence of resistant clones during therapy is to individualize treatment. An important aspect of this approach is to optimise treatment

results while at the same time minimising drug concentration-dependent adverse effects. Optimising treatment results was based on pharmacokinetic (PK) measurements relative to pharmacodynamics (PD; i.e., drug susceptibility measures). Based on in vitro modelling, efficacy end points following PK/PD calculations would then need to exceed certain cut-off ratios in order to be considered adequate [38]. Dosage adjustment according to PK/PD, the latter based on minimal inhibitory concentration (MIC) test results would provide efficacy for each drug component in the regimen chosen [38,39,40]. The impact of drug susceptibility testing (DST) on outcome has been shown [41]. Molecular identification of resistance genes translating in phenotypic resistance should shorten time to a tailored treatment regimen, but until these molecular tools have been fine-tuned, phenotypic DST results will remain indispensable for definitive PK/PD calculations.

The 2016 update of WHO treatment guidelines for drug-resistant TB advocates a shorter treatment duration for selected patients, i.e. 9-12 months. In our retrospective analysis of our MDR-TB patients we show that nearly half (85/172; 49.4%) of our patients would meet the criteria for the new shorter regimen. Only 4 of our eligible patients still had positive sputum smear microscopy after 4 months of treatment; 81/172 (i.e., 95% of those eligible for shorter treatment duration) would therefore be eligible for 9 months treatment only, according to the new guideline. We have treated patients in whom intuitively, treatment was extremely long, considering the fact that their lesions were limited, as well as their bacterial loads [42]. Therefore we warmly welcome these new recommendations; obviously, shorter regimens should not jeopardize excellent outcomes earlier achieved and therefore vigilance is warranted. A shorter regimen is only applicable in MDR-TB patients without previous exposure to, or resistance to 2nd line TB drugs.

Conclusions and recommendations: we realize that the high success rate in our study is the compound result of many different factors. The relative contribution of each individual component in our approach cannot be determined. All components may ultimately prove essential: selection of adequate empirical initial drug combinations; DST performed in a well-coordinated fashion in a central reference laboratory; adjusting drug treatment to DST, MIC and PK measurements, using certain cut-offs for PK/PD ratios; a committed treatment team that applies the principles of close supervision, under the umbrella of a well-organised national TB program; and TB centres of excellence, thereby merging a developing expertise with a DOTs strategy.
In close cooperation and with support of high resource countries, methods like drug treatment adjusted to DST and PK measurements could and should be made available in high TB burden countries as well.

In **Chapter 4** we describe the efficacy and side effects of aminoglycosides, using PK/PD modeling; see figure.

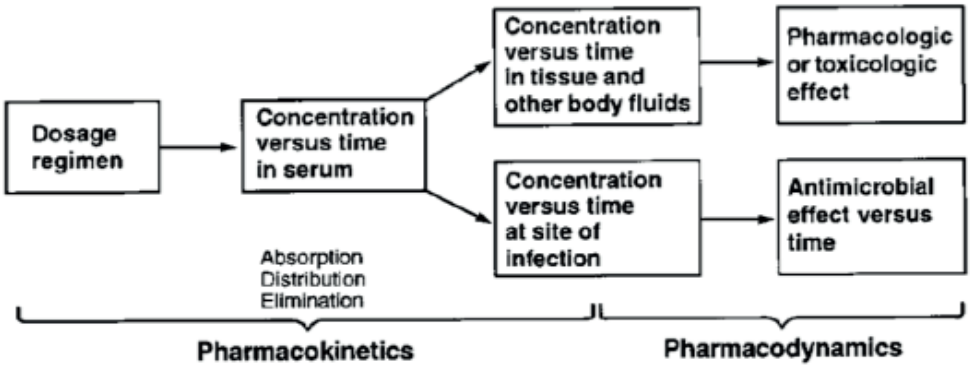

FIGURE 1: Overview of pharmacokinetics and pharmacodynamics in antimicrobial therapy
From: Craig WA. Pharmacokinetic / pharmacodynamic parameters:
rationale for antibacterial dosing of mice and men. Clin Infect Dis 1998; 26: 1–12

The assessment of efficacy of TB treatment is challenging. Typically, using only one single drug is bound to fail, as no single drug is able to kill all different clones within the bacterial population present in one single individual that suffers from TB. No single drug, however effective in most (95, or 99% of organisms present in the specimen isolated from the host) can be dosed high enough to prevent drug-resistant mutants to replace susceptible organisms. Invariably, drug combinations with at least 3 active drugs are required to effectively kill the bacterial population. Although actively replicating, metabolically active organisms are killed rapidly in a log-wise fashion within the first three weeks of treatment, the population of slowly replicating, metabolically inactive phenotype organisms – the so-called persister organisms – require long-term treatment in order to obtain sterilising treatment. Rifampicin in combination with isoniazid and pyrazinamide; and perhaps, clofazimine have the largest activity against persister phenotype organisms. In vitro models like culture may predict efficacy reasonably accurately, but single drug susceptibility assays using agreed breakpoints have been based on consensus rather than robust evidence in clinical trials with patient outcomes as the reference test. These breakpoints have been challenged [43] using more accurate in vitro models like the hollow fiber model that more closely mimics PK variations over time than the traditional solid and liquid culture media [44]. Even the hollow fiber model still simplifies the reality as only variations of drug concentration in the blood stream over time are modelled; drug penetration in sanctuary sites may further complicate matters [45,46,47].

For most drugs in TB treatment, the optimal dosage has not yet been established. Even with excellent compliance, simulation studies show that up to 1% of patients will develop drug resistance due to variability in drug concentrations [48,49,50,51,52]. Amikacin and kanamycin were classified until 2016 as WHO group 2 (injectable agents) and in the new classification in group B for the treatment of MDR-TB and are administered in a dose of 15 mg/kg/day with a maximum of 1000 mg daily in MDR-TB treatment [53]. The pharmacodynamic index of aminoglycosides is usually quantified as the ratio of the maximum blood concentration (Cmax) to the MIC. The area under the concentration–time curve might be a more appropriate pharmacokinetic parameter in comparison with the Cmax or Cmin [54]. Cmax / MIC is a key predictor of the antibacterial activity of aminoglycosides; in gram-negative and gram-positive bacteria there is consensus that it should reach a value of > 10 in order to prevent the emergence of resistant clones, and to ensure clinical efficacy [55,56].

In our approach in using aminoglycosides for MDR-TB treatment, [57,58] we have applied individualised treatment based on the Cmax/MIC ratio using a limited sampling strategy. The importance of individual dosing in relation to toxicity and effectiveness of aminoglycosides was stressed by Zhu et al [59]; the AUC of streptomycin in 19 patients varied from 124 – 680 µg•hr/ml while the Cmax varied from 9 – 107 µg/ml. Since the pharmacokinetics of aminoglycosides like streptomycin, amikacin and kanamycin are assumed to be comparable, variations in AUC and Cmax of amikacin and kanamycin can be expected.

Dosages of aminoglycosides in patients with MDR-TB in our observational study (a median dose of 400 (IQR; 350.0 – 500.0 mg) were more than two-fold lower than the dose recommended by WHO, while nonetheless, outcome (clinical efficacy) was favourable in the vast majority of our patients. Besides, no treatment failures or documented relapses were observed using this lower dose of aminoglycosides in an analysis of all MDR-TB patients diagnosed and treated in the Netherlands [60]. Like in earlier studies treatment duration and the cumulative dose were correlated with side effects, but not with the dose or the dosing frequency [61,62,63].

This limited sampling strategy provides a good estimate of the AUC_{0-24h} and is therefore suitable for daily patient care and use in outpatient clinics, but also during therapeutic drug monitoring (TDM) in prospective clinical trials [64]. TDM using standard or alternative sampling techniques, such as dry blood spot (DBS) sampling or oral fluid sampling, could improve acceptability by doctors and patients, thereby contributing to limiting adverse effects by tapering the TB-drug dose to the lowest possible effective dose [65]. Therefore ultimate goals should perhaps be: either optimizing doses for specific populations by

taking into account pharmacokinetic variability or, better still, individualization of each patient's doses if resources are available [64].

WHO recommendations underscore that countrywide, comprehensive and effective implementation of the WHO-recommended Stop TB strategy (developed from the DOTS framework), is an important approach for preventing drug-resistant TB [66]. However there are suggestions that monitoring the levels of TB drugs in a patient's blood could be as important as monitoring compliance with therapy -- in contrast to current WHO guidelines [50,53].

Conclusions and recommendations: to monitor efficacy of the aminoglycosides in treating MDR-TB, in future studies the use of the AUC/MIC ratio instead of the Cmax/MIC ratio to monitor efficacy and the use of a limited sampling strategy needs to be validated in an in vitro model for infection and subsequently tested in a prospective clinical trial [67]. Nevertheless, evidence in animal models suggest that the $AUC_{0\text{-}24h}$/MIC ratio predicts the efficacy of aminoglycoside therapy [54], and we speculate that this ratio can also be applied to humans [68]. This needs to be confirmed in a hollow fiber model as has already been done for moxifloxacin [49].

Chapter 5: Ertapenem.
In the battle against TB, the development of new and/or the exploration of repurposed drugs are urgently needed, with a quest for few(er) drug-drug interactions, high(er) efficacy, few(er) side effects and low(er) cost. This is not only required for DR-TB but also for *M tuberculosis* sensitive for the first line drugs in order to facilitate and shorten therapy as much as possible. In particular, as treatment of DR-TB is costly and requires prolonged use of many drugs, administration of safe and effective drugs is crucial [69]. All this will improve patients' compliance even for patients with drug susceptible *M tuberculosis*, after all a so-called standard "short" course treatment for TB is lasting 6 months. In regard to treatment duration in patients with MDR-TB, the shortest treatment with acceptable outcome and minimal side effects still lasts 9 months [70,71,72]. This is a considerable improvement compared to the 20-24 months recommended in the former guidelines by WHO for DR-TB, while the new 2016 WHO guidelines recommend a period of 12 months for a shorter regimen for selected patients. However 9 months is still asking a lot from the patient and the supporting, providing system. Generally speaking, many patients do not experience clinical signs of their disease anymore after several weeks of treatment, and this will contribute to 'treatment fatigue' and ultimately non-adherence. Therefore: the shorter the duration of the therapy, the better. Additionally, it would be most useful when these therapies are beneficial for drug-susceptible as well as for DR-TB [73].

In adding new drugs to a TB regime there are 2 possibilities: (a) repurposed drugs 74,75,76 and (b) new drugs, like bedaquiline (TMC 207), delamanid (OPC 67683) and pretomanid (PA-824) [77].

There are more than a dozen new or repurposed tuberculosis drugs under clinical investigation. In the revised 2016 WHO guidelines on DR-TB, two new drugs, delamanid and bedaquiline are recommended as add-on agents. The first reported patient with both these add-on agents was described in 2016 [78].

In Chapter 5, we focus on one of the repurposed drugs, ertapenem, one of the carbapenems, labelled for other bacterial infections and potentially useful in the treatment of DR-TB (MDR- as well as XDR-TB) [79].

Ertapenem has not yet been labelled as a group 5 drug to be used as part of MDR-TB treatment. In case of proven efficacy against DR-TB and because of its relative long half-life time which enables once daily dosing, ertapenem may be an attractive carbapenem. The drawback is its parenteral use. Parenteral drug administration has a price, both in terms of logistics and finance as well as in terms of risks – bleeding, nosocomial transmission of drug-resistant organisms, bleeding and thrombosis [80]. An inhaled formulation of injectable TB drugs might be advantageous, provided that such treatment would be tolerated and would result in acceptable and adequate bioavailability.

In our retrospective study among MDR- and XDR-TB patients, safety and pharmacokinetics of ertapenem were evaluated. Ertapenem treatment was well tolerated during MDR-TB treatment and showed a favourable PK/PD profile in MDR-TB patients [81]. Although our study did not focus on efficacy, sputum smear and culture were converted in all patients. In the review study by Sotgiu et al [79] the efficacy/effectiveness profile of the carbapenems was promising.

In another retrospective study of our group the aim was to develop a model to predict the area under the concentration-time curve, measured over the first 24h after ertapenem drug administration ($AUC_{0\text{-}24h}$) during steady state, using a limited sampling strategy in patients with MDR-TB. Whether TB drug concentrations influence clinical outcome is still a controversial issue [82]. Although not yet proven for patients with TB, %T>MIC is expected to be an important pharmacodynamic parameter. According to the population model, most of the patients reached a minimum of 40% time above MIC of 0.5 mg/L and eleven patients exceeded %T>MIC of 0.25 mg/ L. We had previously shown in a population pharmacokinetic model based on healthy volunteers that $AUC_{0\text{-}24h}$ of ertapenem can be estimated in with 2 sampling time points using a limited sampling strategy [83]. This study

shows that $AUC_{0\text{-}24h}$ of ertapenem can be estimated also in patients with MDR-TB with only 2 sampling time points using a limited sampling in a population model and in a linear regression model [82].

Indeed, ertapenem safety and pharmacokinetics in combination with TDM as described above make us believe that ertapenem in combination with clavulanate is a highly promising drug for the treatment of MDR-TB that warrants further investigation [79,82, 84].
Ineffective or incomplete treatment, slow drug responses leading to prolonged infectiousness, acquired drug resistance, treatment failure and early relapse, as well as the emergence of MDR- TB that all thrive in the absence of TDM, call for a change to bring TDM to the forefront [85]. TDM could be cost-effective even in high incidence, low resource settings. With the novel tools and procedures in place, TDM should no longer be a remote possibility but rather be adapted as an integral component of national TB programs similar as TB diagnostics and first- and second-line TB drug supply. We therefore propose that methods like limited sampling strategies and optimized by TDM supported by high resource countries, can already be very useful in high TB burden countries, to optimize outcome, minimize side effects and toxicity, and prevent emerging drug resistance.

Conclusions and recommendations: we advocate a prospective study in patients with DR-TB with ertapenem, dosing by estimating the $AUC_{0\text{-}24h}$ translated in a %T>MIC with 2 sampling time points (a limited sampling strategy); parameters being efficacy and safety.

Future perspectives and conclusions

To defeat TB (including MDR-TB) by 2030, which is the current target set by the United Nations in their Sustainable Development Goals (SDG), a combination of effective vaccination, immunomodulation and improvement of current drug treatment is needed. To achieve the ambitious goals, the recently revised guidelines on MDR-TB (WHO, 2016) have already agreed that in selected patients, treatment duration might be shortened to 9-12 months. This thesis describes several approaches, like effective vaccination, immunomodulation and improving current drug treatment as valuable options in the battle against MDR-TB.

To reach the TB-related SDG, we advocate and emphasize aspects of vaccine development (pre- and post-exposure; and therapeutic), finding ways of boosting the immune system during active disease, the promotion of precision diagnostics and treatment with a combination of active drugs killing rapidly replicating bacteria and of drugs targeting persister organisms. Initial drug treatment with relatively high efficacy and low toxicity

should be guided by a selection based on mutations detected by PCR (Xpert-TB RIF; Haine Line Probe Assay), tailored if possible on phenotypic DST (i.e., below or above the EUCAST breakpoint) and preferably MIC, allowing for optimized by TDM, instead of a shot gun approach with all the collateral damage, like (avoidable) drug-induced toxicity, and preventing the emergence of drug resistance. The proposed strategy is currently not evidence-based; it should be possible to test the hypothesis that individualised, PK/PD driven treatment for MDR-TB yields improved outcome with reduced toxicity compared to programmatic standardized treatment; a study design using wedge-stepped randomisation might be the best way forward.

A close collaboration between all stakeholders in TB is mandatory in reaching the TB-related SDG, both in high-resource and in high-burden countries. This approach should be (made) affordable for national programs in endemic regions and countries. The required resources combatting global TB – both in manpower and in financing - are huge. Solving the TB-problem will perhaps be extremely costly, however in the light of an earlier experience with AIDS, these problems must and should be overcome [86]. Besides, few health interventions have been as cost-effective as fighting TB [87,88].

Last but not least, in order to be able to compare studies on MDR-TB a common set of core research definitions (like on efficacy and safety) is needed to ensure there is comparability in clinical trials on MDR-TB and to maximize policy impact [89].

NOTE

From 2011 until 2016 the aminoglycosides were represented in group 2 of the five groups of drugs of the WHO list for treating DR-TB. These five groups were:

WHO recommended grouping of anti-TB drugs (until 2016)

Group		Anti-TB agent	Abbreviation
1	First-line oral anti-TB drugs	Isoniazid	H
		Rifampicin	R
		Ethambutol	E
		Pyrazinamide	Z
		Rifabutin	Rfb
		Rifapentine	Rpt
2	Injectable anti-TB drugs (the injectables)	Streptomycin	S
		Kanamycin	Km
		Amikacin	Am
		Capreomycin	Cm
3	Fluoroquinolones (FQs)	Levofloxacin	Lfx
		Moxifloxacin	Mfx
		Gatifloxacin	Gfx
		Ofloxacin	Ofx
4	Oral bacteriostatic second-line anti-TB drugs	Ethionamide	Eto
		Prothionamide	Pto
		Cycloserine	Cs
		Terizidone	Trd
		p-aminosalicylic acid	PAS
		p-aminosalicylate sodium	PAS-NA

5	Anti-TB drugs with limited data on efficacy and/ or long term safety in the treatment of drug-resistant TB (This group includes new anti-TB agents)	Bedaquiline	Bdq
		Delamanid	Dlm
		Linezolid	Lzd
		Clofazimine	Cfz
		Amoxicillin/Clavulanate	Amx/Clv
		Imipenem/Cilastatin	Ipm/Cln
		Meropenem	Mpm
		High-dose isoniazid	High dose H
		Thioacetazone	T
		Clarithromycin	Clr

In 2016, WHO changed these groups in Updated guidelines on MDR-TB.

Current Medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant TB (Table 6 in the Updated 2016 WHO Guidelines on MDR-TB) [1]

A. Fluoroquinolones	Levofloxacin Moxifloxacin Gatifloxacin		Lfx Mfx Gfx
B. Second-line injectable agents	Amikacin Capreomycin Kanamycin (Streptomycin)		Am Cm Km (S)
C. Other core second-line agents	Ethionamide / Prothionamide Cycloserine / Terizidone Linezolid Clofazimine		Eto / Pto Cs / Trd Lzd Cfz
D. Add-on agents (not part of the core MDR-TB regimen)	D1	Pyrazinamide Ethambutol High-dose isoniazid	Z E H
	D2	Bedaquiline Delamanid	Bdq Dlm
	D3	p-aminosalicylic acid Imipenem-cilastatin Meropenem Amoxicillin-clavulanate (Thioacetazone)	PAS Ipm Mpm Amx-Clv (T)

1 http://www.who.int/tb/areas-of-work/drug-resistant-tb/MDRTBguidelines2016.pdf?ua=1

The second line injectable agents were and still are an important component of a DR-TB regime.

Of the carbapenems imipenem (in combination with cilastatin[2]) and meropenem were classified as group 5. In the new guidelines they are considered as one of the add-on agents.

Ertapenem is not included yet in the new WHO guidelines.

2 Cilastatin is a chemical compound which inhibits the human enzyme dehydropeptidase. This enzyme is situated in the kidney and is responsible for degrading the antibiotic imipenem. Cilastatin is considered a beta-lactamase.

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Chapter 7

Appendices

Nederlandse samenvatting

Lijst met afkortingen / list with abbreviations

Dankwoord

Curriculum vitae

List of publications

*In **hoofdstuk 1** van dit proefschrift geven wij een probleemschets van TB, mondiaal en in Nederland, met de nadruk op het ontstaan van, en de omvang van het probleem van geneesmiddelresistentie.*

Tuberculose (TB) is een infectieziekte veroorzaakt door *Mycobacterium tuberculosis* (MTB). Naast de meest voorkomende lokalisatie in de longen komt de ziekte in circa 20 % van de gevallen elders voor in het lichaam. Dat kan in alle organen en weefsels. Verspreiding van de ziekte gebeurt vnl. via een aerosol uitgehoest door patiënten met (open / besmettelijke) longtuberculose. Na inademen van MTB kan een gezond iemand besmet raken. Na besmetting treedt actieve tuberculose op bij minstens 10 en mogelijk bij 18 % van de geïnfecteerden. Naarmate de cellulaire immuniteit lager is, neemt de kans op actieve tuberculose toe.

Zonder behandeling komen veel patiënten aan de ziekte te overlijden. Circa 70 % van patiënten met besmettelijke long-TB overlijdt binnen 10 jaar en bij de minder ernstige gevallen van long-TB is het percentage dat komt te overlijden binnen 10 jaar nog altijd circa 20%.
Vaccinatie (BCG) is nog weinig effectief gebleken in de preventie van TB. Bij jonge kinderen zou het bijdragen aan een tegengaan van sommige ernstige vormen van buiten de longen optredende TB, zoals hersenvliesontsteking en verspreiding van MTB door de bloedbaan waarna diffuus in vele organen TB uitzaaiingen ontstaan - vaak aangeduid als miliaire TB.

Streptomycine was het eerste medicijn tegen TB. Het werd in 1943 geïsoleerd uit de bacterie *Streptomyces griseus* door Albert Schatz in het laboratorium van Selman Abraham Waksman. Streptomycine bleek niet alleen effectief maar ook schadelijk te zijn zowel voor de nieren als voor het gehoor- en evenwichtsorgaan.

Ondanks de (aanvankelijke) verbetering die zich voordeed bij streptomycine therapie verslechterde het klinische beeld na enkele maanden alweer. Dit bleek te berusten op een verworven / secundaire resistentie van MTB bij deze monotherapie. Dit probleem deed zich later ook voor bij andere TB-middelen gebruikt als monotherapie.
Para-aminosalicylic acid (PAS) was al gauw het volgende medicijn tegen TB in 1944.
De British Medical Research Council toonde in 1948 aan dat combinatietherapie met streptomycine en PAS niet alleen effectiever was dan monotherapie maar dat ook daarbij nog steeds verworven / secundaire resistentie voorkwam.
Van primaire resistentie wordt gesproken wanneer de patiënt wordt besmet door al resistente mycobacteriën.
Indien bij een patiënt met resistente TB slechts 1 nieuw medicament aan de medicatie

wordt toegevoegd zal verdere resistentie tegen dit laatste middel worden ontwikkeld waardoor de MTB steeds resistenter wordt. Belangrijk om deze verworven resistentie te voorkomen is daarom dat bij iedere vorm van actieve TB zo snel als mogelijk een resistentie- (c.q. gevoeligheids-)patroon van de MTB wordt verkregen zodat daarop een adequate therapie in kan worden gesteld.
Een stam van MTB wordt resistent genoemd als de benodigde concentraties van het geneesmiddel zo hoog zijn dat het in de praktijk, met gangbare doseringen niet mogelijk is om die remmende concentraties te bereiken.
Niet altijd is resistentie dus een absoluut begrip; de test die in de reageerbuis wordt uitgevoerd probeert in feite een voorspelling te doen van wat in het lichaam van de TB-patiënt gebeurt. Men meet de laagste concentratie van het geteste middel waarbij nog juist de groei van de bacterie geremd wordt. Deze geneesmiddelconcentratie wordt de minimale remmingsconcentratie (MIC) genoemd; op andere terreinen van de farmacologie heet dit de farmacodynamiek of PD. Theoretisch is het denkbaar dat ook al is deze MIC relatief hoog, het middel toch nog effectief is als de patiënt maar een hogere dosering krijgt toegediend; de juiste uitspraak over de vraag of een middel nog effectief bijdraagt aan de behandeling hangt dus af van twee vragen: hoe hoog is de MIC van de bacterie? en: hoe hoog is de effectief gegeven geneesmiddelconcentratie in de aangedane weefsels van de patiënt? Er van uitgaande dat de meeste weefsels goed van bloed worden voorzien, kunnen we voor de weefselconcentraties ook de bloedconcentratie in de loop van de tijd direct na inname van het middel nemen. Het meten van bloedconcentraties van geneesmiddelen in de tijd noemen we farmacokinetiek of PK. We noemen deze benadering waarbij we de individuele dosering toepassen op basis van de MIC en de gemeten bloedconcentraties: Therapeutische Geneesmiddel-Monitoring of TDM; de onderliggende berekeningen en metingen heten: PK/PD.

In de loop der jaren is resistentie van MTB steeds meer toegenomen. Van Multi-Geneesmiddel Resistente TB (MDR-TB) wordt gesproken wanneer de MTB resistent is voor tenminste isoniazide en rifampicine, de twee sterkste TB-medicijnen. Een nog ernstiger vorm van resistentie is Extensief Geneesmiddel Resistente TB (XDR-TB). Hierbij bestaat er naast de resistenties die voorkomen bij de MDR-TB óók resistentie tegen een van de fluorochinolonen en tevens tegen minimaal één van de drie via injectie toegediende middelen uit de tweedelijnsmedicatie tegen TB (amikacine, kanamycine of capreomycine).

Bij normaal gevoelige MTB bedraagt ondanks de naam 'short-course' de behandelduur nog altijd zes maanden. De WHO gaf tot voor kort als aanbeveling bij MDR-TB een therapieduur van 20 maanden. De tweedelijns medicijnen die bij MDR-TB worden toegepast zijn minder effectief dan eerste lijns-middelen, hebben meer bijwerkingen en zijn duurder.

Wereldwijd ontwikkelden in 2012 circa 8 miljoen mensen actieve TB en circa 1.3 miljoen kwamen aan de ziekte te overlijden. De hoogste aantallen nieuwe TB gevallen doen zich voor in de ontwikkelingslanden. Immigratie vanuit landen met hoge prevalentie maakt dat TB ook in de westerse rijkere landen een probleem blijft.
Van de geschatte 9 miljoen patiënten in 2013 was circa 56% afkomstig uit de regio zuidoost Azië en het westen van de Stille Oceaan. Afrika telde circa 2 miljoen patiënten (bijna 25%), en gezien het totaal aantal mensen in Afrika, heeft dit continent de hoogste incidentie en mortaliteit cijfers per 100.000 inwoners. India en China hadden respectievelijk 24 en 11 % van het totale aantal TB gevallen.
Van de geschatte 9 miljoen TB patiënten in 2013 had circa 13% (1.1 miljoen patiënten) co-infectie met HIV. Het aantal mensen dat komt te overlijden aan de combinatie TB en HIV is de laatste 10 jaar dalende.
Van de nieuwe TB patiënten in 2013 had naar schatting 310.000 MDR-TB, ongeveer 3.5% van de nieuwe TB patiënten en 20.5% van de patiënten die eerder waren behandeld voor TB. Dat komt neer op 480.000 patiënten met MDR-TB in 2013.
De schatting is dat gemiddeld 9% van de MDR-TB patiënten in feite XDR-TB hebben. MDR-TB bedreigt de voortgang in het bestrijden van TB, het ontstaan van XDR-TB heeft dit probleem nog groter gemaakt. Sinds 2006 is XDR-TB gediagnosticeerd in alle werelddelen. XDR-TB is door de Wereldgezondheidsorganisatie (WHO) benoemd als een ernstige opkomende bedreiging voor de wereld gezondheid, speciaal in landen waar veel mensen met HIV geïnfecteerd zijn.
In veel gebieden, zoals Afrika, is de werkelijke omvang van het resistentie probleem onbekend en in de meeste ontwikkelingslanden is therapie voor MDR-TB onvoldoende of zelfs volledig afwezig.

In 2013 waren in Nederland op een bevolking van 17 miljoen inwoners 848 nieuwe gevallen van TB, 5 nieuwe TB gevallen per jaar/100.000 inwoners (NTR[1]). De laatste 10-jaar daalde het aantal patiënten met 38%. De meesten waren van buitenlandse komaf (74%). Het percentage dat werd getest op HIV nam toe van 28% in 2008 tot 51% in 2013. Het percentage TB-patiënten geco-infecteerd met HIV daalde de laatste 10-jaar tot 2.0% in 2013.

De laatste 5 jaar varieerde het aantal MDR-TB patiënten in Nederland tussen de 10 en 20, circa 1-2% van het totaal aantal TB-patiënten. In 2013 waren 17 patiënten geregistreerd met MDR-TB, allen oorspronkelijk afkomstig uit het buitenland.
Alle TB gevallen in Nederland worden behandeld volgens nationale richtlijnen en aangegeven bij de afdeling TB bestrijding van de GGD. Deze afdeling behandelt ook de

1 NTR: Nederlands Tuberculose Register. 'Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Tuberculose in Nederland 2013 Surveillancerapport'. (pg 9-10)

ongecompliceerde gevallen, doet contactonderzoek en houdt zich bezig met screening. Bij de dalende TB cijfers werden afdelingen samengevoegd, terwijl kennis op peil werd gehouden met trainingen.
De NTR wordt bijgehouden door de KNCV. Vanaf 2012 gebeurt dit onder auspiciën van het Rijksinstituut voor Volksgezondheid en Milieu (RIVM).
Patiënten met complexe TB, met bijkomende infecties en andere ziektes kunnen terecht in een van de twee specialistische TB centra in Nederland – in Beatrixoord, Haren, onderdeel van het UMCG, Groningen; en in Dekkerswald, Groesbeek, onderdeel van het Radboud UMC, Nijmegen.

*In **Hoofdstuk 2** (immunologische aspecten van tuberculose) wordt het afweersysteem tegen TB beschreven.*

MTB geeft in het lichaam aanleiding tot verschillende immuunreacties. Deze reacties zijn belangrijk in het kader van het bedwingen, voorkomen of indammen van de ziekte. Soms kan de afweerreactie zo heftig zijn dat het lichaam meer schade heeft van de eigen afweer dan van de MTB zelf. Het bijsturen van deze reacties is daarom soms belangrijk om lichaamsschade te beperken. In dit kader kunnen ijzer en vitamine D als corrigerende stoffen van nut zijn.

Bij veel ziekten is vaccinatie het belangrijkste middel gebleken ter bestrijding. In de preventie van TB heeft het enige vaccin dat op dit moment ter beschikking staat, BCG, weinig bijgedragen om de TB epidemie een halt toe te roepen, hoewel vaccinatie van pasgeborenen wel enige kortdurende bescherming biedt tegen miliaire TB en TB meningitis. Om de verspreiding van TB in de bevolking te verminderen is de hoop gevestigd op nieuwere, betere vaccins.

Tijdens therapie kunnen de symptomen van TB verergeren. Dit kan berusten op een falen van de therapie, bijvoorbeeld doordat de medicatie dosering onvoldoende is of MTB resistent is tegen de gegeven medicijnen. Een toename van symptomen kan echter ook berusten op een paradoxale reactie: de ontstekingsreactie neemt toe terwijl de MTB effectief onderdrukt en gedood wordt. Bij deze paradoxale reactie zijn geen er dus therapiefouten of tekortkomingen, maar reageert de afweer te heftig. Het onderscheid tussen beide aandoeningen kan moeilijk en vaak onmogelijk zijn. Omdat beide aandoeningen een totaal andere aanpak nodig hebben is een zo goed mogelijk onderscheid hierin van groot belang.
Bij falen van de therapie zijn meestal andere TB-medicamenten nodig; bij een paradoxale reactie kan worden afgewacht of zijn afweer onderdrukkende medicamenten zoals

corticosteroïden nodig om de lichaamsafweer te dempen; dit laatste zou funest kunnen zijn bij therapiefalen.

Een paradoxale reactie bij HIV positieve patiënten wordt het 'Immuun Reconstitutie Inflammatoir Syndroom' (IRIS) genoemd. Zeker bij deze populatie, met verminderde weerstand, is het van belang snel te weten of we te maken hebben met therapiefalen of met een overreactie van de zich herstellende eigen afweer, door de gegeven antiretrovirale (anti-HIV) therapie.

De interacties tussen gastheer (patiënt) en ziektekiem zijn gebaseerd op een samenspel van vooral erfelijke eigenschappen van beide partijen. Deze eigenschappen kunnen gunstig maar ook ongunstig zijn voor de patiënt.

*In **Hoofdstuk 3** wordt een overzicht gegeven van behandelresultaten van patiënten met MDR-TB in Nederland.*

Twee observationele studies van Nederlandse patiënten worden besproken. De eerste studie betreft MDR-TB patiënten geïncludeerd van 1 januari 1985 tot 1 september 1998, die tot 1 augustus 1999 gevolgd werden. De 2e studie betreft eveneens MDR-TB patiënten, die met therapie waren gestart in de periode 2000–2009. Patiëntkenmerken, klinische gegevens en gegevens van de Medische Microbiologie (kweek, PCR, gevoeligheidstesten) worden besproken. In beide studies waren de percentages (86%) van succesvolle behandeling zeker in vergelijking met die van andere landen gunstig. Deze percentages behaald bij MDR-TB patiënten zijn in feite de streefwaarden voor patiënten met geneesmiddel-gevoelige TB. De behandelschema's zijn niet gebaseerd op vergelijkend geneesmiddelonderzoek; de keuze en dosering van de gebruikte middelen zijn zoveel mogelijk individueel gekozen op basis van geneesmiddelgevoeligheidstests, in toenemende mate gecombineerd met bepalingen van bloedconcentraties van geneesmiddelen in de tijd. Hoe logisch deze benadering ook lijkt, echt goed vergelijkend wetenschappelijk bewijs dat deze benadering de beste is ontbreekt in feite. Het is dus nog onduidelijk wat de beste therapie is bij MDR-TB; dit geldt voor toepassing van de gebruikte middelen, de combinaties, de dosering van die middelen en de therapieduur. In een grote meta-analyse met 9153 patiënten waren de uitkomstresultaten met een succes percentage van gemiddeld 60%. gemiddeld genomen duidelijk minder gunstig dan in onze twee studies, Een succesvolle behandeluitkomst was vooral gerelateerd aan een langere behandelduur, aan het aantal effectieve middelen dat werd ingezet zowel in de eerste intensieve fase, als het aantal MTB dat actief deelt nog hoog is, alsook in de

continuatie fase - de fase waarin de nog aanwezige levende MTB in een soort winterslaap verkeert en vaak binnen bepaalde cellen overleeft ('persisteert'). Sommige middelen m.n. de chinolonen van de modernere latere generaties lijken een belangrijke factor voor succes. Of de verschillen in uitkomst alleen verklaard moeten worden door verschillen in de behandeling of wellicht in belangrijke mate ook door van de samenstelling van de patiëntengroep, de bijkomende ziektes, de ziekte-ernst, de voedingstoestand en de ernst van de TB, blijft in dit soort cohortonderzoek onduidelijk. Niet alle gerapporteerde onderzoeken die in deze meta-analyse zijn betrokken zijn nauwkeurig in het vermelden van alle belangrijke gegevens, en voorzichtigheid is geboden bij de interpretatie van de resultaten. Voor sommige TB-middelen is nog steeds onduidelijk wat hun bijdrage is aan genezing van patiënten. Resistentievorming tijdens therapie kan uiteraard de uitkomresultaten slechter maken.
Een door ons gehanteerde strategie ter voorkoming van het ontwikkelen van resistentie en tegelijkertijd het minimaliseren van bijwerkingen is het toepassen van maatwerk in de behandeling, TDM, zoals hierboven beschreven. TDM is belangrijk enerzijds ter voorkoming van bijwerkingen en anderzijds om te waarborgen dat MTB effectief bestreden wordt, door PK/PD doelen na te streven vooral voor die TB-middelen die een beperkte therapeutische bandbreedte van bloedconcentraties hebben waarbinnen de bijwerkingen laag blijven, en de effectiviteit voldoende hoog is.
In deze twee studies werden PK metingen in bloedmonsters verricht. De MIC testresultaten werden toegestuurd vanuit het nationale TB referentielaboratorium van het RIVM in Bilthoven. De gevoeligheidsbepalingen via een kweekmethode zijn goed maar vergen relatief veel tijd. Via snelle moleculaire tests kunnen genetische afwijkingen in MTB worden aangetoond die voorspellend zijn voor resistentie. Of deze tests de huidige tijdrovende kweek-gebaseerde gevoeligheidsbepalingen helemaal zullen vervangen lijkt op dit moment niet erg waarschijnlijk maar nu al worden deze tests in toenemende mate gebruikt wat tijdswinst geeft in de keuze van de juiste middelen.

We zijn ons bewust dat de hoge succescijfers van onze studies meerdere oorzaken hebben en het is moeilijk aan te geven hoeveel iedere factor hieraan heeft bijgedragen. Uiteindelijk kunnen alle factoren hierin essentieel zijn: de selectie van de combinatie van geneesmiddelen, gevoeligheidsbepalingen, DST uitgevoerd door een centraal referentie laboratorium, het aanpassen van de therapie aan de gevoeligheid van MTB en de dosering van de medicatie op geleide van PK metingen. Een sterk gemotiveerd en goed getraind behandelteam van artsen, verpleegkundigen, diëtisten, fysiotherapeuten, maatschappelijk werkers en ander ondersteunend personeel; enthousiaste artsen-microbioloog en andere medisch specialisten die in consult of medebehandeling zijn; vloeiende samenwerking met de verwijzende medisch specialisten, GGD-artsen en

leidinggevenden en bestuurders van de specialistische centra en de samenwerkende ketenpartners, allen spelen een onmisbare rol in het bereiken van de goede resultaten.

Of en in hoeverre de TDM-principes ook toepasbaar zijn in lagelonenlanden is voorlopig nog afhankelijk van goede samenwerking met gespecialiseerde centra in de Westerse wereld. Bloedconcentratiemetingen kunnen met behoud van voldoende precisie aangepast worden, door het aantal af te nemen monsters te beperken tot slechts twee of drie; en door het monster via een vingerprik af te nemen en als druppel op een vloeipapier op te brengen en als gedroogd monster per post op te sturen naar een referentielaboratorium. Waarschijnlijk loont het de moeite om de moeilijke kweekmethode voor vaststellen van geneesmiddelgevoeligheid in die landen te vervangen door snelle, makkelijk uit te voeren genetische tests.

*In **hoofdstuk 4** beschrijven wij drie studies over het gebruik van aminoglycosiden bij MDR-TB.*

Aminoglycoside antibiotica zijn middelen die niet als tablet via het maagdarmkanaal in het lichaam worden opgenomen. Er kunnen alleen voldoende bloedconcentraties bereikt worden als ze direct in de bloedbaan (of als injectie in de spier) worden ingespoten. Het zijn belangrijke maar ook bezwaarlijke middelen: bij te lage concentraties werken ze niet, en bij te hoge blootstelling van het gehoor- en evenwichtsorgaan en de nieren kunnen duizeligheid, doofheid en nierfunctieverlies ontstaan. Bij deze middelen is het dus enorm belangrijk dat serumspiegels worden afgenomen om de effectiviteit te waarborgen en de ernstige bijwerkingen en vergiftigingsverschijnselen te voorkomen. Op basis van gepubliceerde gegevens en onze eigen opgebouwde ervaring zijn we er in ons onderzoek steeds van uit gegaan dat we bij TDM bij aminoglycosiden met een beperkt aantal bloedafnames (en niet met minstens 10 bloedafnames in 1 dag - volledige dagcurves) kunnen volstaan om die doelen te bereiken.

De aminoglycosiden (streptomycine, kanamycine, amikacine) en het andere daarop lijkende middel capreomycine worden als belangrijke, effectieve medicamenten beschouwd bij de aanpak van MDR-TB. De Wereldgezondheidsorganisatie (World Health Organization; WHO) heeft deze middelen eerder geclassificeerd in groep 2 - nu groep A (injiceerbare middelen) voor de behandeling van MDR-TB met een vaste dosering van 15 mg/kg/dd en een maximum van 1000 mg per dag.
Bij toepassing van aminoglycosiden kunnen zich echter twee belangrijke bijwerkingen voordoen: nefrotoxiciteit (met een prevalentie van 7.5% tot 15%) en gehoorverlies (met een prevalentie van 18% tot 37%). Daarom ligt de grote uitdaging bij toepassen van

aminoglycosiden bij de behandeling van MDR-TB in een minimaliseren van deze ernstige bijwerkingen met behoud van effectiviteit.

Om te proberen deze ernstige bijwerkingen tegen te gaan werden in onze (retrospectieve) studie van MDR- en XDR-TB patiënten aminoglycosiden gedoseerd op basis van PK/PD en MIC bepalingen. Op grond van onze bepalingen kwamen we uit op een mediane dosering van 6.5 mg/kg/dd, in plaats van de door de WGO geadviseerde dosering van 15/ mg/kg/dd, een dosisreductie van meer dan 50 %. Hierbij is onze aanname dat effectiviteit behouden blijft met minimalisering van bijwerkingen.

In deze studie waren 113 patiënten (69 mannen en 44 vrouwen), waarvan 104 met therapie startten. Om diverse redenen werd geen therapie gestart bij 9 patiënten: 1 asielzoeker kon niet meer worden getraceerd; 4 patiënten hadden Nederland al verlaten voordat de diagnose werd gesteld; 1 kind van 9 jaar met klier-TB werd na klierextirpatie vervolgd zonder therapie; en 3 patiënten werden post-mortem gediagnosticeerd met MDR-TB.

De uitkomstresultaten (klinische effectiviteit) waren goed; 86% van de patiënten was genezen (de kweken werden negatief) of had een succesvol voltooide behandeling (zonder kweek-bewijs). Deze getallen zijn vergelijkbaar met streefcijfers bij patiënten met normaal gevoelige TB. Falen van de behandeling werd niet gezien, evenmin als een terugkeer van ziekte na voltooiing van de behandeling bij deze relatief lage dosering van aminoglycosiden. Net als bij eerdere studies deden bijwerkingen zich voor bij langere behandelduur en bij oplopende totale (cumulatieve) dosering, maar er was geen samenhang met de dagdosering en evenmin met doseringsfrequentie.

Bij 80 patiënten met MDR- en XDR-TB werd eveneens gekeken naar de bijwerkingen en de effectiviteit van de aminoglycosiden. De dosering was als hierboven beschreven: op geleide van PK/PD en MIC bepalingen met een beperkt aantal afgenomen bloedmonsters. De mediane (meest voorkomende) dosering was 6.5 mg/kg/dd i.p.v. de door de WGO geadviseerde, meer dan 2x hogere dosering van 15 mg/kg/dd.
Gehoortesten werden iedere 3-4 weken uitgevoerd bij 250, 500, 1000, 2000, 4000 en 8000 Hz. Gehoorverlies werd gedefinieerd als een vermindering van 20 decibel (dB) in vergelijking met de test voor de start van aminoglycoside-behandeling.
Bij 9 patiënten (11%) werd een gehoorverlies gevonden, vooral bij de hogere frequenties (4000 en 8000 Hz). Er werd geen correlatie gevonden tussen gehoorverlies en één van de andere medicamenten.
In andere studies werden veelal hogere waarden gevonden van patiënten met gehoorverlies: vanaf 21.3% tot 37%. Naarmate de dosering per kg lichaamsgewicht hoger

was, trad meer gehoor-schade op, dit was de enige correlatie die werd gevonden.
Het voorkomen van nierbeschadiging in onze studie was vergelijkbaar met andere studies. Een verhoogd creatinine wordt hierbij als maat gebruikt maar is deze maat is discutabel omdat TB patiënten tijdens adequate therapie vaak in spiermassa aankomen – en creatinine wordt aangemaakt in de spieren, waardoor een toename van het creatinine juist een gunstig teken kan zijn.
Wij zagen dat het met onze patiënten goed ging; kennelijk was de behandeling effectief.

Om de effectiviteit te behouden en de bijwerkingen zo gering mogelijk te houden hebben we een populatie farmacokinetisch (PK) model ontwikkeld en gevalideerd. Daarbij hebben we het aantal bloedmonsters voor het bepalen van amikacine en kanamycine bij (MDR-)TB zo klein mogelijk gehouden. Dat is prettig voor patiënten die dan minder bloedafnames hebben, en het bespaart kosten in de laboratoriumbepalingen, zowel voor de dagelijkse patiëntenzorg als bij wetenschappelijk onderzoek.

De beste maat voor effectiviteit van aminoglycosiden is waarschijnlijk de maximale concentratie in het bloed (Cmax) gedeeld door de MIC (Cmax / MIC). Dit is bij andere bacteriën dan MTB een belangrijke voorspeller van de antibiotische activiteit van aminoglycosiden. Bij gramnegatieve en grampositieve bacteriën is men het erover eens dat de waarde > 8 zou moeten zijn voor klinische effectiviteit en ter voorkoming van resistentievorming. Het lijkt aannemelijk dat de PK van streptomycine, kanamycine en amikacine vergelijkbaar is

Wij beargumenteren dat het oppervlak onder de concentratie-tijdcurve (*the area under the curve*) gedeeld door de MIC (AUC_{0-24h}/MIC) tenminste net zo belangrijk is hoewel dit nog niet is bevestigd bijvoorbeeld in een proefopstelling waarin de wisselende concentraties in de tijd in een holle buis worden nagebootst. Dit idee moet nog wel in toekomstig onderzoek bij mensen worden bevestigd.

Een beperkte afname strategie van aminoglycosiden werd specifiek bestudeerd in een andere studie. Daarnaast werd in deze studie het hierboven beschreven populatie PK model ontwikkeld en gevalideerd om een optimum wat betreft de effectiviteit en de bijwerkingen te vinden in de dosering. Voor amikacine was dat een mediane (meest voorkomende waarde) voor de AUC_{0-24h}: 77.2 mg h/L en voor kanamycine was dit 64.1 mg h/L.

Variatie van AUC_{0-24h} tussen verschillende personen kan soms groot zijn en daarbij

bijdragen aan zowel onderdosering met daarbij mogelijk verworven resistentievorming, als aan overdosering met meer bijwerkingen.

Toekomstperspectieven en aanbevelingen

Na meer dan 30 jaar voorschrijven van aminoglycosiden bij TB in een dosering van 15 mg/kg lichaamsgewicht lijkt de tijd rijp voor een prospectief vergelijkend onderzoek van behandeling met de klassieke dosering (15 mg/kg) aminoglycosiden in vergelijking met dosering op basis van de Cmax / MIC m.b.v. TDM met als primaire uitkomstmaten: effectiviteit en bijwerkingen.

TDM stuit zowel bij artsen als bij patiënten op bezwaren en bedenkingen. Wij pleiten voor een ruimere toepassing. Als de methode om geneesmiddelconcentraties te meten wat makkelijker wordt, zoals uit een op filterpapier gedroogde bloeddruppel uit een vingerprik, of concentratiemeting in speekselmonsters zal TDM misschien makkelijker ingang vinden in de praktijk.

Het bewaken van medicatiespiegels bij de patiënt zou wel eens een belangrijk wapen kunnen zijn in de strijd tegen de oprukkende geneesmiddelresistentie wereldwijd.

*In **hoofdstuk 5** bespreken we twee studies over ertapenem, een carbapenem antibioticum, en als bèta-lactam antibioticum verwant aan penicillines en cefalosporines, medicijnen die bij vele andere infectieziekten (veroorzaakt door gram positieve, negatieve en anaerobe bacteriën) worden gebruikt.*

Om nieuwe medicamenten aan het arsenaal van middelen tegen TB toe te voegen worden geheel nieuwe medicamenten zoals bedaquiline (TMC 207), delamanid (OPC 67683) en Pretomanid (PA-824) ontwikkeld; daarnaast bestaat de mogelijkheid om te kijken of middelen die al voor andere indicaties op de markt zijn, misschien ook werkzaam zijn bij TB. Op dit moment zijn van alle carbapenems twee middelen: meropenem en imipenem (samen met cilastatine) die door de WHO als mogelijk effectieve anti-TB-middelen worden genoemd. Tot nu toe maakt ertapenem er nog geen deel van uit. Het middel wordt al wel gebruikt bij diverse gram positieve en negatieve en anaerobe infecties. Een carbapenem gecombineerd met clavulaanzuur toonde bij muizen activiteit tegen TB. Een gunstige eigenschap van ertapenem is dat het langzaam uitgescheiden wordt in de urine: het heeft een relatief lange halfwaardetijd, en dat er geen omzetting in de lever plaats vindt, waardoor er ook minder botsingen met andere medicamenten ontstaan. Bij andere infecties dan TB kan ertapenem heel goed werken als het slechts eenmaal per

dag wordt toegediend. Het nadeel is dat het middel niet via het maagdarmkanaal wordt opgenomen; het moet als injectie worden toegediend.

Het bactericide (bacterie-dodende) effect van alle bèta-lactam antibiotica, en dus ook van carbapenems, is gecorreleerd aan de tijd dat de concentratie in het serum – niet aan eiwitten gebonden - hoger is dan de MRC (MIC): T_{free} > MIC. Zodra de concentratie onder de remmende concentratie daalt, houdt het effect op; bij andere infecties moet die concentratie minstens 70% van de tijd boven de MIC zijn om optimaal te werken. Bèta-lactam antibiotica hebben een tijd-afhankelijke bacteriedodende werking. De carbapenems krijgen antibacteriële eigenschappen wanneer de plasmaconcentratie minimaal 40% van de tijd uitkomt boven de MIC (%T > MIC). Hoewel dit nog niet is bewezen voor TB patiënten, is de verwachting ook hier dat %T>MIC een belangrijke maat voor effectiviteit is.

In ons (retrospectieve) onderzoek werden de bijwerkingen en farmacokinetiek van ertapenem bestudeerd bij 18 patiënten met MDR- en XDR-TB. Naast ertapenem was de andere DR-TB medicatie gebaseerd op een individueel regiem op basis van gevoeligheidsbepalingen van de gekweekte MTB stam in het laboratorium. Het middel werd uitstekend verdragen tijdens langdurige behandeling. Het PK/PD profiel was gunstig (zoals beschreven hierboven); de gemiddelde $AUC_{0\text{-}24h}$ was 544,9 (tussen 309 – 1130) mg h/L. De gemiddelde C_{max} was 127.5 (73.9 – 277.9) mg/L.

Onze studie richtte zich niet op effectiviteit; wel bleek dat bij alle patiënten het microscopisch en kweekonderzoek van het sputum een fraaie omslag naar negatief liet zien.

De studieopzet had beter gekund – als de gegevens niet retrospectief maar prospectief waren verzameld waren de uitslagen waarschijnlijk betrouwbaarder; ook was er geen controlegroep met voor het overige gelijke behandeling om zuiverder het effect van de toevoeging van ertapenem te kunnen beoordelen. We moeten daarom voorzichtig zijn met het doen van harde uitspraken, vooral over de effectiviteit. Een ander en groot probleem is het definiëren van de MIC van ertapenem bij MTB.

Het doel van onze andere ertapenem-studie was een model te ontwikkelen dat het gebied onder de concentratie tijd curve gemeten over de eerste 24 uur ($AUC_{0\text{-}24}$) na toediening van ertapenem kon voorspellen met gebruik van een beperkte bloedafname bij patiënten met DR-TB. De $AUC_{0\text{-}24}$ kan 'vertaald' worden naar %T>MIC en de verwachting is daarmee

dat deze waarde voor wat betreft de anti-mycobacteriële activiteit gebruikt kan worden bij prospectieve studies.
Frequente afnames van het gebied onder de curve (AUC) is tijdrovend, kostbaar en een last voor de patiënt daar gedurende 24 uur afnames plaatsvinden. Gebruikmakend van het populatie PK model werden strategieën met beperkte afnames geëvalueerd. In een simulatie gaven de tijdstippen 1 en 4 uur na dosering de beste voorspellende ertapenem-uitslagen, weergegeven in de $AUC_{0\text{-}24h}$. Deze tijdstippen zijn ook geschikt zijn voor afname van andere medicamenten.

De gemiddelde $AUC_{0\text{-}24h}$ voor ertapenem was 621.3 (403.4 - 1101) mg*h/L. Twee patiënten hadden hoge plasma-top-concentraties (> 900 mg/L). Volgens het populatie PK model (PKK) werd bij de meeste patiënten een minimum bereikt van 40% van de tijd boven de MRC (MIC) van 0.5 mg/L, bij 11 patiënten kwam de %T>MIC boven de 0.25 mg/ L.

Dit is de eerste studie bij patiënten met DR-TB waarin een PK-model van ertapenem werd gebruikt. Het is een goed bruikbaar model, met een onderschatting van 14 % vergeleken met directe precieze metingen in het bloed.

Toekomstperspectieven en aanbevelingen

Ertapenem in WHO D3 ('*add-on*'; voorheen: groep 5: '*unknown significance*') van de MDR-TB medicatie?
Wij denken dat ons werk met ertapenem, samen met dat van anderen, reden is voor verder onderzoek naar de klinische effectiviteit en veiligheid bij DR-TB. daarvoor is eerst nodig dat een betrouwbare gevoeligheidstest voor MTB beschikbaar komt; een gerandomiseerd onderzoek met een controlegroep (RCT) met een effectief achtergrondschema, waarin liefst een aminoglycoside (amikacine / kanamycine) wordt vergeleken met ertapenem.

TDM en beperkte bloedafname

Bij gebruik van ertapenem bij DR-TB (dit geldt evenzo voor andere medicamenten in gebruik bij DR-TB) is TDM van groot belang om ineffectieve therapie (zoals verworven resistentie, therapie falen en een terugkeer van de ziekte) en (vermijdbare) bijwerkingen tegen te gaan.
Zowel in landen met lage als met hoge TB incidentie dient TDM (met beperkte bloedafname) een vaste plaats te krijgen in het nationaal TB programma (vergelijkbaar met TB diagnostiek en 1e en 2e lijns medicatie voorziening).

In landen waar veel TB voorkomt kan TDM met beperkte bloedafname misschien kostenbesparend zijn; technische ondersteuning is daarbij gewenst en noodzakelijk vanuit partners in de westerse wereld.

Om DR-studies met elkaar te kunnen vergelijken zijn vaste, eensluidende definities nodig, zoals bv over effectiviteit en bijwerkingen.

*In dit **Hoofdstuk 6** worden de belangrijkste bevindingen van dit proefschrift in 't kort vermeld: wat voegt het toe aan wat we al wisten, hoe kunnen we de resultaten gebruiken voor patiënten met MDR-TB en wat zijn toekomstverwachtingen bij de aanpak van TB?*

De WHO 2035 visie is het aanzienlijk terugbrengen van ziekte en sterfte door TB. Vooral voor de zgn. 'low-resource, high burden countries' is dit een enorme uitdaging. Diagnostiek en therapie moeten optimaal zijn om dat doel te bereiken. Vanuit die visie doen we in dit proefschrift enkele aanbevelingen m.b.t. de therapie, vooral van DR-TB.

Een benadering met de beste aanpak lijkt ons het ontwikkelen van een werkzame vaccinatie (toe te passen voorafgaand aan- en na besmetting; en als TB is ontstaan: als therapeutisch vaccin), naast eventueel nog andere vormen van beïnvloeding van de afweer van de patiënt zoals beschreven in hoofdstuk 2.

Hoofdstuk 3 geeft onze behandeluitkomsten weer van DR-TB in Nederland met zeer goede resultaten. De methodes waarmee deze werden behaald zijn medicatie gedoseerd op resistentiepatroon (PCR (Xpert-TB RIF; Haine Line Probe Assay), en met behulp van TDM; een goed geoutilleerd TB centrum met een gemotiveerd behandelteam en een uitstekende TB organisatie in Nederland, in dit laatste komt zeker een goede politieke wil als belangrijke factor naar voren.

In hoofdstuk 4 beschrijven wij drie studies over het gebruik van aminoglycosiden bij MDR-TB. We tonen aan dat met behoud van effectiviteit de dosering veelal kan worden verlaagd (< 50 % van de aanbevolen dosering) dit ging gepaard met een aanzienlijke afname van de bijwerkingen. Dosering was op basis van PK/PD en MIC met een beperkte afname strategie.

Ertapenem, een medicijn in gebruik bij andere infecties wordt besproken in hoofdstuk 5, als zodanig is het (nog?) niet toegevoegd aan de WHO lijst van anti-TB middelen. Een model werd ontwikkeld dat het gebied onder de concentratie tijd curve gemeten over

de eerste 24 uur (AUC_{0-24}) na toediening van ertapenem kon voorspellen met gebruik van beperkte bloedafname bij patiënten met DR-TB. De AUC_{0-24} kan 'vertaald' worden naar %T>MIC en de verwachting is daarmee dat deze waarde voor wat betreft de anti-mycobacteriële activiteit gebruikt kan worden bij prospectieve studies.

Een belangrijke volgende stap zou zijn om PK-studies in 'low income high-burden settings' te starten. 'Echte' DST met kweekproeven zijn misschien voorlopig nog een brug te ver, moleculaire testen zijn daarbij de beste benadering voor die kweekproeven. Naast en na PK studies kan ook een vergelijkend onderzoek tussen ertapenem en een aminoglycoside bij patiënten met DR-TB worden uitgevoerd, al moet dan wel met een panel van moleculaire tests worden gewerkt om ervoor te zorgen dat de gevoeligheid voor alle vergeleken geneesmiddelcombinaties bekend zijn. Met andere woorden, het achtergrond-regime moet bij alle deelnemende patiënten effectief zijn om therapiefalen te voorkomen.

Van belang voor het behalen van alle doelen is een nauwe samenwerking tussen alle deskundigen en medewerkers betrokken bij TB. Dit geldt zowel in landen met hoge als met lage incidentie.

Chapter 7

Appendices

AE	Adverse effects / adverse events
AUC	Area Under the Curve
BCG	Bacillus Calmette-Guérin
CI	Confidence interval
CIb	Centrum voor infectieziektebestrijding
DBS	Dry blood spot
DOTS	Directly Observed Treatment Short-Course
DST	Drug sensitivity testing
DR-TB	Drug resistant tuberculosis
GGD	Gemeentelijke Gezondheidsdienst
HIV	Human immunodeficiency virus / Humaan immuundeficiëntie virus
IRIS	Immuun Reconstitutie Inflammatoir Syndroom
KNCV	Koninklijke Nederlandse Centrale Vereniging tot bestrijding der tuberculose
MDR-TB	Multi-drug resistant tuberculosis
MIC	Minimal inhibitory concentration
Mtb (MTB)	*Mycobacterium tuberculosis*
NTR	Nederlands tuberculose register
PAS	Para-aminosalicylic acid
PD	Pharmacodynamische / farmacodynamische
PK	Pharmacokinetische / farmacokinetische
PPK	Een populatie farmacokinetisch model
RCT	Randomised controlled trial (onderzoek waarbij door loting patiënten of proefpersonen hetzij aan de actief behandelde groep, hetzij aan de controlegroep worden toegewezen)
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
RMSE	Root mean square error
SDG	Sustainable Development Goals
TB	Tuberculose / Tuberculosis
TDM	Therapeutic drug monitoring
WHO	World Health Organization (Wereld gezondheidsorganisatie)
XDR-TB	Extensively drug resistant tuberculosis

Chapter 7

Appendices

Mijn eerste promotor: Prof. dr. T.S. van der Werf, beste Tjip. Jouw kennis en inzet voor patiënten, collega's en onderzoek was niet-aflatend. Ondanks je drukke werkzaamheden was je, indien nodig, altijd aanspreekbaar voor overleg over patiëntenzorg en onderzoek. Ook als promotor was je voor mij zeer waardevol, altijd opbouwend kritisch, gelardeerd met ironie, en altijd komend met suggesties, zowel tekstueel als inhoudelijk, waarin uitkwam dat je de literatuur vaak al kende voordat die werd gepubliceerd. De wekelijkse, gezamenlijke visites met jou op de TB-afdeling werden door mij erg op prijs gesteld naast het feit dat je altijd andere relevante disciplines en interne en buitenlandse stagiaires aanmoedigde daarbij aanwezig te zijn, allemaal facetten waarmee deze visites naar een hoger plan werden getild. Dit was patiëntenzorg en onderwijs tezamen met tevens altijd suggesties voor onderzoek. Het was voor mij een eer en plezier met jou te hebben gewerkt.

Mijn tweede promotor: Prof. Dr. H.A.M. Kerstjens, beste Huib. Ik heb ontzettend veel waardering voor niet alleen je vele capaciteiten maar ook hoe je die inzet voor de longafdeling. Ondanks de meerdere long-subafdelingen was je er altijd voor de TB-afdeling en wist je de lijnen nadat je informatie had ingewonnen, niet alleen uit te zetten maar hierin ook veel te implementeren. Onder jouw leiding hebben we ontzettend veel bereikt, om er een paar te noemen: een fusie, een goed draaiende afdeling en een geheel nieuw TB-gebouw. Beatrixoord was aanvankelijk wel betrokken qua medische activiteiten bij toentertijd nog het Academisch ziekenhuis Groningen (AZG), het latere Universitair Medische Centrum Groningen (UMCG) maar was daarvan een onafhankelijke instelling. Bij het fusieproces, waaraan vele haken en ogen, was je nauw betrokken en kon je goed hoofd- en bijzaken van elkaar onderscheiden. Vanaf 1988 was ik werkzaam in Beatrixoord en had al vele fusiegesprekken meegemaakt, tijdrovend en zonder enig succes, mogelijk door onvoldoende motivatie van sommigen? Mijn scepsis over deze (achteraf laatste en gelukkig succesvolle) fusie wist je goed te hanteren. De bouw van een volledig nieuwe TB-afdeling, de drive bij het tot stand komen hiervan, komt grotendeels op jouw conto.
Onze eerste promotiegesprekken hebben we jaren terug gehouden tijdens een diner in Kaapstad. Het is er nu eindelijk van gekomen.

Mijn co-promotor: Dr. J.W.C. Alffenaar, beste Jan-Willem. Vanaf je komst in Beatrixoord, waar we elkaar leerden kennen, kreeg ik veel waardering en bewondering voor je vooruitziende blik en inzet die je toonde voor onderzoek m.b.t. de farmacologisch gerelateerde patiëntproblematiek op de TB-afdeling. Wij konden komen met vragen en problematiek vanuit de klinische kant, jouw farmacologische kennis en drive voor onderzoek vulde dit goed aan. Tijdens de patiëntbesprekingen en ook tijdens onze dinsdag en donderdag lunchbesprekingen waarin veelal TB-problematiek werd bediscussieerd, kwamen vrijwel

altijd suggesties met onderzoeksvragen en hoe daarmee verder vooral (maar niet alleen) vanuit farmacologische benadering. Dit was altijd erg stimulerend. Veel onderzoek en promovendi heb je in korte tijd succesvol begeleid. Je ziet snel kansen, kunt delegeren en blijft tegelijk goed coachen en 'in the lead.' In dit alles heb ik veel aan je gehad en daarmee bedoel ik ook de TB-afdeling die daarmee en dankzij jou op een veel hoger plan is gekomen.

I am grateful to Professor GB Migliori for being a member of the assessment committee. Ook Professor Frank Cobelens, en Professor Bhanu Sinha, de twee andere leden van de beoordelingscommissie dank ik voor het beoordelen van het manuscript.

Daarnaast zijn er veel mensen die op directe en indirecte wijze een belangrijke rol hebben gespeeld of tot grote steun zijn geweest bij het tot stand komen van dit proefschrift. Aan hen ben ik veel dank verschuldigd.

Alle patiënten die deelnamen aan onderzoek, en dat zijn er veel geweest in al die jaren, ben ik erg dankbaar dat ze hieraan en altijd belangeloos, wilden deelnemen. Een openheid en uitleg van onze kant, aangepast aan het niveau van de patiënt, over het waarom van onderzoek zal hieraan zeker hebben bijgedragen. De verpleging heeft hierin, onder leiding van ons (mijn) aller Tineke een belangrijke rol in gespeeld. Nu ik Tineke heb genoemd, je was geweldig en erg belangrijk voor de afdeling. Onze reis naar Canada was onvergetelijk. Je was me pas jaren later dankbaar dat je je presentatie ook uitstekend beheerste voor het geval de elektriciteit zou zijn uitgevallen. Iets wat daar trouwens onwaarschijnlijk was maar waar ik nu ben is het tegenovergestelde meer het geval.

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Dr. O.W. Akkerman, beste Onno, de afdeling kan zich geen betere opvolger van mij wensen. Deze mooie tak van sport wordt door weinig (klare) longartsen geambieerd, m.i. omdat het te onbekend is. Dankzij de UMCG-Beatrixoord fusie zijn er voor deze functie ook meer mogelijkheden en kansen geschapen om het vak breder te kunnen uitoefenen. In de korte tijd dat we hebben samengewerkt was je een voortreffelijke en heel plezierige

collega, begaan met je patiënten en met onderzoek. Heel mooi dat jouw boekje is afgerond. Begrijpelijk is dat je als domicilie voor het dorpje Altena hebt gekozen.

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Na de totstandkoming van het UMCG was er vanuit psychiatrie altijd een vaste consulent. Voor de TB-afdeling is dit een must. Er zijn veel patiënten met psychiatrische problematiek, het herkennen daarvan en indien mogelijk hulp bieden zijn belangrijke pijlers. Voor het team kan niet-herkennen en vaak daarmee gepaard een verkeerd hanteren van sommige psychiatrische problematiek grote negatieve gevolgen hebben. Ondersteuning was er niet alleen voor patiënten maar vooral voor de teamleden hoe hiermee om te gaan, enigszins vergelijkbaar met dog whisperer Cesar Millan: niet de hond maar vooral de baas veranderen om beter om te gaan met de hond. Beste Drs C. Jiawan, beste Carel, de teamleden waar ik toe behoor hebben ontzettend veel aan je gehad, veel dank voor je humoristische en wijze inbreng.

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werk. Dit lijkt me een goede eigenschap voor een apotheker, maar hopelijk blokkeert dit niet dat het afronden van jouw boekje al te lang op zich laat wachten. Aan onze samenwerking op de afdeling heb ik goede herinneringen.
Dear Hoa, I really enjoyed our cooperation in particular taking (limited?) samples from patients on the TB-department. I still remember your Groningen-home-made Vietnamese spring rolls and according to your family legends, your mother can make them even better. Although we live close by since a few years, we have not teamed up yet after Groningen.

Vanuit het laboratorium van het RIVM was er goede samenwerking richting medicijn-gevoeligheidsbepalingen bij MTB maar ook nauwe betrokkenheid bij meerdere onderzoeksprotocollen. Prof. dr. D. van Soolingen, beste Dick dank voor alle samenwerking, je inbreng en kritische opmerkingen bij de referaten en je bereidwilligheid altijd mee te denken over de overlappingen in ons vakgebied. Onze reis naar Turkmenistan staat me nog diep in het geheugen gegrift. Na een reis van bijna 2 dagen zonder slaap bleek het hotel niet beschikbaar waarna we naar een leeg gebouw werden gereden, zonder drinkwater, en zonder water in de toiletten. We konden slapen op houten britsen zonder enige vorm van bekleding. Jij weigerde subiet, hoewel hun aanbod een welgemeende traditie kon zijn en mogelijk zelfs door hen werd gezien als uiterst eervol, gaf je aan de Turkmeniërs te verstaan direct terug naar Nederland te willen gaan waarop na wat morren adequate logies voor onze groep werd geregeld. Ook hier toonde jij je talenten, met veel verve en succesvol!
T. van der Laan, beste Tridia. Ondanks de afstand kon ik altijd (en vaak) een laagdrempelig (telefonisch) beroep op je doen met vragen over gevoeligheidsbepalingen of gerelateerde zaken. Veel dank voor al je hulp en inzet en altijd prettige samenwerking.

Dr. G de Vries, beste Gerard, de belangrijke combinatie van jouw klinische kennis eerder opgedaan en je huidige inzichten en visie vanuit de epidemiologische en de public health sector waren voor mij erg waardevol en stimulerend. Zonder jouw hulp en inzet was mijn dissertatie nooit zover gekomen. Veel tijd heb je gestoken in het opdiepen en analyseren van Beatrixoord-data. Je bereidheid om mee te willen lezen en denken vond ik een prachtig aanbod. Je bent voor mij een geweldige vriend en collega.

Drs J. A Dijkstra, beste Koos en Msc. S. P. van Rijn, beste Sander en J. F. Borjas Howard, beste Jaime, jullie veel dank voor al je inzet bij het vergaren en interpreteren van vele, vele data, een monnikenwerk (waar ik nu ben is 1 op de 100 mannen monnik) met uitkomsten goed voor mijn en binnenkort ook voor de dissertaties van Koos en Sander. Het was ontzettend plezierig met jullie de vele data uit te spitten en te interpreteren. Werkzaam vooral als clinicus was dit met jullie kennis en uitleg, voor mij een aanvullende eye-opener.

Mijn dank gaat uit tevens uit naar alle co-auteurs die hebben bijgedragen aan deze dissertatie. Niet ongenoemd wil ik laten de gedreven dokters uit het TB-centrum te Dekkerswald, Dr Martin Boeree en Dr Cecile Magis-Escura, ook zij verdienen een nadrukkelijke vermelding. Het was en is plezierig met hen samenwerken!

Alle personeelsleden van de TB-afdeling waren mij erg dierbaar. Het zijn, zeker in de loop der jaren, te veel personeelsleden om ze allemaal te benoemen en bedanken, zowel in de disciplines verpleging, maatschappelijk werk, fysiotherapie als diëtetiek en last but not least: de voedingsassistenten. Maar wel: mw I Rinsma, teammanager / hoofdverpleegkundige, beste Iris, heel veel jaren hebben we met veel reilen en zeilen de afdeling geleid. Er was niet altijd even veel houvast, met iedere nieuwe manager werden nieuwe managementstijlen ingevoerd, niet gebaseerd op logica maar op persoonlijke voorkeur. Jij wist hier goed mee om te gaan en verdiepte je weer in onwerkbare controlesystemen met allerlei 'checklisten' met geduldig papier. Ik wachtte vaak weer op de volgende manager voor een 'nieuwe aanpak'. Jij wist me dan in zo'n periode goed uit de wind te houden zodat ik mijn werk in patiëntenzorg en onderzoek kon doen. Hierin waren we zeker aanvullend. Waar we vooral naar keken was niet zozeer de methode met het systeem maar waar liggen de kwaliteiten van de teamleden en waar kunnen we die het beste inzetten. Onze patiënt-populatie was een bijzondere met alle mogelijke lichamelijke en psychosociale problematiek en leeftijden. Onze teamleden konden hier goed mee omgaan, mede te danken aan jouw leiderschap. Zowel mijn ontgroeningsexamen (in 1994?) als mijn afscheidsfeest waren voor mij onvergetelijke gebeurtenissen, en zolang ik 'ertussen werd genomen', leek de fantasie van de teamleden ongebreideld te zijn. Om over de tussenliggende periode maar te zwijgen. Vaak denk ik met veel plezier en met goede herinneringen terug aan mijn periode in Beatrixoord. Voor mij was het een voorrecht om van dit team deel uit te mogen maken. En zonder de hulp, inzet van al deze vele teamleden onder jouw leiding zou ik het eindresultaat van deze dissertatie nooit hebben kunnen bereiken.

Het secretariaat: naar mijn mening is een secretariaat helaas een sterk ondergewaardeerd discipline. De drie secretaresses: Alie, Natascha en Marja ben ik erg dankbaar voor alle hulp gegeven aan de afdeling, zowel qua patiëntenzorg als qua ondersteuning in onderzoek maar ook richting de menselijke kant naar menig personeelslid. Beste Alie, een afdeling en de personeelsleden daar werkzaam, kunnen zich geen betere secretaresse wensen. Iedereen kon altijd op je inzet rekenen. Mijn eerste stappen op de TB-afdeling waren in '93-'94, jij werkte er al veel langer. Door je kennis van de afdeling heb je me toen veel geholpen en veel werk uit handen genomen. Ik stelde prijs op inhoudelijk goede brieven zonder taalfouten; het nakijken en corrigeren van de taal werd altijd zorgvuldig door jou gedaan en je wist altijd wel verbeteringen aan te brengen. Verder heb ik van jou geleerd op buienradar te kijken voordat je naar huis fietst. Dit laatste heb ik nu niet meer nodig,

het weer is hier vrijwel 100% (en zelfs maanden van tevoren) voorspelbaar. Enkele jaren terug heb je het besluit genomen vervroegd uit te treden en thuis (en vooral uit) met je Arjen, je man te gaan genieten. Slechts herinneringen blijven over aan onze goede samenwerking die ik altijd heb gewaardeerd.
Beste Natascha, je was altijd plezierig aanwezig en met veel inzet. Doordat jouw Russisch nog altijd beter is dan 'GOOGLE translate' kwamen we erachter dat voordat we een trip maakten naar een van de ex-Russische staten voor een TB-meeting, niet al onze dia's juist waren vertaald. Het Engelse woord 'stable' was voor meerderlei uitleg vatbaar: *'Shortly after start therapy the patient came in stable condition'* zou zonder jouw hulp in het Russisch zijn veranderd in: *'Shortly after start therapy the patient came in a horse stable.* Geen idee hoe onze gastheren zouden hebben gereageerd, echter nadat we de omstandigheden voor patiënten in het desbetreffende land hadden bekeken zou de paardenstal niet eens een slecht alternatief onderkomen zijn geweest.
Beste Marja, niet alleen als secretaresse was je voortreffelijk, ook als mens en ook nog eens met humor. Je was handig in het regelen en organiseren en had hierin veel goeie tips, en soms zelfs diplomatiek. Ook ben je heel creatief, mijn (papier-maché) hond is prachtig geworden.

Als laatste wil ik mijn belangrijke paranimfen bedanken voor hun steun en inzet; mijn dochter Lydia, geweldig zoals jij kunt multitasken waardoor veel werk werd verzet en ogenschijnlijk met plezier en haar vriend Maarten Roorda die altijd klaarstond als een schaaktoren in de branding.

Last but not least, lieve Naing Naing, jouw aanmoedigingen met het proefschrift te beginnen en je verdere ondersteuning gedurende de hele periode waren voor mij belangrijk dit tot een goed einde te kunnen brengen. Ongetwijfeld komen er nog vele andere mooie projecten op onze weg waarin wederzijdse ondersteuning meer oplevert dan de som der delen.

Hiernaast zijn er natuurlijk ook velen die indirect hebben bijgedragen aan dit proefschrift. Onderzoek doen en een dissertatie schrijven waren voor mij twee verschillende eieren die gelegd zijn. Het doen en vooral bedenken van onderzoek is voor mij een must en hoort bij patiëntenzorg. Het bediscussiëren en komen met ideeën en niets op voorhand aannemen is fascinerend. De uitvoering in een dissertatie wordt al wat minder en het opschrijven is weer wat verder voor mij weg.

Hoe verder na dit proefschrift?
Ik geloof dat ik voorlopig iets geestelijks ga doen, voor mijn gedachtenleven. Maar wat?
(Marten Toonder, Heer Bommel uit: De Viridiaandinges)

Chapter 7

Appendices

Curriculum vitae

Richard van Altena was born on December 17th 1947 in Haarlem, the Netherlands.
In 1966 he attended Fairfield College for 1 year in Hamilton, New Zealand.
He graduated from Petrus Hondius lyceum, a secondary school in Terneuzen, in 1968.

Medical (and Philosophical) training period

1968 - 1977	Medical training (Graduated at the Free University of Amsterdam)
1972	Master in Philosophy (Graduated at the Free University of Amsterdam)

Medical working experience

1974 - 1975	Junior Medical Doctor at Westmoreland County Hospital in Kendal, Great Britain.
1978 - 1981	Medical Doctor in Bennekom Hospital (The Netherlands) respectively in the departments of orthopedics, casualty, obstetrics and gynecology, internal medicine, cardiology, neurology, paediatrics and psychiatry. During evening, night and weekend shifts working on all departments.

Pulmonary Physician training period

1981 - 1983	St Franciscus Hospital Rotterdam; Department of Internal Medicine.
1984 - 1986	University Medical Centre Utrecht; Department of Pulmonary diseases.

Pulmonary Physician working period

1987 - 1988	University Medical Centre Utrecht; Department of Pulmonary diseases.
1989 - 1993	Medical Head of the Asthma Centre Beatrixoord; University Medical Centre Groningen, the Netherlands.
1994 - 2012	Medical Head of the Tuberculosis Centre Beatrixoord; University Medical Centre Groningen, the Netherlands.

Participation in national Dutch guidelines

Medical treatment of tuberculosis
Diagnostics and treatment of latent tuberculosis
Prevention, diagnosis and treatment of tuberculosis and HIV
Diagnosis and therapy of tuberculosis in immunocompromised patients
Latent tuberculosis and TNF alpha therapy

WHO activities

2004 Ethiopia, one week training session in the approaches of MDR-Tb.
2007 Geneva, Switzerland; disabilities resulting from tuberculosis infection.
2008 Kiev, Ukraine one week training session in the approaches of MDR-TB.
2011 Turkmenistan, one week training session in the approaches of MDR-TB.

Invited lectures

Yearly, in 2007, 2008 and 2009 during a one week session, lecturing on different aspects of tuberculosis in Cape Town, South Africa.

Commission memberships

From 1994 until retirement president of the schooling committee of the Foundation of Dutch Tuberculosis Doctors (VvAwT).
Member of the committee tuberculosis of the KNCV Tuberculosis foundation.
Member of the tuberculosis committee of the Dutch Pulmonary Physicians society.

Rewards

2008 Pulmonary Physician of the year in the Netherlands.

Retirement

December 2012 he retired from his work at the UMCG, since then he lives part of the year in Myanmar and part of the year in Glimmen, Groningen.

Chapter 7

Appendices

List of publications related to this thesis

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