

From THE DIVISION OF INFECTIOUS DISEASES  
DEPARTMENT OF MEDICINE SOLNA  
Karolinska Institutet, Stockholm, Sweden

# TUBERCULOSIS CONTROL IN SWEDEN

Jerker Jonsson



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# Tuberculosis control in Sweden

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

**Jerker Jonsson**

*Principal Supervisor:*

Ass. Prof. Judith Bruchfeld  
Karolinska Institutet  
Department of Medicine Solna  
Division of Infectious Diseases

*Co-supervisor(s):*

Med. Dr. Ingela Berggren  
Karolinska Institutet  
Department of Medicine Solna  
Division of Infectious Diseases

Ph.D. Ramona Groenheit  
The Public Health Agency of Sweden  
Department of Microbiology  
Unit for Laboratory Surveillance of Bacterial Pathogens

*Opponent:*

Prof. Bertie Squire  
Liverpool School of Tropical Medicine  
Department of Clinical Sciences  
Faculty of Clinical Sciences & International Health

*Examination Board:*

Prof. Anna-Mia Ekström  
Karolinska Institutet  
Department of Public Health Sciences  
Division of Global Health (IHCAR)

Prof. Per Björkman  
Lund University  
Department of Clinical Infection Medicine  
Division of Translational Medicine

Med. Dr. Einar Heldal  
WHO and  
Norwegian Institute of Public Health  
Department of tuberculosis, blood borne and sexually  
transmitted infections







## ABSTRACT

Sweden is a low tuberculosis (TB) incidence country with an incidence of 5.3 cases per 100 000 inhabitants 2017. The majority of new TB cases in Sweden are diagnosed among migrants from high TB incidence countries. Data from TB surveillance is analysed to identify risk groups, follow trends, discover new risk groups or outbreaks and update policies and guidelines, with the ultimate goal of reducing the number of new cases.

Today all isolates from TB cases diagnosed in Sweden are genotyped to detect clustering which may reveal unknown links between cases and risk of transmission that has been overlooked. The methods of molecular genotyping have improved over time and reduced the risk of false clustering. In **paper I** we compared two different methods of molecular genotyping, Restriction fragment length polymorphism (RFLP) and Mycobacterial interspersed repetitive units – variable number of tandem repeats (MIRU-VNTR). There was an 82 percent concordance between the two methods. Compared to epidemiological data, around 50 percent of the clusters were judged as being false with both methods. As turn-around time for MIRU-VNTR is much faster than for RFLP it was the preferred method.

Latent tuberculosis infection (LTBI) is defined as having a detectable immune response towards TB but no signs or symptoms of active disease. The tuberculin skin test has gradually been replaced by Interferon Gamma Release Assays (IGRA's) to detect immune response towards the *Mycobacterium tuberculosis* (*M.tb*) specific antigens ESAT-6 and CFP-10. With QuantiFERON Gold In tube, the most widely used IGRA, there are challenges in interpreting results close to cut-off (0.35 IU/mL) due to the variability of the test method. In **paper II** we investigated the effect of a borderline range (0.20–0.99 IU/mL) around cut-off where repeated testing was recommended. Of negative (0.20–0.34 IU/mL) and positive (0.35–0.99 IU/mL) results in the borderline range, 66.1 percent and 42.5 percent respectively were negative below the borderline range (< 0.20 IU/mL) when retested. None of the subjects with initial result in the borderline range and a negative second test, developed incident active TB during a period of minimum two years of follow-up. We recommend re-testing of individuals with a result in the borderline range for a more reliable result.

Multidrug resistant TB (MDR-TB) is an increasing problem worldwide and also in Sweden but on a smaller scale. In **paper III** we analysed a Swedish cohort of 158 MDR-TB cases diagnosed from 1992–2014. Treatment outcome was successful in 83.5 percent which is similar to treatment outcome for susceptible TB in Sweden. Treatment with pyrazinamide (PZA) in cases with PZA susceptible *M.tb* strains, shortened time to sputum culture

conversion (aHR 2.25 (95% CI 1.27–3.99) p=0.005) (median difference 30 days). Increasing minimum inhibitory concentration of levofloxacin was correlated to unfavourable outcome (aHR 1.77 95% CI 1.15–2.71 p=0.009), as was diabetes (aHR 5.52 (95% CI 1.42–21.55) p=0.014) and increasing age (age >40 years, aHR 4.51 (95% CI 1.74–11.67) p=0.002).

The majority of people with LTBI will not develop active TB but there are factors increasing this risk, like for example human immunodeficiency virus or any other condition affecting the normal immune response. As pregnancy temporarily alters the immune response, we wanted to investigate if the risk of active TB increases during pregnancy and postpartum. In **paper IV**, a retrospective register-based cohort study of all women who gave birth in Sweden during the study period 2005–2013, we showed an incidence rate ratio of 3.0 (95% CI 2.3–3.9) and 4.2 (95% CI 3.2–5.5) of active TB during pregnancy and postpartum respectively compared to outside these risk periods. The increased risk was concentrated to women from high TB incidence countries. We recommend that women from high TB incidence settings should be screened for both active and latent TB when pregnant.

## LIST OF SCIENTIFIC PAPERS

- I. Jonsson J, Hoffner S, Berggren I, Bruchfeld J, Ghebremichael S, Pennhag A, Groenheit R. Comparison between RFLP and MIRU-VNTR genotyping of Mycobacterium tuberculosis strains isolated in Stockholm 2009 to 2011. PLoS One. 2014 Apr 14;9(4):e95159. doi: 10.1371/journal.pone.0095159. eCollection 2014. PMID: 24733167
- II. Jonsson J, Westman A, Bruchfeld J, Sturegård E, Gaines H, Schön T. A borderline range for Quantiferon Gold In-Tube results. PLoS One. 2017 Nov 2;12(11):e0187313. doi: 10.1371/journal.pone.0187313. eCollection 2017. PMID: 29095918
- III. Davies Forsman L, Jonsson J, Wagrell C, Werngren J, Mansjö M, Wijkander M, Groenheit R, Hammar U, Giske C, Schön T, Bruchfeld J. Minimum inhibitory concentrations versus clinical outcome in MDR-TB patients - a nationwide Swedish cohort study over two decades. Submitted
- IV. Jonsson J, Kühlmann-Berenzon S, Berggren I, Bruchfeld J. Increased risk of active tuberculosis in pregnancy: a retrospective register-based cohort study. Manuscript

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## LIST OF ABBREVIATIONS

AM	Amikacin, a second-line injectable drug
BCG	Bacillus Calmette-Guérin, vaccine against tuberculosis
BDQ	Bedaquiline, new second-line drug
DST	Drug susceptibility testing
CFZ	Clofazamide, second-line drug
CS	Cycloserine, second-line drug
E	Ethambutol, first-line drug
ETO	Ethionamide, second-line drug
EU TB PAN-NET	A European research group
H	Isoniazid, first-line drug
HIV	Human immunodeficiency virus
IGRA	Interferon gamma release assay
IRR	Incidence rate ratio
LFX	Levofloxacin, second-line drug, a fluoroquinolone
LPA	Line probe assay
LTBI	Latent tuberculosis infection
LZD	Linezolid, second-line drug
MDR-TB	Multidrug resistant tuberculosis
MXF	Moxifloxacin, second-line drug, a fluoroquinolone
MIC	Minimum inhibitory concentration
MIRU-VNTR	Mycobacterial interspersed repetitive units - variable numbers of tandem repeat
<i>M.bovis</i> BCG	Strain of <i>Mycobacterium bovis</i> which is used to produce BCG
<i>M.tb</i>	<i>Mycobacterium tuberculosis</i>
NTM	Non-tuberculous mycobacteria
PAS	Para- aminosalicylic acid, second-line drug
PCR	Polymerase chain reaction
PPD	Purified protein derivate

PTO	Prothionamide, second-line drug
PZA	Pyrazinamide, first-line drug
QFT	QuantiFERON Gold in Tube, commercial name for one type of IGRA
QFT Plus	New version of QFT with an extra tube for measuring reactivity of CD8+ cells
R	Rifampicin, first-line drug
RFLP	Restriction fragment length polymorphism
SLID	Second-line injectable drug (amikacin, capreomycin and kanamycin)
SmiNet	Web-based system for reporting cases of notifiable diseases
SNP	Single nucleotide polymorphism
TB	Tuberculosis
TDM	Therapeutic drug monitoring
TNF-alfa	Tumor necrosis factor alfa
tSCC	Time to sputum culture conversion
T.SPOT.TB	Commercial name for one type of IGRA
TST	Tuberculin skin test
WGS	Whole genome sequencing
WHO	World Health Organisation
XDR-TB	Extensively drug resistant tuberculosis



# 1 BACKGROUND

Tuberculosis (TB) is one of the most important infectious diseases in the world and caused 10.0 million new cases and killed 1.3 million people in 2017, according to the Global tuberculosis report 2018 published by the World Health Organisation (WHO)(1). Like many other infectious diseases, TB has always been associated with poverty, crowded living conditions and poor nutrition. Effective medical treatment for TB has been available since the mid 1940's but economic growth in a country with an increased spending on social support, has a stronger association with reduced national TB incidence(2, 3) than any biomedical tool has ever had. Being able to seek health care when feeling poorly, without restriction due to lack of resources or availability, being able to afford treatment and to get sick-leave from work, is still far from a universal reality. In WHO's End TB Strategy(4) which was launched in late 2014, one of the targets is a 90% reduction in TB incidence rate by 2035 compared with 2015. The current annual decline in global TB incidence is about 2 percent, but to reach the set target the annual decline needs to increase to 10 percent by 2025. The global incidence 2017 was 133 cases per 100 000 inhabitants and year and Africa carries the heaviest burden with an average of 237 cases per 100 000(1). Another target of this strategy is to reduce the number of TB affected households facing catastrophic costs to zero percent. For low-income countries with a high incidence of TB, addressing universal health coverage(5), social protection and major social determinants of TB, are the most important measures to reduce the TB burden(6).

In high income countries like Sweden, which have already transitioned to a low-incidence stage, focusing on risk groups where pockets of disease remain is the priority according to the End TB Strategy. In order to achieve this, a surveillance system is needed through which reliable data can be acquired and the risk groups identified by analysing this data. The main goal is to find new TB cases and their contacts early on, in order to provide treatment and prophylaxis to interrupt the chain of transmission as efficiently as possible. However, to reach the End TB final goal there are still important tools lacking.

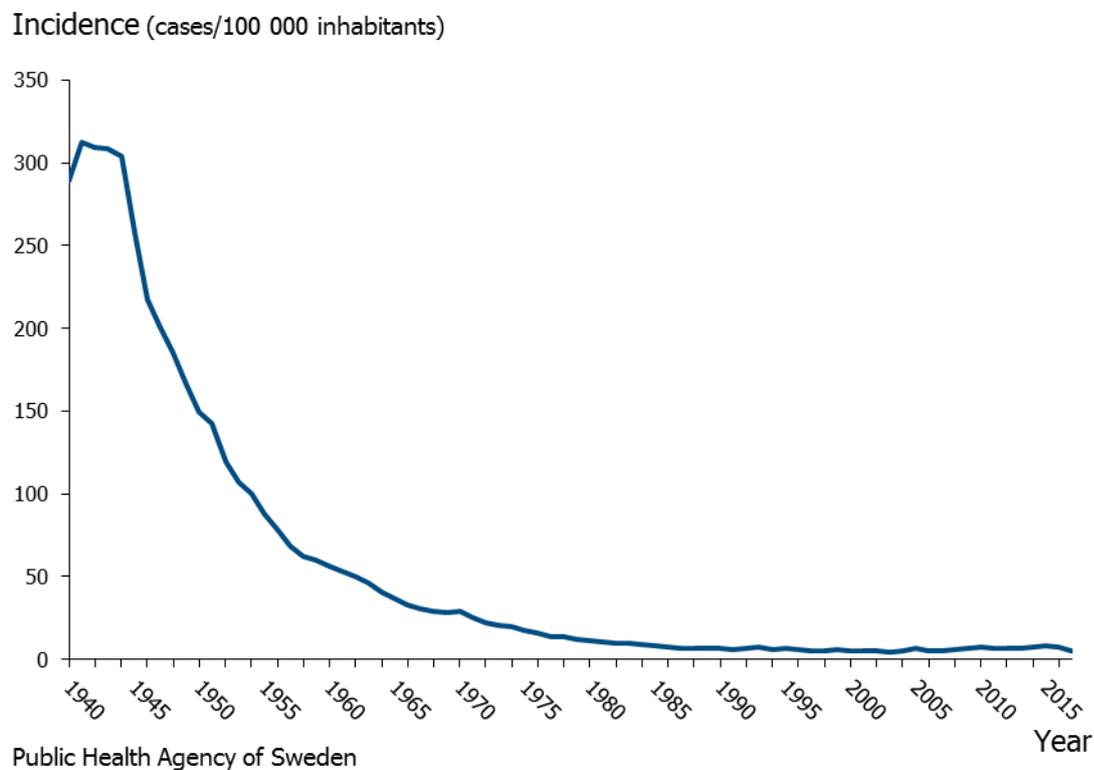
A more effective vaccine for better prevention would make a huge difference, as it already has for many vaccine preventable infectious diseases. There are quite a few TB vaccine trials ongoing but it is a challenge to develop a vaccine against a disease that does not induce immunity even after active disease(7-10).

Development of simple and reliable point of care diagnostic methods are of outmost importance, in particular for use in poor resource settings where many cases still do not get diagnosed. There have been improvements in early diagnosis of TB recently, such as for example polymerase chain reaction (PCR)-based tests like GeneXpert, which has been the subject of a WHO campaign in order to facilitate the introduction of the test in low income countries(11).

New and effective medications enabling shorter treatment regimens to increase the number of successful outcomes are also important in the quest of TB elimination. There are trials for new and shorter regimens ongoing, both for susceptible and drug resistant TB(12).

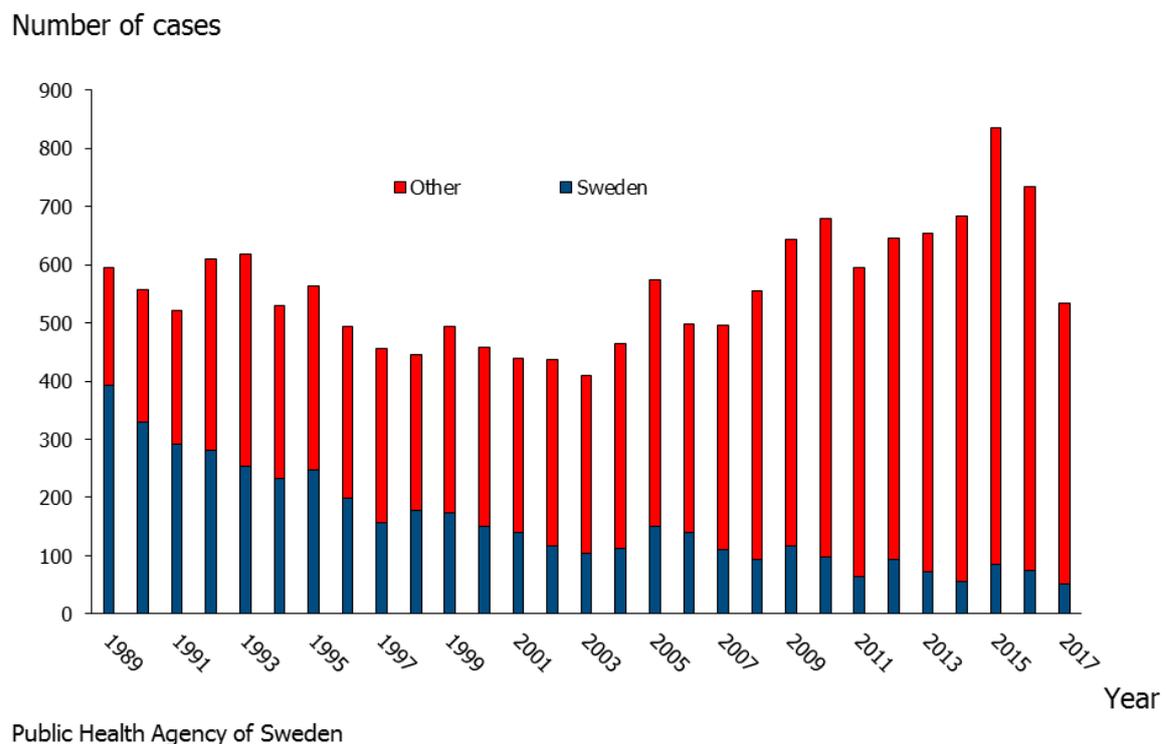
## 1.1 THE TB SITUATION IN SWEDEN

**Figure 1. TB incidence in Sweden 1940 to 2017**



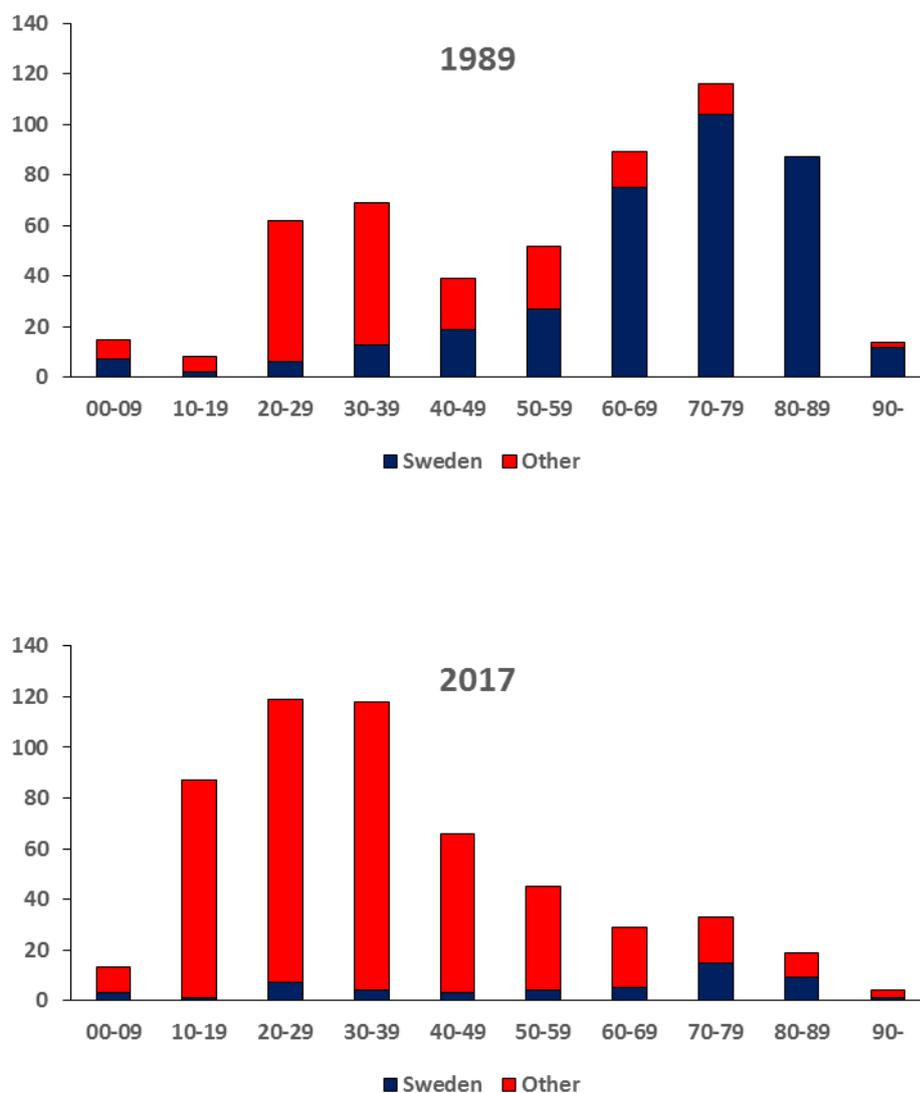
TB was once a common disease in Sweden with incidence rates reaching > 300 cases per 100 000 inhabitants per year. The Swedish TB incidence started decreasing rapidly at the end of World War II, before there was effective treatment available (Figure 1). Sweden is a very good example of how economic growth, improved living and social conditions played an important part in reducing TB incidence(13). In 2003 Sweden reached an incidence of TB as low as 4.6 cases per 100 000 inhabitants per year and among Swedish-born the incidence is now less than 1 case per 100 000 and still decreasing(14). Since the early 1990's Sweden has had a continuous increase in migration from countries with a high TB incidence (>100/100 000 inhabitants and year) and an increasing total incidence of TB in Sweden, reaching 8.5 in 2015. In 2015, 90 percent of all TB cases diagnosed in Sweden were born outside of Sweden and the vast majority came from high TB incidence countries (Figure 2). Since 2015 migration to Sweden as well as to other European countries, has decreased and in parallel, so has the TB incidence which in 2017 was 5.3 cases per 100 000 inhabitants.

**Figure 2. Number of TB cases in Sweden per year and country of origin, 1989 to 2017**



TB epidemiology in Sweden is a small reflection of the TB situation in the rest of the world but with limited domestic transmission in risk groups(14) and even less outside these. Along with the changing epidemiology of TB in Sweden, the majority of cases today are found in the age groups 15–40 years, which was not the case in the late 1980`s when a majority of TB cases were older than 40 years (Figure 3).

**Figure 3. TB cases in Sweden per age group and country of origin, comparison between 1989 and 2017.**



This development renders contact tracing much more important as TB cases in reproductive age groups usually have more social contacts, including family contacts, and therefore are much more effective in transmitting their TB infection. In the late 1990's and early 2000's a large isoniazid resistant *Mycobacterium tuberculosis* (*M.tb*) cluster in Stockholm (called cluster 49), was identified through routine genotyping of *M.tb* isolates, which indicated ongoing domestic transmission(15). A cluster is defined as two or more *M.tb* isolates with an identical or very similar genotypical profile, indicating a possible epidemiological connection between the cases. Since the majority of cases in cluster 49 originated from high TB incidence countries, it was initially assumed that these cases had been TB infected before coming to Sweden. When the cluster was investigated in detail, it was noted that cases came from several different countries but lived in the same neighbourhoods in Stockholm. Further investigation showed that in many cases contact tracing had been completely neglected(15). The need to centralise TB care in Stockholm was recognised and resources were allocated to improve preventive interventions such as intensified contact tracing and screening of risk groups like asylum seekers from high incidence countries. Sporadic cases belonging to cluster 49, which consists of 133 cases in total so far, are still detected but are mainly thought to be due to reactivation from remote transmission. There were also 17 culture negative cases with pulmonary radiographic changes among children attributed to cluster 49, associated with an outbreak at a day care centre in 2005 where one of the staff members was diagnosed with TB after having symptoms for five months(16).

Smaller clusters have been detected among homeless people and drug abusers since then but no large clusters indicating uncontrolled transmission have been identified. In Denmark, which in many ways is similar to Sweden, the largest cluster identified at present is found precisely among homeless people and alcohol abusers, a group where contact tracing can be very complicated. This cluster was first detected in 1992, but transmission is still ongoing with new cases in the same risk group every year and a total number so far of around 1000 cases of active TB(17-20), underlining the importance of effective active case finding and contact tracing to stop transmission.

## 1.2 MULTIDRUG RESISTANT TUBERCULOSIS

The first two antibiotics effective against TB, streptomycin (S)(21) and para-aminosalicylic acid (PAS)(22), were discovered in the mid 1940's. It was soon noted that TB needed to be treated with a combination of at least two anti TB drugs to avoid the development of drug resistance(23). The first combination treatment consisted of S and PAS for up to two years in order to avoid recurrence. The most bactericidal drug at present is isoniazid (H), in particular towards rapidly replicating bacteria. When pyrazinamide (PZA) and rifampicin (R), both with a sterilising effect and an ability to kill non-replicating or semi dormant bacteria, were added to the standard regimen in the early 1970's, it was possible to reduce treatment length to six months(24, 25). The two most important TB drugs today are still H and R. The definition of multidrug resistant TB (MDR-TB) is an *M.tb* strain resistant to both of these drugs. Less effective second-line options are then used and with current recommendations, a treatment regimen should contain at least four, preferably five different anti TB drugs for a period of up to two years(26). The order of inclusion of drugs has recently been revised and WHO now recommend the inclusion of bedaquilin (BDQ) in a MDR-TB regimen instead of using BDQ primarily for extensively drug resistant TB (XDR-TB) cases. WHO issued a rapid communication in August 2018 to inform that new guidelines on MDR-TB treatment are to be published during 2018(27) (table 1).

**Table 1. The latest MDR-TB treatment recommendations according to a rapid communication in August 2018 from WHO.**

New recommendations for MDR-TB treatment 2018		
Group A	Include all three medicines if possible	Levofloxacin (LFX) or Moxifloxacin (MFX) Bedaquiline (BDQ) Linezolid (LZD)
Group B	Add both medicines if possible	Clofazimine (CFZ) Cycloserine (CS) or Terizidone (TRD)
Group C	Add to complete the regimen when medicines from Group A and B cannot be used	Ethambutol (E) Delamanide (DLM) Pyrazinamide (PZA) Imipinem-cilastatin (IPM-CLN) or Meropenem (MPM) Amikacin (AM) Ethionamide (ETO) or Prothionamide (PTO) para-aminosalicylic acid (PAS)

With such a long and difficult regimen the problems with side-effects and adherence increases significantly(28). An alternative option was suggested in May 2016 when WHO published recommendations for a shorter MDR-TB treatment for cases of pulmonary TB with no suspicion of other resistance than towards H and R(29). In order to shorten the duration of treatment to 9–12 months, the intensive phase includes seven different drugs; high dose H, PZA, ethambutol (E), moxifloxacin (MFX), amikacin (AM), clofazimine (CFZ) and prothionamide (PTO) for 4-6 months and then a five months continuation phase of Z, E, MFX and CFZ. There is an ongoing multinational study, the STREAM trial, evaluating economic impact of the shortened regimen both on patients and health systems(30). In the Swedish MDR-TB cohort in study III, 30 percent of the cases were eligible for this short course regimen. It is likely that the new recommendations for MDR-TB treatment will change also the recommendations for the short course treatment and possibly substitute AM with BDQ. The ongoing STREAM2 trial is comparing three short course regimens to standard MDR-TB treatment, two of these containing BDQ out of which one is all oral(31).

The increasing number of MDR-TB cases world-wide is also reflected in Sweden and since the late 1990's more than 210 cases of MDR-TB have been reported. During the 1990's a few domestic cases of MDR-TB occurred in Sweden due to poor adherence to treatment and a failing TB control system. Since then support to improve adherence to treatment has been strengthened and during the last fifteen years, no patient with drug susceptible TB has developed MDR-TB during standard first-line TB treatment in Sweden. The majority of MDR-TB cases diagnosed in Sweden originate from high incidence countries and are diagnosed with *M.tb* isolates often resistant only to the four first-line drugs H, R, PZA and E. Of the 154 cases of MDR-TB diagnosed in Sweden during the last 10 years (2008–2017), 57 percent were (88/154) MDR-TB with resistance only to first-line drugs. Another 16 percent had MDR-TB with resistance also to ETO/PTO and/or PAS. XDR-TB is defined as MDR-TB with resistance also to the most important second-line drugs, aminoglycosides (referred to as second-line injectable drugs (SLID)) and fluoroquinolones (OFX, MFX and LFX). Of the remaining 27 percent (42/154), 20 percent had pre-XDR (additional resistance to either any SLID or any quinolone) and 7 percent (11/154) had XDR-TB, the largest part of them coming from Eastern Europe or former Soviet Union countries. In the early 2000's a national advisory group for MDR-TB cases was formed by physicians and microbiologists with a special interest in TB. This has been extended to include also complicated cases of TB. As TB in general and MDR-TB in particular is an uncommon disease in Sweden, it is difficult

for an individual clinician to gather experience on how to treat both MDR/XDR-TB and complex drug sensitive TB. In Sweden treatment of MDR-TB is always individualised in accordance with available drug susceptibility testing (DST) results(32). Testing of plasma drug concentration of first-line options for TB-treatment and the second-line option AM has been available for years. Regarding other second-line options, testing of plasma concentration of LZD has recently been made available and testing of quinolones will hopefully be made available sometime in the near future. Plasma concentrations can be monitored and compared with the minimum inhibitory concentration (MIC) to ensure adequate dosing and to avoid toxicity. The rate of successful treatment outcome for MDR-TB cases in Sweden is on average 85 percent which is excellent in an international comparison and does not differ from treatment outcome of sensitive TB(33). Occurrence of adverse drug reactions is definitely higher with MDR-TB treatment compared to standard treatment but hopefully this will improve with the new recommendations for MDR-TB treatment from WHO in which newer medications with less side effects have been favoured before SLIDs(27). A common adverse reaction to SLIDs is impaired hearing which is not reversible.

### **1.3 GENOTYPING OF *M.TB* AND CLUSTERING**

Molecular epidemiological typing of *M.tb* isolates is performed to detect clusters of TB cases in time and geographical space. Genotyping results are used to monitor and detect signs of domestic transmission as part of a TB control strategy. Typing is useful to rule out an epidemiological connection between cases but with genotyping methods with low discriminatory power, there is a risk of false clustering between cases without any real connection.

In Sweden genotyping of drug resistant *M.tb* isolates started in 1994 and from 1998 drug susceptible strains were also included. The first method used was restriction fragment length polymorphism (RFLP)(34, 35) combined with spoligotyping(36) but in 2012 the genotyping methodology was changed to mycobacterial interspersed repetitive units – variable number of tandem repeats (MIRU-VNTR)(37) combined with spoligotyping. These methods only analyse a part of the bacterial genome but if identical genetic patterns are identified, it suggests a possible epidemiological connection(20, 38). When genetic clustering of isolates is detected, the corresponding cases are investigated for epidemiological connections in order to detect if recent transmission has occurred (39, 40), and if it is still ongoing. If genotyping is

performed in a timely manner it can be very useful in extending contact tracing to others who may have been exposed and are at risk of developing active TB. Typing can also reveal laboratory contamination between clinical samples. In Sweden >95 percent(14) of the isolated *M.tb* strains are at present analysed with molecular genotyping and results are added to data of the individual TB case in the TB register.

Routine whole genome sequencing (WGS) of *M.tb* isolates was introduced in Sweden in September 2016 and has a higher discriminatory power than both RFLP and MIRU-VNTR(41). WGS is as rapid as MIRU-VNTR since it requires small amounts of genetic material and the result is often available within a month from diagnosis as compared to several months with RFLP. Strain similarity is usually measured in number of differences in single nucleotide polymorphisms (SNPs) where so far, five SNPs difference or less, defines a cluster in many countries(42, 43). It can be difficult to compare data from different sequencing platforms and work is ongoing to define specific loci to use to facilitate comparison. Instead of 24 standardised loci like in MIRU-VNTR, it will probably be closer to 3000 different loci, to maintain a high discriminatory power(44). In clusters including several cases you can potentially see in which order they have been infected as the change of SNP's is thought to be in the order of less than one per year(45). However, compared to other bacteria, it is still difficult to fully assess transmission between cases because of variable incubation periods of TB. The switch to WSG has resulted both in more rapid investigations of clusters with suspicion of domestic transmission(45), as well as increased precision as clusters are fewer with this method. In the majority of clusters, already known epidemiological links are confirmed but unknown connections are sometimes discovered. Feedback to the clinician is provided in order to extend the contact tracing if deemed necessary and/or improve TB contact tracing routines if needed. With genotyping nosocomial spread of TB has also been detected and faulty infection control routines corrected(46). When it comes to detection of laboratory contamination the reduced time in obtaining results is very helpful as unnecessary or erroneous medication can be corrected sooner. The result of genotyping is also very helpful when trying to determine the proportion of ongoing transmission within a country. The proportion of cases infected in Sweden can be used as an indicator of how well our TB control programme is performing. The goal is to reduce the number of cases infected in Sweden to a minimum and find cases of active TB through screening or contact tracing before they become infectious. Based on genotyping-results, 14 percent (75/531) of the reported TB cases in 2017 were considered as infected in Sweden(14). One third of the cases were born in Sweden more than 60 years ago and most likely had reactivated a remote infection from their youth. Of the remaining 50 cases, 29 were

known contacts of infectious TB cases and the majority were found during contact tracing, many of them with family from high incidence countries.

## 1.4 TB RISK GROUPS

**Contacts of cases with recently diagnosed active TB:** Individuals with the highest risk of developing active TB are persons who have been in close contacts with infectious TB cases recently, which explains why contact tracing is mandatory in Sweden (and in many low TB incidence countries). As it often takes time to develop active TB, there is a good chance of identifying recently infected persons among contacts and reduce their risk of falling ill by giving preventive treatment or diagnosing active TB early, before they become infectious. Only about 10 percent of people infected with TB will develop active disease some time during their lifetime but of those, at least 50 percent do so within the first two years after being infected(47, 48). Ideally, anyone who is considered at increased risk of developing active TB should be offered preventive treatment to reduce this risk. A second option is clinical follow-up to detect any sign of active TB early, so treatment can be initiated before they become infectious.

**Migrants from TB endemic countries:** The largest risk group for developing active TB in Sweden today are recent migrants from high TB incidence countries, as they are more likely to have had recent contact with a TB case. The risk of TB activation decreases with time after infection which corresponds with the observation that 73 percent of cases among migrants diagnosed in 2017 had lived in Sweden for less than five years(14). Subgroups within this population may have co-morbidities that make them more prone to develop TB, like for example co-infection with HIV (49). A British study published in 2012 also presents evidence for an increased risk of active TB in connection with pregnancy(50). According to this study the risk was significantly increased (incidence rate ratio 1.95 CI (1.24–3.07)) in the six months post-partum period. Although an increased risk could not be demonstrated during pregnancy, the authors suggested that the increased risk postpartum was mainly because of delayed diagnosis due to the pregnancy itself inducing a shift in the immune response, reducing the symptoms of active TB. Few previous studies regarding possible increased risk related to pregnancy have been performed and with conflicting results(51, 52), indicating a research gap.

TB-screening of newly arrived migrants from high incidence countries does not have a satisfactory coverage in Sweden as only about 45 percent of asylum seekers attend the health examination they are offered(53). Family members of asylum seekers who arrive later, with a prearranged permit to stay, are not offered free health examination. For many migrant women the first contact with Swedish health care occurs when they get pregnant. If pregnancy indeed constitutes an increased risk for women with LTBI to develop active TB, this stresses the need for TB screening of pregnant women belonging to this risk group(54). Some counties in Sweden have implemented this strategy but the underlying evidence for this measure has not been evaluated in Sweden. WHO guidelines recommend careful consideration before initiating screening of new risk groups for LTBI(55).

**Immunosuppressive treatment:** An expanding risk group for active TB are individuals with medically induced impairment of their immunity. Treatment with anti TNF-alfa inhibitors is becoming increasingly common for systemic inflammatory diseases such as rheumatism, inflammatory bowel disease and psoriasis, and carries a statistically significant increased risk for TB activation(56). Other groups of patients with medically induced immunosuppression to consider for screening according to WHO-guidelines are transplant recipients and persons undergoing regular dialysis(55).

**Other risk groups:** Globally an important risk group for TB is drug-abusers (including alcohol) and homeless people. Not only may they be prone to develop active disease due to impaired immunity(57) and co-infections, they may also have problems with adherence to treatment, of which the latter has to be anticipated and properly addressed by health care providers. Homelessness may add to the problem and temporary housing in crowded conditions could also increase the risk of transmission and make contact tracing very difficult. In Sweden drug abusers have so far not been overrepresented among TB cases. However, as mentioned earlier, in neighbouring Denmark a large *M.tb* cluster in this risk group has been known since 1992 and new cases are diagnosed every year, indicating ongoing domestic transmission(20). In total 23 cases belonging to this particular MIRU-VNTR cluster have been identified in Sweden, the majority with direct epidemiological links to Copenhagen. When analysing the isolates with WGS, 20 of the cases were identical and the last one was diagnosed in 2016. At present few indications of further transmission from these cases have been observed within Sweden(14).

Risk groups currently screened for TB in Sweden are:

- contacts to TB cases
- asylum-seekers from high TB incidence countries (>100/100 000)
- patients who are to be started on immunosuppressive treatment
- in some counties; pregnant women born in high endemic countries (>100/100 000)

For asylum seekers and quota refugees, TB screening is included in a free general health examination where questions are asked about previous and current health and symptoms, TB testing is performed as well as HIV and hepatitis screening. Those with a positive TB test or symptoms compatible with pulmonary TB will be sent for pulmonary radiography. But far from all migrants from high incidence TB countries are asylum seekers. People immigrating for family reunion with prearranged residential permits, guest workers and guest students are not offered any health examination.

## 1.5 TB-SCREENING

For many years the only test to indicate LTBI was the tuberculin skin test (TST)(58). It is a sensitive but non-specific test in populations with a low prevalence of TB as prior BCG-vaccination or infection with non-tuberculous mycobacteria (NTM) also can induce a positive TST. A standardised amount of purified protein derivate (PPD) from *M.tb* is injected intradermally and the patient has to come back for measurement of the reaction 72 hours later. An induration of more than 10 mm is usually interpreted as a positive reaction. To make the test even more sensitive in specific groups like unvaccinated children, the cut-off is normally set to 6 mm. In immunosuppressed individuals the test may be falsely negative due to anergy, which cannot be determined with this method. The TST requires trained staff both for a standardised intradermal injection and reading of the result(59). Interferon Gamma Release Assay (IGRA) is performed on whole blood and analysed according to the manufacturer's instruction in the laboratory. Since the introduction of the IGRA's, the TST has gradually been replaced in Sweden. In IGRA's the CD4+ T-helper lymphocytes are stimulated with the *M.tb* specific antigens ESAT-6 and CFP-10, which are not present in *M. bovis* BCG or most NTM, avoiding positive results due to earlier BCG-vaccination or NTM infection. This makes IGRA's more specific in low TB incidence settings where BCG and NTM infections can cause a substantial part of all positive TST results. If the white blood cells recognise the

TB antigens they respond by releasing interferon-gamma and the amount is measured, quantifying the response. The IGRA's include a positive and a negative control, making it possible to distinguish negative results from those not able to respond due to immunosuppression. As it is a blood test which is analysed in the laboratory, there is no need for any return visit. The positive predictive values for progression to active TB within two years is slightly higher than for TST although still low at 1.5–15 percent, depending on the setting(60, 61). IGRA's include a positive control which if negative, indicates anergy as opposed to the TST, where a negative result cannot be interpreted in this sense. As there is no reference method for LTBI, the true sensitivity of these tests is unclear. IGRA's are blood-based tests and since the analysis is performed in a laboratory, this reduces the risk of individual variations in the reading of the result.

Two commercial tests are available, the QuantiFERON-TB Gold (QFT) (which has been replaced by QuantiFERON-TB Gold Plus (QFT Plus)) and the T.SPOT.TB. QFT is the most extensively used commercial IGRA for detecting an immunological response towards TB in whole blood test. The magnitude of the response is measured by ELISA. If higher than 0.35 IU/ml it is considered as positive according to the manufacturer's instruction and is thus by definition a qualitative test. Still, there are problems also with the interpretation of IGRAs. T.SPOT.TB includes a borderline zone around cut-off where results should be interpreted as indeterminate but QFT does not. When you get test results close to the given cut-off, the variability in the test method itself may cause reversions and conversions between positive and negative test results(62) in repeated testing, not correlated to biological variability. This makes results close to the given cut-off difficult to interpret, for example in the assessment of whether individuals with current or planned immunodeficiency should be offered treatment for LTBI or not(63). There is a clinical need to define a borderline zone for QFT and not routinely interpret any positive or negative result close to cut-off as definite(62). In general, a positive test indicates that your immune system recognises TB antigens, confirming previous TB exposure and infection, which then implies a risk of developing active TB in the future(64, 65). Unfortunately, none of the immune reactive tests can differentiate between active or latent TB and they are not useful for monitoring treatment effect(66). Both the TST and IGRA's indicate exposure to *M.tb* but say very little about the individual risk of developing active TB. The difference in positive predictive value is not significant(61, 67). A test that could predict the individual likelihood of developing active TB would be a valuable diagnostic complement, and research in this field is ongoing(68). The new version of QFT that was released in 2015, QFT Plus, includes an extra test tube testing for CD8+ T-cell reactivity. The manufacturing company claims an increased sensitivity(69) and there have

been some studies published trying to validate this(70, 71). An Italian study showed a concordance of 90 percent between QFT and QFT Plus with 12/119 subjects having a negative QFT but positive QFT Plus. In the discordant tests the average QFT Plus result in Tube1 and Tube2 was 0.83 IU/mL and 0.73 IU/mL respectively and the cut-off is 0.35 IU/mL. None of the samples negative in QFT Plus were positive with QFT. They used Tube2 minus Tube1 as an estimate of CD8+ stimulation in Tube2 and values of > 0.6 IU/mL was significantly associated with proximity to the index case. In a Japanese study QFT Plus was compared to T.SPOT.TB when screening immunosuppressed persons with rheumatoid arthritis. There were more positive results with QFT Plus compared to T.SPOT.TB but there was little additional effect of Tube2 in the QFT Plus. Where the results were discordant with T.SPOT.TB the positive QFT Plus results were < 0.7 IU/mL.

A study published in 2017 and sponsored by Qiagen, the manufacturing company, suggested that the QFT Plus could be used for monitoring treatment response(72). The study included 38 subjects treated for susceptible TB during 6-9 months. They were all sampled at months 0, 3 and 6 and 36/38 had an initially positive QFT Plus result. The change in Tube1 response was significant from month 0 to month 3 but not from month 3 to 6. The change in Tube2 minus Tube1 (CD8+ response estimate) was not significant between month 0 and 3 but from month 3 to 6. In total 15 percent of the initially positive QFT Plus result reversed to negative during treatment.

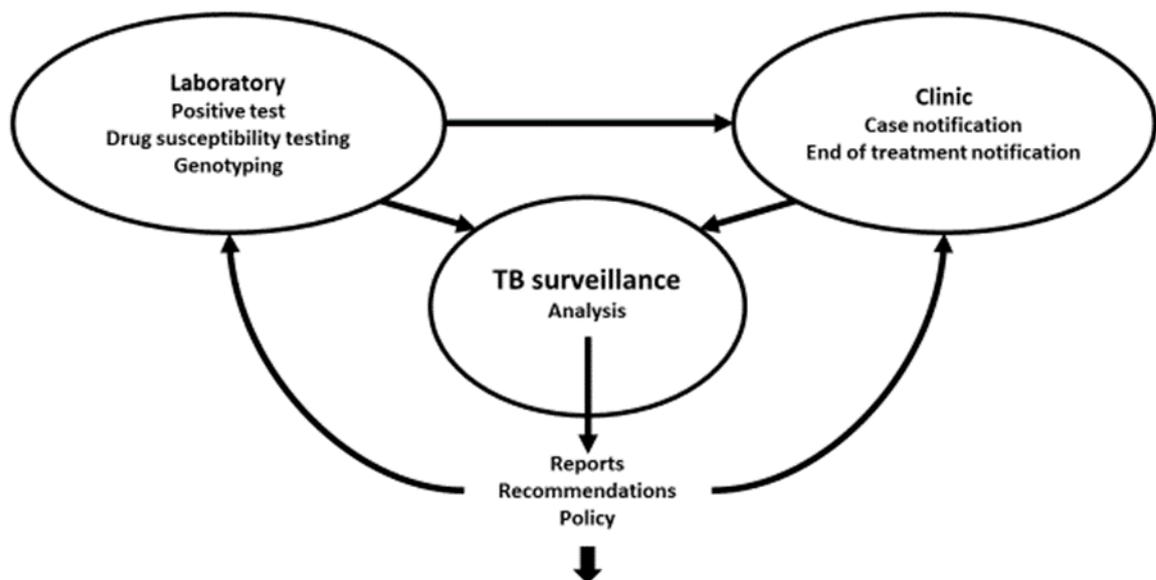
## **1.6 TB SURVEILLANCE**

TB surveillance is of importance in order to follow trends, define risk-groups, monitor signs of domestic spread and identify failing or insufficient TB control measures. Reporting of new cases of active TB is mandatory in Sweden, both for mycobacterial laboratories detecting positive microbiological samples and for clinicians diagnosing TB or treating a patient based on clinical suspicion of TB(73).

Since 2005 cases are reported through a web-based system called SmiNet where laboratory reports and clinical reports are merged into cases through their unique personal identity number. The data is checked for accuracy by the regional County Medical Offices and completed if necessary after contact with the treating physician. It is also checked for duplicates at national level.

The register/database is analysed thoroughly at both regional and national level at least once a year but surveillance is also continuous as reports on cases and laboratory results are entered in the system daily. Data on DST-results and molecular epidemiological typing of *M.tb* isolates are entered as soon as they are available. When the TB treatment of a case has been completed, treatment outcome is reported in the system as one of the following: cured, treatment completed, treatment failed, died before or during treatment (whatever the cause), lost to follow-up or not evaluated, according to current WHO definitions(74). Treatment outcome is an indicator used to evaluate the performance of the TB control and treatment programs globally. The five mycobacterial laboratories in Sweden have a close collaboration with the national and supranational reference laboratory for TB at the Public Health Agency where the majority of the molecular genotyping as well as all the extended DST is performed. The reporting from the laboratories on positive *M.tb* findings is close to 100 percent. Clinical reporting is not always as timely and cases who are not verified by culture might not always be reported, although they should be. For any case with a positive laboratory test but without a clinical report, the treating physician will be reminded by the County Medical Office to report it and the same will occur if the report of treatment outcome is lacking a year after the initial report.

**Figure 4. TB surveillance in Sweden**



## 1.7 TB CONTROL MEASURES

- Isolation of infectious individuals until effective treatment has cleared the risk of transmission.
- Support to secure adherence to treatment.
- Mandatory reporting for laboratories on all positive test results regarding active TB and for clinicians on diagnosis of active TB. Treatment initiation and outcome is also reported.
- Mandatory contact tracing.
- Screening of risk groups.
- Offering of BCG vaccination to children with family origin from countries with a TB incidence above 25 per 100 000 inhabitants.

The most important measure to reduce TB transmission is early diagnosis and treatment. Since TB is no longer a common disease it is often a challenge for physicians to consider TB early in the diagnostic work-up. Delayed diagnosis greatly increases the risk of spread since it increases exposure of non-infected individuals to TB when infection control measures are not applied(75). In low incidence countries active case finding is usually a deliberate search for both active and latent TB(76), performed by screening of high-risk groups. One such risk group is known contacts of an index case. Being exposed recently is the most important risk group as the risk of falling ill if infected, is highest during the first two years(48). Preventive treatment in this group requires the lowest number needed to treat in order to prevent a secondary case(77). Contact tracing of all TB-cases is mandatory in Sweden(73). Even if a case is not infectious, contact tracing might identify a source case or others exposed to the same source as the index case. This is especially important regarding children who almost always have been infected recently and usually by an adult in their vicinity. The largest risk group to screen is new migrants from high TB incidence countries as they might have been exposed recently. There are also individuals with immunosuppressive conditions that greatly increases the risk of developing active TB if they are infected, where screening for TB is indicated(78). Another important measure to reduce TB transmission is to make sure that adherence to treatment is good in order to avoid recurrence and development of resistant strains of *M.tb*. It can be a challenge to motivate cases to complete their treatment as many (around 20 percent of hospitalised patients)(79) have problems with side effects from the medication, and symptoms of TB often resolves long before the treatment is completed.

Patients need to be supervised and supported through the whole treatment and ideally helped with any psychosocial problem that could jeopardize their adherence to treatment, something they are entitled to by law in Sweden(73). The mandatory reporting of cases of active TB, both by the treating clinician and the laboratory confirming the diagnosis, makes it possible to follow trends over time, define risk groups to include in guidelines and recommendations and keep track of treatment outcome to evaluate performance of the TB programme. In Sweden LTBI is not a notifiable condition as opposed to for example in Norway, where LTBI is notifiable if preventive treatment is initiated.

## **2 AIMS**

The overall aim of this thesis is to improve TB control in Sweden. Various tools that can help improve TB-control are included and cover different parts of this wide area. The national TB register has an excellent coverage of the TB cases in Sweden, contains reliable basic data of good quality and has been used in all studies included in this thesis.

### **2.1 SPECIFIC AIMS**

- To compare different methods of molecular genotyping, strengths and weaknesses, in order to find out which one gives the most reliable and useful information
- To improve interpretation and thus usefulness of Quantiferon-results close to the manufacturers recommended cut-off, when screening for LTBI
- To describe the cohort of Swedish MDR-TB cases and evaluate the treatment outcome of individualised treatment guided by DST and if MIC is associated to treatment outcome
- To investigate if the risk of active TB is increased during pregnancy and postpartum

## 3 RESULTS AND DISCUSSION

### 3.1 MOLECULAR GENOTYPING OF *M.TB* ISOLATES, COMPARING RFLP AND MIRU-VNTR – PAPER I

#### 3.1.1 Background

Molecular genotyping has been performed on drug resistant *M.tb* isolates in Sweden since 1994 and since 1998 drug susceptible isolates as well, to monitor signs of domestic spread. The initial method used was RFLP but as it is a very labour intensive and time-consuming method, the reference genotyping technique was changed to MIRU-VNTR in 2012. Different methods analyse different parts of the genome, which means that cluster results with the two methods do not always correspond. Changing methodology entails losing the reference to older strains for comparison unless you analyse some strains retrospectively with the new method.

Due to a European network study of clinical management of TB drug resistance, the EU TB PAN-NET, all *M.tb* isolates from Stockholm during 2009–2011 had been analysed with both RFLP and MIRU-VNTR, in combination with spoligotyping. A study was performed to compare clusters of *M.tb* isolates with the different methods with epidemiological data from the contact tracing, in order to evaluate strength and weaknesses of each method.

#### 3.1.2 Material and methods

Molecular genotyping of *M.tb* isolates analyses the genome of the bacteria. Depending on the method, either different parts of the genome with high variability are analysed or the whole genome, for comparison with data from other isolates, to decide the degree of similarity. Identical (or very similar) isolates form clusters and indicate that there could be a connection between the cases from whom the isolates come. The different methods used have different discriminatory power which means that they can rule out epidemiological connection if isolates do not cluster, but if they cluster, with low discriminatory power the cluster might be false.

The Hunter-Gaston index of discriminatory ability, can be calculated for different typing algorithms in order to compare their discriminatory power(80). The index is based on the probability of unrelated strains being characterized as similar.

Spoligotyping is based on PCR-amplification of a highly variable region in the *M.tb* genome(36). The method only requires a little amount of material, is rapid and cheap, but with a low discriminatory power that improves if combined with other methods of genotyping.

Restriction fragment length polymorphism (RFLP) uses insertion sequence 6110 (IS6110) as a probe, cuts the genome in fragments and then visualises the number and size of the fragments through diffusion in a gel(34, 35). It has a fairly high discriminatory power for *M.tb* strains with more than five IS6110 bands but require a large amount of genetic material, which makes it slow as the bacteria have to grow for at least a few weeks before DNA-extraction is possible. It is also a very labour-intense method.

Mycobacterial interspersed repetitive units - variable numbers of tandem repeat (MIRU-VNTR) is based on PCR-amplification of 24 different standardised loci in the bacterial genome with a variable number of tandem repeats(37). The result is presented as a series of 24 numbers which can easily be compared with results from other laboratories. The 24 digit code can be further shortened into a so called MLVA *Mtb*C15-9 code using a public web application(81) that provides a universal nomenclature, simplifying communication between laboratories. As it is PCR-based, only a small amount of genetic material is required, which makes the method faster than RFLP. The discriminatory power is similar to that of RFLP, but low for strains of the Beijing genotype.

In this study the clusters formed with RFLP plus spoligotyping versus MIRU-VNTR plus spoligotyping were compared and correlated to epidemiological data available in the TB register. This data was complemented when needed with data from the cases contact tracing files. The idea was to better understand the differences in clustering with the different methods. Confirmed contact between clustering cases was considered as true clusters, clustering between cases with no confirmed contact in Sweden but from the same geographical region was considered as possibly true clusters and clusters without any of the former were considered as false clusters.

### **3.1.3 Results**

In total 405 *M.tb* isolates were included in the study. When comparing RFLP and MIRU-VNTR, both in combination with spoligotyping, the global concordance in isolates defined as belonging to clusters or being unique was 333/405 (82%). There were fewer clusters with MIRU-VNTR compared to RFLP (32 vs 41) but they were larger (2-10 vs 2-8). When combining the two methods the number of isolates clustering was reduced by 54 percent for

RFLP and 34 percent for MIRU-VNTR. For the 17 true clusters with epidemiologically confirmed contact between cases, all methods coincided except in one cluster where MIRU-VNTR did not coincide but, the difference was only in one of the 24 loci. To compare discriminatory power the Hunter-Gaston index (HGI) of discriminatory ability was calculated. For each method alone, MIRU-VNTR performed slightly better than RFLP, but when combined with spoligotyping, RFLP performed somewhat better, in our material. The study confirmed findings from earlier studies showing the reduced capacity for RFLP to distinguish between strains with few *IS6110* copies and for MIRU-VNTR to distinguish between isolates belonging to the Beijing family. In our material 13 percent of the isolates were of the Beijing genotype and 15 percent were strains with five or less *IS6110* copies. The study supported the decision to include an extra four hypervariable loci when analysing Beijing isolates with MIRU-VNTR, to reduce the risk of false clustering.

#### **3.1.4 Discussion**

The study was very useful at the national level in the interpretation of genotyping data in Sweden when differentiating between true and false clustering. It visualised the limitations of the methods and that their ability to rule out connections are good but that the level of false clustering is quite high. The much shorter turn-over time for MIRU-VNTR compared to RFLP is a huge advantage as results are available while contact tracing is still ongoing, which at least in Sweden was rarely the case with RFLP. In general, genotyping of isolates is very helpful to detect outbreaks, laboratory contamination and to keep track of the amount of domestic transmission. At the time of the study, WGS was not an option due to costs and limitation in software capacity to store earlier data for comparison. Since then these issues have been resolved and in September 2016 the method for molecular genotyping of tuberculosis in Sweden was changed to WGS. It has reduced the number of clusters substantially and more effort can be put into investigating unexpected clusters, revealing unknown occasions of transmission or laboratory contamination(82). However, even with results from genotyping it is not always possible to exactly determine where or when transmission has occurred. There are still clusters of cases where the only obvious connection between cases is the same country or region of origin. The strain may be common in that region or, in some cases, along a migratory route. As an example, of the 87 clusters detected with WGS in Sweden during two years since September 2016, seven of the clusters consists of more than 10 cases. Of these seven larger clusters, three include only recently arrived migrants from Eritrea and one includes only recently arrived migrants from Afghanistan. One cluster consist mainly of not recently arrived migrants of Somali origin living in the same

small town in Sweden, revealing a problem of insufficient contact tracing. Another advantage with WGS is the obtained information about resistance mutations(83). In a near future WGS may be used to predict phenotypic susceptibility and phenotypic DST may possibly only be performed on isolates where genetic resistance has been detected. Public Health England has decided to stop phenotyping isolates that are predicted to be susceptible to all first-line drugs and similar decisions have been made in the Netherlands.

The problem with communicating results from one laboratory to another is being addressed by a suggestion of close to 3000 standardized loci to compare in order to maintain the discriminatory power, and not only 24 loci as with MIRU-VNTR. Asking the patient about contacts is still the most important measure in contact tracing but genotyping is a useful complementary tool.

## **3.2 QUANTIFERON AND BORDERLINE RESULTS – PAPER II**

### **3.2.1 Background**

LTBI is defined as having a detectable immunological response towards TB but no sign or evidence of active disease. To detect this immunological response, TST has in high income countries like Sweden, more or less been completely replaced by IGRAs during the last ten years. The increased specificity of IGRAs compared to TST is especially valuable in low-incidence settings where the relative risk for a false positive result is higher.

QFT was introduced in Sweden 2006, gradually replacing TST little by little. With a negative or positive result far from the cut-off, precision is usually maintained, but awareness grew that some unanticipated positive results had values very close to cut-off(62, 63). This is logical as it is a quantitative test and the variability in the test method makes any result close to cut-off unreliable(62, 84, 85). The problem has repeatedly been pointed out as especially troublesome when it comes to screening of health care workers in high income countries where serial testing has shown conversions and reversions for results close to cut-off(86-88). In the other commercially available IGRA, T.SPOT.TB, there is a borderline range included in the test and any result in that range should be interpreted as indeterminate. Borderline ranges have been suggested for QFT by others but have, in contrast with T.SPOT.TB, not been incorporated in the manufacturer's instructions(89, 90), most likely in order to reduce the risk of false negative results.

In Sweden a recommendation to take a follow-up sample was introduced in 2010, by laboratories performing the test, if the result was within a borderline range of 0.20–0.99 IU/ml. A pilot study including 4000 clinical tests for QFT showed that almost 300 of these had results in the borderline range. For many of these tests there were also follow-up tests available and they showed that around 45 percent of the results were still in the borderline range, another 45 percent were negative and only 10 percent were convincingly positive. A larger retrospective register-based study was performed to evaluate the reliability of QFT results in this tentative borderline range.

### **3.2.2 Material and methods**

Data on results of all clinical routine QFT samples during 2009-2014 were collected from four laboratories in Sweden, serving more than 50 percent of the population with this analysis at the time. The follow-up samples of those with a first result in the borderline range were analysed, to see how many were completely negative at follow-up versus still in the borderline range or positive above the borderline range. Data was also compared with the national TB-register to investigate how many in each group developed incident active TB (within 90 days to two years) after QFT sampling.

### **3.2.3 Results**

According to the recommended cut-off at 0.35 IU/mL, 75.1 percent of the initial tests were negative, 21.4 percent were positive and 3.5 percent were indeterminate. In total 9 percent of the more than 40 000 tested individuals had a first QFT result in the borderline range. Of negative (0.2-0.34 IU/mL) and positive (0.35-0.99 IU/mL) results in the borderline range, 66.1 percent and 42.5 percent respectively were negative below the borderline range (< 0.20 IU/mL) when retested and none of them developed incident active TB in a period of minimum two years of follow-up. Of 6712 individuals with a positive initial test above 0.99 IU/mL, 65 (0.97%) developed incident TB within 3-24 months after the test.

### **3.2.4 Discussion**

Findings of variability in the test method have been presented in other studies where for example testing of the same sample in different laboratories showed a standard deviation of 0.16 IU/mL (-0.46–0.43 IU/mL) for results in the zone 0.10–0.60 IU/mL(62). Besides laboratory variability many other factors can cause variability between samples from the same individual without true TB infection(91). Pre-analytical factors in the handling of the sample may influence the result, like for example insufficient or too much shaking of the test tubes. Immunologic reactivity in the same individual may vary depending on if sampling has

been performed in the morning or in the afternoon. Physical exercise or stress just before the sampling can also temporarily influence immunologic response.

We recommend re-testing of individuals with a QFT result in the borderline range for a more reliable result, to avoid unnecessary treatment and worry in individuals who do not have LTBI. In 2015 a new version of QFT called QuantiFERON-TB Gold Plus was launched and has replaced the former version. It includes an extra test tube to analyse CD8+ cells in order to make it more sensitive and possibly able to differ between remote and recent infection. The cut-off is still set at 0.35 IU/mL without any borderline range indicated by the manufacturer and results close to cut-off should thus be interpreted with caution. In studies comparing the new QFT Plus with the old QFT and also with T.SPOT.TB it is evident that discordance between results often includes results within the borderline range (0.2-0.99 IU/mL) that are applied in Sweden(70, 92).

The specificity of IGRAs compared to TST has made screening for LTBI more efficient in low incidence settings but if they could also differ between active and latent TB, it would be a great improvement. A test that could differ between active and latent TB could not only be used for screening contacts, but theoretically also for monitoring treatment response and possibly allow an individualised treatment length. In addition a test is urgently needed which could identify individuals with LTBI who are more likely to progress to active TB. The positive predictive value (PPV) of both TST and IGRAs for progress to active TB is low.

According to the manufacturer, the new version of QuantiFERON-TB Gold Plus (QFT-Plus), with an extra tube for detecting a CD8 response, should be able to differ between recent and old infections. Theoretically this is encouraging, but it still has to be proved if the new version of the QFT-plus can do this. Individuals with proven recent infection could then be targeted for preventive treatment(48) , as they have a higher risk of developing active TB.

### **3.3 A SWEDISH COHORT OF MDR-TB 1992-2014 – PAPER III**

#### **3.3.1 Background**

MDR-TB treatment in Sweden has always been individualised according to the DST result. As new medications have been introduced for which an established breakpoint for determining susceptibility has not been determined, the reference laboratory has started to measure the MIC instead, also for some of the drugs with an established breakpoint. There is an ambition to introduce therapeutic drug monitoring (TDM) for all MDR-TB cases In

Sweden. The MIC may then be correlated to the drug concentration in the patient, to avoid overdosing and reduce the risk of adverse events as well as avoid under-dosing for increased efficacy. In the Swedish context so far, this is mostly done for LZD but hopefully it can be extended to more drugs shortly. If the level of resistance is quantified, it increases the options to possibly treat with increased dosing.

### **3.3.2 Material and methods**

There are five laboratories in Sweden performing routine mycobacterial analysis and then the national and supranational reference laboratory at the Public Health Agency performs extended DST for all MDR-TB isolates. All MDR-TB isolates are frozen and stored at the reference laboratory after analysis.

MGIT is an automated mycobacterial detection system where mycobacterial growth are surveyed continuously after inoculation in test tubes with different concentrations of antibiotics. It is used to test for susceptibility for any antibiotics where critical concentrations (CCs) have been established for growth in liquid media, giving a binary result of susceptible or resistant.

MIC – minimum inhibitory concentration, is the lowest concentration of an antibiotic that inhibits visual growth of the tested bacteria. This may coincide with the established critical concentration for determining susceptibility but is not necessarily the same. This is measured by inoculating bacteria in a series of antibiotic dilutions of different concentrations and as a result quantifies the level of resistance.

Molecular DST – may rule in but not rule out resistance. With molecular methods it is possible to detect DNA sequences known to be associated with phenotypic resistance. Line probe assays (LPA) has been in use for years at all five routine laboratories mentioned above to detect resistance to above all R and H but also E, quinolons and second-line injectable drugs (SLIDs), producing a result in a few days. The WHO endorsed GeneXpert is also a PCR based method to detect R resistance which is a marker for MDR-TB.

In this study MIC's of first- and second-line TB drugs were correlated with time to sputum conversion (tSCC) and treatment outcome in a cohort of Swedish MDR-TB cases. The MDR-cases diagnosed in Sweden 1992 to 2014 were identified through the national TB register. All their medical records were reviewed and data collected on sociodemographic information: gender, age, country of origin, years in Sweden, asylum status, use of interpreter, and clinical data: smoking, alcohol/drug use, height, weight, comorbidities, radiology reports, treatment

regimens and duration, adverse drug reactions, tSCC, treatment outcome and microbiological data including routine DST. The isolates were retrieved from the freezer, cultured and MIC determination for the different first and second-line anti-tuberculosis drugs was performed. Treatment outcome was analysed in relation to the data collected and specifically to MIC for the different drugs.

### **3.3.3 Results**

In total 163 cases of MDR-TB were identified in the TB register as diagnosed in Sweden during the study period. For five of the earlier cases it was not possible to retrieve the medical files and the final cohort consisted of 158 cases. A majority of cases (94%) were born outside of Sweden and 77 percent had pulmonary TB. There were few comorbidities, 10 patients with HIV co-infection and seven with diabetes mellitus type 2. The age ranged from 1-85 years with a median age of 29 years and 42 percent of the cases were women. According to DST performed in MGIT the resistance to PZA amounted to 52.5 percent, to E 43.7 percent, to SLIDs 15.2 percent and to ofloxacin (OFX) 10.1 percent. Thirty percent of the isolates were resistant only to R and H. The most common treatment regimen used was E, PZA, LFX and AM combined with PTO, CS or LZD. Overall treatment outcome was favourable in 83.5 percent of the cases, which is in level with treatment outcome for susceptible cases in Sweden. Analysis of MIC was possible for 142 of the 158 isolates and in a multivariate model, including all patients regardless of use of or resistance to fluoroquinolones, adjusting for age, gender and year of diagnosis, an increase in MIC for LFX was associated with poor treatment outcome (aHR 1.77 95% CI 1.15–2.71 p=0.009). Diabetes (aHR 5.52 95% CI 1.42–21.55 p= 0.014) and increasing age (age >40 years aHR 4.51 95% CI 1.74-11.67 p=0.002) was also associated with an increased risk for poor outcome. Treatment with PZA in cases with PZA susceptible isolates was associated with shorter tSCC (aHR 2.25 95% CI 1.27-3.99 p=0.005) (median difference 30 days), but not with treatment outcome.

### **3.3.4 Discussion**

The finding of increasing LFX MIC levels being associated with poor treatment outcome confirms findings in other studies where resistance to fluoroquinolones has been associated with treatment failure(93-95). Regarding the association of diabetes and age above 40 years being associated to poor treatment outcome, this also supports earlier findings in other studies(96, 97). Drug resistance testing of PZA is complicated, among other reasons due to low pH requirement, and often not available. The finding that treatment with PZA shortens tSCC significantly in patients with susceptible isolates, stresses the importance of including

DST for PZA and hopefully it will be generally more available and also more reliable if genotyping DST becomes the standard method(83). Since the overall treatment outcome was as good as 83.5 percent, there were not enough cases with poor treatment outcome to find much significant association to MIC levels.

This study shows that with resources and individualised treatment according to DST-results it is possible to reach successful treatment outcome of MDR-TB at the same level as with susceptible TB. Adding measurement of MIC and TDM to also individualise dosing has the potential to reduce the risk of adverse drug reactions and increase efficacy of the treatment. As new and repurposed drugs is being introduced in MDR-TB treatment this becomes even more important, to maximise the impact of these new drugs and hopefully be able to reduce treatment duration.

Regarding MDR-TB treatment in general there have been many interesting changes recently such as the new WHO recommendations for second line options and also reduced length of treatment. There are clinical trials ongoing with new drugs in combination with older ones, like for example SimpliciTB (BDQ, Pretomanid, MFX, PZA), in order to study reduced treatment length also for pan-sensitive TB(12). Injectable TB-drugs have been downgraded to add-on options due to toxicity and administration route, and an all oral shortened regimen are being studied in the STREAM2 trial(31).

### **3.4 RISK OF ACTIVATING TB DURING PREGNANCY – PAPER IV**

#### **3.4.1 Background**

Earlier studies on risk of active TB in relation to pregnancy have not been conclusive. It is known that pregnancy induces a temporary shift in the immune response of women(98-100). There are also studies that found asymptomatic and extrapulmonary TB to be more frequent in pregnancy related TB, something that could be attributed to a difference in immune response(101-104). There is no doubt that delayed diagnosis of TB during pregnancy might affect the outcome of the pregnancy and increases the risk of transmission to the child(105). Considering the access to different registers with reliable data on childbirths and TB diagnosis where a unique personal identifier allows for linkage, it was decided to do a new study to investigate the possibly increased risk of active TB occurring during pregnancy or during a six months postpartum period.

### 3.4.2 Material and methods

In Sweden all childbirths, occurring after gestational week 22, are registered in a medical childbirth register, Medicinska födelseregistret, which apart from the outcome, includes data on the mother and the pregnancy.

The Swedish TB-register collects data on all notified cases of active TB which includes date of diagnosis, date for start of treatment and date of laboratory report.

A cohort study allows for following individuals in a defined group until an event of interest or censoring for any reason like death, reaching a certain age, end of study period etcetera. As different subjects will contribute different amounts of time it is common to count the ratio of events occurring per person-years included in the study. Incidence of TB is usually presented as number of cases per 100 000 inhabitants and year in a geographically specified area, most often a country. In a cohort study the equivalent will be number of cases occurring per 100 000 person-years.

This was a register-based retrospective cohort study of all women between 15 and 49 years of age, who gave birth in Sweden at some point during the study period of nine years, 2005 to 2013. The cohort data from the childbirth register was linked to the national TB register to see when any women in the cohort had been diagnosed with TB. Since the risk of reinfection and recurrence of TB in Sweden is very low, TB diagnosis was used as an endpoint. Time contribution started at the start of the first pregnancy during the study period since reaching an endpoint before that excluded the actual exposure (pregnancy). Time contributed was divided in three different periods; during pregnancy, postpartum (6 months) and outside these two risk periods. For all events of TB diagnosis, the time period when they occurred was registered. The women were stratified into three groups according to TB incidence in their country of origin:

- Low                      less than 25/100 000
- Medium                 25-99/100 000
- High                     more than 100/100 000

Incidence per 100 000 person-years were calculated for the three different time periods and for the three strata of incidence in country of origin. The incidence risk ratio (IRR) was calculated comparing the incidences during pregnancy and postpartum to outside these two risk periods. As a reference a population based calculation was performed, including all women in Sweden in the age group during the study period, not using TB diagnosis as an endpoint for contributing time.

### 3.4.3 Results

The study included 649 130 women with 951 054 pregnancies and 344 cases of TB. The rates of TB (incidences) for the whole cohort were 4, 12 and 17 per 100 000 person-years for the three time periods; outside pregnancy and postpartum, during pregnancy and during postpartum respectively, resulting in an IRR of 3.0 (95% CI: 2.3–3.9) during pregnancy and 4.2 (95% CI: 3.2–5.5) during postpartum compared to outside these risk periods. When stratifying per TB incidence in country of origin the increased risk was concentrated to women from countries with high TB incidence. In the low incidence group there was no detectable increase in risk during any period and for the medium incidence group there was no increase in risk during pregnancy but a small increase in risk postpartum. In the high incidence group the increased risk was statistically significant for both periods. The result from the population based analysis gave an IRR of 1.6 (95% CI: 1.5-1.7) during pregnancy and of 2.3 (95% CI: 2.2-2.4) during postpartum.

### 3.4.4 Discussion

TB in pregnancy is often detected late and it is always better to detect and treat as early as possible to reduce the risk of complications for both the woman and the child(99, 106-109). Therefore many studies recommend screening of women from risk groups during pregnancy(101, 102, 105). As pregnancy temporarily alters the immune defence, an increased risk of developing active TB seems plausible for women who have been infected with TB before or during their pregnancy(100).

The best way to study the increased risk of TB related to pregnancy would be a prospective cohort study including only women with known LTBI who have never received any preventive treatment nor treatment for active TB before being pregnant. For ethical reasons it is not possible to screen for LTBI and then not offer preventive treatment if positive. In our study, the only women who were considered as not having LTBI when pregnancy started were those treated for active TB before ever being pregnant. Most probably, the majority of women in the low incidence and medium incidence groups did not have LTBI either and stratification by incidence in country of origin confirms this assumption, as there were very few TB cases among women from low and medium incidence countries. The population-based analysis showed a less pronounced increase in risk of active TB during pregnancy and postpartum, but the increase was still statistically significant. This is probably due to a diluting effect of including many women who never were pregnant during the study period. When comparing the amount of time during pregnancy and postpartum in our study to all

person-years contributed by the whole population of women, only six percent of the total amount of person-years occurred during pregnancy and postpartum.

Based on our results we recommend that women from high incidence countries should be screened for TB when pregnant due to an increased risk of active TB compared to when not pregnant. Diagnosing active TB in pregnant women early and treating them, reduces the risk for the infection to affect the pregnancy and for transmission to the child, and if LTBI is detected preventive treatment can be given. In some counties in Sweden screening for TB at maternal health care clinics in pregnant women from risk groups, has already been introduced. In Stockholm County there is an evaluation of the yield of this screening ongoing and the data will be used for a health economic evaluation. Pregnant women from high TB incidence countries should be considered in the WHO recommendations as a risk group to be screened for TB.

### **3.5 ETHICAL CONSIDERATIONS**

The most important consideration from an ethical perspective in a study is to avoid harm to any subject of the study, whether it be physical or psychological. For none of the studies there has been direct involvement of or interaction with the study subjects as all the papers are of retrospective character. Where clinical data has been retrieved from medical files, all patients had already finished their treatment, and for that reason the studies could not in any way affect the outcome for the subjects. All data was anonymised before analysis.

Study I: The study was retrospective with all cases cured at the time the study started, meaning that it could not interfere with treatment outcome. Data was anonymised before analysis and the only one involved in retrieving data was I. At the time I also worked at the clinic and had been involved in several of the contact investigations, reducing the added breach in confidentiality. Informed consent was waived by the ethics committee.

Study II: This was a retrospective register-based study where data from the four laboratories involved were sent in password protected files to the Public Health Agency where I linked data to the TB register before removing the id-numbers from the data set. No one else was involved in handling the data which contained results from more than 40 000 individuals. Informed consent was waived by the ethics committee.

Study III: This was also a retrospective cohort study of all MDR-TB patients in Sweden over 22 years. They had all finished treatment when the study started and the problem was limited

to potential breach of patients' integrity. It would have been impossible to reach all individuals included as some have left the country and some are diseased. To reduce the breach of integrity, only three medical staff bound by confidentiality were involved in the data collection from the medical files. As the knowledge gained from the study was considered to exceed the harm to the study subjects, informed consent was waived by the ethics committee.

Study IV: This was the largest of the studies and also a retrospective cohort study, including data from almost 650 000 women. The cohort was extracted from Medicinska födelseregistret at The National Board of Health and Welfare and then complemented with data on migration and death from Statistics Sweden, before being linked to data from the TB register and then anonymised. The linkage was done at The National Board of Health and Welfare and the anonymised data set was sent to the Public Health Agency of Sweden for analysis. Informed consent would have been impossible due to the size of the study and was waived by the ethics committee.

### **3.6 FUTURE PERSPECTIVES**

Universal health coverage is the most important measure to combat TB and unfortunately the most difficult to achieve. In Sweden TB is a small problem but there will always be TB cases here as well, as long as TB exists somewhere else in the world. This is true for any low TB incidence country and one reason for why continued research for better tools to fight TB matters to everybody.

Improvements in diagnosing of TB, including more rapid DST is right around the corner, though it will probably take a while before it is available where it is most needed.

New and repurposed drugs will shorten treatment and hopefully reduce the amount of adverse events, also in a not too far away future.

A vaccine that is more efficient than BCG might take a bit longer.

## 4 POPULÄRVETENSKAPLIG SAMMANFATTNING

Den här avhandlingen bygger på fyra helt olika studier vars minsta gemensamma nämnare är TB kontroll i Sverige.

Studie nummer ett är en jämförelse mellan olika metoder för molekylär genotypning som använts i Sverige. Genotypning görs för att kunna jämföra bakterieisolat från olika patienter då identiska eller mycket lika isolat talar för att det finns en koppling mellan fallen. I de flesta fall så verifieras en redan känd koppling mellan fall men ibland upptäcks oväntade kopplingar vilket underlättar för att avgöra var smittan skett och om fler individer kan ha blivit exponerade utan att känna till det. Man har då chansen att upptäcka fler smittade som kan erbjudas förebyggande behandling för att minska risken att insjukna. Olika metoder för typning har olika urskiljningskapacitet vilket innebär att fall som liknar varandra med en metod kan vara olika med en annan och då inte är relaterade. De två metoder som jämfördes stämde överens till drygt 80 procent men om man kombinerade metoderna så minskade antalet fall som var likadana med 35 till 55 procent vilket då betyder att nästan 50 procent av klustren som bildades var falska kluster. En nyare metod med bättre upplösning har börjat användas efter att studien genomfördes och antalet kluster har sedan dess minskat med ungefär 50 procent.

I studie två studerade vi en ny typ av immunologiskt test som kan påvisa om man smittats av TB. Vid tydligt negativa eller positiva svar är det inga problem men vid resultat nära brytpunkten mellan negativt och positivt är svaret mindre säkert. Det finns en variabilitet i metoden som gör att resultat nära brytpunkten vid upprepad provtagning kan hoppa mellan positivt och negativt. Vi analyserade drygt 40 000 provsvar från fyra olika laboratorier som tagits i klinisk verksamhet vid både screening och utredning av misstanke om aktiv TB. Under studieperioden introducerades rekommendation om upprepad provtagning vid resultat i en gränzon runt brytpunkten. Resultatet visade att en majoritet av provtagna med resultat i gränzonen blev negativa vid upprepat prov och att ingen av dessa utvecklade aktiv TB de närmaste två åren. Således ledde resultatet till att vi rekommenderar nytt prov vid resultat i gränzonen för en säkrare tolkning.

I studie tre analyserades alla fall av multiresistent TB (MDR-TB) i Sverige under åren 1992 till 2014. Alla MDR-fall i Sverige får en individualiserad behandlingsregim med ledning av resistensbestämning. Vi relaterade behandlingsresultat med ålder, kön, sociala faktorer, en del andra sjukdomar samt med graden av resistens mot olika tuberkulosläkemedel genom

bestämning av lägsta koncentration som förhindrade bakterietillväxt (MIC). Generellt var behandlingsresultaten utmärkta med 84 % som tillfrisknade. Vad gällde faktorer associerade till sämre behandlingsresultat så var det bara tilltagande resistens mot fluorokinoloner som var statistiskt signifikant samt diabetes mellitus typ 2 och ålder över 40 år. Således fungerar nuvarande strategi med individualiserad behandling bra men vi vill ändå arbeta för att MIC görs på alla relevanta läkemedel så att doseringen kan individanpassas för högre effektivitet och minskad risk för biverkningar.

I fjärde och sista studien tittade vi på alla kvinnor som fött barn i Sverige under studieperioden 2005 till 2013. Medicinska födelseregistret gav dessa data som sedan jämfördes med TB-registret för att få fram vilka av dessa som insjuknat i TB under samma period. Tiden som alla kvinnor bidragit med delades in i tre perioder; tid under graviditet, 6 månader efter förlossning samt övrig tid. Därefter tittade vi på under vilken av dessa perioder som fallen insjuknat och räknade sedan ut antal fall per 100 000 personår under respektive period. Det visade att risken att insjukna i TB i samband med graviditet var tre gånger så hög som under övrig tid samt att risken under sex månader efter förlossning var fyra gånger så hög. Vi hade också delat in kvinnorna beroende på hur vanligt TB var i deras födelseland. Det var då tydligt att all riskökning var koncentrerad till kvinnor från länder med en TB incidens på >100 per 100 000 invånare och år. Vi rekommenderar därför att kvinnor från högincidensländer bör screenas för TB i samband med graviditet.

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