

From the Department of Medicine in Solna
Unit of Infectious Diseases
Karolinska University Hospital
Karolinska Institutet, Stockholm, Sweden

TUBERCULOSIS IN STOCKHOLM

**STUDIES ON TRANSMISSION,
PREVENTION AND CONTROL**

Boris Kan



**Karolinska
Institutet**

Stockholm 2013

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ISBN: 978-91-7549-193-6

ABSTRACT

The first priority of tuberculosis control programs is diagnosis and treatment of all individuals with active tuberculosis. Contact tracing constitutes the second priority of tuberculosis prevention and control. Patients' inclinations to cooperate are essential for its results. Treatment of latent infection with *Mycobacterium tuberculosis* effectively reduces future activation and transmission of tuberculosis. However, patient adherence to preventive treatment influences its effectiveness. Early diagnosis and treatment as well as prompt isolation of infectious patients are essential for protection of health-care workers and patients in hospital settings.

We analyzed a nosocomial outbreak of tuberculosis in a hospital ward where the number of cases with active tuberculosis among contacts was unexpectedly high. The outbreak was not revealed until the *Mycobacterium tuberculosis* genotyping results from the first two secondary tuberculosis cases were available. Seven contacts including three health-care workers developed tuberculosis within 10 months after the death of a HIV positive patient from pulmonary tuberculosis. Six out of seven cases were verified by culture and all six *M. tuberculosis* isolates were confirmed by restriction fragment length polymorphism to cluster with the *M. tuberculosis* isolate from the index case. For the health-care workers there was a correlation between number of working hours and risk of acquiring tuberculosis infection and disease. TB outbreaks often originate from clinical mistakes in diagnosing and treating the disease. Spread of a unique, isoniazid resistant strain of *M. tuberculosis* in Stockholm and Sweden resulted in 121 active tuberculosis cases between 1996 and 2012. Several deficiencies in the case-management of patients were identified. Non-adherence was significantly associated with poor outcome, defined as failure, relapse and death. In this thesis we investigated transmission of this cluster and evaluated results of contact tracing performances. Out of 109 cases from Stockholm, 91% were included in this study. In 16 % of infectious index cases, the contact tracing was not executed. Non-adherence to treatment was recorded in 40% of index cases. There was a strong association between not executing contact-tracing and non-adherence of index cases. There were significantly more active TB cases in contacts to non-adherent index cases.

Our results indicate that the cooperation of patients with health personnel regarding performance of contact tracing could be used to predict future non-adherence and TB transmission. In another study we tried to determine factors associated with failure to complete preventive treatment. Association between treatment completion status and patient characteristics was assessed using logistic regression. We found that younger patients, patients originating from Somalia and asylum seekers were more likely to interrupt treatment. The proportion of those who completed treatment increased from 71% in 2002 to 87% in 2007. However, this trend appeared to be caused mostly by an increase in the proportion of European patients. In conclusion, our studies have revealed serious pit-falls in the control of tuberculosis in Stockholm and provide recommendations about how to improve case-management of patients and preventive measures.

Key words: contact tracing, immigrants, health-care workers, adherence

”Я подох на задах, не при старых свечах в канделябрах
Не к Мадонне прижат Божий Сын, а в хоромех холоп
В дивных райских садах просто прорва мороженных яблок,
Но сады сторожат и стреляют без промаха в лоб”.

Высоцкий

LIST OF PUBLICATIONS

- I.** Kan B, Berggren I, Ghebremichael S, et al. Extensive transmission of an isoniazid-resistant strain of *Mycobacterium tuberculosis* in Sweden. *Int J Tuberc Lung Dis.* 2008 Feb; 12(2): 199-204.
- II.** Kan B, Vikman A, Freidlin B, Kalin M, Bruchfeld J. Contact tracing and adherence to tuberculosis treatment: is there an association? Manuscript.
- III.** Jonsson J, Kan B, Berggren I, Bruchfeld J. Extensive nosocomial transmission of tuberculosis in a low-incidence country. *J Hosp Infect.* 2013 Apr; 83(4): 321-6.
- IV.** Kan B, Kalin M, Bruchfeld J. Completing treatment for latent tuberculosis: patient background matters. *Int J Tuberc Lung Dis.* 2013 May; 17(5): 597-602.

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LIST OF ABBREVIATIONS

TB	Tuberculosis
<i>Mtb</i>	<i>Mycobacterium tuberculosis</i>
EPTB	Extrapulmonary tuberculosis
PTB	Pulmonary TB
BCG	Bacillus Calmette-Guérin
LTBI	Latent infection with <i>M. tuberculosis</i>
DTH	Delayed hypersensitivity reaction
TST	Tuberculin skin test
STR	Streptomycin
INH	Isoniazid
PZA	Pyrazinamide
RIF	Rifampicin
PAS	Para-aminosalicylic acid
PPD	Purified Protein Derivative
IGRA	Interferon-gamma release assay
QTF-G	QuantiFERON TB Gold In-Tube
HIV	Human immunodeficiency virus
GLA	Gastric lavage aspirate
CoTr	Contact tracing
HCW	Health-care worker
DOT	Directly observed treatment
IC	Index case
MDR-TB	Multidrug-resistant TB
XDR-TB	Extensively drug-resistant TB
IFN	Interferon
CDC	Centers for Disease Control and Prevention
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
CXR	Chest X-ray
NTP	National Tuberculosis Control Programme
WHO	World Health Organisation
AFB	Acid-fast bacilli
TNFBS	Anti-tumour necrosis factor- α (TNF- α) agents (blockers)
ESDR	End stage renal disease receiving hemodialysis
CAP	Community-acquired pneumonia
LM	Light microscopy
BAL	Bronchoalveolar lavage (bronchial washing)
GLA	Gastric lavage
IS	Induced sputum
DST	Drug susceptibility testing
SAT	Self-administered therapy
PHA	Public health authorities
RFLP	Restriction-fragment-length polymorphism
MIRU	Mycobacterial interspersed repetitive units
VNTR	Variable number tandem repeats

1 GENERAL BACKGROUND

1.1 TUBERCULOSIS: A SINOPSIS

1.1.1 Personal

When I was about thirteen, my father took me to Leningrad. We visited various people, including the family of a dear old friend of my grandmother. We talked about many things but there is only one story that I still remember today. They told me that Rafael Galperin, her son-in-law had died of tuberculosis (TB). He died because of some “plastic balls” which were used to treat him. I was told that the balls made a hole or holes in Rafael’s lungs. Later, I found out that Rafael had been a physician who specialized in radiological phthisiology (phthisis means pulmonary TB). He died in 1951 after being ill with excavating pulmonary TB for three to four years. The direct cause of death was probably a suppurating infection, which was a recognized complication of the ball plombage, the treatment he received. The “balls”, often made of cellulose, were used for the collapse therapy of the cavitary TB. This was a plausible alternative to thoracoplasty which was contraindicated in poor risk patients. The procedure was later abandoned by most chest surgeons because of a high incidence of complications (1, 2). It was a strange subject to talk about with a teenager like me, but I believe that if I had not heard this sad story, I would have never have written this thesis.

There are two other people I would like to mention.

I met Brita 6 years ago. She was admitted to a TB clinic due to a suspected reactivation of an old TB.

When she was in her early twenties she studied dentistry in Stockholm. First year students studied anatomy, pathology, and so forth at the Karolinska Institutet before they moved to the dentistry school “Käftis” on Holländargatan. She told me that in 1940s together with her fellow-students, she had to attend a necropsy. It was a “famous” professor himself who performed it. He was dressed in protecting clothes while students had their regular garments. The patient had died of pulmonary TB. The necropsy took a long time, as the professor was eager to demonstrate the body. Brita

remembered that she received a splash on her dress from the prosecutor's knife. Three months later she got TB. In total 20 students became ill, and two of them died. Anna Brita went to different sanatoria (Dalarna, Söderby, Switzerland). She was getting worse. On her wedding day she had a high fever. In 1950, her condition deteriorated, and she had to be operated. A two-stage surgery was performed by "one of best thoracic surgeon in the world" at the Red Cross Hospital, one of the places where Crafoord performed his magic. She was also treated with streptomycin. Brita had been going for check-ups for 30 years. All because of a necropsy! However, though she had been badly hurt by TB, it could not break her spirit. In the 1960s she went back to school, became a dentist and worked in Farsta until retirement.

I have heard that there were some publications on the matter and decided to dig a little deeper. First I called Gunnar Dahlström in Uppsala who had heard this story but could not give me any details. He sent me to his colleague Erik Berglund in Gothenburg who knew a little more. The "famous pathologist," who performed that necropsy was Folke Henschen, professor of pathological anatomy at Karolinska until 1946. According to Erik, Henschen told the students something like that: "don't be afraid, TB won't jump at you." Unfortunately, Erik did not remember how he had heard this story. So, I had to find out little more. I talked to three dentists and to a granddaughter to the fourth one, who died last year. No information. But history doesn't die, and if you are not looking to it, it might be looking for you. My mobile rang. A voice of an old man asked, "Are you Boris Kan?", "Yes, I am." "Are you searching for students at Karolinska?" It felt at once, that I was getting close.

The man who called me was Sune Lidén from Karlstad. He came to "Käftis" from Lund 1946, because dentistry school in Malmo was not yet finished, and students had to move to Stockholm to continue their education, after their preparatory year in Lund. It was the class of 1945, who got decimated after that necropsy. Students at Käftis were collecting money for "tuberkulosstudenterna." Sune told me that at the necropsy, the professor sent around the tuberculous lungs in the bowl, so the student could learn its special smell. After what had happened, the TB screening at Karolinska was intensified.

George Orwell, a Burmese policeman and a soldier in Barcelona, gave TB a fight, which went on for many rounds, but at the end it was hard to be with him, because his

breath was so terrible, as his lung were decaying (3). Henschel was an experienced pathologist, and he knew exactly what a tuberculous lung smelled like.

Long before the general risk for TB among health-care workers (HCWs) had been acknowledged (1920-1930s), the particular risks of necropsies in relation to TB were well known. Erik Hedvall published an article in 1940 where he showed how dangerous it was to study pathology at Lund University (4).

When I studied pathology in Uppsala in 1994, our group of 6-7 students attended a necropsy of an old woman. When we looked at her lungs we saw a well-confined, “lump” which resembled of a ping-pong ball, situated in the parenchyma of one lung. When the teacher (EL) came in and was asked a question about what it was, he took the lung to the sink, poured water on it, and after a little while, the “ball” dissolved completely. The teacher told us that it was nothing. This is what I remember, “Nothing”. I don’t recall that we asked any other questions; I guess we just wanted to get it done and go home. But there is one thing I do remember, when I looked on the “washed” lung, there was a big hole in it, the size of a ball, also well-confined, but already collapsed; and I also saw a fine lining in the bottom of the excavation.

The third person that I would like to mention is Hans Difs (1904-1952). He was a doctor at Serafimlasarettet and suffered from TB for many years but he managed to publish some articles (5). According to his daughter, his lungs were severely damaged. Together with Gunnar Dahlström he published one of the finest work on TB written in Sweden “The efficacy of B.C.G vaccination: A study on vaccinated and tuberculin negative non-vaccinated conscripts” (6). For this publication Dahlström and Difs were awarded the highest prize by the Swedish Society of Physicians. In his thesis (1953) Dahlström wrote the following words about Difs: “I shall remember him and his work with deepest sense of loss and gratitude.” He kept his promise and he told me about his friend.

So now, you can remember him too.



Hans Difs

1.1.2 Historical overview

Modern history of tuberculosis (TB) probably started on December 5, 1865 when the French physician Jean-Antoine Villemin notified the Paris Academy of Medicine that rabbits inoculated with TB developed disease, thereby demonstrating its infectious nature. In 1882, Robert Koch isolated *Mycobacterium tuberculosis* (*Mtb*) and in 1895, Wilhelm Konrad Röntgen discovered X-rays. Only several months after Röntgen made his findings public, French doctor Bouchard already used x-rays to examine the lungs of his patients, so that around 1905, doctors could diagnose TB with improved accuracy.

Koch made in 1890 an extract of cultures of *Mtb* which he thought would cure and prevent TB. This agent, later known as Old Tuberculin was “a most confusing mixture of substances”, only parts of it had been derived from mycobacteria. It is believed that Old Tuberculin cause many deaths due to vicious allergic reactions, so called tuberculin shock described by Koch himself (8, 9) . It was a precursor of “purified protein derivate” (PPD), separated by Florence Seibert in 1924, PPD was adopted as the standard by the United States in 1941 and by the World Health Organization (WHO) in 1952, and is still in use today. Seibert, crippled by polio, turned into academic career because she

could not go out and dance, as she later recalled, was probably one of the first women in the “TB hall of fame” (10).

Also, Koch studied how infection with *Mtb* could be diagnosed; he never recognized the diagnostic potential of the skin reaction at the site of injection. The first mention of the diagnostic value of the injection site was made independently by Epstein and Escherich in 1891. The latter was a teacher of Moro, Pirquet and Hamburger, all of whom made own contribution in the field of tuberculin (11). First TST survey was conducted by Mantoux using intradermal technique, which was first reported by Mendel in 1909. Calmette received a strain of *Mycobacterium bovis*, which later became the source for the BCG (Bacillus Calmette–Guérin) from Nocard, who isolated it 1902 from a cow with tuberculous mastitis. This originally virulent strain, attenuated miraculously, was first given orally to an infant in 1921. One of the earliest studies on sub- and intradermal administrations of BCG were published in 1928 by Heimbeck in Norway and few months later by Wallgren in Sweden (12, 13). The “Lubeck catastrophe” in 1930, when 250 babies were given what supposed to be an oral BCG vaccine and 72 died within a year, resulted in considerable loss of public faith in BCG (9). Despite the later controversies around its efficacy, BCG remains a part of control program in countries where TB is frequent among young people (14). In 1943, two drugs were discovered, first PAS by Lehmann in March (15, 16) and in October, streptomycin was isolated by Schatz and Waksman (17) which gradually led to an unprecedented cure of TB (18). However, 60 years later, TB still remains the leading cause of death among infectious diseases.

1.1.3 TB globally

TB is second only to human immunodeficiency virus (HIV), causing an estimated 1.7 million deaths in 2011, as the greatest contributor to adult mortality. According to the WHO, there was an estimated 8.7 million new cases of TB worldwide and 1.4 million deaths from the disease in 2011 (19). TB is also the most common cause of morbidity and mortality in individuals with HIV in sub-Saharan Africa. Mortality rates of up to 40% per year have been reported in patients coinfecting with TB and HIV who are receiving treatment for TB, but not for HIV (20).

People living with HIV infection are 6--50 times more likely to develop active TB than are people without HIV infection. It has been estimated that at least 12 million people are coinfecting. The annual incidence rate of TB is five times higher in countries where the prevalence of HIV infection among adults is high, compared to countries where the

prevalence of HIV infection is low. The TB case rate has increased 5- to 10-fold in many sub-Saharan countries since the identification of HIV, and the prevalence of HIV among TB cases exceeds 80 %.

The risk of TB infection has decreased by roughly 10% annually in the majority of Western countries between 1900 and 1970; however, the rate of decline differed in individual countries (21). Overall, TB mortality rate has decreased globally around 41 % since 1990 (19). Nevertheless, the true incidence trends may be unknown since the estimates rely heavily on the quality of case-finding, diagnostics and reporting. Under-reporting and misdiagnosis of TB is a large problem, especially in countries in which TB is endemic, not least because many cases may be managed outside national TB control programmes (NTPs) by private, voluntary or corporate health sectors (22).

1.1.3.1 Drug resistance

MDR-TB, defined as resistance to both isoniazid (INH) and rifampicin (RIF), has reached levels of up to 14% among new patients, and levels as high as 50% among previously treated patients in some settings (23-25). XDR-TB, first reported in November 2005 and defined as *Mtb* strains with resistance to at least isoniazid and rifampicin, plus resistance to any fluoroquinolone and any aminoglycoside (amikacin, kanamycin) or capreomycin, are being reported in increasing numbers across the globe (26). Southern African nations, having the world's highest HIV infection rates, are especially in danger for XDR TB which has a mortality rate of more than 85% (27).

MDR TB is also associated with a very high mortality rate among HIV-infected individuals (28, 29). It has been anticipated that more than 300 000 patients with pulmonary TB (PTB) in 2011 had MDR-TB. The majority of these cases were diagnosed in India, China and the Russian Federation. The average proportion of XDR resistance pattern among MDR-TB cases was 9 %. In several countries in Eastern Europe and central Asia, 9–32% of new cases are MDR-TB and more than 50% of previously treated cases have MDR-TB (19, 30). WHO stresses the importance of using molecular testing methodologies to detect drug-resistant strains (31).

1.1.4 *Mycobacterium tuberculosis*

Besides *Mtb*, TB is also caused by *Mycobacterium bovis* and *Mycobacterium africanum*. All are primarily intracellular parasites and in normal hosts induce a granulomatous response in tissue. These slowly growing, rod-shaped bacteria do not form spores and are obligate aerobes. Humans are the only known reservoir for *Mtb* but several animal species are susceptible to *Mtb* infection.

Once stained, *Mtb* resist decolorization by acid or alcohol and are therefore called “acid-fast” bacilli (AFB). The acid-fastness is due to the robustness of its multilayered cell wall that contains a variety of lipids and has very low permeability. These properties of *Mtb* facilitate survival of the bacilli within macrophages. Mycobacteria can be demonstrated by yellow fluorescence after staining with auramine, which is more sensitive than classical Ziehl-Neelsen stain. Growth on solid media is visible from 3 to 8 weeks after inoculation (32, 33).

1.1.5 Historical perspective on infectiveness

Sputum of TB patients has long been recognized as the main source of infection (34). but it was not until 1930s that the essential role of air-borne transmission as cause of TB became clear (35). Segal wrote in 1939 that “any object previously handled or contacted by an unclean patient may be an indirect means of transmitting sputum” (36). He mentioned the most common “objects” which could spread TB, such as knives, forks, glass-ware, napkins, towels, wash-basins, money, door knobs, telephones, books, toys, vegetables and candy. Even common house-flies were believed to aid the spread of sputum containing bacilli. Cornet in 1888 was the first to “clearly established that the dust in the vicinity of consumptives, especially dirty ones, frequently contains tubercle bacilli”. He inoculated the dust from hospital bedrooms, homes and workplaces of TB in animals, and 20 % of these developed TB (37). Similar results were demonstrated by others though even much higher proportions (60-90 %) of animals, inoculated with dust, developing TB have been reported (34).

M. bovis used to be an important pathogen for TB in humans, mostly before World War II. Infection is otherwise contracted either through inhalation, via cut on the skin (“Butcher’s wart”) or through consumption of contaminated milk, in which case the

bacilli lodge in the tonsil or intestinal wall. According to Griffith (1937), 90 % of cervical node and 28 % of meningeal TB in children < 5 years in England and Wales 1901-1932 were caused by *M. bovis* (38). In a study by Sjögren and Sutherland it was investigated whether the risk of TB infection in different areas of Sweden was related to changes of the prevalence of TB infection in cattle. The authors suggested that bovine infection in childhood provided a subsequent prolonged protection against adult infection with *Mtb* (39).

1.1.6 Pathogenesis and immune response

The vast majority of otherwise healthy people infected with tubercle bacilli do not develop clinically evident disease. Approximately 5 % of the infected develop symptomatic primary TB, often during the 18 months after exposure, and a further 5 % subsequently develop the disease later in their lifetime (40). The usual source of *Mtb* infection is a person with pulmonary TB (PTB) whose sputum contains AFB visible by microscopy. Patients with cavitory lung disease are particularly infectious because their sputum usually contains 1--100 million bacilli per mL and they cough frequently (41, 42).

A person with PTB who coughs, sneezes and talks aerosolizes infectious droplets nuclei which may remain suspended in the air for hours and may then be inhaled (43). Only the smallest droplets contacting 1-3 bacilli are capable of reaching the alveolar space. Therefore, only a small portion reaches the terminal alveoli where bacilli are ingested by alveolar macrophages (44). In practice, prolonged exposure and multiple inocula are required to cause infection, though theoretically one droplet can be sufficient (33). Genetic factors confer virulence to *Mtb* and also determine innate resistance to infection. Intracellular growth of bacilli takes place in non-activated macrophages and extracellular growth in liquefied caseous (acellular) substances produced by necrosis (44). Killing of infected macrophages releases tissue-liquefying enzymes that can cause caseous necrosis and cavitation (32).

A TB lesion starts as an acute inflammatory reaction, with edema fluid, polymorphonuclear leukocytes, and monocytes around tubercle bacilli. In the lungs, it resembles bacterial pneumonia. In this phase the lesion may heal, may lead to massive necrosis of tissue, or can evolve into a chronic granuloma, termed the

primary focus. The central area of a granuloma undergoes caseation necrosis, the true tubercle, which may form a cavity or subsequently heal by calcification. Once bacilli are trapped inside macrophages, they are either killed, or start to multiply leading to lysis and death of macrophages which attracts monocytes from the bloodstream. Macrophages stimulate proliferation of CD4+ T lymphocytes which are crucial to containing growth of *Mtb*. Production of interferon (IFN) - γ , interleukin (IL) 2, 4, 5, and 10 by CD4+ lymphocytes promotes immunity, including intracellular killing of bacilli. Tumor necrosis factor (TNF) is essential to control *Mtb* infection by stimulating phagocytosis and to form granulomas. It is produced by macrophages. Mycobacteria surrounded by T lymphocytes are found in the center of granulomas together with macrophages (45). However, the theoretical basis for understanding the immunity to *Mtb* may be oversimplified: human immune response to *Mtb* is very complex, and that existing animal models (mice, guinea pigs, rabbits) utilized in experimental studies do not imitate it sufficiently (46).

Through complex interactions involving mononuclear phagocytes various T cell subsets and cytokine production, host defenses are enhanced by mechanisms related to delayed-type hypersensitivity (DTH). DTH is associated with the development of the tuberculin reaction, 48--72 hours after the intradermal injection of protein antigens (e.g. purified protein derivative (PPD)). Adaptive immune response to *Mtb* is delayed for approximately 2 weeks and it can be measured in experimental animals by tuberculin skin test (TST) approximately 42 days after *Mtb* infection is established (33, 46). In clinical practice, this time is estimated to be 8 to 10 weeks. Most humans and animals develop apparently appropriate immune responses after *Mtb* infection but these immune responses are not always sufficient to eradicate the bacteria. Instead, such responses cause *Mtb* to adopt an asymptomatic, latent state of infection, which can reactivate in the future (46). In clinical practice, the term latent *Mtb* infection (LTBI) refers to a condition in which the individual is infected with *Mtb* and who, at the time of examination, has no symptoms but is considered to be at risk for progressing to TB disease. After *Mtb* infection is established, it can remain clinically silent for decades before active disease develops. Waning of cellular immunity may permit the *Mtb* population to start growing again, which is denoted as reactivation TB, the far commonest form of adult TB, as an opposition to primary TB. Thus, people with LTBI comprise a reservoir of future TB cases (47).

1.1.7 Epidemiology

Risk factors associated with infection and with development of disease are two main topics that epidemiology of TB deals with. Little is known about endogenous factors associated with acquisition of infection; on the other hand risk factors related to development of disease are well studied, with HIV and age being probably most renowned (48).

1.1.7.1 Risk of becoming infected

Due to practical reasons, within the realm of epidemiological studies, acquisition of infection is considered has taken place when a positive TST has been developed. There are several methodological pit-falls in estimating infection rate, related to standardization, sensitivity and specificity of TST (49). In his Frost lecture, George W. Comstock brought up the issue of discrepant results of many studies caused by so called “two-step testing”. This problematic procedure, using tuberculin in two strengths, led to an overestimation of true reactors, since 90 % of older children had positive reactions upon retesting. Comstock stressed the importance of analyzing the frequency distributions of reaction size instead of identifying a “potentially misleading” proportion of positive reactors which will miss the bimodal distribution of false and true positives (7).

Characteristics related to the contagiousness of the index case (IC), exposure, delays in diagnosis, social and behavioral patterns (exogenous factors) determine the risk of *Mtb* infection (50). Proximity to and duration of contact with a coughing sputum smear-positive individual are critical factors for infection to occur (49). The risk of *Mtb* infection in a close contact to an infectious case is 25–50%. The review of the literature on TB in children published in the pre-chemotherapy era showed that following lengthy household contact with a sputum smear-positive index case, 60–80% of children became infected. When the source case was smear negative, 30–40% of children became infected. Most children (80%) who became infected before the age of 2 were infected by a household index case. Older children were probably infected in the community, since the majority of children who became infected after 2 years of age had no household contact identified (51). The relatively small family size in high income countries today and the sporadic nature of social contacts between elderly people

(potential cases) and their grandchildren (the most susceptible group) have reduced the opportunity for exposure.

It has been estimated that up to 20 persons who have been in contact with a smear-positive case may be infected and eventually one of them will become smear-positive, thus spreading TB further (33). Grzybowski, et al. demonstrated a strong association between pulmonary involvement and infectivity of contacts (52). This study from Canada also showed the association between risk of infection and race, the latter, however, confounded by the fact that Indian households were more crowded. Indian contacts were more likely to be infected than white contacts, but the contribution of casual contacts (as opposite to domestic or close contacts) for acquisition of infection was more pronounced among Indian children (37 % vs. 10 %). Transmission due to casual contact makes a sufficient contribution to the dynamics of TB in endemic areas where a significant proportion is infected outside households (53). The majority of household contacts were infected within 3 months of symptom onset in the adult index case (54).

Chapman et al. showed that severity of disease in the patient with active TB (index case) was the strongest predictor of occurrence of infection in children in the proximity, but poverty and crowding were also significant (55). The latter observations were confirmed by two other North-American studies (56, 57). In a study on TB rates in twins, intensity of exposure was shown, contrary to original findings by Comstock, to outweigh the importance of hereditary factors. The authors' conclusion was that "environmental factors and the context of transmission should be given more emphasis when studying interindividual and population differences in susceptibility to infectious diseases such as TB" (58).

Delays in the patients seeking care and in obtaining the diagnosis of an index case may influence the rate of infectiousness by prolonging exposure.

Multiple studies have described TB transmission in schools, nursing homes, hospitals, shelters, churches, bars, ships as well as other congregate and confined settings (59, 60). Although some TB patients can be very infectious (61), but when the duration of exposure is taken into consideration, the average patient with TB has a low degree of infectiousness per unit per time (48).

The risk of becoming infected has been declining most rapidly in industrialized countries. The most remarkable decline in the risk of infection was demonstrated

among the Inuit population in certain parts of Alaska: 62 % of children ≤ 6 years were infected with *Mtb* in 1949-51 while there were only two reactors among 1535 tested children in 1969-70. This decline was due to an effective control program for diagnosing and treating active TB cases and cases with LTBI (48).

Little is known about the association between mycobacterial genetics and risk for acquisition of infection but there are indications that some strains have an enhanced capacity for transmission. *Mtb* strains of the so called Beijing family may have a higher propensity to cause disease, as for example did the spread of strains *W* and *N* in the US caused many TB cases (62). Moreover, the capacity to cause infection vs. disease may be a different phenomenon.

Age and sex of the patient also influence the risk of infection with *Mtb*. The risk increases with age to early adult life and after that probably levels-off. Though adult men are more likely to have been infected as compared with women, the prevalence of infection, as demonstrated by TST positivity, among boys and girls is believed to rise equally in childhood but increases disproportionately in men after the age of 13-15 y (49). This could be due to a larger number of social contacts in men (48, 63). Though social factors do play a role in exposure to infection, similar disparities in the TST positivity due to gender have been recorded in different societies and time periods, suggesting that gender related differences in infection rate may be influenced by biological factors (64). However, according to a classical British textbook "Pulmonary tuberculosis" (1948) "there is no evidence that the rate of infection shows any difference in the two sexes at any age period" (65).

Treatment of TB has been shown to reduce infectiousness rapidly (66). Drug resistance may oppose this effect, though certain *Mtb* strains expressing drug resistance may lose some of its capacity to spread (67). However, the earlier hypothesis that drug resistance always correlated with a loss of bacterial fitness, was questioned already in the 1950s (68). Steiner et al. described a TB outbreak caused by an *Mtb* strain isolated from a 16 year old girl from Brooklyn in 1969. The isolate was resistant to isoniazid (INH), streptomycin (STR) and para-aminosalicylic acid (PAS). All 23 household contacts were found to be TST positive, and in six of them active disease developed. A significant proportion of non-household contacts of the source case were also TST positive (69). Also episodes of treatment interruption may interfere with reduction in infectiveness.

Other factors that have been mentioned in the literature to influence incidence of *Mtb* infection are urbanization/population density, family size, age of index case, occupation and institutionalization(48, 49, 65, 70).

1.1.7.2 Development of disease: endogenous factors

The risk of developing TB is associated with the background prevalence of TB and varies according to diverse host factors such as patient's age, time after infection, social situation, genetic or acquired susceptibility including grade of immunosuppression and various medical conditions.

1.1.7.3 Time after infection and age

Children less than 1 year old are at higher risk of developing disease after being infected. Early in life, the effects of age and time after exposure can barely be separated. Incidence falls in 8—12 old children, followed by a second peak in 15--25-year-olds (7). According to recent estimates derived from studies conducted by Ferebee and Comstock, the lifetime risk of reactivation of TB is about 10--20 % among children < 5yrs of age when infected and who have TST \geq 10 mm (71). The temporal association is most striking for early forms of TB. Most cases of tuberculous meningitis and pleurisy in children were diagnosed 1--3 months and 3--7 months after occurrence of primary TB, respectively. The latter was characterized by conversion of TST and in individuals with symptoms, fever, increase in ESR (erythrocyte sedimentation rate) and sometimes development of erythema nodosum (72). Recent infection (< 2--5 years after exposure) is 10 times more likely to cause active TB than a long-standing infection (49).

The age distribution of TB reflects the degree of ongoing transmission in a given population. TB in the elderly is generally due to reactivation of infection acquired in the past, while TB in young children and adults indicates ongoing active transmission in a community.

1.1.7.4 Sex and ethnicity

The incidence of TB among women peaks at 25--34 years of age. It was believed, based on clinical experience, that TB was a more acute disease in women as compared to men (73). Analysis of TB mortality in the US during 1924 to 1962 showed, that though the median age at death of TB was constantly increasing, the life-shortening effect of TB was 5 % for white men and 30 % for non-white women in 1962. The non-

white women died of TB 15 years earlier than the white women (74). Interestingly, familial contact with TB had more severe consequences for the non-white children than for the white though non-white had already a higher community attack rate from TB compared to the white children. The study of mortality due to TB in 138 white and 147 nonwhite families exposed to TB, showed that non-white children had a mortality from TB that was 3-4 greater than the white children, once exposure has been established (54). Drolet (1935) suggested that the differences in mortality between males and females were due to “differences in labour conditions and the burdens carried on daily by one or other sex” (75). There was a decline in TB mortality among adults in England and Wales in 1939-1943 in all groups except for men of 35 and older. Up to 10 years of age the mortality was greater among boys, but between 10 and 30 the female rate was distinctly higher and after 30 the male rate was again higher. This was particularly evident in cities and industrialized communities (65).

According to Comstock, when age, closeness of contact and infectiousness of index case had been taken into account, the race per se seems to have a little impact on the risk of reactivation once infection was acquired (48).

1.1.7.5 HIV

To my knowledge the first report on development of TB in patients with “a new acquired immunodeficiency state” was presented in 1983 (76), the years when the causative retrovirus was described first by researchers from the French Pasteur Institute (77). Disseminated TB preceded the other (opportunistic) infections by 2 to 15 months in seven patients from Haiti which according to the authors “may have been secondary to a T-cell immunodeficiency in some.” Studies from Florida and New-York suggested that in most cases TB occurred before other opportunistic diseases (78, 79). HIV markedly increases the TB progression and aggravates the severity of TB but TB also accelerates HIV replication in the lungs and other organs (50). Before antiretroviral therapy (ART) was introduced, the annual risk of progression from LTBI to active TB in HIV-infected individual was estimated to be 5 --15 % (49). Among HIV-infected, not only is the risk of TB correlated with CD4⁺ lymphocyte count but also the manifestations of TB depend on the CD4 counts (80). Atypical presentation of PTB and an increased proportion of extrapulmonary TB manifestations (EXTB) are seen in approximately 50 % of coinfecting patients with CD4 counts below 200 mm³ (81).

1.1.7.6 Medical conditions other than HIV

Prolonged treatment with *corticosteroids* is generally considered to be a risk factor for reactivation, though few adequately performed studies demonstrated that this risk must be small (49).

The association between pulmonary TB and *lung cancer* become evident in the late 1950s when TB was diagnosed with increasing frequency in patients over the age of 50 (82, 83). TB associated with *silicosis* has been reported in foundry and mine industry and silicotic workers have an approximately 3—5 greater risk of developing TB and dying from it than in the general population (84, 85). *Smoking* and smoking-related chronic lung disorders are responsible for a significant proportion of TB cases globally. According recent estimates, smokers have up to three times higher risk of TB (86).

The risk of TB has been reported to be increased in patients with *haematological malignancies*, *squamous cell carcinoma of head and neck* and certain *sarcomas* (49, 87). The risk estimation is confounded by factors like the weight loss, cachexia and extent of cancer (88). Increased risk for TB has been reported in *solid organ transplant recipients* (89) and after *bone marrow transplantation* (90).

The importance of the *tumour necrosis factor* (TNF) in the pathogenesis of TB was recognized more than 10 years before infliximab, the first monoclonal antibody to TNF-alpha was approved (91, 92). Estimates of the relative risk of developing TB, while receiving *tumour necrosis factor- α blockers* (TNFBs), range from 2 to 25 fold (93). TB rates among TNFBs users in the UK, Sweden and France were 4 –10-fold higher than the background general populations (86, 94).

Patients with *end stage renal disease receiving hemodialysis* (ESRD) are also at an increased risk of TB, especially within 2 years of onset of ESRD (95). It has been estimated that incidence of TB in patients with ESRD is more than 10 times greater than in the general population (49).

The association of *diabetes mellitus* and TB has been recognized at least since 1859, when the first report of 250 diabetic cases with special reference to pulmonary TB was presented by Griesinger (96). In a study by Silwer and Oscarsson, 3.6 % of diabetic patients in Kristianstad County had pulmonary TB compared with 0.88 % in a control group (97). The incidence of TB is two to five times higher in patients with diabetes than in those without (98).

1.1.8 Pulmonary TB

1.1.8.1 Pulmonary TB in the pre-modern era

Sputum and X-ray examination of the individuals with known or suspected TB have been cornerstones of diagnosis since around 1900, which should be considered as the beginning of pre-modern era. The modern era probably started in the late 1940s when STR and PAS became available. Information on clinical and physical signs of TB is however scarce in modern textbooks (14, 99-101).

Segal wrote in 1939 that pulmonary TB can be compared to lobar pneumonia with one important difference: lobar pneumonia leads to recovery or death within 2 or 3 weeks while PTB goes through different stages of infection and disease “at a much slower tempo extending over a number of years” and that the course of TB “is punctuated by periods of exacerbations and remissions, as one force or the other predominates for the time” (36). Turban classification of PTB in adults (1899), named after Karl Turban – the “tuberculosis tyrant” of Davos and the father of strict rest cure, was recognized of many as the best and most practical system. PTB was later subdivided in 1) *Exudative* benign type, or *incipient* TB, characterized by the absence of sputum, symptoms and distinctive physical signs and the wooly shadow (infiltrate) on the CXR near the clavicle. Later, Simon’s foci (well-known apical fibro-nodular shadows) would sometimes appear in the place of these first shadows 2) *Caseous pneumonic* type was characterized by a dense shadow in one lobe and beginning cavitation in a patient already being aware of the disease. Failure to absorb caseous process leads to 3) *Fibro-caseous* type, corresponding to the third stage of Turban-Gerhardt or “far advanced” (Trudeau) stage of PTB. This type embraces all grades of pulmonary involvement including multiple cavities with fibrosis. At that stage the symptoms and physical signs are distinct and sputum is positive for tubercle bacilli. The cavity was considered a great danger for patients and was the “keystone of the whole arch of modern tuberculosis therapy “(Segal), this opinion can be commonly found in the literature.

TB doctors of the past paid great attention to the symptoms of TB and believed that for an experienced eye, TB often became visible already in its incipient (early) form: patients usually feel well but may look “little of color, may have lost a little weight and may tire easily” (36). Hundreds of pages of the books published long before the invention of streptomycin are devoted to symptoms and signs of TB. Should we

dismiss our sires as unscientific and terribly outdated or should we try to listen to their distant voices? Would Cornet and Klebs laugh, hearing me say to a fellow colleague that we found a case of advanced TB without apparent physical signs and symptoms? For them the absence of clinical findings in this case was probably equal to the absence of clinical skills. However, the well-known textbook on PTB published in 1948 indicates a paradigm shift “symptoms have not the same significance in the diagnosis of pulmonary tuberculosis as they used to have” (65). The authors continue stating that there is a little doubt that even active PTB can occur “without what at one time were considered the essential symptoms of disease.”

Ruehle (1887) says that there is no consumption (PTB) without cough which according to Klebs, with some exceptions is a “fair statement” (102). Klebs writes that “there is no typical cough of tuberculosis” and “When large cavities exist, the cough is usually very loose and free and may be intermittent, coming on in paroxysms until the cavity is emptied, the sputum coming up in large amounts and then ceasing until the cavity is full again.”

Klebs says that “of constitutional symptoms, fever occupies the most important from a diagnostic and prognostic point of view and as a guide of treatment” (102). He believed strongly that “subnormal morning temperature”, referring to $t < 36\text{ }^{\circ}\text{C}$, was of real diagnostic value. It was widely acknowledged that TB patients have an increase of the temperature in the evenings. Klebs devotes 12 pages to “Fever” in his book while Cornet suggests, “after fourteen years of observation directed to this point” that temperatures should be taken every two hours (37)

In the second, caseous stage, sweats, closely associated with fever, become common symptoms. Klebs writes that “they generally occur in the night, usually shortly after going to sleep, and are often repeated in the early morning.” It was known that sweats of this stage, stopped within 2 weeks of the beginning of an outdoor rest cure. Profound, so called colliquative, sweats were seen only in late stages of PTB, were always resistant to any treatment and very disturbing for the patients.

Tachycardia was another sign commonly discussed in the elderly literature but careful recordings of pulse-rate were abandoned in 1940-s (Gunnar Dahlström, personal communication). Tachycardia (in a person whose normal rate is known) was believed

to appear early in the disease and often exists for a long time before pulmonary lesion can be discovered and when a lesion become evident the pulse rises above 85.

In the 1940s, diagnosis of many patients were made in out-patient settings and it was difficult to obtain reliable temperature and pulse records. There was also a growing understanding after the World War II that “the significance of constitutional symptoms has altered in recent times owing to an altered mode of life...competition is keener and anxiety follows... the result is that people very often suffer from symptoms similar to those of tuberculosis: fatigue, nervousness, loss of appetite, loss of weight” (65). The authors come to a conclusion that “symptoms can no longer be treated purely objectively; the mode of life of the patient for a preceding period must be examined.”

1.1.8.2 PTB today

Today, the symptoms of TB are most often regarded as “nonspecific”, and since TB is associated with other illnesses that have similar symptoms, the lack of specificity can result in a delayed diagnosis. In industrialized countries around 60 – 90 % of TB cases have respiratory disease (81). Though TB remains an important differential diagnosis in a patient with community acquired pneumonia (CAP), it comprises only 0.3 % of CAP in industrialized countries (103). This proportion is evidently higher in those with medical conditions that increase a patient’s risk for TB, including a history of HIV infection, foreign-born patients from countries with high prevalence of TB, and patients with a prior history of TB. Low prevalence of TB in patients from industrialized countries with CAP, cause a delay in diagnosis of TB. A high index of suspicion for TB should be maintained in any patient with cough for >3 weeks, anorexia, night sweats, unexplained weight loss, or hemoptysis (104). It has been shown that physician experience with TB positively influenced the survival of patients (105). Constitutional symptoms may be the only presenting feature of PTB.

The insidious onset of cough, fatigue and other symptoms should suggest TB rather than “common” CAP (103). Duration of cough at the time of diagnosis was more than 1 month in 70 % of smear-positive cases from Kolin , Czechoslovakia (106).

Hemoptysis and weight loss are more common in TB than in patients with CAP. TB should be suspected in the presence of prominent radiographic abnormalities and, at the same time, in the absence of dyspnea. Serious hypoxemia due to PTB is uncommon and seen in cases of pneumothorax or advanced cavitary disease. A

substantial proportion of patients with PTB seek medical care due to non-pulmonary symptoms but when specifically questioned, only a small minority did not have cough and expectorates (103).

Physical examination is no longer considered as an important diagnostic tool for PTB and certain findings can be attributed to other chronic diseases and physiological changes related to aging.

Primary TB infection typically occurs in the lower lobes after inhalation, followed by bacteremia, which explains the presence of *Mtb* bacilli in nearly any organ. Primary infection causes clinical pneumonia in 5--10% of adults and in an even higher percentage of children and HIV-infected individuals. A lobar or segmental infiltrate, characteristically with ipsilateral hilar adenopathy, sometimes leading to compression of the bronchus, may be seen on chest radiographs (104). Primary TB may progress in a few patients, resulting in pleurisy, extensive pneumonia, and even disseminated disease. Miliary involvement at onset occurs in less than 3% of cases, most commonly in children under 3 years of age. The chest radiograph of HIV-infected immunosuppressed individuals most commonly resembles those with primary disease (14).

Secondary TB is referred to as reactivation disease and is responsible for 90% of TB in patients not infected with HIV. The chest radiograph typically shows fibronodular changes in the upper lobes, cavity formation, or volume loss. The cavities in TB usually lack an air-fluid level. Computer tomography (CT) may be helpful in visualizing a difference between active and inactive TB but cannot be used to exclude activity (107).

Immunocompromised patients with pulmonary TB may show a varied pattern of abnormality in chest radiographs. In HIV infection, atypical presentation is seen at CD4 counts of less than 200 mm³ such as hilar lymphadenopathy, pleural effusions, fuzzy infiltrate (81). In a study from New York, 14% of patients with coinfection had a normal X-ray, and this rose to 21% when patients with low CD4 counts were considered (108). Subclinical TB with negative sputum smears and CXR but positive findings on a sputum culture is seen in approximately 10 % of HIV positives in TB endemic countries (81).

Elderly patients with TB are more likely to have lower lung lesions on chest radiographs as they age and are less likely to have cavitory disease. In a meta-analysis comparing features of pulmonary TB in older and younger patients, both groups had

similar prevalence of cough and sputum production, but older patients were less likely to have fever, sweating, hemoptysis, and a positive TST (109).

1.1.8.3 Smear-negative PTB

Studies prior to the HIV epidemic estimated that there were 1.22 cases of smear-negative and EPTB for each smear-positive case. In general, smear-negative cases had a lower mortality but approximately 50 – 70 % of cases with smear-negative PTB progressed, and a substantial proportion became smear-positive (110). This form of PTB is more common among children and in the elderly. Molecular-epidemiologic studies have shown that, whereas overall less infectious, 13% of secondary cases has been a result of transmission from smear-negative TB index cases (111).

1.1.8.4 Extrapulmonary TB

EPTB is seen in approximately at least 20% of immunocompetent individuals but is far more common in HIV-positives (up to 70% in some studies). The frequency of EPTB, mycobacteremia and “atypical” radiographic findings increases with low CD4 count. The most common sites of EPTB are peripheral lymph nodes, the pleura, the bones and joints, the genitourinary system, the abdomen, and the central nervous system (CNS). The term *miliary* usually describes any progressive disseminated hematogenous TB. Originally, “military” was “applied to the appearance of the numerous small, gray or grayish-red tuberculous nodules about the size of a millet-seed and approximately at the same degree of development in an organ, especially the lung.” Besides the lungs, these nodules are distributed fairly regularly in liver, spleen, kidney, brain, bone-marrow, etc (37). There is usually no prior history of TB, and the onset is often subtle. Typical chest radiographic findings may not be seen until late in the course of the disease (14).

1.2 DIAGNOSIS OF TUBERCULOSIS

Physical examination usually underestimates the extent of the illness and adds little to the evaluation of TB patients because the classical pulmonary sounds are frequently absent. Occasionally, an amphoral sound can be heard over a large cavity. Even when symptoms and signs are present, they are not specific for TB. Routine laboratory examinations are rarely helpful in suggesting diagnosis. Therefore, sputum microscopy and chest radiography still remain the most useful diagnostic tools for initial diagnosis (112).

1.2.1 Smear microscopy

All patients suspected of having PTB should have at least two sputum samples collected for examination using light microscopy (LM), preferably in the early morning. The patient may also submit three specimens collected at home on consecutive mornings. If compliance is doubtful, the patient should be admitted to an airborne infection isolation room to obtain samples (23).

The sensitivity of microscopy on sputum in HIV-negative individuals with culture-confirmed TB is about 60-70 % (113) but may vary considerably: 34- 80 % (114). Microscopy is dependent on production of good quality sputum (purulent bronchial expectoration) with the highest catch in patients with cavitory disease, and the lowest in those with feeble cough or minimal disease (115, 116).

Most of the advantage in testing serial sputum specimens is gained with the second specimen collected. Examination of a third specimen provides a small increase in sensitivity (2--5%). Therefore, reducing the recommended number of specimens examined from three to two (particularly to two specimens collected on the same day) could probably benefit TB control programs (117, 118). Performance of smear microscopy can be optimized by applying sputum processing with bleach, sodium hypochlorite or by use of overnight sedimentation and fluorescence microscopy (119-121).

Front-loaded microscopy is a relatively new diagnostic approach in which two smears are made from one or more sputum specimens obtained on the first day a patient is evaluated. Many patients do not provide all specimens needed for sputum examination or to return because standard sputum collection requires 2-3 clinic visits. In a systematic review and meta-analysis same-day sputum smear microscopy was shown to be as accurate as standard methods, for example 3 sputum specimens collected on 2 consecutive days (122). In a study from Uganda, microscopic examination of 2 smears prepared from a single sputum specimen was as sensitive as standard two-specimen microscopy and that fluorescence microscopy (FM) using a light-emitting diode (LED) light source was more sensitive than conventional LM. The authors suggest that these strategies could both increase TB case detection rates and decrease the burden on patients and providers in low-income countries (123). In a study of adults with cough \geq 2 wk from 4 high-incidence countries, LED-FM had higher sensitivity but lower

specificity than LM for diagnosis of PTB (124). LM examination of two sputum specimens collected on the spot the first day of consultation, followed by the examination of a third specimen collected the following morning has similar sensitivity as the examination of one sputum specimen collected on the spot the first day of consultation, followed by the examination of a second specimen collected the following morning and a third specimen collected on the spot when the patient brings the morning specimen to the health center (125, 126).

Though sensitivity of microscopy can be increased by using these methods (116), positivity rates for microscopy smears and for cultures are first and foremost reliant on background TB prevalence rather than method of measurement (115). Quality assurance of smear microscopy is an imperative activity, especially in resource-poor settings, where it is still the main laboratory technique used for the diagnosis of TB (127).

1.2.2 Induced sputum, gastric lavage and bronchoscopy

If the patient is unable to produce sputum, then sputum induction with saline should be attempted in a room with negative pressure ventilation (128). According to Olsen, published evidence suggests that induced sputum (IS) is a less disturbing and more cost-effective method of diagnosis of TB than bronchoscopy. Despite published evidence recommending IS collection before bronchoscopy in suspected PTB patients, bronchoscopy is often preferred, where it is easily available, over IS. This practice is less cost effective and exposes patients and health care workers to greater risk (129). The use of IS samples with molecular tests seems to have promising future, especially in children (115). A meta-analysis of diagnostic yield of IS showed that this method will detect approximately three-quarters of *Mtb* culture-positive cases, at least under study conditions (130).

Another effective technique is the early morning gastric aspiration (GLA) of 50 mL of fluid after an 8 hour fast. A prospective study from Zambia recently demonstrated that analyses of GLA samples with the GeneXpert(®) MTB/RIF (Cepheid, Sunnyvale, CA, USA), a molecular biological assay for the detection of TB and MDR-TB, was a sensitive and specific method (131).

In most cases bronchoscopy for alveolar lavage (BAL) is not required but it may be appropriate when sputum smears are negative in the case of a known radiographic

lesion. IS and BAL can be used together, and combining GLA and BAL could also increase culture positivity in suspected PTB patients with negative sputum smears (132). Collection of post-bronchoscopy sputum can be another valuable source of diagnostic material (133). Thomas suggests that chest physiotherapy, instead of bronchoscopy, could possibly be used to increase the diagnostic yield of expectorated sputum samples. This approach is less invasive and costs less and has already been studied in the HIV population (134). Simple instructions can increase sputum smear yield (135). Providing such instructions may be particularly important in females (136, 137).

1.2.3 Culture and drug sensitivity testing

Culture remains the cornerstone of the diagnosis of TB. For detection of mycobacteria by culture, the current gold standard consists of a combination of solid and liquid media. In addition to protracted procedure of culturing *Mtb*, which usually takes 4-8 week, drug susceptibility testing (DST) takes another 2-3 week to accomplish (138). The new culture concepts are based on broth-based liquid media, capable of detecting as few as 10-1000 bacilli, and higher sensitivity of this method (BACTEC MGIT 960) compared to solid media (BD, Franklin Lakes, NJ, USA) has been reported (138). Another important advantage of liquid culture method is that it reduces the time for getting DST results, roughly by 10 days, which can be important for clinicians (138). The use of solid culture medium is, however, still more cost-effective in poor countries according to recent review by Zumla, et al (81). Also, risk for contamination by other bacteria in the specimen is alleged to be higher when liquid culture systems are utilized (138).

DST testing is recommended for all initial *Mtb* isolates because of the great significance and possible severity of undetected drug resistance for the patient, the health care workers and the society. DST can be performed on both solid- and liquid-culture based methods or by use of DNA-based techniques but the proportion method on solid media remains the reference standard. This involves inoculating one or more dilutions of cultured mycobacteria on drug-free media and on media containing antimycobacterial agents (32). The proportion method permits quantification of the proportion of drug resistant bacilli in the clinical isolate by the comparison of the number of bacterial colonies growing on the drug free control and drug containing media. In the 1960s, the criteria for defining clinically relevant drug resistance were set

from clinical and bacteriological studies. Consensus for a cut-off of 1% was decided. This is the proportion of resistant bacteria above which therapeutic success had been demonstrated to be unlikely (139, 140). Therefore phenotypic drug-susceptibility testing (DST) aims to determine if 1% or more of the bacterial population in clinical specimens is drug resistant.

Therefore, drug resistance is generally considered to be present when the growth on the drug-containing medium is more than 1% of the growth in a drug free media (the control growth) (141). At present, drug susceptibility testing by the proportion method in Sweden and other high-income countries is mostly done in the broth-based BACTEC MGIT 960 system.

About 15% of cases of active TB in industrialized countries are culture-negative. A clinical response to multidrug therapy over a course of 1-3 months is often sufficient to make a diagnosis of TB in the face of negative cultures (112). Histopathological examination of biopsy samples can be a supplement for diagnosis of culture-negative TB but plays a much more important role in detection of EPTB.

1.2.3.1 Molecular diagnosis of TB and drug-resistance

Nucleic acid amplification NAA by PCR is applied to confirm the identity of a smear-positive or culture-positive sample, preferably from a respiratory specimen, and for sequence-based detection of drug-resistant mutations. The rapid techniques (≤ 24 h) of polymerase chain reaction (PCR) are standard methods for detecting *Mtb* directly in various clinical specimens. In a study from New York, for 158 patients whose specimens tested negative for AFB on smear, the NAA test had a sensitivity of 79.3%, a specificity of 80.3%, respectively (142).

False positive PCR results, usually due to contamination, may occur and a cautious interpretation of a single, PCR positive and smear negative sample have been suggested (32).

Certain mutations are associated with acquiring drug resistance, for example more than 95 % of rifampicin-resistant isolates are in the region of the *rpoB* gene, while 70-80 % of isoniazid resistance is caused by mutations in the *katG* and *inhA* genes. The Xpert(®) MTB/RIF assay detects *Mtb* (+/- rifampicin resistance) within 2 hours, with overall pooled sensitivity of 90.4% and a pooled specificity of 98.4%. The pooled sensitivities for sputum smear-negative and smear-positive disease were 75.0 and 98.7%, respectively (143). The importance of this method for the high-endemic settings (where it has been mostly evaluated) can be illustrated by the notion that in the resource

poor settings only 10 % of MDR- and XDR-TB are diagnosed (81). The updated review on the diagnostic accuracy of Xpert(®) MTB/RIF in different subgroups has recently been published by the Cochrane Library (144).

A PCR-based, hybridization assay the Genotype MTBDRplus (Hain Lifescience, GmbH, Germany), is labeled for use on isolates from solid and liquid culture as well as directly on smear-positive pulmonary specimens. This assay is simultaneously detecting *Mtb* complex and specific mutations in the *rpoB* gene conferring rifampicin resistance, and in the *katG* gene conferring high-level isoniazid resistance as well as those in the *inhA* gene conferring low-level isoniazid resistance. This probe assay has been approved by WHO and is one of the most widely used, also in Karolinska hospital (145-147).

There are also commercially available assays that are able to rapidly detect resistance against fluoroquinolones, aminoglycosides and ethambutol, second line drugs utilized in treatment of MDR-TB (148).

1.3 TREATMENT OF ACTIVE DISEASE

The combination of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB) for 2 months, followed by INH and RIF for 4 months, is a conventional course of therapy for almost all forms of TB without known drug-resistance (81, 149, 150). Before effective drugs were available, 50% of patients with active pulmonary TB died within 2 years, but with the introduction of modern therapy almost all patients without MDR resistance can be cured(151).

Intermittent administration of treatment, preferably three times a week, enables supervision to be provided more efficiently and economically with no reduction in efficacy (150).

INH kills dividing bacteria, while PZA kills dormant bacteria. RIF is bactericidal against actively replicating MTB but is also active against intracellular slowly replicating bacilli and somewhat active against nearly dormant organisms in necrotic foci (152). Chemotherapy has the greatest impact on the duration of infectiousness since it shortens the period of transmissibility to at most around 2 weeks for patients with drug-sensitive TB who take their medications (153).

Treatment of TB in special clinical situations, for example in the presence of liver disease (154), pregnancy (155), and HIV infection (156), may require the modification of the standard regimen. HIV coinfection has little demonstrable impact on outcome,

leaving aside non-TB-related deaths during treatment, but likely accounts for occasional early TB-related deaths and late recurrences (156, 157).

1.4 BCG

Approximately 100,000,000 children receive BCG vaccine annually throughout the world today. International expertise recommends only a single dose of BCG, given at earliest the contact with the health service. The protection imparted by BCG appears to be high against childhood TB meningitis and military disease (158, 159). A conclusion about average protection seems problematic since the observed range in protection is very wide (0--80%). On average, BCG vaccine reduced the risk of TB by 50% according to one meta-analysis (160). The practice of giving repeated doses of BCG, used for example in former socialist states, lacks scientific verification (161).

1.5 LATENT INFECTION

1.5.1 Tuberculin skin test and interferon-gamma release assays

Positive results of TST and IGRAs indicate an immunologic response towards mycobacterial antigens that cannot automatically be interpreted as existence of live *Mtb* (162). TST is primary an epidemiological tool and not a diagnostic test for active TB (163). Both TST and IGRAs have little use in the evaluation of reactivation TB as these tests do not differentiate between LTBI and active TB.

Approximately 10-25 % of patients with active TB do not react to TST (163, 164). In a large study from Peru, negative reaction to TST, detected in almost 43 % of children upon admission, was highly predictive of death among children with active TB (165).

The authors have suggested that a negative TST result may be a substitute for the compromised immunity resulting from malnutrition, while severe protein-energy malnutrition has been reported as an independent predictor of TB mortality.

The sensitivity and specificity of TST depends on the cut-off used. A higher sensitivity is reached with a lower cut-off and a higher specificity with a higher cut-off. In close contacts or patients suspected of active TB, reactions less than 5 mm indicate lower probability of active or inactive disease, but above that edge, size of TST was not more likely to indicate active disease (166).

In other studies, the increased TST reactivity was associated with a bigger risk of TB, but the magnitude of the association between TST reactivity and the risk of TB varied substantially (167).

The usefulness of the TST as a screening method for LTBI depends on the age of the patient and the incidence of TB in the country of origin. The sensitivity of TST is limited in individuals with deficient cell-mediated immunity (162). The effect of BCG vaccine given once in infancy on results of TST is regarded as minimal. BCG vaccine received after infancy, in particular after the age of 2, frequently induces more persistent and larger TST reactions (168). A case-control study from Guinea-Bissau showed that risk factors for a positive TST are closely related to TB exposure. Having a BCG scar did not increase the risk of positive skin test in unexposed individuals (169). Nontuberculous mycobacteria (NTM) are an additional important cause of false-positive TSTs in populations with a high prevalence of NTM sensitization and a very low prevalence of TB infection (168, 170). Also errors in placement and reading of the TST can also yield false-positive results (171).

Neither TST nor IGRAs distinguish between recent and remote infections. Thus, a case of LTBI detected in a contact investigation may result from remote exposure years earlier with low risk of progression to active TB disease. CDC recommends that a positive TST in contacts of persons with infectious TB should be interpreted as evidence of recent infection with *Mtb*, independently of BCG vaccination and country of origin (172).

The two commercially available IFN- γ release assays (IGRAs), differ mainly in the technique for detecting responses by T cells to MTB. QuantiFERON-TB Gold (QFT-G) from Cellestis uses enzyme-linked immunosorbent assay (ELISA), and T-SPOT.TB (Oxford Immunotec) uses enzyme-linked immunosorbent spot assay (ELISPOT) to detect the release of IFN- γ in blood from sensitized persons when it is incubated with the *Mtb* specific antigens ESAT-6 and CFP-10 (173). These proteins are absent in all BCG vaccine strains and in most environmental mycobacteria. The diagnostic specificity of IGRAs is thus higher than TST as they are not influenced by prior BCG vaccination (174).

A systematic review demonstrated unsatisfactory sensitivity of 69%–83% and specificity of 52%–61% for IGRAs in the diagnosis of active TB in low- and middle-income countries (175). There is a substantial range in the recommendations on IGRAs

for testing of LTBI, with four methods usually suggested: 1) a two-step approach with TST first, followed by IGRA either when the TST is negative (to increase sensitivity, mainly in immunocompromised individuals), or when the TST is positive (to increase specificity, mainly in BCG-vaccinated individuals); 2) either TST or IGRA, but not both; 3) IGRA and TST together (to increase sensitivity); and 4) IGRA only. However, these recommendations are not strictly evidence-based (176). The area of uncertainty concerning both IGRAs and TST lie in the test variability, both conversions and reversions can occur in the same patient (177).

The questions if IGRAs outmatch TST performance in the diagnosis of LTBI in TB contacts and HIV positive individuals have not yet been compellingly answered, but IGRAs are believed to be more cost-effective (178-182) In end-stage kidney disease, a positive IGRA result was more associated strongly with radiologic evidence of past TB and contact with active TB than a positive TST (183).

In a meta-analysis, commercial IGRAs had a somewhat higher positive and negative predicting value for progression to active TB compared with the TST in persons recently infected by *Mtb* (184). Still, the relationship between an initial IGRAs test result and the future development of TB disease has not yet been studied in depth. For example, a study from Holland demonstrated comparable predictive values of QFT-GIT, T-SPOT.TB and TST for progression to TB disease among immigrant close contacts (185).

1.5.2 Intention to test is intention to treat!

LTBI is defined as an asymptomatic state of continual response of specific *Mtb* oriented T cells as assessed by TST and IGRA. In practice, this response is interpreted as presence of live *Mtb*. Therefore positive TST or IGRA are regarded as indicators of a risk for TB and necessity of a preventive treatment. The primary objective of testing for LTBI is to provide preventive therapy to persons identified at a risk of TB (162, 173).

In low-incidence settings: screening is recommended for individuals from high-incidence countries, contacts to infectious TB cases and or those with a high probability of progression (186). In high-endemic setting, the preventive treatment should be primarily considered for young household contacts to infectious cases and HIV positives (187, 188).

The presently suggested favored regimen is 9 months daily self-administrated INH, has efficacy of more than 90 % if taken properly. Yet 12 months of INH have the strongest evidence of efficacy (189-192), followed by 3 months of Rif plus INH (193) or 4 months of rifampicin (194).

Hepatotoxicity of INH is its most common adverse effect, posing one of the limitations of its effectiveness (195). Other, more frequent obstacles are low rates of treatment acceptance, among both clinicians and patients, and completion (196-198).

The WHO is currently recommending INH given for at least 6 months and ideally 9 months (187). The latter should be given to persons with verified contact with an index case with proven resistance to INH (173, 199). Recently, a randomized control trial showed that a DOT with a combination of rifapentine plus INH, given for 3 months, was as effective as self-administrated treatment (SAT) with 9 months INH (200). Prior to treatment of LTBI, a careful evaluation to rule out active TB is compulsory; if not the patient may develop drug-resistant strains and disease progression (187).

There is a much lower threshold of accepting adverse drug events during the treatment of LTBI compared to treatment of active TB. Patients have to be educated about symptoms consistent with hepatotoxicity (nausea, abdominal pain, jaundice etc.) and should be monitored baseline and monthly by testing for liver enzymes and clinical evaluation. This is particularly important in pregnancy, alcoholics, HIV-infected and in the elderly (201).

1.6 MOLECULAR EPIDEMIOLOGY AND TB CONTROL

The technique of restriction-fragment-length polymorphism (RFLP) analysis of the distribution of the insertion sequence IS6110 has been a standard approach to genotyping *Mtb* since the beginning of 1990s (202). It was fortunate that DNA typing was more or less available already in 1989-1992, when the number of TB cases increased in the US. RFLP played a significant role, when scientists and public health officials strengthened TB research and control programs. The data provided by *Mtb* genotyping showed that active TB in susceptible groups of HIV positives and homeless developed within few months after infection, thus confirming that immunosuppression can change the natural history of TB disease, highlighting the need to recognize these individuals prior to TB activation (203).

Pioneering work in the 1980s demonstrated that repetitive DNA sequences display species specificity (204-207). Thierry and coworkers described IS6110 and suggested its use for genotyping of *Mtb* (208, 209). This development was possible thanks to advance of the technique for radiolabeling DNA restriction endonuclease fragments in the early 1980 (210), and studies on how sequences in the DNA fragments could be separated by gel electrophoresis in the 1970 (211).

The RFLP technique involves cutting DNA by a restriction enzyme. The resulting DNA fragments are then separated by length by means of a gel electrophoresis and transferred to a membrane via the Southern blot process. Hybridized DNA fragment can be displayed as a specific band pattern on a gel surface. This pattern is then passed on to a radiograph which is analyzed by software (212). Numerous copies of IS6110 are present in most *Mtb* isolates, and at highly variable positions, making RFLP one of the most discriminatory genotyping methods. The patterns mostly remain stable over time, with an estimated half-life between 3 and 8 years and can therefore be used for epidemiological purposes. Unfortunately, RFLP is a slow, complicated and time-consuming technique and it also has a low discriminatory power in isolates with less than 6 copies of IS6110 (213). In the latter case RFLP analysis is usually supplemented with a secondary typing method such as a spacer oligonucleotide typing (spoligotyping). This is a PCR-based, rapid technique that investigates the DNA polymorphism observed in spacer sequences present within the direct-repeat (DR) region of the *Mtb* genome (214). There is both an RFLP database and an international spoligotyping database which provides useful information on the overall multiplicity of *Mtb* strain patterns, with potential clues to the prevalence of endemic versus ubiquitous strains, and more theoretically to the historical versus recent transmission of *Mtb* between different geographic locations (215).

A newer PCR-based genotyping method is the MIRU-VNTR (mycobacterial interspersed repetitive unit - variable number of tandem repeat analysis) which has promptly become the golden standard for molecular typing and has replaced RFLP in many laboratories (216).

Typing methods that are utilized at present, do not have perfect sensitivity and specificity. Therefore, estimates of recent transmission that account for genetic heterogeneity and fingerprint pattern change rate need to be developed. Studies comparing the results of RFLP and whole-genome sequencing (WGS) have shown that isolates exhibiting identical DNA fingerprinting patterns can possess substantial

genomic diversity. Because this heterogeneity is not apprehended by traditional genotyping, some aspects of the transmission dynamics could be missed or misinterpreted (217). WGS has been used for identifying transmission events that were genetically indistinguishable by RFLP or MIRU-VNTR typing (218-220).

1.6.1 Transmission in low-incidence countries

Most of the published molecular epidemiological studies have been employing RFLP. When interpreting molecular epidemiological data it is important to remember that the genome of *Mtb* and other species are continuously evolving. Identical isolates, with regard to RFLP patterns, are called clustered and are thought to represent the same *Mtb* strain. Two cases yielding the same strain type, and hence part of the same cluster, are usually considered likely to be directly 'linked' in the following sense: either one case or another is the "descendant" (221).

Three sources for the bias influencing estimates of the proportion of transmission have been suggested: 1) the reported cases do not represent a random sample of all active TB cases in the society, 2) the cases with identified genotypes are not necessarily a random sample of the reported cases, 3) frequency distribution bias (221), e.g. a predominant group of strains which is common in Southern Africa but is seldom isolated in other parts of the world, doesn't automatically indicated recent transmission if found in Cape Town patients (222). Sometimes, isolates from patients infected with epidemiologically unrelated strains may have an identical RFLP patterns, because they share a common "ancestor", which is more common for the communities with limited genetic diversity (223).

Still, most of the clustering is regarded as a result of recent transmission indicating reactivation within two years after the infection was established. Reactivation of a remote infection, often from a distant past, often the dominating cause of TB in low-endemic settings, is a result of *Mtb* infection with unique, non-matching RFLP patterns (224). Due to different methodological problems (bias, variance etc.) even a complete coverage of genotyping, will not be able to provide a complete picture of the actual transmission network (221). Epidemiological studies that include relatively few samples or when many isolates are collected during relatively short time period tend to underestimate the extent of recent transmission. Due to the phenomenon of latency, it is

difficult to predict how many *Mtb* strains will cause clinical disease after the study is completed, and how many strains existed before the study was initiated (225).

By determining the relative proportion of clustered TB cases in a community, an estimation of the efficacy of a local TB control policy can be attained (224). In high-endemic communities, especially those with high prevalence of HIV as in South Africa, the majority of new cases are due to recent (ongoing) transmission: 72 % of cases in Cape Town area were clustered and 58 % of those were due to ongoing transmission (226). For example, in a study by Marais et al. no significant association between the *Mtb* genotype and transmissibility within the household was demonstrated, probably because of unidentified exposure outside the domestic environment (227).

RFLP studies in the early 1990s revealed that a surprisingly high proportion of cases was a result of a casual contact with an infectious TB case, frequently outside the traditional household.

In a case of relapse, RFLP helps to distinguish between exogenous reinfection and endogenous reactivation. South African HIV-infected gold miners, particularly those who were more immunosuppressed, were at higher risk of TB recurrence, which in many cases was a result of newly acquired infection and not a relapse (228).

1.6.2 Risk factors for clustering

Risk factors for being involved in ongoing transmission events have been studied in a number of studies combining DNA typing with a collection of sociodemographic patients' data. Age, ethnicity, socioeconomic situation, HIV status have all been associated with transmission in predominantly urban areas. In San Francisco, TB patients younger than 60 years, of African American or Hispanic ethnicity, and those who were HIV-seropositive were more likely to have been recently infected (229, 230). An outbreak study showed that visiting a bar and not alcohol use was a risk factor for *Mtb* transmission in this setting (231, 232). The proportion of clustered cases depends on study duration and it has been suggested that the proportion of clustering increases over time and reaches a plateau at 3-4 years (225, 233).

A systematic review found a large variation in clustering proportions (7-72%) among 36 studies in 17 countries (234). TB incidence rate, mean cluster size, and CoTr were found to be significantly positively associated with clustering. In 17 studies the median

proportion of cases identified through CoTr was 26% (0-87 %;). Lengths of the studies were associated with higher clustering, but this association disappeared after adjusting for other factors. Pooled estimates of ORs were obtained using different studies from areas with low (< 25/100 000) and high/intermediate incidence of TB ($\geq 25/100\ 000$) that analyzed risk factors for clustering (**Table 1**).

Table 1: Risk factors for clustering (ORs)

	Low-incidence areas ($< 25/100\ 000$)	High/intermediate-incidence areas ($\geq 25/100\ 000$)
Male sex	1.4	1.1
Local birth	2.9	1.8
Pulmonary TB	1.5	1.6
Smear-positive	1.3	1.4
HIV-seropositive	1.4	1.4
Alcohol abuse	2.6	1.4
Injection drug use	4.0	1.9
Homelessness	3.6	2.0

Another review and meta-analysis of TB clustering, excluded the variables homelessness and alcohol abuse from the analyses due to data insufficiency (235). According to Houben and Glynn (235), 28% of the variation between studies was explained by study design including duration, sampling fraction, proportion of strains with low band numbers unless secondary typing methods were applied and the local TB incidence. The proportion of clustered increased with increasing length of study, sampling fraction and TB incidence and decreased with increasing patient age and proportion of immigrants in industrialized countries. However, it changed little with increasing study size. Only one of 21 studies included in the meta-analysis demonstrated a positive association between proportions of foreign-born individuals and clustering (236). These findings were tested in a follow-up study. Country of origin (Somalia, Peru and Senegal) and city of residence were risk factors for clustering (237). According to Houben and Glynn, lower than expected clustering, as e.g. in Japan could

reflect TB reactivation in an old population while low proportion of clustering in Bangladesh was probably due to undersampling since only a small fraction of notified cases in the studied area was confirmed by culture (235).

1.6.3 Genotyping studies and TB control

Most studies have used genotyping data, retrospectively because of the long delay, usually measured in months between actual transmission' events and data analysis. Therefore, the direct influence of the method on TB control activities is hard to estimate. The major impact of DNA typing results on TB control has been identification of cross-contamination in the laboratories and unknown sites of transmission in the communities (213). Genotyping also confirms the need to prioritize immunocompromised contacts for prompt assessment (238).

All-encompassing genotyping of isolates in five areas in the US, where conventional CoTr was executed, revealed additional epidemiological linkages in 38 % -- 57%, undisclosed by conventional CoTr (239). In addition, genotyping can be used to evaluate the performance of a TB control programme. A decreasing proportion of clustered cases can for example be indicative of efficient measures to reduce transmission. An excellent guideline on application of genotyping to TB control has been published by the CDC (240). Molecular genotyping is one method that can be used to improve contact and outbreak investigations. The development of real-time molecular genotyping techniques will further improve our ability to investigate outbreaks and enable profounder understanding of TB epidemiology, leading to more costs-effective strategy (238). During 2008-2010, a total of 23,108 TB cases in the US had at least one genotyped isolate; 7,942 (34 %) were part of 2,184 county-based genotype clusters. Of these clusters, 1,679 (77%) clusters consisted of two or three cases, compared with 100 (5%) clusters with ≥ 10 cases. Only 17% of the 2,184 genotype clusters had geospatial concentrations indicating a potential outbreak. CDC's conclusion is that increases in genotype surveillance coverage facilitates prompt outbreak detection (241). Combining genotyping with prompt and repeated interviews with index cases, the results of routine CoTr can be substantially improved (242).

1.6.3.1 Outbreak studies

Cases with indistinguishable genotypes that are close in place and time are regarded as part of a genotype clusters and might represent an outbreak (241). CDC states that

outbreak increases the urgency of investigations and places greater demands on the health department. Therefore, whenever possible, an alleged linkage between cases should be supported by genotyping results before escalating CoTr. A conventional epidemiologic investigation is necessary for defining probable transmission linkages, even if genotypes match (172).

The capability to discriminate between *Mtb* strains has allowed uncovering multiple outbreaks in different settings: hospitals (27), mines (243), prisons (244, 245), schools (246) and communities (247, 248). Many outbreak reports mention a prolonged period of infectiousness of the source case as a contributing factor for the expansion of the outbreak. This can be due to a delay in the diagnosis of TB which can be attributed either to patient's delay in initially seeking care or health-care delays in recognizing TB after the patients first contact with medical care, or a combination of both factors (249). In a review on outbreak investigations in the US in 2002-2008, 24 of the 27 total outbreaks involved primarily US-born patients. TB outbreaks among immigrants in the US were all associated with crowded environments and lack of access to medical care, whereas outbreaks involving mainly US-born persons were associated with substance abuse, homelessness and incarceration (250). A number of outbreaks with MDR strains have been described in foreign-born people in low prevalence countries (249, 251, 252).

Statistical techniques, when applied retrospectively to routinely collected TB data, can successfully detect outbreaks, earlier than local public health authorities become aware of the problem (253). Routinely reported data may identify small clusters that are likely to become outbreaks and which are therefore candidates for intensified contact investigations. Althomsons et al. showed that clusters, in which at least 1 of the first 3 patients reported homelessness, substance abuse or were in prison at the time of TB diagnosis, were most probable to become large (≥ 6 cases) (254). Independent predictors for the emergence of large clusters (≥ 5 cases) in Holland were less than 3 months' time between the diagnosis of the first two patients, one or both of these below 35 years of age, both patients living in an urban area and coming from sub-Saharan Africa (255). Most of the clusters are aborted in a short time period while others grow to contain more than 100 cases in 1 decade (256-259). Famous outbreak in town of Harlingen (Holland) encompassing 49 cases diagnosed between 1993 and 1996 and found through vigorous CoTr when in total 6519 persons were screened (260). This outbreak continued to grow to 104 cases in 2008 (261). Newly diagnosed cases in a

larger cluster have been shown to infect more people than the corresponding cases in smaller clusters, while INH resistance was associated with a reduced number of positive contacts (261). The authors' conclusion was that strain-specific factors influence spread of TB. Also another study shows that strains that were resistant to INH were less likely to result in secondary cases, than were drug-susceptible strains (67). However, a unique strain that caused several, often related outbreaks in Stockholm was INH resistant (257, 262). The size of clusters was not related to the duration of symptoms of most patients who belonged to clusters and there some correlation between being part of a small cluster and having HIV or being old (263). CDC suggests which cluster investigation should be prioritized (240). In Table 2 the 4 most prioritized types of clusters to perform cluster investigation are presented.

Table 2. Investigation of which cluster should be prioritized?

Priority (from high to low)	Type of cluster	Rationale for priority
1	Suspected false-positive culture	Need to determine which patients do not have TB and stop treatment
2	Cluster of three or more high-risk* patients with possible epidemiologic links	Need to confirm or exclude recent transmission in large clusters of high-risk* patients
3	Cluster of two high-risk* TB patients with possible epidemiologic links	Smaller clusters less likely to yield epidemiologic links, but presence of high-risk patients deserves attention
4	Cluster of three or more low-risk TB patients with possible epidemiologic links	Investigation of low-risk patients less urgent than high-risk* patients, but larger clusters may deserve attention

* "High risk" is defined as patients living in congregate settings (e.g., correctional institutions and nursing homes), persons infected with HIV or having other immunocompromising conditions, children, patients with cavities on chest radiographs or with MDR TB, and the homeless.

1.7 TB IN LOW-INCIDENCE COUNTRIES

Low TB incidence countries are usually defined as those with TB case notifications less than 10-20 per 100.000 and in decline. Typical for these countries is a falling TB incidence in the native population, an increasing number of TB cases in the risk groups comprising of the foreign-born, HIV-positives, prisoners and the homeless (264). TB incidence in these risk groups often considerably exceeds the background TB incidence (265). The prospect of TB elimination in low-incidence countries has been broadly discussed (266, 267). TB elimination is considered to have been accomplished when there is less than 1 smear-positive case per 100.000 population and year. Treatment of LTBI is required for TB elimination since the pool of TB infection constitutes possible future TB cases. Provision of preventive treatment, primarily to contacts to smear-positive cases and immunocompromised individuals is prioritized (268). In addition to treatment of LTBI, scaling up interventions related to TB/HIV and MDR-TB is required if the WHO goal of TB elimination by year 2050 will be achieved (267).

When TB incidence subsides, a shift towards outbreak management of TB control takes place. Clustering of TB cases in high-risk groups and the absence of LTBI in the great majority of young people in low-incidence countries are necessary conditions for the occurrence of “microepidemics” (269). TB outbreaks in low-incidence settings are typically characterized by a single source of infection, diagnostic delay, and a congregated environment, which facilitates transmission (270).

1.7.1.1 The European perspective

Most European countries fulfill the criterion for low-incidence settings with several exceptions here listed according to decreasing rate: Romania with an incidence of 98/100.000, followed by Lithuania, Latvia, Bulgaria, Portugal, Estonia, Poland, Hungary, Spain and finally the UK with an incidence of 13.5/100.000. Cyprus and Sweden are the only low-incidence countries in Europe with increasing incidence trends mainly driven by cases of foreign origin (271). More than 70 000 cases of TB are reported yearly in EU/EEA countries, but declining about 4% per year. However, low-incidence countries continue to report an increasing TB incidence in the foreign-born. The proportion of foreign-born TB patients is highest in Norway and Sweden and there constitute more than 85 %. The proportions of bacteriologically confirmed cases (61%) and effectively treated cases (79%) remain disappointingly low. The import of MDR-TB from former USSR to low-incidence countries has taken place since the early

1990 (272). The proportion of MDR-TB cases in 2010 was 4.6%, slightly lower than 2009, but at the same time there was an increased number of cases with XDR-TB. MDR and XDR TB in Europe are especially prevalent in Romania and the Baltic states. National reference centers in five countries representing different settings of TB in Europe were surveyed with the purpose to evaluate the management of MDR and XDR TB. This survey revealed divergences from international standards of TB care in the following areas: no information available on patient outcomes, shortage of resources for respiratory isolation, inadequate bacteriological diagnosis, inadequate drug-regimen selection and treatment duration as well as lack of laboratory support (273).

The European Centre for Disease Prevention and Control (ECDC) outlines areas with a need of improvement: 1) case detection and treatment especially of MDR cases 2) enhancement of TB surveillance systems and collaboration between laboratory and reporting physicians 3) case-management of TB among migrants, prisoners, HIV positives, and marginalized people in inner cities such as drug users, and homeless people (271).

Active case finding (ACF) is a process of seeking methodically for cases of active TB and LTBI in high-risk groups known, or thought to be, at higher risk of TB, rather than waiting for people to become ill and present themselves for medical attention, so called passive case finding (274). ACF was widely implemented in industrialized countries during most of the twentieth century, with mass chest radiography being probably the most well-known method (275). The effectiveness of ACF among the homeless has been demonstrated in low-incidence countries, while benefits of immigrant screening, both on an individual and societal level are more difficult to confirm (265).

The *International Standards for Tuberculosis Care* (ISTC) guideline, revised in 2009, forms a basis for the EU TB framework (149). However, due to a need to adapt recommendations for the European context, EU published its own guideline in 2012. Some differences exist between the two documents such as the overpowering importance of DST, implementation of rapid testing for MDR-TB in risk populations etc (23).

1.7.2 Case-management of TB patients

According to WHO, there is a huge variability in the quality of TB care in the world (276). The patient-centered standards to ensure optimal diagnosis, treatment and prevention of TB have been recently published (23, 149). In this thesis I will

concentrate on three vital issues of case-management: health-care and patients' delay, adherence and treatment administration.

1.7.3 Problem of delay

Delayed diagnosis and treatment may aggravate the patient's condition, increase the odds of complications and facilitate transmission (277). Treatment delay has also been associated with increased mortality in patients with AIDS (278). High mortality-rate among TB patients (60% HIV positive) in Johannesburg in the first weeks of hospitalization was associated with delay in admission (279).

In low-incidence countries, the diagnosis and treatment of patients with pulmonary TB is often delayed because of atypical presentation (280) and physician lack of experience with TB (281). In a study from Canada, delayed diagnosis and other complications were more common in hospitals with few TB admissions, compared to hospitals with a high admission rate ($> 10/10.000$). In addition, institutional risk of TB transmission increased due to delayed diagnosis (282). Delay in TB diagnosis (≥ 90 days) among 54 US-born patients was associated with spread of TB infection to contacts (283). The authors' suggestion was that diagnostic delay in the index case could be used to identify contacts who are at increased risk of being infected. An increased patient and doctor delays in Dutch patients have been reported. Meanwhile, shorter delays for foreign-born could indicate greater awareness among physicians of TB being a problem among foreign-born persons but could also be consistent with active case finding in immigrants (270, 284).

In a report from California, there were few migrants who feared that seeking health care might lead to reporting to immigration authorities, but those who did fear reporting, were almost 4 times as likely to delay seeking care for more than 2 months, a period of time likely to result in increased disease transmission. The conclusion of this report was, that "any legislation that increases undocumented immigrants' fear that health care professionals will report them to immigration authorities may exacerbate the current TB epidemic (285).

A global TB situation assessment reported by the WHO suggested that delays in diagnosis were common. The delay was more common in the diagnostic work-up rather than in seeking care, although both circumstances often occur (149). Many factors have been linked with diagnostic delay such as HIV, chronic lung disease, negative sputum smears, EPTB, difficulties related to availability of health-care (long distance, rural

residence), drug abuse, initial visit to a private practitioner, poverty and female gender (286). However, despite facing greater stigma and difficulties, women notified by a rural DOTS programme in India were more likely than men to access health services and adhere to treatment (287).

Several studies have shown that health-care professionals, especially clinicians who work in the private sector, frequently diverge from internationally recommended care practices (288-290). Increasing the capacity of TB services and improving their cooperation with the general health-care may reduce both diagnostic and treatment delays (284). Patients receiving antibiotics prior to TB verification may experience a process-related delay in starting treatment, partly due to transient improvements of symptoms (291).

It has been suggested that educating people about TB symptoms and the importance of timely medical consultation would decrease patient delay (292, 293). It is not clear if education influences health-seeking behavior for example a media campaign in Hong Kong failed to attract noteworthy numbers of patients (294).

Studies from Kenya showed that a proportion of patients, identified through screening for cough \geq 1 month, decreased with increasing distance from their home to hospital/health-care facilities, probably due to patients' dissatisfaction with health-staff and weakness of primary-health care in the periphery (295). Availability of services and equality of access to care had a bigger impact on delay in obtaining treatment, than severity of illness (296). An innovating Japanese study has investigated if the financial crisis 2009 in Japan has had an influence on patients' delay. Higher number of cases among non-homeless persons in Osaka, were sputum smear positive, had respiratory symptoms and showed advanced disease in CXR than those in 2008, with a longer patients' delay. On the contrary, in health examination for homeless people, fewer cases of advanced pulmonary TB were found in 2009 than in 2008, with a shorter patients' delay (297).

1.7.4 Adherence

In TB patients, the problem of not following given recommendations was recognized as early as in the 1930s, approximately 40 % (25-80 %) of TB patients in the US who were leaving hospitals and sanatoria against doctors' advice. The problem was considered to

have serious implications such as increased mortality compared to patients whose discharge was approved and danger to the public health.

Irregular intake of PAS was very common in the early years of chemotherapy era, partially because PAS had an unpleasant taste and frequent side-effects such as nausea and diarrhea. For example, during the winter in TB hospital Uttran (Stockholm), yellow spots in the snow were visible, the result of throwing or spitting PAS through the windows (Elsa Tynell, personal communication). The problem of “non-adherence” concerned researchers already in early trials on PAS (1950) and a urine test to detect PAS was developed and used to monitor patients in secret (298, 299). According to Wallace Fox, one of the most prominent figures in the history of TB treatment, this problem was particularly common in France and to a much lesser extent in Britain! Moreover, 43 of 100 patients who had no side-effects of PAS did not comply with treatment (300). Fox reported problems with self-administration of therapy (SAT) in a study from Madras. Patients picked medicine supplies of PAS and INH once a week for at least one year. The drugs were taken at home in tasteless cachets twice a day and patients were visited at home once weekly during the first 1 or 2 months of treatment. The monitoring of drug intake was done through urine tests for PAS and by counting remaining cachets. Recurrent irregularity of intake was noticed in a minority of patients. Fox also noticed that there was no difficulty in getting a hospital group of 81 patients on the same treatment regimen as the outpatients, to take medicine regularly under direct observation. This was probably the birth of DOT.

Adherence can be defined as the extent of agreement between a person’s health-related behavior and recommendations from providers of health care. In the context of TB, this behavior primarily encompasses taking medications, coming in time to the scheduled visits and cooperating in CoTr by disclosing names of contacts. The basis of adherence is the functioning relationship between the patient and the health practitioner. To make this work, mutual expectations, the regimen and schedule should be negotiated and discussed. Garner et al. write that “if a large proportion of people do not complete treatment, then it is a health-care system that has failed” (301). Accurate assessment of adherence permits planning and evaluation of treatment. Obviously, a regimen’s efficacy cannot be validated if the medicine has not been taken properly (302). There is no golden standard for measuring adherence (198) and it has been shown that both patients and health-care providers tend to overestimate the extent to which patients follow recommendations. Patients who report problems with adherence are believed to

be more trustworthy than their counterparts who deny it (303). Estimates of patients' characteristics and personality traits have proven to be poor predictors of adherence behavior (304). Health practitioners may believe in their own ability to judge certain patients as more likely to be non-adherent but studies have shown that non-adherence is hard to predict (150).

Reviewing the report on successful TB programme in Bangladesh, Grange and Zumla wrote that "the success of the Bangladesh programme may well stem from a partnership of equals, with well-informed patients being guided (rather than commanded) by health workers selected from their own community" (305). WHO stresses that "patients need to be supported, not blamed" suggesting that focus should be moved from patient-related factors to health-care provider and system determinants. The importance of taking into account patients' preferences in relation to treatment is also emphasized. Systems of health-care delivery influence patients' behavior by directing length of visits, and by putting up schedules (302).

A Cochrane review demonstrated that educational or counseling interventions may improve completion of treatment for LTBI but found no trials that assessed the effect of these interventions on adherence to treatment for active TB (306). Another Cochrane review, only found some evidence in support of the use of material incentives to improve adherence to preventive therapy, among male drug users and prisoners in the US. Currently, it is not known if incentives can improve long-term adherence and completion of treatment for active TB (307).

1.7.5 DOTS

DOT was first implemented by the Tuberculosis Chemotherapy Centre in Madras and in Hong Kong, in the trials conducted by Fox and his associates from Medical Research Council programme (308). In the 1960s Poole and Stradling reported that it was their practice in wards in Hammersmith (W Fox used to work there too), London, to watch every TB patient take every prescribed dose (309).

Today, WHO recommends DOT as one of a variety of measures to endorse adherence. It is well known that defaulting from treatment is one of the most important problems in TB control (310). Starting in the mid-1990s, efforts to improve TB care and control intensified at national and international levels. WHO developed the DOTS strategy, comprising political commitment, passive case finding using sputum smear microscopy, a regular supply of first-line TB drugs, standard short-course

chemotherapy given under proper case-management conditions including DOT, and a system for recording and reporting case-detection and treatment outcome (311, 312). The key individuals contributing to the roll-out of DOTS were Annik Rouillon and Karel Styblo, both from the International Union Against Tuberculosis and Lung Disease (IUATLD) (3). Styblo introduced standard short-course therapy in low-income countries and created a prototype of the National Tuberculosis Programmes (NTP). The basis for DOTS was laid between 1978 -1991 in low-income, high incidence countries where nine NTPs operated successfully with the support from the Union. These programmes were highly effective, had very low rate of failures and acquired drug resistance. Low-income countries with relatively undeveloped infrastructures such as Tanzania, Malawi, Mozambique and Nicaragua reached above 85 % documented cure rates (3, 313, 314). In Nicaragua, war in 1983 - 1987 had an extensive impact on health services, and health economics (315), TB control was however very successful (316, 317). In a publication comparing the TB situation in New York and Managua, the Nicaraguan TB program cured nearly 80% of the patients enrolled in the mid-to late 1980s, whereas New York City's program rarely cured 50% and in some areas cured less than 15% (318). In 1992, TB programme in Alberta (Canada) was proud to achieve comparable cure rates as the Nicaraguan NTP (319). Another highly successful TB program, employed DOT throughout the course of treatment, was in refugee camps in Thailand (320).

Despite obvious success in some settings, the global TB situation worsened in the end of 1980 due to poverty, the concurrent HIV epidemic, political instability followed by upheaval of health care and infrastructures. In 1993, WHO declared that the upswing of TB around the world was a “global emergency”. In the same year, the World Bank suggested the use DALYs (disability-adjusted life-years) to measure the cost-effectiveness of a given health intervention. DALYs takes into account morbidity, mortality, and age to determine which health interventions to support. Consequently, short-course chemotherapy for TB was declared a highly cost-effective intervention and the WHO subsequently formed and endorsed the DOTS. This was launched as a central strategy for effective TB control in 1994 (68, 321, 322).

Global implementation of the DOTS reduced the anarchy that ruled in TB treatment during the early 1990s, (323). The appearance of MDR-TB in the 1990s was especially noticeable in countries with weak DOTS or without DOTS programmes

such as the former USSR. Subsequent reports revealed a growing geographical spread of MDR TB, by definition resistant to the two most important drugs used in DOTS, INH and rifampicin. The spread of MDR TB showed that “only very rarely does a one-size programme fit all” (3). Some experts argued against the institution of programmes against MDR-TB in low-income countries fearing that MDR would steal resources from the more widespread, pan-sensitive TB. In Ivanovo (Russian Federation), the percentage of MDR TB in previously untreated TB cases more than doubled after the implementation of DOTS, and only one patient out of 19 with MDR was cured with standard short-course regimen augmented with STR (324). It was suggested that a lot of smear-negative patients, declared “cured” by DOTS criteria, were in fact transiently suppressed. According to Farmer and Walton, “the driving force behind the pressure for a single treatment strategy [*DOTS*] that did not rely on laboratory [*culture and DST*] and radiographic data was its low cost” (325). The MDR-TB situation in Peru became important for the debate and decisions on treatment and management of MDR-TB in low-income countries, leading to implementation of DOTS-Plus. The latter relies on DST and the use of second-line drugs and is aimed at treating MDR TB. Experience from Peru suggests that MDR TB can be effectively treated under unfavorable field conditions in countries with poor resources (326)

In the end of 1990s, publications scrutinizing DOT started to emerge. Hill and co-workers reviewed reports describing the outcome of DOT in cohorts treated in 1990–2000 and showed that non-adherence remains the principal barrier to success, despite implementation of DOT. Though authors agreed that well-implemented DOT is superior to unsupervised pill-intake, they could not quantify its contribution to improvement in TB control (327). In 2002, WHO recommended flexibility in implementing DOTS (328). Thiam and co-workers (310) describe a variety of strategies intended for better treatment outcome, mostly by targeting treatment delivery. These strategies have been evaluated and showed variable success (329-331). A study from Senegal demonstrated that decentralization of treatment and other interventions led to a significant enhancement of treatment success compared with the usual DOTS procedures. In addition, the study found that the choice of a DOT supporter among the patients' family members provided better treatment outcome than other DOT supporters (310). In a current meta-analysis, DOT was not significantly better than SAT in preventing microbiologic failure, relapses or acquired drug resistance (332).

1.8 IMMIGRATION, TB CONTROL POLICIES AND STIGMATIZATION

The majority of TB cases in Western countries are diagnosed in foreign-born people whereas native TB is affecting mainly elderly individuals and groups of socially marginalized such as homeless and drug users, though the latter two are often a mix of native- and foreign born. Screening for LTBI and CoTr, followed by preventive treatment are necessary to reduce TB burden in these high risk groups (333). Four out of five TB cases among foreign-born persons in the US can be ascribed to reactivation TB (334). Only 25% of 2660 pediatric patients with TB diagnosed in the US 2008-2010 had no known international connection through family or residence history (335). This study indicates that 75 % of pediatric patients have potential TB exposures through foreign-born parents or residence outside the US. Non-immigrant visitors such as exchange students, travelers and temporary workers also contribute to the burden of foreign-born TB in the US (336). This situation shows that to achieve the goals of TB elimination in developed countries, global TB control should be improved and resources allocated to TB control programmes in low-income high-endemic countries. In this text the term “immigrants” encompass all foreign-born people, both legal and undocumented, including refugees, asylum seekers, migrant workers, students and tourists (333). The accurate fraction of undocumented immigrants among the foreign-born in industrialized countries is unknown. This marginalized people live in even larger poverty than their legal counterpart. They have limited access to treatment and difficult to trace due to contact investigation (100). In a study by Achkar et al., undocumented status was associated with an increased frequency of cough and hemoptysis and a longer duration of symptoms in 194 patients with PTB admitted to a large hospital in New York (337). An article (47), following the publication of the mentioned study, raises a difficult and much needed question about the prospect that presenting these results could contribute further to the stigmatization of the immigrants, and thus, worsen the problem identified in the previous study. However the authors’ conclusion is that “the importance of the knowledge obtained both for the health of the public and for the undocumented immigrants themselves, warrants wide dissemination of the results”.

TB rates among immigrants are highest in the first year after arrival. The reason for this might be a result of improved screening and access to health care in a host country and

might also be due to war, refugee conditions, for example living in refugee camp (333). These extreme conditions probably increases risk for reactivation of LTBI due to stress, hunger, other diseases, and also risk of being infected, with subsequent risk to reactivated within following year. Among the 46970 TB cases reported among foreign-born persons in the United States in 2001-2006, 28 % were found within 2 years of arrival. TB case rates dropped with increasing time since US entry, but continued to be higher than among US-born persons--even more than 20 years after arrival (338). This study showed evident differences in TB rate and drug resistance with regard to country of origin. In total, 53% of TB cases occurred among 22% of immigrants born in sub-Saharan Africa and Southeast Asia. INH resistance was approximately 20% among recent entrants from Vietnam and Peru.

Indigenous populations of low-prevalence countries demonstrate generally lower rates of drug resistance compared to immigrants often due inadequate TB drug supplies or ineffective TB control programs in low-income countries (333). In industrialized countries, MDR-TB is primarily seen in immigrants from former USSR and its allies (339-341).

Dahle and coworkers showed that despite 12 years of immigration to Norway from high-incidence countries, there had been little influence on transmission in the low-incidence country (342). A commonly raised concern regarding immigrants with TB is the risk they pose to the population of the host country (333). Though most TB patients in Switzerland are foreign born, but TB transmission is not more common comparing with Swiss-born patients (343). US-born cases generated more secondary cases than immigrants, according to a study from San-Francisco (344). To my knowledge, no significant TB transmission from immigrant to native population of host country has been demonstrated (345, 346), though a number of outbreaks where the source case was from high-incidence country have been reported (257, 269, 347). The most likely reason is a low degree of integration of immigrants with the indigenous population (345, 348). In Italy, dissimilarities in TB transmission were observed among immigrants in from different countries and even within national groups, where living conditions have been found to exercise an effect. Enhanced social integration of immigrants would probably limit risks of TB transmission in low-incidence countries (237). Increased transmission among immigrant groups in the Netherlands was largely attributable to the relatively young age of immigrant source cases (349).

In a recent meta-analysis of screening activities at entry, the yield for diagnosed active TB cases was low making cost-effectiveness and importance of the current screening strategies unclear. However, LTBI reactivation in immigrants plays a central role in shaping national TB incidence, though LTBI screening remains inadequate (350), and there is no international agreement on which immigrants to screen and how to screen (351, 352). It has been suggested that immigrant screening could cost-effectively and safely abolish compulsory CXR on arrival and instead screen for LTBI with IGRA (353).

A study of Waldorf and co-workers demonstrated high rates of non-adherence with existing guidelines on immunization and screening at primary care facilities in Boston. Only 43 % of immigrant patients were screened for TB, 36 % were screened for HIV and hepatitis B, and 33 % received tetanus vaccinations (354). In a study from Norway, only 30 persons out of 2.127 asylum seekers with TST \geq 6 mm were treated for LTBI. There was an apparent uncertainty among physicians about this condition, since only 41% of those seen with a TST \geq 15 mm were “diagnosed” with LTBI (350).

A study from Holland on the discriminatory value of TST to differentiate between immigrants at low and high risk of disease found that newly arrived immigrants with a positive TST result at entry were at considerable risk of progression to active TB, whereas for TST negatives (< 10 mm) this risk was limited. However, the discriminatory ability of the TST was somewhat lower than that of the QFT-GIT (355). While in another Dutch study, the predictive values of QFT-GIT, T-SPOT.TB and TST for progression to TB disease among immigrant close contacts were comparable (185).

Western Europeans carried TB to native populations that they traded with or colonized, from the 1700s and as late as in the 1950s, foreigners were still transmitting TB to the previously unexposed natives in Canada and Papua New Guinea (100). TB incidence was constantly falling through the 20th century in the Western Europe and other rich countries. Though relatively short periods of upswing have been recorded due to impact of World War I and II (356), and more recently in the US (1985-92) due to HIV epidemic, underfunding of public services, failures of healthcare and criminal justice system (357).

TB was almost unknown in sub-Saharan Africa in the beginning of 20th century. The incidence of TB increased dramatically in several sub-Saharan African countries in the early 1990s because of the HIV epidemic (333). This has coincided with growing numbers of African immigrants to Western countries, not least to the UK which has experienced an increase in TB notifications rates, especially in London (100). The political instability in the Horn of Africa also contributed to growing numbers of TB cases in the West, initially due to the war zones in Ethiopia and Eritrea (358), and thereafter being due to the large immigration from Somalia from the 1980s and ongoing (359-361).

Approximately 41-85 % of TB cases in industrialized countries are diagnosed in foreign-born (100). High rates of TB among immigrants from Mexico, Philippines, Vietnam and other countries have been reported since the 1970s in the US (100). Increases in immigration to Canada, Western Europe, New-Zeeland and Israel, roughly from 1990, have been also reflected in the national TB trends. According to Albert and Davies, Australia, known for its harsh approach towards immigrants “has managed to maintain low TB incidence rates and has not experienced the increases seen elsewhere in the world” (100). Bashford presents a less flattering account on the topic by stating that between 1901 and 2001 TB has been “the one disease for which entry to Australia is always denied” and that “not every national history has such a deep and explicit connection between racial exclusion, communicable diseases, and medico-legal border control” (362). TB control policies may also preserve health and social inequities. Common assumptions about minority populations, seldom questioned by anyone, are deeply rooted in health policies (363).

1.8.1.1 Past and present situation

Some of the earliest publications to be found on the PubMed when searching for “tuberculosis, immigrants” have been written by Canadians after the World War II (364). Many of the post-war immigrants were war and civilian prisoners of fascist and Nazis concentration camps where TB was thriving (365). Adamson describes risks of bringing TB with immigration to Canada: “The importation of infected people would be particularly dangerous to Canada for the following reasons. Presumably a large proportion of new settlers will be sent to the less settled parts of Canada -- the Prairie Provinces. In these areas the death rates from tuberculosis (excepting isolated Indian settlements) are among the lowest in the world, and the younger generation is growing

up quite unprotected by previous infection. The introduction of many foci of infection into such communities could have a devastating effect “(364). Adamson continues by telling us about 2,876 Polish soldiers who were brought to Canada in 1946 and who fought with the British since early in the war. They were sent to Canada without having had a CXR performed. Approximately 3 % of them were diagnosed with active TB soon after arrival. The report’s conclusion is that the history of Polish veterans suggests the need of both pre-arrival and post-arrival (annually for 2 or 3 years after arrival) examinations by radiogram, in order to prevent “an enormous amount of tuberculosis”...”among our own people” and to avoid great costs related to treatment. We are informed that “this problem has been studied by the Canadian Tuberculosis Association, and it has been recommended that all prospective immigrants have a chest radiogram before embarkation for Canada, and that those with active or doubtful lesions be rejected” (364).

The view that TB was imported by immigrants became prevailing in the 1950s and since that TB in immigrants, primarily from India, Pakistan and Ireland, has been a public health concern in Britain. Medical examination of migrants at the check posts of entry was considered to be the best approach to deal with the problem of increasing TB incidence.

In 1956 there was a recommendation from the municipal authorities that all migrants to Britain should be examined before departure and that those having TB, other infectious diseases, mental illness, and deficiency be barred from entering (366). Springett, an influential British TB expert wrote in 1964 that it was in some way fortunate that immigrants from high-endemic (Asian) countries in Birmingham had a little mixing with other resident groups (367). Also in his later report from Birmingham (1973), Springett was still preoccupied with chest examination in the port of entry and underlined the importance of BCG vaccination of children in “Asian households” but did not mention prevention through improved living conditions (368).

In 1964, a publication by Edgar prized the act temporally restricting immigration providing “breathing-space in which some of the problems could be reviewed and tackled” and “if immigration continues at this rate the problem will be almost insoluble”(369). The essential of the problem, according to Edgar is that Pakistani immigrants with active TB arrived to Bradford “without medical check” and that there was a mixture of TST-positive and –negative reactors ($\approx 15\%$), the latter constituting the susceptible group. TB incidence in Pakistan and India at that period was estimated

to about 2500 / 100.000. The annual rate of TB incidence in the Pakistani population of Bradford was calculated to be about 2 % or 30 times higher than in the local population. Edgar states that susceptibility of Pakistanis is aggravated by “their inadequate diet”, though “every facility for good meals being available” and the overcrowding. Yet, he stresses that reducing overcrowding is not a remedy. According to Edgar’s seemingly biased description, Pakistani work 5-day week and have “long hours of leisure” and “often crowd together to pass the time of day since inadequate social facilities exist”. The rooms where they gather together “in large numbers” often lacks in ventilation.

Welshman reproduces two citations, characteristic for that time, from the annual reports of Bradford Health Committee: “no case has yet come to light where a locally born resident acquired tuberculosis from an immigrant” (1970) and “the detection, or better, the prevention of tuberculosis occupies perhaps 99 percent of the time devoted, in general, to the health of immigrants” (1971). Such proclamations indicate the unaffected lack of interest of the realities of immigrant existence and broader health issues. Many of these studies did not have consistent data to draw reliable conclusions about the relative impact of social deprivation (366).

Besides the stress of migration, poverty contributes significantly to reactivation of LTBI. High TB rates in selected immigrant groups can be regarded as a consequence of marked poverty in this population rather than a reflection of the preexisting exposure to TB in the countries of origin. Regrettably, even today TB control policies do not acknowledge the role of poverty or other social determinants in the reactivation of TB. It is important to visualize the ideological assumptions about race, immigration, and social status which permeate the current TB policies within the immigrant population (363).

According to King, immigration has often been pointed out as a “cause” of TB which on one hand forced lawmakers to subsidize TB control activities in low-income countries but on the other hand fueled nationalistic and protectionist tendencies in Western societies (370). Focusing on border controls has its political reasons but it has also steered to blaming o migrants for social problems. In some cases there have been attempts to make physicians act as a border police. For example, the state of California passed an edict in 1994 that forced public health-care facilities to notify authorities if they suspected that a patient was an illegal immigrant (370). The Italian parliament

passed a bill in 2009 which forces medical staff to contact the police if they believe that the patient does not have a valid visa (The Telegraph, February 5, 2009). There have been reports that such policies have denied care of persons who had infectious diseases and discouraged immigrants of seeking medical care due to fear for being arrested and deported (370).

1.9 CONTACT TRACING

1.9.1 Basic principles

The first priority of TB control programs is diagnosis and treatment of all individuals with active TB. Contact tracing (CoTr) constitutes the second priority of TB prevention and control. When the number of active cases declines, the importance of CoTr, as a principal method to identify people with secondary TB and LTBI, increases. CoTr is an epidemiological investigation conducted in the proximity of a case of TB in order to identify individuals who are infected with *Mtb* (371). An individual who is believed to have transmitted infection to another person is referred to as the “index case” (IC). CoTr should be executed if the IC has TB localized in the upper airways, lungs or pleura (172, 173). In terms of CoTr, an infectious case is defined as either smear-positive in respiratory specimens or smear-negative, later confirmed by mycobacterial culture. If microscopy of three sputum smears does not reveal AFB, an investigation still is suggested if the CXR demonstrates cavities in the lung. However, small cavities that can be detected only by computerized imaging tomography scan are not included in this commendation (172).

In most cases, an IC constitutes the starting point of CoTr but sometimes CoTr is initiated from a contact with a primary TB or LTBI, so called “source-case investigation” which seeks the source of recent *Mtb* infection. This type of investigation has low efficiency but can be considered for children with active TB and less than 5 years. However, CoTr is not generally recommended around children less than 10 yrs because transmission is rare at that age unless a child has PTB similar to an adult (172, 372).

1.9.1.1 Active TB and LTBI in contacts

Most people seek medical attention when they become ill and therefore the goal of CoTr is mainly to identify and treat individuals with LTBI. However, about 1 % of

contacts have already progressed to active disease when CoTr is performed (373, 374). Contact studies in the UK showed that up to 10 % of new TB cases were diagnosed through CoTr and that TB was usually discovered on the first visit (274). In a meta-analysis, the prevalence of active TB in contacts in low and middle-income settings was 3.1 %, microbiologically proven TB 1.2 % and LTBI 51.5 %. The prevalence of active TB and LTBI in high-income settings was 1.4% and 28.1%, respectively (375). In a large-scale study (374), encompassing 52% of reported cases in the US 1999, in average 10 contacts were listed for each smear- and/or culture-positive TB case, while approximately 8 % of smear-positive cases had no contacts reported. Listing few or no contacts was associated with homelessness and an early death of the IC (374). In another study from the US, a median of 4 close contacts was found for a smear-positive case and 36% of contacts had a positive TST (376). In two studies 1 % of contacts had active TB and around 25 % had LTBI (374, 377).

1.9.1.2 Priority and infectiousness

The extent and priority of CoTr should ideally depend on infectiousness of the IC, degree and duration of contact exposure and the exposed contact's susceptibility to infection (172). All these factors should be taken into account when CoTr is organized and executed (173). The number of contacts and the intensity of exposure may be increased in relation to socializing habits of an IC (172). Loudon showed in studies during the 1950-60s that cough frequency and severity of disease are not prognostic of contagiousness while singing and cavitary TB is associated with transmissibility (378-380).

An innovative study by Jones-López and co-workers can be regarded as a complement to the classical studies of Loudon (381). Their study included 96 sputum culture-positive ICs and their 442 contacts, *Mtb* was cultured from cough aerosols using a cough aerosol sampling system (382). *Mtb* was isolated from 45% of aerosols from TB patients though 98 % of the cases were smear-positive. LTBI was significantly more frequent in contacts to TB patients who produced high mycobacterial density aerosols (≥ 10 colony forming units - CFU) compared to contacts from low density aerosol (1-9 CFU) and aerosol negative cases (69%, 25% and 30%, respectively). There was considerable variation in CFU among aerosol positive patients. Patients with high aerosols had median cough peak flow rates that were 60-80 L/min higher than patients with negative or low aerosols. A high density aerosol TB patient was the only

significant predictor of new *Mtb* infection. This study shows that smear-positive cases should not be regarded as homogeneously infectious (381).

1.9.2 Length and location of exposure

When the start of the infectious period cannot be defined accurately, 3 months before TB diagnosis is regarded as a starting point of the disease (172). In extraordinary cases the illness may last a year or longer.

Even staff with a broad experience in CoTr has different perceptions of the meaning of close and prolonged contact and size and ventilation of the exposure environment (383). The variations in definitions and types of data collected, limit the ability to analyze data on CoTr (373, 376). Essential factors such as determination of priority for contact investigation, characteristics of ICs associated with infectiveness, extent of exposure and contact' risks for reactivation of LTBI are often not recorded (373). It has been suggested that only a standard approach to CoTr has the potential to improve outcomes (373, 383).

However, none of these recommendations are based on robust evidence and therefore CDC does not provide cut-off points for duration of exposure when assigning priorities to contacts, (172). Instead, administratively determined durations derived from local experience are recommended, with frequent reevaluation on the basis of results. The shortest estimated exposure period in a contact with confirmed infection can be used as an approximate, which then will define high-priority non-household contacts and should apply only to the members of a particular outbreak. The degree of proximity to the IC is important with regard to transmission, primarily in large and/or efficiently ventilated locations, due to prompt diffusion of *Mtb* containing droplets with air currents. In narrow, poorly ventilated settings, air volume and circulation are crucial for transmission to occur (172).

1.9.3 More about contacts

High-priority contacts are 1) persons in close contact with the IC and at increased risk of developing TB following infection 2) other individuals in close contact or vulnerable persons with less than close contact. In most cases a high-priority label is applied to persons with prolonged exposure, but there are some exceptions: if exposure has taken

place in location/situation when high concentration of *Mtb* can be expected. It can be a poorly ventilated room or exposure during bronchoscopy, sputum induction or autopsy (173). CDC regards contacts who have been exposed for TB during a medical procedure as a high-priority group (172). Exposure locations can be graded by e.g. size of a car, bedroom and house. This classification improves quality of CoTr by providing more distinct information (172).

The use of different definitions to describe grade and nature of contact, which can be found in the literature, as “close”, “less-than-close”, “sporadic”, “casual”, “non-household” and “household” contact is tiresome for a reader. In addition also high- and low-priority contact (172, 173, 371, 384)! CDC advises against using the terms “close” and “casual” due to lack of a uniform definition (172). However, both the NICE guidelines and the Europeans consensus statement use these definitions (173, 274). “Close” contact is defined either as living together with IC or having regular and prolonged contact, or only sharing breathing space (if confined). For “other-than-close” contacts, a term also used in Sweden, two definitions are presented: 1) persons with less frequent and less intense exposure than close contacts (371) 2). This group is called “sporadic” by Erkens and co-workers and is defined as those who spent less time with the infectious case including visitors, friends, work colleagues, class mates etc.

The traditional approach to CoTr is the “concentric circle method” which is also called “stone in the pond principle”, classifies contacts within the inner social circle as “close” and more sporadic contacts, of the outer circle, as “casual” (384). Erkens et al. suggest that inner circle of contacts should include persons with cumulative exposure time of 8 hours if the IC is smear-positive, or 40 hours if the IC is only sputum culture-positive (173). This recommendation has been incorporated also in the Swedish guidelines (385). NICE/UK guidelines also suggest applying the “8 h rule” when a cumulative exposure to a smear-positive IC is calculated in non-household contacts (274).

“High-priority” contacts or “close” household and non-household contacts (according to Erkens classification) should be evaluated first. When a “close” contact is found to be infected, CoTr is extended to include less close contacts.

Small children and immunocompromised individuals are especially vulnerable and required an immediate evaluation, which in practice indicates that the contact is to be examined within 1 or 2 working days after identification. According to the CDC, the initial contact encounter should be performed within 3 working days of the contact being listed. Medical evaluation should be completed within 5 days for high-priority

contacts to smear-positive ICs or if there are cavities on CXR and within 10 days for high-priority contacts to smear – negative cases and medium-priority contacts regardless of smear and culture results (172). Lienhardt (IUATLD) proposes that the evaluation of “high-risk” (another term!) contacts should be completed within a month (384).

1.9.3.1 Contact evaluation, treatment and follow-up

CDC provides comprehensive instructions on how to interview an IC (172). A minimum of two interviews are recommended because the patient may experience stress related to the illness and or the TB diagnosis. The patient also needs to adjust and be familiar with the interviewer. The second interview is ideally conducted 1-2 weeks after the first one. CDC underlines that interviewing skills demand training and periodic tutoring and that only trained personnel should interview ICs. Approximately 75-80% of all useful information related to contacts is revealed in the initial interview, though valuable information is sometimes gained on the subsequent home visits. TB field workers reported that contacts who were not mentioned by the IC in the initial interview were later identified upon home visits. This type of information may be lost without the continuity of TB field workers (383).

The interval between infection and a detectable test result, the so called “window period”, is usually 8-10 weeks with 2 weeks being the lower limit. A negative TST result obtained before 8 weeks after exposure is regarded as unreliable. A new test at the end of the window period is recommended. For low-priority contacts a single TST at the end of window period is suggested (172).

Contacts with a positive TST/IGRA result should undergo CXR. A positive test result and the absence of clinical symptoms and radiological findings suggesting TB, is the basis for diagnosis of LTBI. If the likelihood of *Mtb* infection is high, a negative result should be followed by a repeated test 8 weeks after the last exposure to infection, hereby taking into account the incubation period for immune reactivity (173).

In a situation with a low pretest probability of infection, increasing the cut-off point of TST beyond 14-15 mm is advised. In high-priority, immune-competent contacts, TST indurations’ cut-offs of ≥ 15 mm and ≥ 10 mm are suggested in BCG- vaccinated and –unvaccinated, respectively. In immunocompromised persons, IGRAs, in particular T-spot TB, is generally regarded as a more sensitive and specific test than the TST (173).

IGRAs are more cost-effective than TST screening in both close contacts and immigrants (180, 386).

Other diagnostic tools for LTBI include chest X-ray, case history/symptom screening, and physical examination. In addition, contacts with symptoms suggestive of TB and those who have an abnormal CXR should have their sputum examined. If a patient is unable to produce a sample spontaneously, it is recommended to obtain induced sputum which has a similar yield as specimens obtained with BAL (173). Treatment for LTBI should be initiated only after active TB has been excluded. Failure to rule out TB may result in development of drug resistance. For most patients, treatment with INH for 9 months is chosen. Contacts to IC with INH-resistant TB should be offered 4 month RIF (387). Contacts to MDR TB should be followed by clinical follow-up for a period of \geq 2 years, if no specific drug regimen is recommended (173).

In Sweden, contacts with LTBI who have not received preventive treatment should be scheduled for follow-up visits with CXR at 2 to 3, 6 and 12 month after exposure. For immunocompromised contacts and for children < 5 yrs, 24 months of follow-up is recommended. The importance of educating persons about TB symptoms and providing contact information to health-care facilities if symptoms develop are stressed (385).

In the US, routine longitudinal CXR monitoring has not been recommended for contacts and there are no guidelines for person with LTBI who do not take treatment. The emphasis is instead on teaching patients the symptoms of TB disease. In unusual circumstances, and on an individual basis, immunodeficient patients or contacts who are infected after exposure to MDR/XDR TB are monitored radiologically, but the CDC has not prescribed a monitoring schedule (John Jereb, Division of Tuberculosis Elimination, CDC; personal communication). In the European consensus statement follow-up with radiographic evaluation among persons not receiving preventive therapy is described as of doubtful effectiveness since the majority of new cases in previous studies occurred between examinations (173).

1.9.4 Complexities of CoTr in urban settings

Outbreak studies in recent years have shown the influence of TB transmission outside the customary family setting. Molecular epidemiologic studies have frequently revealed routes of transmission that were not identified by traditional CoTr (242, 388, 389). In addition, identification of locations frequented by persons involved in an outbreak is

necessary to uncover transmission outside households (390-392). The limitations of conventional CoTr become evident in particular when homeless drug users or recent immigrants residing in large cities are involved (390). While limitation of DNA genotyping with regard to CoTr is that genotyping has previously only been carried out on culture-positive cases. The delay between CoTr and availability of genotyping results has reduced the operational value of molecular analysis. Even if genotyping gives a deeper insight in the transmission, its practical impact on the CoTr is often limited (274). Shorter delays are achieved by using MIRU-VNTR and spoligotyping instead of RFLP and their results are expected to be reported within 10 working days of receipt of the isolates (240).

Identification of social links between contacts and a presumed IC may provide drug-sensitivity patterns before culture results of secondary cases are available or in culture negative TB cases and may also assist in choosing appropriate drug regimens for treatment of LTBI. However, it has also been found that CoTr occasionally overestimates the rate of *Mtb* transmission, especially when contacts have more than one risk factor of acquiring TB/LTBI. The main cause of TB in foreign-born is a reactivation of a remote *Mtb* infection acquired in the country of origin (333). Dahle et al. showed that concomitant reactivation of TB in contacts that took place during an outbreak investigation, involving 15 individuals with origins in high-endemic countries, revealed six different genotypes in 14 culture positive cases (393). This study highlights that CoTr has to be confirmed by genotyping and that incorrect assumptions may lead to erroneous drug regimens.

A prospective survey of social mixing patterns in a poor township near Cape Town, South Africa, demonstrated that the number of close contacts were 40% higher than in the corresponding population in industrialized countries ($P < 0.001$). Of all contacts, 86% took place indoors of which 27%, 20%, 20%, and 8% were in transport, own household, school, and work settings, respectively (394). In high-endemic settings, a substantial proportion of TB cases in children may be caused by transmissions from visitors or other adults not living together with the child. Another study from the same South African township demonstrates that although the risk of TB in preschool children was mainly determined by the number of resident adults, transmission risk outside the home increased with increasing number of households visited (395). To some extent, the lessons drawn from high-endemic settings can probably be applied to sites of

intensive transmission in low-endemic places where “microepidemics” occasionally occur (269).

Homeless people present a special challenge for public health providers and failure to identify contacts (missing addresses) for TB cases among homeless people is an ongoing problem (396). CXR, including mobile digital chest radiography, has been used for screening of this high-risk group (397). TB incidence among homeless in Houston, Texas was 41.1/100 000 compared to 9.5 among housed persons. The homeless were more likely to be US-born and their *Mtb* isolates to belong to clusters as well as larger-sized clusters. TB rates among the homeless were driven by social factors and not by comorbidities (398). Overcrowding in shelters for the homeless facilitates transmission. An investigation of a large outbreak in Hamburg identified alcoholism as a high-risk factor for the spread of TB. It is important to evaluate the life style of ICs since it may reveal places of transmission other than domestic or occupational such as clubs, cinemas, shelter and other locations (274).

A high prevalence of LTBI among foreign-born contacts complicates the interpretation of the results of CoTr and probably overestimates the degree of recent infection. The traditional concentric circle approach to CoTr, operating outward from the household, is likely to be unsuccessful in urban settings. A study from Montreal shows that contacts outside the home were rarely evaluated though secondary cases due to casual transmission have occurred (390). The high proportion of immigrants among urban cases largely explains the shortages of conventional CoTr. Coinciding attendance of classes where immigrants study, ethnic clubs, use of public transportation, homeless shelters and other poorly ventilated locations predispose for recent transmission in urban settings. Several studies have also shown that non-foreign-born TB cases generate larger CoTr compared to foreign-born IC (390, 399).

The limitations of existing CoTr methods have been revealed by several studies (242, 388, 389). Regardless of discouraging finding in a study by van Deutekom et al., that the best CoTr could achieve was to identify 30% of the clustered cases, when genotyping data was combined with prompt second interview additional information on environments where transmission had occurred, was collected (242, 400). It is only through the combination of genotyping and CoTr, adjusted to different settings that “we can identify specific patient behaviors, sites, or circumstances” (238).

1.9.5 Novel methods for CoTr

1.9.5.1 Social network analysis

Important information on actual trends of TB transmission in the community can be overlooked because data from many separate CoTr are collected disjointedly, without placing the combined results into a broader context. The outcome of each contact investigation is usually recorded on paper with no organized evaluation. Conventional statistical methods often fail to reflect complex interactions between cases, contacts and places (401). In the places where transmission is active and ongoing, change of practices for CoTr is needed; including a social network approach to CoTr (238). The term “social network” is used to describe a set of persons (nodes) and the connections (ties) among them. Social network analysis (SNA) measures the nature of these ties, for example leisure activity, and can discover the social circumstances relevant to transmission (402). The ties (edges) may represent any relevant social interactions. SNA is a mathematical approach that includes visualization of nodes and ties. The basic SNA units are the ties linking “person-person” and “person-place” nodes (402). When data collected by CoTr is analyzed by new computerized methods, a system of linkages among TB patients, their contacts, and the geographical settings is created. The networks are visualized as graphs or measured quantitatively. Its aim is to identify the most vital nodes that are responsible for transmission and, based upon their location in the network, predict which nodes (persons) are likely to be infected (403). In an outbreak report by Gardy et al. combining use of SNA and the whole genome sequencing (WGS) outclassed CoTr in identifying a probable source case as well as several locations and individuals (404). Initially it was assumed that this outbreak was clonal because all isolates exhibited identical MIRU-VNTR patterns. However, whole genome sequencing (WGS) revealed that that the outbreak was the merging of two outbreaks, each with its own causative lineage of *Mtb*. Through integration of WGS and SNA, key members of a high-risk social network were recognized and the study identified crack cocaine use as a cause of the concurrent expansion of two existing lineages of *Mtb*. Genotyping and contact tracing alone did not capture the true dynamics of the outbreak. This report shows that both SNA and WGS have important implications for CoTr.

1.9.5.2 Geographic information systems

Geographic information and global positioning systems, GIS and GPS have been applied for studying TB transmission and can probably be used for identification of “hot spots” for targeted screening (403). Residential addresses at the time of TB diagnosis can be mapped according to strain characterization. Evaluation of the distribution of cases within zip-code confines has identified distinctive geographical areas of distribution of identical strains indicating increased probability of transmission (405-407).

The excellent review by Cook et al. provided valuable suggestions about how to apply model approaches to existing CoTr programmes (403).

1.10 NOSOCOMIAL TRANSMISSION OF TB

1.10.1 Historical flashback

The great French doctor Laënnec was doubtful of the contagiousness of TB but when he became ill, Laënnec was certain that he had acquired TB by performing autopsies. Both Valsalva and Morgagni avoided autopsies of those dying from TB. However, it was a general view that there was little danger of acquiring TB from expired air of TB patients and that TB hospitals were very safe in that aspect (408). The TB rate in 17,000 German health care workers (HCWs) in 1906-1910, was equal to the general population. On the contrary, Cornet found that 63 % of total deaths among German Catholic nurses were due to TB (409). In a study from 1909 from Mount Vernon Hospital in England, the occurrence of TB among nurses was either equal to the rest of the community or even lower. The risk of employing someone with active TB was seemingly higher than contracting TB at the hospital (410). Similar results were found in the concurrent studies on TB in doctors in sanatoria and hospitals for consumption. In Bromton hospital 1846 – 1882 only 8 out of 150 physicians contracted TB, while in a study of famous Danish doctor Saugman (1902), who sent questionnaires to approximately 60 European sanatoria, 3 doctors out of 174 confirmed contracting TB during their service (34). The possible reason for less TB morbidity in HCW at that period, if we believe that the numbers are correct, was probably due to the better living conditions for the employees at sanatoria and TB hospitals than for the general population as TB infection was more or less unavoidable for the vast majority. Many older studies held the opinion that working with TB patients “involve no special

hazards among those who engage in it” (408). Interestingly, that as late as 1947, the American Public Health Association stated that “conclusive evidence is not available at present that the air-borne mode of transmission of infection is predominant for any particular disease” (411).

It is believed that around 1900 in Europe, when the above-mentioned studies were published, practically everybody become infected at the age of 20 years (412). However, compared to beginning of 19th-century, the TB mortality was already diminishing in several European countries and its peak was climbing to the higher ages: more than 50 years in 1936 as compared to 20 years in 1851, and only 10 % of all deaths in England and Wales 1901-10 caused by TB. Also in the US, TB mortality went down by more than 50 % between 1900 and 1925 (8).

According to Dufault (409), there was a decided change both in figures and in the tone of articles on TB in HCWs from 1925, the year when Britton and Bollman demonstrated that 2.2% of all nurses in Chicago had TB. This rate was only exceeded by telephone operators and seamstresses (408).

When TB rates diminished acquisition of infection took place later in life, and that was probably the reason for observing the increased risk of contracting TB among medical students in the USA (413) and undergraduate nurses in Norway around 1930. To find out whether or not TB was more frequent among the student nurses than among other groups Olaf Scheel, in 1924, began making Pirquet's TST in the nurses' training school at Ullevaal. Annually, about a hundred young women begin a three year course there (414, 415). About 95 % of the initially TST-negative student nurses at Ullevaal converted their skin test by graduation, and 22 % developed active TB. Scheel, who was not known to be a man of emotion, was so devastated about the fact that young, healthy country girls were coming to Oslo to study and later had been dying of TB, that he suggested, in the damn 1940, that Norwegian Nasjonalforeningen stopped schooling nurses (416). His and Heimbeck's findings were later confirmed by the British Prophit survey (1935-44) demonstrating that TB was 3 times more likely in initially TST negative nurses compared to TST positives and that the morbidity rate among nurses (1370 / 100,000) was twice as high as among the controls (417).

As in a case of nurses, the increased risk of TB in doctors and medical students was first denied by the majority (though there were always some who objected!) and recognized later, with autopsies being acknowledged as dangerous first (408).

According to Meade, who worked in the Trudeau Sanatorium between 1920 and 1953, around 10 % of all new admissions were medical students and physicians. Many more were treated in other sanatoriums (418). Meade provides strong evidence that participation in autopsies was the prime source of infection among medical students (419).

The first large survey on infections acquired in laboratories, covering the period 1930-1950, showed that *Mtb* was responsible for 11% of all of infections caused by 32 different microbial agents, second only to *Brucella* (420, 421).

The guardian of HCWs with the respect to TB was Jay Arthur Myers who began to study TB in medical students as early as in 1920 (413). Some of Myers recommendations have been enormously influential e.g. to perform TST and chest X-ray on all new employees, to do follow-up skin testing every 6-12 months and exclude TB in new hospital patients, including initial assessment with CXR already on admission (422, 423).

I should also mention the study of Erik Hedvall "The Incidence of Tuberculosis among Students at Lund University" (4). Among 3, 336 students and probationary nurses examined, TB incidence was as following: in medical students 11 %, in probationary nurses 5 %, and in students from other faculties 1- 3 %. In the medical students the period of greatest risk was during the 3rd-- 5th years of studying. In 16 of the 47 cases of primary TB in the medical students there was an association between the courses in general pathology and the appearance of the primary TB. Hedvall's investigation also showed that live *Mtb* were found in the post-mortem rooms and on different-objects 24 hours after necropsy on cases of PTB. "It has therefore been decided to limit post-mortem work on tuberculous persons as much as possible, and so far, for two 'terms, there have been-no new tuberculin reactors in the students taking the course".

Another issue discussed in the literature was the danger of spreading TB from medical staff to patients. In an the *Editorial* from *Lancet* (1973) we can read that junior medical staff in contact with young children pose a biggest danger, since "about half the junior doctors in Britain come from overseas, most of them from India or Pakistan, where the frequency of tuberculosis is much higher". *Lancet* encourages them to have an annual chest X-ray but admits that "doctors are notoriously remiss about their own health and apt to ignore requests to attend for a routine radiograph" (424).

1.10.2 Modern times

Transmission of TB within hospitals often involves immunocompromised hosts and multi-drug resistant (MDR) strains (425). Even though rates of TB began to increase in USA in 1985, the reports on nosocomial TB transmission were infrequent before 1989, when a significant connection between nosocomial TB outbreaks and spread of TB in the community became apparent. An upswing of TB in the US in the late 1980s was due to a dismantling of previous public health resources, a growing HIV prevalence and widespread homelessness and illicit drug use. In addition, the resurgence of TB in the US, with a 20 % increase in the number of TB cases in 1985-1992, took place as hospital policies that had been developed to prevent nosocomial transmission fell into neglect (423). Lack of adequate infection-control measures in hospitals and many hospitalised patients with advanced HIV before effective antiretroviral treatment (ART) was discovered fuelled several large nosocomial TB outbreaks in New-York and other cities (79, 426-428). During these years, tuberculin conversion in HCWs was frequent, and several of these developed active drug-resistant disease (429, 430). In addition to insufficient infection control measures, a delayed diagnosis due to “atypical clinical presentation of TB among HIV positives was an important factor for the increase in nosocomial TB outbreaks (426, 429, 431, 432).

The problem of nosocomial TB transmission today is particularly manifest in places where resources are limited (433). More recently, the studies from South Africa indicate that nosocomial transmission is an important factor in the spread of extensively drug-resistant TB (XDR-TB) (244, 434).

HCWs in low-income countries are at particular risk of acquiring TB, due to frequent exposures and limited resources. Prompt isolation of infectious TB patients is often not possible in resource-poor settings. It has been estimated that in countries with low, intermediate, and high TB incidence, 49%, 27%, and 81% of TB cases among HCWs, respectively, were attributable to exposure in health care settings (435). Meanwhile, in high-income countries mainly ineffective control measures in hospitals pose HCW at the risk for TB (436, 437).

Fronteira et al. evaluated ten cohort and six cross-sectional studies, conducted in several countries and published during 1991–2006. They found an increased risk of TB for nurses working in hospitals, pulmonary clinics, medicine, TB or HIV wards

compared with nurses working in other locations. However, 2 studies included in the analysis, demonstrated a lower risk of TB in nurses compared with other HCWs, the general population and in nurses working in psychiatric wards (438).

The most important infection control measure is a timely isolation of patients who may be contagious. Early treatment further reduces infectivity and improves prognosis (439). It has been repeatedly shown that ineffective control measures increase the risk for TB among HCWs (437, 440).

Harris and co-workers described a recent outbreak that occurred at a long-term care facility in New York and highlight the importance of considering active TB in aging and immunocompromised patients including those with diabetes. Several factors contributed to the transmission: diagnostic delay, infectious characteristics of the source case such as cough and sputum smear positivity), aerosolization of secretions from repeated suctioning, and inadequate ventilation (441).

1.10.2.1 About guidelines

According to CDC (442) implementation of so called “administrative measures” is the most important stage of TB control in hospitals. The measures include handing over responsibility for TB control to a group with expertise in TB, performing a TB risk assessment, implementing effective practices for the management of patients with suspected TB and educating HCWs. TB Risk Assessment implies that the number of patients with suspected or confirmed TB disease, especially with unrecognized TB, who have been encountered in the setting during, at least the previous 5 years is reviewed. After “administrative” follow “environmental” (ventilation, air filtration) and “respiratory-protection” controls, including use of protective equipment and educating patients about respiratory hygiene.

There are no specific national guidelines concerning TB-control in Health-care settings in Sweden. However, in Stockholm there has been published and recently updated a framework of TB control activities in hospital wards and other health-care settings (443). According to this document, both HCWs who have been exposed to TB should be informed of TB risks “as soon as possible”. After that HCWs should fill-in a questioner (Contact tracing form) and a person who is in charge of the medical staff should compile a list of those who are considered to be exposed. This

list is sent to a physician who treats the presumed index case (IC). This physician will then initiate an investigation of the listed HCWs.

All new health care employees/students should be informed about TB including being educated on its clinical and infection control aspects, according to County Medical Officer in Stockholm. Large nosocomial outbreak in our hospital in 2008 (440) forced us to revise and update the local guidelines concerning issue of infectivity, respiratory isolation, hygiene and CoTr etc (444). These local guidelines follow the CDC guidelines including the key issue to discontinue isolation of the IC only when the possibility of TB is excluded. There are several requirements in the CDC guidelines that we are not able to fulfil, for example that outpatients with suspected or confirmed infectious TB disease should remain in AII rooms until they are transferred or until their visit is complete (442). In TB outpatient clinic in Solna, there are no such rooms and TB patients shared the same waiting rooms with other visitors.

1.10.2.2 Ventilation and other measures

A review of interventions to prevent health care-associated TB in a large public hospital in Chicago indicates that the annual TST conversion rate among HCWs can be substantially minimized by educating HCWs to recognize patients with TB and engineer negative-pressure respiratory isolation rooms (445). The annual rate of TST conversions in HCWs dropped from 1.18% to 0.6% in an urban hospital in California after CDC-recommended control measures were applied (446).

The CDC recommends (442) Airborne Infection Isolation (AII) for all patients with suspected and proven infectious TB. This includes negative pressure rooms, i.e. lower pressure than in attached corridor, with an anteroom. AII rooms should have airflow of ≥ 6 air changes per hour (ACH). Moreover, all new construction or renovation of health-care settings should be designed so that AII rooms achieve airflow of ≥ 12 ACH. CDC provides in depth recommendations on engineering controls, ventilation maintenance, use of air-cleaning methods such as filters and air irradiation etc. Unfortunately, negative pressure rooms are very costly to build and to run and require frequent inspection and maintenance; therefore many hospitals, even in the EU lack such facilities (273). In addition engineering mistakes occur. Medical staff has to be trained in the use of these rooms and an operational policy should be prepared. Visualization of airflow directions between rooms by smoke testing should be undertaken, also for educational purpose (447). However, improved ventilation had a much smaller effect on transmission than timely isolation and treatment (436).

A relationship between ventilation and indoor airborne transmission of infectious agents is supported by strong evidence. However, the scientific proof in support of existing ventilation guidelines in hospitals, including efficacy of personal protective devices are not convincing (448, 449). The evidence comes from reports on outbreaks in which inadequate ventilation was identified as a contributory factor (436).

It has been recommended that HCWs wear N95 filtering facepiece respirators (N95-FFR) when HCWs for example is entering an AII room or any room with a patient with infectious TB disease, to minimize occupational exposure to aerosols, such as TB and influenza. Regrettably, neither these recommendations are strictly evidence-based. On the contrary, when an N95FFR is used for short-duration procedures such as airway suctioning, etc., the likely added respiratory protective benefit may be so minimal as to be counterweighted by its negative impact on the personal performance of the wearer and the risk of self-contamination or dissemination of pathogens (450). WHO and CDC considers personal respiratory protection as the third line of defense for TB control, indicated when TB risk cannot be adequately reduced by administrative and engineering controls (451).

1.10.3 Screening of HCW

A prevalence of LTBI in hospital staff has been reported from high-endemic countries: 50-68 % (452-454). In the study from India, TST positivity was strongly associated with time spent in health care (453). HCW who developed TB in NY in 1994-2002 (at the time when control measures in the US hospital were reinforced) were often foreign-born (50-77.5 %), indicating that the cause of TB was chiefly a reactivation of a remote infection and not a result of work-related transmission (455).

The TST remains a first-line screening test for LTBI in high-incidence settings (456). In contrast to the TST, IGRAs showed good correlation with occupational risk factors for TB exposure in low-TB incidence settings, while in high-incidence settings there were no reliable differences in the prevalence of positive tests, although few studies have been performed in high-endemic settings (179, 456). However, the use of IGRAs for serial testing is complicated mostly due to a considerable rate of both conversions and reversions (457: Gran, 2013 #1211). The use of a single cutoff point criterion for IGRA may lead to overdiagnosis of new TB infections. For example in Cleveland, the majority of HCWs who were identified as converters with serial QTG-G tests had values ≤ 1 IU/mL, though none of them were part of a contact tracing (CoTr) or had active TB (458). Hospital TB programs are organized around timely detection and

isolation of possible TB patients and the use of proper ventilation and other environmental controls. In settings in which a significant proportion of HCWs are foreign born, preventing reactivation of TB disease should be regarded as an evenly essential part of TB control (459).

1.10.3.1 Treatment of LTBI in Health care workers

The CDC recommends that HCWs who take care of TB patients should be tested for LTBI every 12 months. A § HCW with a previously negative test result who have an increase of ≥ 10 mm induration when examined on follow-up testing should be treated for LTBI when active TB is excluded (442). The NICE guidelines in the UK recommend that new employees of any age who are from high-endemic countries should be tested with an IGRA and that preventive treatment should be offered to HCW with LTBI, independently of age (274). In a prospective study by Colson and co-workers, HCWs were less likely to accept treatment of LTBI than other professionals (197). In contrast, HCWs in a hospital in St Louis had a high rate of initiating INH therapy for LTBI, and a high rate of compliance (460). Many HCWs are TST positive as a result of exposure at work and some regard it as a benign occupational hazard (461). A small study from Ohio indicates that use of IGRA instead of a TST for screening may increase acceptance of preventive therapy among HCWs (462). A recent review on LTBI in HCWs recommends that those who decline chemoprophylaxis or when close monitoring cannot be set up should be advised to observe for symptoms of active TB and scheduled for annual CXR screening. European guideline on CoTr does not support CXR controls, with the exception of contacts to cases with MDR-TB (173).

1.11 TB IN SWEDEN

TB mortality in Sweden reached its peak of 324/100.000/ year in 1871-1880. Already in 1911 it fell to 191 / 100.000 and 1942 the corresponding number of TB deaths was 68 (463). Hedvall and Hillerdal showed that in the late 1940s the majority patients who died of TB were older than 50 yrs. Hedvall published also one of the first Swedish reports on treatment with streptomycin (STR) where he described outcomes in 17 patients of PTB in the ages 20—38 years, with first case treated in October 1947. Considerable improvement was achieved in 12 cases, in 2 cases STR was switched to PAS after about 10 days and in 3 cases STR had no or little effect (464).

After that TB situation in the country was constantly improving. TB patients in Sweden were managed by “centaldispensärer” until 1986 when TB care was “spread out” to departments of pediatrics, pulmonary and infectious diseases (465).

A large study from Stockholm investigated the precision of clinical TB diagnosis between the two time periods 1977 to 1978 and 1987 to 1988. The analysis was based on 3,042 autopsies at Huddinge University Hospital. The autopsy rate decreased from 80% to 39% between the two time periods. There was a significant increase in the proportion of infectious diseases from 27% to 32% and the number of cases with clinically missed TB was twice as high in the later period as compared to the initial period (466).

The risk of having been infected by the age of 6 years went down from 4.5% / year in 1925 to 0.75 % / year in 1945 (467). Among persons born in Sweden, TB incidence decreased from 700 /100.000 in 1920 to 1.4 in 2009 (468). A study by Winqvist et al. demonstrates 85 % decline in TB cases that occurred in birth cohorts 1920-1950, after 1967. The authors reach an interesting conclusion: given that very few cases are diagnosed in Swedish-born individuals and since the majority of cases in Swedish-born persons today are a result of infections occurred before 1967, that this indicate a spontaneous clearance of LTBI in most of the cases, since more than > 50 % of cases occurred within 2 years of infection (81).

I believed that the first large of refugees, with supposedly high prevalence of TB, who came to Sweden were The Finnish war children. In 1939-48 approximately 70.000 Finnish children (up to 14 years of age), including 6,000 who were ill, were evacuated to Sweden and about 20 % of them were during some time period was placed in hospitals and institutions, including sanatoria, for example in Rävlanda Sanatorium near Göteborg (469). In 335 children who died in 1941-49, the major cause of death was probably diphtheria, followed by TB (470). I was not able to find any reports specifically on TB in Finnish children in Sweden.



Memorial to children from Finland who died in Rävlanda sanatorium.

The photograph, taken by Tapani Rossi, published with permission of Kai Rosnell, Riksförbundet Finska Krigsbarn.

Stig Cronberg evaluated medical charts of refugees who were treated in Epidemisjukhuset, Malmö 1945 (471). First patients were Norwegians and Danes who flew to Sweden from the occupation and who suffered from similar infections as Swedes. In the end of the war came members of Swedish-German families and Estonian refugees to Skåne, TB was diagnosed in few. Among 700 Danish policemen, who came to Sweden from prisoner camp in Denmark in April 1945, 29 were admitted because of TB and diphtheria. Later, came prisoners of Buchenwald, Bergen-Belsen and from the women's concentration camp Ravensbrück. In total 423 refugees were admitted to Epidemisjukhuset i Malmö, 4 % of them had TB.

Only 5 % of TB patients in Sweden foreign-born in 1951, meanwhile < 4 % of the Swedish population was born abroad with the majority was originated from neighboring countries (468). The proportion of TB cases born abroad has been increasing in Sweden since 1970s: 13% in 1974, 22% in 1984, 25 % in 1987, 59% in 1993 and 85% in 2012 (472, 473). In 1971-1984, the annual rate of decrease in the number of TB cases was 9% for Swedish-born (almost unchanged since 1946) and 5% for foreign-born persons, respectively (474). In many industrialized countries including Sweden, the previously declining incidence of TB have leveled off or even increased from the 1980s. As expected political situation in the world and international agreement determine and control migration. Because of the war in Yugoslavia, the majority of foreign-born TB patients in Sweden in 1992-93 were from this country. Since 1994 more than 40% of the foreign-born in Sweden have been of African origin (475).

1.11.1 Situation in Somalia and migration

Somalia is a country located in the so-called Horn of Africa. The country has been continuously ravaged by war for over two decades. Because of its destroyed infrastructures, widespread food crisis and limited access to healthcare it is considered one of the countries where living conditions are extremely difficult. Epidemiological indexes in Somalia are the worst in the world, and the Somali citizens are entirely dependent on foreign humanitarian assistance (476). Somalia was ruled by General Siyaad Barre in 1969-1991, after a military coup brought him to power. Clan-based opposition militias were formed under his rule and were manipulated by Barre's regime. In 1990, an unrestrained civil war broke out and ultimately led to the collapse of the central government (477). The civil war and ongoing clan violence have handicapped the country's infrastructure and economy (478). Because of continued anarchy, clan warfare, and border disputes, civilians have suffered much violence, including torture and rape (479). Additionally, at least one million people have fled the hunger (480) and turbulence in Somalia to the neighboring countries, mainly Kenya and Ethiopia (481). An estimated 400,000 Somalis have died. The estimated number of internally displaced persons (IDP) in the whole Somalia reached 2 million people at the height of fighting in 1992 (482, 483).

Dadaab, the world's largest refugee camp, in northeast Kenya, which dwelling over 451,000 Somali refugees, has been struggling to stop cholera since September 2011 (484, 485). Somalia has the worst access to water indicators and is one of the five worst places in the world to be a woman (484). Somalia has the highest mortality rates in the world. It also has the highest rates of child malnutrition (486). Probability of dying under age of 5 is 180 per 1.000 live births, compared to approximately 75 in Kenya and Ethiopia. Estimated TB incidence in Somalia in 2011 was 300 / 100.000 and case detection rate 43%. However, the treatment success rate according to WHO, was 89 % in 2010 (487).

The levels of MDR-TB are higher in Somalia than in neighboring countries. In the nationwide survey from 2011, comprised of 754 persons with new cases and 96 persons with previously treated cases, MDR TB was detected in 5 % of persons with

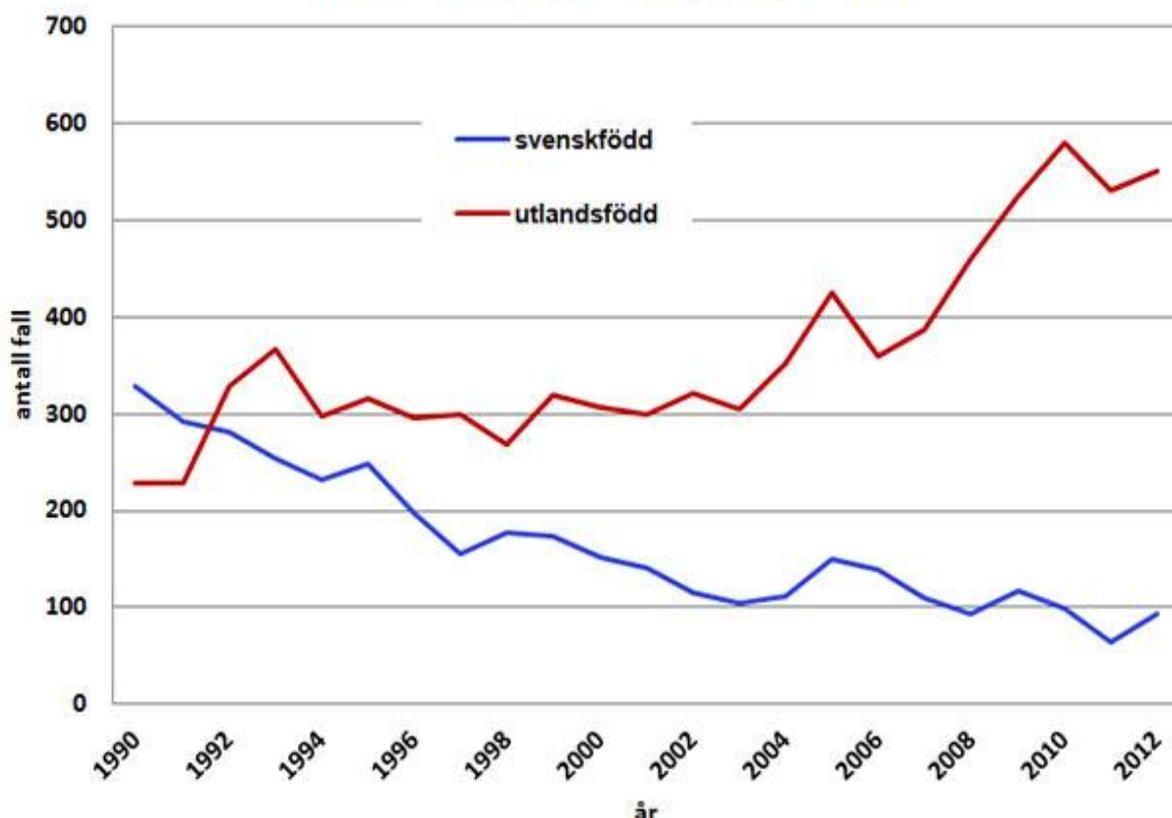
newly diagnosed TB and 41 % of persons with previously treated TB. Overall levels of INH resistance were 6 % (488).

1.11.1.1 High risk of TB in Somalis living in Sweden

In total, 645 cases of TB were diagnosed in Sweden 2012 and 47 % originated from Africa. The majority of Swedish-born patients were old (> 65 yrs) while most of the foreign-born patients were younger than 40 years, with the age group of 20-30 yrs old being the most prevalent one in 2012. TB incidence for foreign-born persons of age 18-24 years was 72 / 100,000 in 2008, compared to < 1 / 100,000 of Swedish-born persons under the age of 65. Somalia was the commonest country of origin of TB patients in Sweden since around 1995 and Somalis comprised approximately 30 % of all TB cases. TB incidence in immigrants from Somalia was estimated to 512 / 100,000 in 2001. The incidence of active TB in Somalis living in Norway at 7 years post-migration was 520 per 100 000 person-years, while the annual incidence rate of TB was as high as 889 per 100 000 persons for Somali immigrants in USA (338). High rates of TB among Somali immigrants also have been reported from Denmark (489, 490), Canada (491, 492), Holland (493) and in the UK (494-497).

Thus, treatment of LTBI should be regarded as an important intervention to reduce the TB burden in this minority-group. According to the Swedish Migration Board, Sweden received 36,766 migrants and asylum seekers from Somalia 1988-2010 In 2010 26% of all new TB cases in Sweden were diagnosed in Stockholm, the capital of Sweden with a population of 2 million. About 25 % of Somali immigrants in Sweden reside in Stockholm. In this context, TLTBI should be regarded as feasible intervention to reduce TB burden in this minority-group.

TB-fall i Sverige per ursprung och år



Annual number of new TB cases in Sweden. Blue line – Swedish born, red line - foreign born (From: <http://www.smittskyddsinstitutet.se/statistik/tuberkulos/>)

In 2012, 645 new cases of TB were diagnosed in Sweden with corresponding incidence of 6.8 / 100,000. Approximately 35-40% of all isolates typed in 2008-2012 belonged to a cluster. The rate of clustering was low in the elderly population epidemic, compared to a young Swedish population (473, 498).

Today Swedish National database of genotyping results at SMI contains following results of 5015 RFLP, 3990 spoligotyping and 1867 MIRU-VNTR. Before the standard method for genotyping was changed from RFLP to MIRU-VNTR in 2012, the proportion of clustering in Sweden was approximately 40 % (Ramona Groenheit, written communication).

1.11.2 TB in Stockholm

Stockholm is the most populous city in Sweden, with a population of more than 2.000.000. As of 2010, the Stockholm metropolitan area was a home to approximately 22% of Sweden's population (<http://en.wikipedia.org/wiki/>). Last year 193 cases of TB were diagnosed in Stockholm, 82 % of them were foreign-born and 13 % lived in wealthy areas (e g Lidingö etc). Among 79 % of cases that were confirmed by culture, only 20 % were sputum-smear positive, though 60 % had PTB. The proportion of drug-resistance was approximately 10 %, including 2 % MDR-TB. Among 152 culture-verified cases diagnosed in Stockholm 2012, 23% were clustered (Ingela Berggren, written communication).

TB was diagnosed in 147 children from Stockholm in 2000-2009, 62 % of who were identified by screening. Majority of the cases were either born in high-endemic countries and or had to parents from such countries (499). The incidence was 451/100,000 for children born in Somalia. The resistance to any first-line drug was present in 25% of the strains, of which 4 were MDR. Of 53 % cases confirmed by culture and, foreign-born cases were clustered, while 18/19 Swedish-born children with Swedish parents belonged to cluster 49 (C49), the subject of my studies (257).

TB cases in Stockholm 2007-2012 (data from PHA)

	Total cases	% Horn of Africa	% Swedish born
2007	174	30	25
2008	230	34	20
2009	243	34	20
2010	178	34	18
2011	149	19^a	13
2012	193	16^a	17

^a Only Somalis

First report on health control of immigrants in Stockholm was, to my knowledge, written by Arhammar et al. from Roslagstull sjukhus (500). Child Welfare Board of Stockholm started an investigation of a health and living conditions of 95 Turkish boys (out of 150 male immigrants from Turkey living in Stockholm) who stayed in “a shabby hotel” in 1969. Two boys were found to have TB. For comparison, in 1968-

69, there was a total 4 cases of PTB among 11.074 students, “thus the difference was substantial, being 2 TB cases per 100 as against 0.04 per 100.” The authors recommended a medical check-up on “this category of immigrants” preferably before working permits are issued, in order to reduce the risk of transmission (500). In 1976 Aurelius and Moëll wrote following recommendations about health screening of immigrants: 1) it should not be mandatory 2) should not be linked with receiving a residence permit 3) should not include only certain nationalities 4) screening should not be restricted to communicable diseases only but person’s general health should be evaluated (501). The authors provide a guideline about how to organize immigrant screening in Sweden.

1.12 ABOUT THIS THESIS

1.12.1 Why write this thesis?

The contribution of TB to overall morbidity, mortality and health-care expenditures in Sweden today is disproportionally small compared to disturbingly emotional and political impact that the Swedish word “tuberkulos” creates. So, the first reason is my interest for the “disproportional” effect. The second reason is the challenge: can we beat it or not? The third reason is close to the second one, i e to find out how good or bad we are at defeating it. The forth reason, is a personal one – revenge. It is about the great-uncle who died young, my uncle who spent one year in bed, and the hunchbacked woman, a family friend, always wearing a corset. The fifth reason is about what everybody knows, that “Old Black Joe's still picking cotton for your ribbons and bows”. It is about politics, inequity, and walls between “us” and “them”.

1.12.2 Objective

The general purpose of this study was to scrutinize and hopefully improve TB control.

Aims:

To understand how and why TB is transmitted in Stockholm

To find out how transmission can be reduced

To evaluate TB control activities in Stockholm

Specific aims:

- To describe routes of transmission of a unique strain of *Mtb* in Stockholm and identify possible causes for its expansion (Paper I and II)
- To examine case-management and control measures related to this cluster (Paper I and II)
- To explore different aspects of non-adherence in relation to TB- and LTBI-treatment
 - o to find out if there was an association between results of contact tracing and adherence of index cases (Paper II)
 - o identify risk factors for not completing treatment of LTBI (Paper IV)
 - o to get better insight in TB situation among Somalis in Stockholm (Papers I, II, IV)

- To describe and analyze causes and consequences of an outbreak of TB in a hospital ward in Stockholm (Paper III)

1.12.3 Specific background

1.12.3.1 The starting point of this thesis

In 2001, I evaluated case-management of 202 new TB patients treated in 1997-99 at two hospitals in Northern Stockholm. These cases constituted 14% of all 1395 cases in Sweden during the study period. This study was presented at the IUATLD meeting in Paris (502).

Results and conclusion

1. The case fatality rate was high (22%) among Nordic patients, who were generally older and had concomitant illnesses. Out of 202 TB cases 140 (69 %) were definitely cured and another 21 (10 %) probably cured. In the study population the problems with drug-resistance were minor and initial treatment regimen seems to be adequate in almost all cases.
2. A three drug regimen was administrated in 57 % of those born in the Nordic countries vs. 12 % for those born in other countries. In comparison 4 drug regimens were initiated in 82 % of cases from other countries vs. 40 % in the Nordic group. Treatment time was less than 6 months in 8 cases (4 %) and not known in 23 cases (11 %).

3. Problems with case holding were demonstrated: 15 % of the patients being treated less than 6 months while 7 patients were lost to follow up.
4. The majority of non-Nordic patients were young adults and 38 % of them had lived in Sweden for more than 5 years. The latter may be interpreted as of ongoing transmission of TB within our community. Using the RFLP we found at least 11 cases with strains belonging to the same cluster.
5. In 21 cases no sputum smear examinations was performed before bronchoscopy, probably due to insufficient efforts in many cases. TB patients in Sweden are usually not followed by repeated smear examinations and in 69% of the cases (15/49) conversion of smear positive sputum specimens was not verified.
6. This study reveals several problems in our TB infection control program. Among those is case holding. DOT should be considered at least in selected cases.

This study was a beginning of the present project. It made me realize that care of TB patients in Stockholm was less than satisfactory, TB was transmitted among my patients, interest for TB in Somalis was awoken.

1.12.3.2 “Follow-up and treatment outcome of tuberculosis patients in Sweden not satisfactory”

An article with this title was published by Romanus et al in *Läkartidningen* in 2000. It showed that of 676 Swedish treated for TB in August 1994--December 1995 and evaluated 12 months after they were diagnosed; only 71 % were reported to have completed the treatment and be cured of TB. Moreover, 7% were lost to follow-up, and information on treatment outcomes was missing in another 8%. The best treatment results were achieved among young foreign-born patients (81%) while elderly Swedish-born patients had a worse outcome (59%). In 5 % of the patients TB diagnosis was first confirmed post-mortem. The article reminded us that according to WHO, at least 85 % of TB patients are expected to be cured and that Sweden is not achieving this goal. This study underlined the necessity to inform public health authorities (PHA) when the patients stop coming to booked appointments. Other mentioned subjects were the loss to follow-up of asylum seekers (who were often moved between refugee facilities) and the difficulties to maintain awareness on current guidelines with respect to TB when TB care is scattered among various care-givers were also discussed. The

conclusion was that there is need for improvement in monitoring patients, if necessary by DOT (465).

According to an unpublished report, written by medical student K. Frisk no contact tracing was performed in 28% of sputum smear-positive cases, and only 25% of identified contacts were screened at chest clinic in Huddinge (Stockholm) in 1998.

1.12.3.3 Outbreak investigation is delayed

Thanks to Victoria Romanus, who was then a national TB coordinator, it became clear that at least 11 patients belonged to an INH-resistant cluster and some of them were non-adherent. I approached PHA in Stockholm County (Department Communicable Disease Control and Prevention) in September 2000 and asked (in vain) about their assistance in investigating the suspected outbreak. Concurrently, Gunilla Källenius, who was in charge of TB diagnostics at the Swedish Institute for Infectious Disease Control in Stockholm (SMI), also contacted PHA in Stockholm about 20 patients with identical RFLP pattern. In January 2001, PHA decided to close this case. In the beginning of 2002, Staffan Sylvan took PHA office, assembled the group in charge outbreak investigation and the first meeting took place in May 2002. A publication in *Läkartidningen* by Källenius in June 2002, “Resistant tuberculosis is spreading in Sweden” had awakened interest of news media (503). In an interview to the most influential Swedish newspaper “*Dagens Nyheter*”, Källenius said that these farsighted words “Sjukvården har inte riktig koll på de här patienterna” (Health-care has really no clue about these patients). And more was there to come.

Attention of the media became an attention of The National Board of Health and Welfare (Socialstyrelsen), a government agency in Sweden which exercises supervision on the national level “to ensure that the standards are observed, and to minimize risk and improve patient safety”. In October 2002, when the number of cases sharing identical DNA patterns reached 54, Gunnar Boman (the author of an early trial of RIF (504)) and Jens Boman were assigned by Socialstyrelsen to inspect how it was possible that a transmission of TB, which started 1996, was allowed to continue uncontrolled for 5 yrs. This inspection led later to an extensive reorganization of TB care in Stockholm, and creation of so called “TB center” in Karolinska hospital in Solna (Stockholm).

It is difficult to understand reasons for initial unwillingness of PHA to investigate this outbreak. It could be partly due to their conviction that, since the majority of cases were from Africa and particularly from Somalia; the cluster was not caused by ongoing transmission but by importation of the strain from high-incidence countries. However,

SMI started to perform RFLP on all drug resistant strains in Sweden and since June 2000 all strains in Stockholm have been genotyped, first at TB laboratory at Karolinska and later at SMI. It was also known from early on that the outbreak started in of the Southern suburbs of Stockholm, at a residential hotel, where the first cases lived and socialized.

Somali patients have been overrepresented in this cluster: among 121 cases, Somalis comprise 39 % (as we have shown earlier the corresponding proportion for whole Stockholm is 32 %) but if look at the period of high transmission which took place between 1996 and 2002, the corresponding proportion was 50 %.

The role of isoniazid resistance in the transmission of this outbreak has not been clarified. As we discussed in the background, previous studies have shown a reduced capacity of INH resistant strains to cause infections and active TB in contacts (67). In a outbreak report by Shorten at al. acquired additional drug resistance and retained virulence was believed to be cause of the difficulty to control the outbreak (505). In our investigation, we could not demonstrate development of drug resistance with a exception of one case, where acquired RIF resistance was probably due to poor absorption and inadequate treatment but a non-adherence (257). Several reports from London have described a large outbreak of INH resistant TB which was first recognized in 2000 (247, 256). Transmission of disease to contacts was found to be high (11%) compared with other documented outbreaks (0.7-2%) (247). Between 1995 and 2006, 293 culture-verified cases with same strain were identified. As in C49, adherence to treatment has been poor and “thus the degree and duration of infectiousness was likely to have been greater than among other TB cases” (256). On the contrary to C49, the high proportion of cases in London was drug-users.

1.12.4 Outbreak investigation (Papers I and II)

1.12.4.1 Study population

The first study comprised 102 individuals from the whole country, diagnosed with active TB, harboring a unique INH resistant TB strain in 1996-2005. Isolates of all patients demonstrated a 100% identical 14-band RFLP pattern. The study started in May 2002 as a part of an outbreak investigation when already 34 patients had

confirmed DNA-identity as “C49”, and the new study objects were included continuously, after the DNA pattern of their isolates became establish. In total, 122 members of C49 were identified by January 2013. In addition, contacts to index cases (IC) with C49 *Mtb* strains, were also included if they had suspected, culture-negative TB and were started on TB treatment. The second study was also a part of C49-investigation, included only patients who were managed in Stockholm: 99 TB cases of 121 diagnosed in 1996-2012. The reasons of exclusion of 22 cases are presented in detail in the Paper II. To sum up inclusion criteria, since we were focused on evaluating control activities in Stockholm, we excluded IC from other cities. Among Stockholm cases only those who had pulmonary TB, with unquestionable requirement for CoTr to be executed, and complete records including contact tracing (CoTr) proceedings, were included.

We used uniform definitions of delays, treatment outcome, adherence, grade of contact and CoTr. There is one topic related to definitions that may require more clarification. When describing CoTr, the variable Expanded *CoTr* was introduced: CoTr executed in a congregate setting such as a workplace, school or hospital.

1.12.4.2 Some clarifications

On contacts that were excluded

The study II also included contacts to 99 IC. We were looking to explore if there was an association between the factual “catch” of CoTr, degree of cooperation (index case) which indirectly, is a measure of the effort executed by TB controllers, and finally adherence of IC towards TB treatment. Therefore we were primarily interested in contacts whose identity could have been uncovered only by the IC. IC is not expected to actively participate in CoTr at their work places. The only information we required on this matter is that IC tells us where he/she studies, work, what hospitals have been attended etc. The rest is our responsibility, sometimes also a PHA duty, and in a case of nosocomial transmission an infection-control team should also be involved. So, the number of contacts identified through “Expanded CoTr” is related to the type of setting (big or small for example) where possible transmission had occurred, and cannot be regarded as a measure of health-care – and patient- related performance. The expanded contacts per se were thus excluded from further analysis. The variable “expanded CoTr executed” remained however though in the analysis as an indicator of performance.

On adherence

As we already discussed in the Introduction there is no gold standard by which to estimate adherence and no measures are wholly accurate: medical staff tends to overestimate adherence, while forgetfulness and reluctance of a patient to admit not taking medication makes self-reporting unreliable. While, poor clinic attendance is most likely a consistent indicator of non-adherence, prompt attendance does not automatically link with treatment completion. Therefore, multiple methods should be used to appraise factual adherence. As in other studies we have employed treatment completion as a functioning measure of adherence. It may appear inconsistent that we use three terms to describe the same phenomenon, “poor adherence” (Paper I), “non-adherence” (Paper II) and “Non-completion” (Paper IV). However, since I was the only person who determined adherence/treatment completion for the purpose of the studies, interobservational bias is eliminated. Moreover, since Paper IV describes a different patient population than Paper I and II, the change of nomenclature or definition should not be a methodological problem. The level of adherence / treatment completion was defined on the basis of patients’ records and information about missing visits; unexplained treatment interruptions or other problems related to treatment were collected. In most of the cases, the estimation of adherence was straight forward, though some cases were excluded due to unfeasibility of determination, and some were more difficult to classify. Since the same criteria were used for the whole study population and only by one person, no systematic bias should have occurred. The consequence of such *Non-differential misclassification* is that “the effect estimate is biased towards the null” (506). According to Webb et al. it means that the true association is likely to be stronger than that observed. I use this explanation to support the accuracy of our findings.

1.12.4.3 Results and discussion

The first three cases of C49 developed TB in 1996. The source case of C49 was a 19-year-old male from Zaire while the next five cases lived in the same residential hotel. In the following two years, an additional 6 immigrants from Somalia developed TB due to C49 strain. Three of them had also previously lived in the residential hotel. The transmission of C49 continued into the suburbs of Stockholm. The spread of C49 went on uncontrolled until 2002, when the first interventions to stop continued transmission were implemented. However in 2005, C49 caused an outbreak in at a day care center where 19 children contracted active TB from their assistant who had epidemiological

links to other cases in C49 and was originally from Somalia. The genomic pattern of the outbreak strain has stayed practically unchanged with regard to drug resistance, IS6110 restriction fragment length polymorphism and spoligotyping patterns. Complete genome sequence analyses of the source isolate and two other isolates, sampled nine years after the source case was diagnosed, demonstrated that this outbreak strain appears to be genetically very stable, yet easily transmissible (507). While the genetic analysis could not clarify the reasons for the expansion of this strain, the epidemiological investigation revealed several social networks, partly interconnected, where extensive transmission has been taking place. Several shortcomings in the case-management of patients were also discovered, as well as a high rate of non-adherence with TB treatment.

The findings may be summarized as follows:

1. Underuse of sputum as a diagnostic material, especially before patients were admitted to a TB clinic but also in the TB clinics within Karolinska hospital in Solna (Hospital N, Paper II). For example, only 71% of TB patients had their sputum examined (Paper I).
2. Non-adherence was recorded in 40 % of cases. There was a significant association between non-adherence and poor treatment outcome and not having CoTr executed when indicated. Also the results of the executed CoTr in non-adherent ICs were inferior, regarding nr of contacts etc.
3. There was also a strong connection between non-adherence and expansion of C49. Though there was no demonstrable difference relating to sputum status between non-adherent and adherent ICs, the proportion of contacts to non-adherent ICs who had active TB was significantly higher compared to adherent ICs.
4. Transmission within a network (group of people, often friends, colleagues, with common social denominator such as place where they meet) undermines the significance of a single IC: this could explain the comparable outcomes of CoTr in smear-positive IC vs. smear-negative/smear not taken. Another explanation is that many patients did not have their sputa examined. Therefore the “true” contagiousness of a case can have been misjudged. In the Introduction (Nosocomial transmission) we reported about a new approach to identify possible “disseminators” by analyzing their capacity to produce aerosol (381). That could also be a possible explanation for the varying proportions of infected contacts.

5. Various routes of transmission and different networks within C49 were identified and described. The finding that 12 individuals from two separate households, who lived in the same building and used the same stairway became part of the cluster, is shocking.
6. Finally, we identify serious pit-falls in case-management of TB patients in Stockholm including inferior performance of CoTr activities.

1.12.5 Pit-falls of TB control in a Stockholm hospital (Paper III)

1.12.5.1 Setting and study population

With the knowledge of serious deficiencies in TB control in Stockholm in relation to C49 investigation (Paper I) it did not come unexpectedly that shortcomings in TB control could occur in other situations and settings in Stockholm. The impact of nosocomial transmission was not examined in the C49 context but unsatisfactory TB control in the community implies also substandard control in health-care setting (423). Seven cases of active TB among HCWs and fellow patients, where 6 out of 7 were confirmed to be identical by Mtb culture and RFLP, occurred within 10 months after the diagnosis of a HIV patient who died of PTB after prolonged hospitalization. The patients and HCWs were part of the outbreak which took place in a ward where 16 patients were hospitalized at the time of the outbreak, with maximum two patients per room. The number of staff per shift varied from 6-8 during the day shifts and 3-4 during the night shifts. The ward belonged to the Unit of Infectious Diseases located at Karolinska University Hospital in Huddinge.

The study population comprised everyone who) was in confirmed or probable contact with the source case both in the ward and outside where the latter included social contacts of the source case. The exposure period was 25 days, equaling the time period starting with admission and ending with the death of the source case. The factual period of transmission in the ward was probably a few days shorter, due to a short period of isolation of the source case initially. Outside the ward the period of transmission

1.12.5.2 Results and Discussion

Delayed diagnosis and treatment of TB is associated with increased in-hospital mortality and further spread of the disease. We describe an outbreak of TB in a tertiary referral hospital in Stockholm resulting in 7 secondary cases among patients and

HCWs. The missed TB diagnosis not only contributed to death of the source case but caused a lot of suffering for both fellow patients and HCWs in the ward.

Most TB outbreaks in hospital can probably be prevented by early diagnosis and prompt isolation of infectious cases. Strict adherence to infection control routines for isolation, diagnostic procedures, staff protective measures as well as CoTr is of outmost importance. The most important infection control measure is timely isolation of patients who may be contagious. Early treatment reduces infectivity furthermore and improves prognosis. Occurrence of nosocomial outbreaks declined sharply in the United States after implementation of infection control guidelines in the mid-1990s.

TB should be investigated and excluded in an HIV-positive patient with injection drug use (IDU) and presenting with cough, weight loss and pulmonary infiltrates. In this context it is difficult to comprehend why isolation was discontinued before TB was ruled out. Immune-compromised individuals regularly treated on the ward where the outbreak occurred were put at danger due to the both delayed diagnosis of the IC and discontinuation of isolation measures. The infectiousness of IC was thereafter misjudged and none of the fellow patients were included in the initial CoTr.

1.12.6 Paper IV: Treatment of LTBI

1.12.6.1 Material and Methods

Study design and setting: The study population consists of 415 consecutive patients treated for LTBI at TB Clinic, Karolinska University Hospital Solna, Stockholm between March 2002 and December 2007. The diagnosis of LTBI was based on TST, CXR and medical evaluation. The chart review assessment of the study subjects was conducted. During the study period 323 cases of active TB were treated at the study clinic. The patients were scheduled for monthly visits to a specialized TB nurse. A standardised case record form was developed to review medical records. All charts were then reviewed by B. Kan in order to provide uniform evaluation of treatment completeness. Patients were identified using a hospital database.

1.12.6.2 Results and discussion

This study illustrates the complexity of the problem with completion of TLTI. Many of the patient characteristics are correlated and thus confound statistical modelling. However, we demonstrated several clinically relevant patients' characteristics that were

associated with non-completion. Cause of screening and referral was an important indicator of completion. Anti-TNF-alpha candidates had better completion rates than other subgroups. The proportion of completers increased during the study period. This improvement appeared to be caused by an increase in the proportion of Europeans over studied period. The estimated ORs for non-completion were highest among “Asylum seekers”, in the age group 15-25 years and in patients from Somalia and the Middle East. In the presence of TD, being Somali represented an additional risk for Non-completion. Our results indicate that delaying TLTBI may affect adherence negatively. Since many dropouts occurred within the first month of the treatment course, close follow-up during the first month seems essential.

To our knowledge this is the first study to investigate the role of delaying LTBI treatment on adherence. This is also one of very few studies on adherence and LTBI in a low incidence European setting

1.12.7 Final conclusions and suggestions (Paper I-IV)

- Previous studies have not investigated the outcome of contact tracing in connection with non-adherence of index cases. This connection might seem obvious but it is known that non-adherence is notoriously difficult to predict (150). We have shown that problematic adherence is usually discovered late in the course of treatment, while patient’s cooperation in contact tracing is usually completed within 1- 2 weeks after treatment initiation. We therefore suggest the outcome of CoTr as an additional tool to predict adherence (Paper II).
- The investigation of multiple outbreaks with C49 summarized and analyzed in our studies has had a direct effect on improvement of TB care in Stockholm: treatment of LTBI was initiated, TB clinics were reorganized and tablet dispensers introduced.
- C49 is probably not the largest cluster previously described but to our knowledge hardly any large clusters has been studied in such detail and followed during such a long period. The results presented provide a unique insight into TB transmission dynamics in a low-endemic urban setting. The unexpectedly high number of patients who originated from high TB incidence countries and who acquired TB in a low-incidence setting is unique.

- Routines for TB control should be continuously updated and proper resources should be allocated as TB control is a resource-demanding endeavor.
- TB strikes again when TB control measures fail. A large nosocomial outbreak has taken place in our clinic which is meant to have a high level of knowledge on how to prevent transmission of air-borne pathogens. Units for high risk respiratory isolation of patients with XDR-TB such as the unit in Linköping University Hospital should be constructed in Stockholm. We recently were forced to send two XDR-TB patients from the former USSR to the high risk isolation unit in unit in Linköping.
- For Swedish health staff that TB is an important occupational risk in a setting where TB and immunocompromised patients are treated. We need to reconsider our routines concerning investigation of TB exposure in hospital settings. I suggest that all exposed HCW should be tested with IGRA adjacent to employment. Those who test negative initially after possible TB exposure should be retested at the end of window period. Those for whom the previous tests results are unknown should be tested when exposure has been discovered, without delay. If they are found to be negative, they should be retested at the end of window period. This is only the way to identify recent converters where a generous attitude towards TLTBI should be taken.
- Screening combined with treatment for LTBI in asylum-seekers from high-incidence countries such Somalia needs a renewed appraisal. This intervention should be prioritised by public health authorities and energetically implemented.
- The absence of a continuous, external evaluation of control measures against TB in Stockholm, not least CoTr activities, and is worrisome. There is need for intervention studies a quality control of CoTr activities including the occurrence of renewed CoTr after results of molecular genotyping of *Mtb* isolates are available? Limitations of traditional CoTr strategies, shown in Paper II have also been demonstrated in other studies. Novel methods, such as social network analysis, genomics and geographic information

system, yet unknown in Stockholm, have had a direct impact on local control programmes in the settings where they have been tested (403).

- All patients in Stockholm receive their medications in tablet dispensers today. Though this measure is perceived positively by medical staff and probably most of the patients, it is not known if this intervention improves adherence. Though, it facilitates monitoring since patients are expected to refill their boxes every week or every second week. However, pill dispensers may also induce a sense of control in medical staff when in reality the treatment completion is unverified. It is timely to discuss the introduction of DOT. The story of C49 indicates that alternative way of treating TB than self-administered treatment might have reduced transmission and improves outcomes. I do not suggest DOT for everyone but we need an option. Limited DOT has been used in Stockholm in selected cases but to my knowledge there is no local guideline on this matter.
- The possible social and political implications of these studies should be mentioned. For a long time transmission was taking place within intensively socializing but still isolated networks. However this “isolation” probably has stimulated persistence of transmission, contributing to delays in diagnosis and non-adherence. Behind closed doors, the treatment and prevention of TB in foreign-born are sometimes regarded as protecting “us” against “them.” We tried to elucidate this painful subject in the Introduction and in Paper **IV**.
- Finally, the social and legal situation of asylum seekers and undocumented migrants with confirmed and suspected TB needs to be improved. There is an urgent need for legal actions to protect them while being investigated and treated for TB will improve TB control in this group as well as in the community.

ACKNOWLEDGEMENTS

I want to thank:

Judith Bruchfeld for helping me to control my weakness and release my strength, for keeping my work and our clinic afloat

Mats Kalin for many things but most of all for teaching me in a discrete way how to conduct myself, giving me the idea to study TB and for always being close and present

Boris Freidlin for Pavlovsk and statistics

Elsa Tynell, Gabrielle Fröberg and Andreas Berge for holding my hand

Ingela Berggren for excellent comments

Anna Vikman, with hope to continue studying TB together

Marie Dahlin for reviewing Paper II

Sergei Kan, Nicklas Johansson, Erja Chryssanthou, Kristian Ängeby, Elisabeth Berg, Ramona Groenheit, Hanna Jensen, Anna James, Jerker Jonsson, Jim Werngren, Gunnar Unge, Lisa Franzen, Katherine Nyberg, Jan Bellbrant, Joachim Rosenquist, Christian Olsson, Maria Engholm, Monica Modin, Anna Färnert, Anna Larsson, Carl Spindler, Gerda Bergström, Lillemor Melander, Marie Källberg, Mikael Blad, for their assistance and advice

Lennart Östlund, Birgitta Hellerqvist, Jan Carlson, Marina Forström, Anne Rasikari, Eva Ka Johansson for help with my schedule and other formalities of working life

To my mentor Sol-Britt Lonne Rahm for our conversations

My co-authors, tutors, advisers Gunilla Källenius, Gunnar Dahlström, Sven Hoffner, Inger Julander, Victoria Romanus, Björn Petrini, Olle Widström, Rutger Bennet, Gunnar Boman, Jonas Hedlund, Britt Noreen, Kai Rosnell, Sune Lidén, Erik Berglund and Lars Frithiof.

Kristina Broliden, Anders Ekbohm, Michael Fored for creating an institution which I am proud to be part of.

My parents Ada and Aleksandr for guidance love and help

My wife Malin for love and support

Swedish Heart-Lung Foundation for more than one hundred year of fight against TB, for taking care of Sweden and its researches.

Medical libraries in Uppsala and Stockholm for tutorship and inspiration

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