General discussion and future perspectives
TB has caused more deaths than any other infectious disease in the history of mankind. In 2013 alone, there were about 1.5 million deaths worldwide. With 9 million new cases annually, the disease burden of TB is also staggering. The epidemiology of sequelae due to TB has been studied less¹. To conquer both TB and its sequelae, more knowledge has to be gained about diagnostics, epidemiology and treatment of TB and its sequelae.

Here we discuss these issues in the context of the studies compiled in this thesis, and we provide future perspectives.

**Transmission of *Mycobacterium tuberculosis***

*Mycobacterium tuberculosis* (Mtb) depends on human hosts for its survival: humans are the dominant reservoir for the organism. Transmission is the critical way for Mtb to survive as a species, as every new human host expands its reservoir. Thus prevention of TB starts by increasing the knowledge about transmission.

Transmission depends on the immunology as well as the behavior of the human host, as we discussed in the introduction. After developing overt pulmonary TB, TB patients start coughing and sneezing, thus producing aerosols containing live Mtb. Once inhaled by a new susceptible host, Mtb may reach the lower airways including the alveolar spaces. Healthy bronchi will clear the Mtb by the ciliary beat driving the tapis roulant; entrapped bacilli are transported to the proximal airways and swallowed or expectorated by coughing. Damaged airways – e.g., as a result of smoking – make the host more susceptible to TB²⁻⁴.

Contact tracing around an index case can tell us about the transmission of TB in a microenvironment⁵. Contact tracing can be done using the tuberculin skin test (TST) or an interferon gamma release assay (IGRA). As latent TB infection (LTBI) is still lacking a clear definition a gold standard is still lacking for the TST and both IGRA. Before interpreting the results of the TST and the IGRA the BCG vaccination status, previous contacts, the immune status of the individual tested and the test properties should be known⁶⁻¹². Normally, active case finding is only performed around a case of human TB. However, *M. bovis* still causes TB – in around 1.0⁻¹.5% of the cases of TB in the Netherlands, the United Kingdom and the United States¹³⁻¹⁵. Our report shows that using an IGRA may help in contact tracing around an index case of bovine TB.

In the macro-environment, the routes of transmission of the strains within and between countries or continents are found using molecular-epidemiological techniques like
fingerprinting techniques. Fingerprinting helps understanding the route of transmission thereby generating knowledge about virulence of certain strains and lineages. Furthermore fingerprinting can provide evidence of unexpected routes of transmission. Moreover, it can provide links that cannot be detected by classical epidemiological techniques. In the past this was done using spoligotyping or IS6110-RFLP, but in recent years it has been replaced by VNTR technology. Another benefit of fingerprinting is that early knowledge of the results may guide clinicians to embark early on an adequate treatment regimen in a patient with an MDR strain. Early knowledge about the origin of the strain and its susceptibility to SLD’s may prevent administering unnecessary drugs or overdosing appropriate drugs, thereby preventing adverse effects of the drugs prescribed.

Therefore the most optimized VNTR technique should be available worldwide with little increase in costs. The combination of the studies comparing 14 different PCR assays and the optimized VNTR technique can guide clinicians in requesting rapid VNTR typing in individual cases.

The knowledge of the routes of transmission and the evolution of Mtb should further increase and results should be known as soon as possible. It can change early knowledge about the strain and thereby not only change management of contact tracing but also treatment for a patient. Whole genome sequencing (WGS) might fill the gap left over by conventional but cheaper techniques like VNTR. So future studies with WGS should make the routes of transmission and evolution of Mtb even clearer and known as soon as possible. For smaller outbreaks, much cheaper optimized epidemiologic techniques should be developed to combat TB worldwide. This means that these techniques should be better affordable without loss of performance using WGS as gold standard.

**MDR-TB**

In the Netherlands, MDR-TB was steady with around 15–20 patients per year in recent years, though in 2014 the number of newly detected MDR-TB cases was slightly lower (i.e., only 6 patients). In 2013, worldwide estimations are that 3.5% of all newly detected cases and 20.5% of the relapses have been reported to be MDR-TB. In Belarus around 75% of the relapses are MDR-TB. These facts make MDR-TB one of the biggest infectious threats globally. MDR-TB probably has similar virulence compared to drug-susceptible TB strains. Though this is still debated and therefore controversial, virulence is probably dependent on factors associated with certain MTB lineages with an inherent potential to elicit cytokine
and inflammatory reaction of the host; loss of drug susceptibilities does not appear to result in an equal loss of virulence factors in most studies 22–24.

Two validated, commercialized molecular tests are currently available for testing for possible MDR-TB, the GenoType MTBDRplus (Hain Lifescience) and the Xpert MTB/RIF (Cepheid). Molecular testing for sensitivity should be performed as early after diagnosis as possible. In the future the molecular sensitivity testing using WGS should be combined with the viability of bacilli. Knowing the resistance of a strain prevents inadequate treatment with its toxicity. By giving adequate treatment for drug-resistant strains, its transmission can be prevented earlier. Importantly, we show that in-house and commercial assays can successfully be compared in a clinically meaningful way. We have shown for the first time, that most of these assays compare favorably using a platform that mimics real-life. Our experiment indeed mimicked the situation often encountered in low incidence settings like ours, with patients having low bacillary loads. In these sputum smear- and PCR negative patients suspected to have TB, we employ bronchoscopic broncho-alveolar lavage for diagnosis. Broncho-alveolar lavage is a procedure that by definition causes dilution of the alveolar bacillary load by around 1,000 fold. Comparing diagnostic thresholds of these 14 different diagnostic assays is highly relevant in clinical practice. In our study comparing PCR assays, with its limitations, we found the Xpert MTB/RIF to have the lowest analytical sensitivity. In high incidence countries this is of less importance due to a high a priori change of diagnosing TB and finding a higher bacterial load in the population of these countries. In low incidence countries there is a need to further evaluate the performance for detecting resistance of the GenoType MTBDRplus and the Xpert MTB/RIF on its own or using both tests together.

In contrast to WHO guidelines, therapeutic drug monitoring (TDM) has indeed been used in the treatment of MDR-TB in the Netherlands, with excellent results 25,26. By adjusting the dose in case of high serum drug levels possible adverse events of second line anti TB drugs (SLD) can be prevented, while detection of low serum drug levels can be adjusted so as to improve efficacy, and hence, potentially enhance cure rate. Indeed, no treatment failures or early relapses have been detected in the last decades in our center 26,27.

One potential drawback of TDM is, that it assumes a near-perfect correlation of TB drug concentrations in the blood stream and at the site of TB infection. Further knowledge about the correlation between serum levels of TB drugs and the levels of the same drugs at the site of infection would fill important knowledge gaps. Obviously, only occasionally will it
be possible to obtain tissue specimens of infectious TB foci; and only in cases of elective surgery, will such simultaneous measurements in blood and in resected tissue be possible. Nonetheless, it would be a great advantage to obtain more specimens of affected tissues or fluids for measuring levels of drugs with simultaneous plasma drug measurements over time. This also applies to measuring concentrations at target sites that are even more challenging and critical, e.g., cerebro-spinal fluid.

The current technology for TDM is burdening to patients and the health care system. One disadvantage of TDM is the large number of serum samples that should be drawn to obtain a reliable pharmacokinetic curve. The reliability and potential benefit of TDM through limited sampling strategy using the dried blood spots (DBS) sent by mail in ambient temperature conditions for processing in a reference lab have to be further examined.

No study has yet compared treatment outcome of MDRTB with individualized treatment using TDM versus standard-dose following the WHO guidelines. Therefore, such a study would be a tremendous asset to evaluate the full potential of TDM using DBS. We hypothesize that this strategy would improve treatment outcome in MDR-TB while also reducing adverse effects and toxicity.

A similar spot card, as used for DBS, might be suitable for detecting molecular genetic mutations in drug targets predicting drug sensitivity in sputum samples, using whole genome sequencing. Both TDM and molecular drug sensitivity might be performed in specimens collected using only one or two spot cards. If this platform provides adequate predictions for outcome, it might be used on a routine basis in national TB programs, and not only for patients that fail on treatment.

**Sequelae of TB**

Those who survive TB may pay health costs that have received little attention. Side effects – especially, liver test abnormalities – may cause delay in the treatment. Other side effects include vestibular and auditive impairment, especially as a result of group 2 second-line TB drugs – aminoglycosides.

Sequelae of extra-pulmonary TB have been identified. TB meningitis has the most profound and severe sequelae. Indeed, if this condition does not result in death, it may result in severe neurological damage, even with anti-inflammatory co-medication, neurological outcome is poor in a large proportion of affected individuals. Future studies in this
population are urgently needed to gain more knowledge of the proper treatment and the levels of the drugs in the CSF. In low incidence countries this can be achieved by transferring such patients to a high care TB center where a wide range of facilities are available, and where protocols are in place to achieve the goals of TDM simultaneously in blood- and CSF specimens over time 29.

Sequelae of pulmonary TB were not systematically recorded when the trials on short-course, rifampicin-based therapy were reported 40. Several authors have published about cavitary lung lesions that were sterilized from TB bacilli following TB treatment but that were subsequently colonized by fungal hyphae, especially, Aspergillus fumigatus leading to aspergillomas 1,41–43. Bronchiectasis after pulmonary TB used to be common although currently, it has become an unusual cause of non-Cystis Fibrosis (CF) bronchiectasis in affluent countries 44–49. In TB-endemic regions however, this condition is fairly common among the sequelae of TB, and clearly TB is the most common cause of structural lung disease world-wide 49,50.

Unfortunately, also due to a paucity of studies, the data base providing the evidence for the management of non-CF bronchiectasis is still limited 51. For that reason, treatments are often based on the relatively large studies on which the CF bronchiectasis guidelines are based.

Aggressive treatment for infectious exacerbations improved the survival of patients with CF dramatically in the last 20–30 years. One important aspect of this treatment is antibiotic therapy directed against Pseudomonas aeruginosa. The persistent presence of this microorganism has been shown to be an independent risk factor for more rapid decline in quality of life and pulmonary function 52,53. Recently a study was performed to test inhalation of tobramycin in patients with non-CF bronchiectasis chronically colonized with P. aeruginosa with an acute exacerbation. Oral ciprofloxacin was added for 14 days. This led to a greater reduction in microbial load at day 14 but no significant clinical effect was found 54.

Inhalation of aminoglycoside antibiotics has several potential advantages over intravenous administration. High intravenous dosing of these agents is usually not well tolerated, with considerable renal and audio-vestibular toxicity. Inhaling these drugs may provide high concentration of the drug at the site of infection circumventing the systemic toxicity.

The first generation of aminoglycoside-inhaled therapy was done using a wet nebulizer. In recent years, several devices containing dry powder antibiotics have become available, for example the Podhaler with tobramycin 55–57. Inhalation of dry powder antibiotics is less
time consuming and easier for patients that prefer a small device to carry on. Indeed low systemic exposure was confirmed in CF patients with the Podhaler 58.

The Cyclops, a device specifically designed for inhalation of dry powder aminoglycosides 59, was tested in patients with non-CF bronchiectasis with excellent tolerability.

Pharmacokinetic results in the population of non-CF BE gained from this study should be compared with results gained from future studies in healthy volunteers and patients with CF-BE. This could help us to understand the mechanism of systemic absorption after inhalation of tobramycin, as there is still a lack of knowledge of what happens after inhalation; no detailed information is available on where particles are deposited, and neither do we know what happens next 60. Such studies could evaluate and improve our described model as well. Also future studies should evaluate microbiological and clinical outcomes of patients with non-CF BE using the Cyclops compared to the Podhaler, or other devices used for inhaled aminoglycosides.

As the Cyclops was developed for inhalation of dry powder aminoglycosides, kanamycin can be used as well. Future studies should explore the question whether inhalation of kanamycin using the Cyclops – or any other dry powder inhalation device – could possibly replace the injectable therapy of kanamycin. The latter has its inherent problems of injected therapy including long hospital admissions and / or risks incurred by intravenous access, including catheter-related thrombosis and infection.

This thesis might be the start of exploring inhalation therapy of anti TB drugs in dry powder formulation 61. Perhaps, a reduction of toxicity might be achieved by inhalation therapy. One other theoretical or potential advantage could be that by providing high concentrations into the airways, the time that patients remain infectious might be reduced, thus providing a public health advantage.

Optimization of our mathematical inhalation model can be useful in predicting serum levels at different time-points using different devices for dry powder inhalation.

**Concluding remarks**

In the Introduction of this Thesis, we started a short history of the long journey of Mtb and humankind. Still, our knowledge about the epidemiology and transmission of TB, and its treatment should be enhanced to effectively fight TB. Not only by working together with
clinicians or molecular biologists but also with molecular epidemiologists, geneticists, immunologists, and people working in the field of inhalation technology, insights can be obtained to improve the fight against TB, and its most threatening variants: MDR- and XDR-TB. Some battles have been won, but winning the war requires a huge concerted effort.

References


