Tampere University

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Quality of Tuberculosis Treatment in Finland

Treatment outcomes, recurrent tuberculosis and risk factors for non-successful treatment

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ACADEMIC DISSERTATION
To be presented, with the permission of
the Faculty of Medicine and Health Technology
of Tampere University,
for public discussion in the auditorium F 115
of the Arvo building, Arvo Ylpön katu 34, Tampere,
on 11th December 2020, at 12 o'clock.

ACADEMIC DISSERTATION
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The originality of this thesis has been checked using the Turnitin OriginalityCheck service.

Cover design: Roihu Inc.

ISBN 978-952-03-1721-8 (print)
ISBN 978-952-03-1722-5 (pdf)
ISSN 2489-9860 (print)
ISSN 2490-0028 (pdf)
http://urn.fi/URN:ISBN:978-952-03-1722-5

PunaMusta Oy – Yliopistopaino
Vantaa 2020

ABSTRACT

Adequate treatment of tuberculosis (TB) is important both to cure the patient and to decrease airborne transmission of this infectious disease. The most common way to evaluate the quality of tuberculosis treatment is by presenting treatment outcomes. Outcomes reflect treatment efficacy in a shorter period; thus, more permanent cures can be assessed by evaluating TB recurrence. It is important to distinguish the two ways of recurrences, relapses and re-infections when evaluating the quality of TB treatment, because only relapses reflect shortcomings in the treatment of the previous TB episode.

The aim of this thesis was to evaluate the quality of tuberculosis treatment in Finland by assessing treatment outcomes at one year and recurrence of tuberculosis in a longer period and to determine risk factors for non-successful treatment. This study additionally aimed to distinguish relapses from re-infections among recurrent cases and to evaluate the validity of treatment outcomes notified and the adequacy of TB treatments prescribed in the episodes preceding recurrence.

This retrospective cohort study is based on TB cases registered in the National Infectious Disease register. Treatment outcomes were evaluated for 1416 microbiologically confirmed pulmonary TB cases, excluding cases with multi-drug resistant strains, in a study period of eight years. TB recurrence was evaluated among the cohort of 8084 TB cases in a study period of 19 years. Relapses were distinguished from re-infections among recurrent cases when the isolates of the causing bacteria, *Mycobacterium tuberculosis,* were available for genotyping from both TB episodes. A novel genotyping method, whole genome sequencing, was used in this study.

The treatment outcome was notified as successful in 75% of cases. Death as outcome was common with 16% proportion, whereas the other most unsuccessful outcomes, failed and lost to-follow-up, constituted only 1% combined. Less than 1% of TB cases recurred during the study period. Among the recurrent cases, more than 80% were due to relapses. Male gender was a risk factor for both death as outcome and for TB recurrence. Older age increased the risk for death, whereas younger age increased the risk for TB recurrence. Comorbidities were associated

with the risk of death in all age groups. Drug resistance predicted non-successful treatment outcomes other than death. Pulmonary site of disease strongly correlated with the risk for TB recurrence in the long run. Furthermore, substantial deviations from guidelines in notified treatment outcomes and prescribed treatments in the episodes preceding recurrence among recurrent TB cases were observed.

In conclusion, the rate of successful treatment outcomes is clearly below the target of 85% set by World Health Organization. This is mainly due to the high proportion of death as outcome. TB recurrence is rare. The high mean age among Finnish-born cases and the presence of comorbidities at least partially explain the high proportion of death as the outcome. However, because most fatal cases die early, better awareness of TB among the population and health-care professionals is needed to reach early suspicion, timely diagnosis and treatment of TB. Furthermore, special attention should be paid to choosing correct treatment regimens and to promoting treatment adherence using directly observed treatment for younger male patients and patients with drug-resistant strains, because they show high relative risks for non-successful treatment. Finally, shortcomings observed in allocation of treatment outcomes in the cohort and in prescribed treatment regimens in episodes preceding recurrence show that a need exists to review national guidelines and to further train the physicians treating TB cases.

TIIVISTELMÄ

Tuberkuloosin tehokas hoito on tärkeää sekä potilaan parantamiseksi että uusien tartuntojen estämiseksi, koska tuberkuloosi tarttuu ilman välityksellä ihmisestä toiseen. Tuberkuloosin hoidon laatua arvioidaan yleisimmin määrittämällä hoidon lopputulos. Tämä kuvastaa hoidon tehoa lyhyellä aikavälillä. Pysyvämpää paranemista voidaan tarkastella tutkimalla tuberkuloosin uusiutumista. Uusiutuminen voi johtua joko aiemmin hoidetun taudin uudelleen aktivoitumisesta tai uudesta tartunnasta. Tutkittaessa tuberkuloosin hoidon laatua on tärkeää erottaa nämä syntymekanismit, koska vain taudin uudelleen aktivoituminen kuvastaa aiemman tuberkuloosiepisodin hoidon riittämättömyyttä.

Tämän väitöstutkimuksen tarkoituksena oli tarkastella tuberkuloosin hoidon laatua Suomessa arvioimalla hoidon lopputuloksia vuoden kohdalla hoidon alusta laskien, tuberkuloosin uusiutumista pidemmällä aikavälillä sekä hoidon epäonnistumisen riskitekijöitä. Uusiutuneiden tapausten kohdalla oli tarkoituksena erotella taudin uudelleen aktivoitumisesta ja uudesta tartunnasta johtuvat tapaukset. Lisäksi arvioimme ilmoitettujen hoidon lopputulosten sekä uusiutuneille tapauksille edeltävässä tuberkuloosiepisodissa annetun hoidon asianmukaisuutta.

Kyseessä oli taannehtiva kohorttitutkimus, joka pohjautui valtakunnalliseen tartuntatautirekisteriin ilmoitettuihin tuberkuloositapauksiin. Tutkimuksessa arvioitiin kahdeksan vuoden tutkimusjakson aikana rekisteriin ilmoitettujen mikrobiologisesti varmennettujen keuhkotuberkuloositapausten hoidon lopputuloksia. Tapauksia oli 1416, kun monilääkeresistentit tuberkuloosikannat oli suljettu pois tutkimuksesta. Tuberkuloosin uusiutumista tutkittiin 19 vuoden tutkimusjakson aikana, ja tässä kohortissa oli mukana 8084 tuberkuloositapausta. Tuberkuloosin uusiutumisen syntymekanismi pystyttiin tutkimaan niiltä tapauksilta, joilla oli tuberkuloosia aiheuttavan bakteerin, *Mycobacterium tuberculosis'n,* kannat tallessa molemmista tautiepisodeista. Erottelu tehtiin käyttämällä kokogenomisekvensointia, joka on uusi genotyypitys-menetelmä.

75 %:lle tapauksista oli ilmoitettu hyvä hoidon lopputulos. Kuolema ennen hoitoa tai hoidon aikana oli toiseksi yleisin hoidon lopputulosryhmä 16 %:n osuudella. Muut erityisen huonot hoidon lopputulokset, epäonnistunut hoito ja hoito jäänyt kesken, olivat harvinaisia yhteensä vain yhden prosentin osuudella. Alle prosentilla

tapauksista tuberkuloosi uusiutui tutkimusjakson aikana. Yli 80 % uusiutumisista johtui taudin uudelleen aktivoitumisesta. Miessukupuoli oli sekä kuoleman että tuberkuloosin uusiutumisen riskitekijä. Vanhemmilla ikäryhmillä oli suurempi kuolemanriski, kun taas nuoremmilla tapauksilla oli suurempi tuberkuloosin uusiutumisen riski, vaikka eroavuudet seuranta-ajoissa ikäryhmien välillä otettiin huomioon. Liitännäissairaudet lisäsivät kaikkien ikäryhmien riskiä hoidon lopputulokselle kuollut. Lääkeresistentti tuberkuloosikanta ennusti muita huonoja hoidon lopputuloksia paitsi kuolemaa. Tuberkuloosin uusiutumisen riski pidemmällä aikavälillä oli voimakkaasti suurempi keuhkotuberkuloosia sairastavilla kuin keuhkojen ulkopuolista tuberkuloosia sairastavilla. Lisäksi havaitsimme, että ilmoitetut hoidon lopputulokset sekä uusiutuneiden tapausten edeltävässä tuberkuloosiepisodissa saama hoito poikkesivat merkittävissä määrin kansallisista ja kansainvälisistä ohjeista.

Yhteenvetona voidaan todeta, että hyvän hoidon lopputuloksen osuus on selvästi pienempi kuin Maailman terveysjärjestön WHO:n tavoitteeksi asettama 85 %. Tämä johtuu pääasiassa kuolleiden suuresta osuudesta. Tuberkuloosin uusiutuminen on harvinaista. Kuolleiden suurta osuutta selittävät ainakin osittain suomalaissyntyisten tuberkuloosipotilaiden korkea keski-ikä sekä liitännäissairauksien yleisyys tuberkuloosia sairastavilla. Koska suurin osa kuolemantapauksista ilmenee joko ennen tuberkuloosin hoidon aloitusta tai kahden ensimmäisen hoitokuukauden aikana, tuberkuloosin tunnettavuuden parantaminen sekä väestön että terveydenhuoltohenkilöstön keskuudessa on tarpeen, jotta tätä harvinaista tautia osataan epäillä ja diagnosoida sekä aloittaa hoito ajoissa. Lisäksi on tärkeää huolehtia oikean lääkeyhdistelmän valinnasta sekä hoidon tukemisesta käyttäen valvottua lääkehoitoa erityisesti nuoremmilla miehillä ja henkilöillä, joilla on lääkeresistentti kanta, koska näillä potilasryhmillä on selvästi lisääntynyt hoidon epäonnistumisen riski. Havaitsemamme poikkeamat ilmoitetuissa hoidon lopputuloksissa sekä uusiutuneiden tapausten edeltävän tuberkuloosiepisodin hoidoissa viittaavat siihen, että tuberkuloosia hoitavat lääkärit tarvitsevat lisäkoulutusta ja kansallisia ohjeita on syytä tarkentaa.

CONTENTS

ABBREVIATIONS

BCG	Bacillus Calmette-Guérin
COPD	chronic obstructive pulmonary disease
DOT	directly observed treatment
DST	drug susceptibility testing
ECDC	European Center for Disease Prevention and Control
EU/EEA	European Union/European Economic Area
HILMO	Finnish Hospital Discharge Register
HIV	human immunodeficiency virus
ICD10	International Classification of Diseases, 10th revision
IGRA	interferon gamma release assay
IQR	interquartile range
IS6110-RFLP	IS6110-restriction fragment length polymorphism
IUATLD	International Union against Tuberculosis and Lung Disease
LTBI	latent tuberculosis infection
MDR	multi drug resistant
MIRU-VNTR	multilocus variable number tandem repeat genotyping
M. tuberculosis	*Mycobacterium tuberculosis*
NAAT	nucleic acid amplification test
PCR	polymerase chain reaction
RR	risk ratio
RRR	relative risk ratio
SNPs	single nucleotide polymorphisms
TB	tuberculosis
THL	Finnish Institute for Health and Welfare
TTR	National Infectious Disease Register
WGS	whole genome sequencing
WHO	World Health Organization
XDR	extremely drug resistant
95% CI	95% confidence interval

ORIGINAL PUBLICATIONS

I Korhonen V., Lyytikäinen O., Ollgren J., Soini H., Vasankari T., Ruutu P. (2020): Risk factors affecting treatment outcomes for pulmonary tuberculosis in Finland 2007-2014: a national cohort study. BMC Public Health 20:1250.

II Korhonen V., Soini H., Vasankari T., Ollgren J., Smit P.W., Ruutu P. (2017): Recurrent tuberculosis in Finland 1995-2013: a clinical and epidemiological cohort study. BMC Infectious Diseases 17:721.

III Korhonen V., Smit P.W., Haanperä M., Casali N., Ruutu P., Vasankari T., Soini H. (2016): Whole genome analysis of *Mycobacterium tuberculosis* isolates from recurrent episodes of tuberculosis, Finland, 1995-2013. Clin Microbiol Infect 22(6):549-554.

1 INTRODUCTION

Tuberculosis (TB) is currently a poorly known disease among inhabitants of the Western world. However, TB still ranks as one of the top ten causes of death globally according to the World Health Organization (WHO) (WHO, 2019a). TB afflicts especially populous and poorer areas in the world (Reid et al., 2019). In high-income settings, most TB cases are among certain more vulnerable risk groups, such as immigrants from countries with a high TB-incidence, persons with social risk factors or with an impaired immune system due to a disease, e.g. HIV-infection and diabetes, or receiving immune suppressive medication (Lönnroth et al., 2015). A major concern in Europe is the emergence of multi-drug resistant (MDR) strains, especially in countries of the former Soviet Union (WHO, 2019a).

The rate of annually diagnosed TB cases in Finland has declined from approximately 7000 to the current 250 during the last 60 years (Jaakola et al., 2018; Tala-Heikkilä, 2003). The major risk group for TB is elderly Finnish-born persons who become sick due to reactivation of an infection transmitted decades ago. However, the epidemiologic situation is changing with increasing immigration and a decreasing TB incidence among the native population (Räisänen et al., 2015). Immigrants constitute more than a third of annual TB cases currently (Jaakola et al., 2018).

TB is caused by a slow-growing bacterium, called *Mycobacterium tuberculosis (M. tuberculosis)*, and its transmission is airborne from human to human. A long treatment period of at least 6 months with multiple drugs is needed to ensure permanent eradication of the bacteria (Migliori et al., 2018). Adequate TB treatment is a key element both to heal the patient and to decrease transmission together with early diagnoses and appropriate contact investigations. WHO recommends evaluating treatment outcomes for all TB cases (WHO, 2014).

Evaluation of treatment outcomes reflects the efficacy of treatment in a shorter period. More permanent cures can be assessed by evaluating TB recurrence (Rosser, Marx, & Pareek, 2018). A recurrent TB case can occur in two ways; as re-infection or as relapse. Re-infection means that a person gets a new infection with a new strain of *M. tuberculosis* after being treated for tuberculosis. Relapse occurs due to

endogenous reactivation with the same strain of *M. tuberculosis* as in the former TB episode, which reflects an insufficient treatment of the former TB episode.

Distinguishing relapses from re-infections can be established by genotyping methods when isolates of *M. tuberculosis* are available from both TB episodes. Whole genome sequencing (WGS) is a new method for genotyping with more discriminatory power compared to traditional genotyping methods (Nikolayevskyy et al., 2019). WGS is expected to replace the currently used heterogeneous methods for species detection, rapid drug resistance prediction and genotyping (Amlerova et al., 2018; Quan et al., 2018).

Evaluating the quality of TB treatments is important to identify high-risk groups and to improve national TB control programs. This dissertation was carried out to evaluate the quality of tuberculosis treatment in Finland by assessing treatment outcomes at one year and recurrence of tuberculosis in a longer period and to determine risk factors for non-successful treatment. Relapses were distinguished from re-infections using WGS.

2 REVIEW OF THE LITERATURE

2.1 Tuberculosis

2.1.1 History

Tuberculosis is an ancient disease among humankind. It has been stated that early hominids in East Africa were infected by an early progenitor of *M. tuberculosis* three million years ago (Gutierrez et al., 2005). Typical skeletal deformities for tuberculosis have been found in Egyptian mummies dating back to 2400 BC, and existence of *M. tuberculosis* complex in mummies has been shown using modern molecular technology methods, nucleic acid amplification and genotyping (Morse et al., 1964; Zink et al., 2003). Additionally, 2300-3300 years old written documents describing tuberculosis have been found in China and India (Barberis et al., 2017).

The incidence of tuberculosis in Europe was proportionately low in the Middle Ages (Amlerova et al., 2018). The incidence subsequently started to rise and was augmented by poor social conditions during the industrial revolution (Barberis et al., 2017). Tuberculosis especially afflicted lower social groups: up to one third of employees and tradesmen compared to one sixth of the upper class died of TB at the beginning of the 19th century (Barberis et al., 2017). The etiology of TB was especially debated during the 19th century. Finally, the infectious etiology was confirmed when the causative bacteria, now called *Mycobacterium tuberculosis,* was isolated by Robert Koch in 1882 (Koch, 1882). This discovery was a milestone for the study of TB and was followed by several inventions, such as the theory of transmission via droplet infection in 1897, the development of vaccine Bacillus Calmette-Guérin (BCG) in 1921 and, finally, the invention of the first anti-tuberculosis drugs, streptomycin in 1944 and isoniazid in 1952 (Daniel, 2006; Netea et al., 2014).

2.1.2 Epidemiology

Approximately 10 million people are estimated to fall ill and almost 1.5 million people to die with TB annually, which ranks TB as the number one cause of death among infectious diseases globally (WHO, 2019a). TB especially afflicts populous and poorer areas in the world: two thirds of new cases occur in eight countries of South Asia and Africa, such as India, China, Indonesia and Nigeria (Reid et al., 2019). TB seems to be more common among males: more than 60% of notified cases are males (WHO, 2019a).

Tuberculosis was declared a public health emergency by WHO in 1993 (Nakajima, 1993). WHO additionally launched the Stop TB Strategy for global tuberculosis control in 2006 and the newest END TB Strategy in 2015 with the target of reducing TB incidence by 90% and TB deaths by 95% by year 2035 (WHO, 2006, 2015). TB incidence has decreased by approximately 1.4%/year and mortality by 3%/year since year 2000, yet the decline is far from reaching the targets (WHO, 2019a). Two particular reasons for this are the emergence of human immunodeficiency virus (HIV) and TB co-infection, especially in sub-Saharan Africa, and the MDR-TB strains, i.e., strains resistant to two of the most effective TB drugs, rifampicin and isoniazid, in India, China and countries of the former Soviet Union (Furin et al., 2019).

The incidence of TB in Finland was about 170/100 000 in 1960, the same magnitude as in many developing countries nowadays (Tala-Heikkilä, 2003). The decline of TB incidence in Finland has been one of the greatest declines in Europe. Finland reached the target of becoming a low-incidence country in 2001 with less than 10/100 000 new cases diagnosed annually (Hulkko et al., 2010). Since then, the incidence has still decreased to current 4-5/100 000 (Jaakola et al., 2018). In contrast to many European countries, such as Sweden, Denmark and the Netherlands, where TB mainly occurs among immigrants, tuberculosis in Finland is still a disease of elderly natives due to the reactivation of an infection transmitted decades ago (ECDC/WHO regional office for Europe, 2019). However, the proportion of immigrants among TB cases has increased from 4% in 1995-1996 to 40% in 2017 (Jaakola et al., 2018; Vasankari et al., 2007). Despite the alarming MDR situation in the Russian Federation, only 5-8 MDR-cases are diagnosed annually in Finland. Cases with extremely drug resistant isolates (XDR), i.e., MDR isolates with additional resistance to at least one fluoroquinolone and one injectable drug, have been sporadic (Jaakola et al., 2018; Vasankari et al., 2012).

2.1.3 Mycobacteria

Mycobacteria are aerobic gram-positive bacteria with about 200 different species recognised so far (Forbes et al., 2018; Tortoli, 2014). Tuberculosis is caused by closely related mycobacteria species belonging to the *M. tuberculosis* complex, which includes, in addition to the most prevalent species, *M. tuberculosis,* also *M. bovis*, *M. bovis BCG*, *M. africanum*, *M. microtii* and *M. canettii* (Forbes et al., 2018). Leprosy, a still existing disease especially in India, Brazil and Indonesia, is caused by *M. leprae* (Naaz et al., 2017). Most of the known mycobacteria species are not transmitted between humans and are called non-tuberculous or environmental mycobacteria.

2.1.4 From transmission to infection and disease

Transmission of tuberculosis can be described as a simplified process: a person with TB in the respiratory tract generates infectious particles that are aerosolised through forceful exhaling, such as coughing or sneezing; another person who inhales these aerosolised particles containing *M. tuberculosis* bacteria may become infected and develop the active disease (Churchyard et al., 2017; Turner et al., 2017). Many factors can affect this process.

Only persons with active pulmonary or laryngeal TB can transmit the disease with the exception that TB outside the respiratory tract may be transmitted in operations or other procedures when aerosolised particles are formed. Infectiousness depends on the state of the disease, and persons with sputum smear positivity and cavitary lung lesions in chest radiography are considered particularly contagious (Churchyard et al., 2017; Zelner et al., 2014). The events in humans after exposure to *M. tuberculosis* are not well understood (Cadena et al., 2016). The alveolar macrophages of an exposed person play a major role in trying to destroy inhaled particles containing *M. tuberculosis* bacteria at first, and T-cell-mediated immune response is developed in about six weeks (Cadena et al., 2016). The bacteria may be destroyed by granuloma formation. If not destroyed, the bacteria may spread to distant parts of the body. Latent tuberculosis infection (LTBI) is a state of persistent bacterial viability, immune control and no evidence of active tuberculosis, and it can last for decades, even throughout the lifetime (Getahun et al., 2015). Interferon gamma release assay (IGRA) and tuberculin skin test (Mantoux) reflect the T-cell response to *M. tuberculosis* antigens and become positive when a measurable T-cell response has been induced (Cadena et al., 2016). LTBI can be detected using the IGRA or tuberculin skin test.

A systematic review including 95 studies from countries with a low or moderate incidence of TB described that the prevalence of LTBI was 28% among contacts of patients with TB (Fox et al., 2013). Active TB disease was diagnosed for 1.4% of contacts during the study period. The period with the highest risk to develop active TB is the first year after exposure, but the risk lasts throughout the lifetime and the overall risk for active TB is about 3% (Fox et al., 2013). Factors increasing the risk of LTBI proceeding into active disease are, e.g., impaired immune system because of a disease, such as HIV-infection and diabetes, undernutrition or immune suppressive medication, smoking and belonging to the under 5 years age group (Al-Rifai et al., 2017; Dowdy et al., 2014; Zhang et al., 2017).

2.1.5 Clinical picture

Tuberculosis infection can affect almost any organ or tissue of the human body. The most prevalent site of the disease is in the lungs. TB is diagnosed only outside the lung parenchyma, called extrapulmonary TB, in approximately 15-20% of cases, with the most common sites of disease recognised as lymph nodes, pleura, bones and joints, genitourinary tract and meninges (Peto et al, 2009; WHO, 2019a). Factors favoring the development of extrapulmonary disease, instead of pulmonary disease, are female gender and origin from high-incidence country, which may reflect a shorter time since infection and the existence of certain lineages of *M. tuberculosis* (Al-Ghafli et al., 2019; Click et al., 2012; Peto et al., 2009).

The TB symptoms are the same as in many other chronic infections and some other diseases, such as fever, night sweats, loss of weight and fatigue. Patients with pulmonary TB often have a cough, which is productive or dry and has lasted for more than 2-3 weeks, dyspnea and chest pain as well as occasional haemoptysis. Extrapulmonary TB manifestations may represent with, e.g., pain and edema in the affected organ. Sometimes patients with active TB do not recognise any symptoms, especially those who are immunocompromised (Storla et al., 2008).

2.1.6 Diagnostic procedure

A chest radiography should always be taken when a suspicion for TB has been awaken due to, e.g., typical symptoms and/or risk factors. Typical findings include pneumonia-like infiltrates, nodules and cavitary lesions, especially in the upper lobes or superior segments of lower lobes. Adenopathy, pleural effusion and widely

distributed small nodules (miliary TB) may also be detected (Rozenshtein et al., 2015). High-resolution computed tomography of the chest is more sensitive and specific than conventional chest radiography for diagnosing pulmonary TB (Raghuvanshi et al., 2016).

An intention to acquire at least three sputum samples is crucial, especially when pulmonary TB is suspected (Migliori et al., 2018). Expectorating sputum samples can be induced by inhaling hypertonic saline. Bronchoscopy is established to obtain samples by aspirating bronchial washings, as well as bronchoalveolar lavage and by needle aspiration when needed, if satisfactory sputum samples cannot be produced (Mondoni et al., 2017). Obtaining samples from affected organs is crucial when diagnosing extrapulmonary TB. Furthermore, HIV should be tested for all persons suspected of having TB (Migliori et al., 2018).

The diagnosis can be based on clinical and radiological features when TB cannot be microbiologically confirmed (Migliori et al., 2018). Tissue samples obtained by biopsy, surgery or at autopsy that show a typical histological picture, granulomas with caseating necrosis, can endorse the diagnosis of TB, even though not specific for TB. Indefinite cases may be confirmed by a positive response to TB treatment.

2.1.7 Laboratory methods for diagnosis and drug susceptible testing

Smear microscopy for acid fast bacilli is the most common way to detect mycobacteria directly from clinical specimens. Smear microscopy is non-sensitive and non-specific even though fast and inexpensive. (Eskola et al., 2004).

M. tuberculosis complex can be directly detected and distinguished with high sensitivity and specificity from other mycobacteria in smear positive samples using nucleic acid amplification tests (NAAT), such as the widely used commercial test, GeneXpert® (Yan et al., 2016). NAA tests are rapid, which is particularly important for both severely ill patients and for pulmonary TB cases with a positive sputum smear, because the patient is considered highly contagious if mycobacteria belong to *M. tuberculosis* complex. NAA tests are also used for rapid diagnostics of smear negative samples, but the sensitivity of NAA tests is lower and depends on the type of sample (Altez-Fernandez et al., 2017; Pormohammad et al., 2019; Shaw et al., 2019; Tian et al., 2015). Commercial NAA tests, such as Xpert MTB/RIF®, can also detect mutations in the most common rifampicin resistance conferring gene, *rpoB*, which can be used as a proxy for MDR-TB.

Culture is still the gold standard for laboratory diagnostics of tuberculosis despite the advances in laboratory methods. It is rather sensitive and enables identification of species, drug susceptibility testing and the use of genotyping methods for, e.g., the evaluation of transmission chains (Forbes et al., 2018). However, culture is a slow method, because it takes usually 2-6 weeks for *M. tuberculosis* to grow sufficiently.

The species of mycobacteria, when cultured, can be identified rapidly using commercial tests such as Line-probe assays, based on direct probe hybridisation and sequence-based techniques (Forbes et al., 2018). When MDR is suspected on a clinical basis, e.g., immigrants from countries with a high proportion of MDR strains, or the result of Xpert MTB/RIF® shows resistance to rifampicin, an additional commercial rapid test, such as Hain genotype MTBDR plus®, can be performed (Dominguez et al., 2016). It detects mutations in the most common rifampicin and isoniazid resistance conferring genes, *rpoB*, *katG* and *inhA*. An additional test can be performed when MDR is strongly suspected for rapid detection of resistance to fluoroquinolones and injectable drugs (Dominguez et al., 2016). A consensus statement given by the Tuberculosis Network European Trials Group and Research Excellence to Stop TB Resistance announces that the final interpretation of drug resistance should currently be made by phenotypic drug susceptibility testing (DST) based on minimal inhibitory concentration analysis of cultured specimens (Dominguez et al., 2016).

2.2 Genotyping for TB isolates

The DNA sequence of an isolate is analysed by molecular methods and compared to the sequence of another isolate or a reference sequence in bacteria genotyping. Genotyping methods have been used since the early 1990s to distinguish strains of *M. tuberculosis* for epidemiologic research (Das et al., 1993; Hawken et al., 1993; Kato-Maeda et al., 2011). Genotyping methods have been applied for several purposes, such as investigation of TB outbreaks, assistance in contact tracing, detection of laboratory cross-contamination, characterisation of molecular epidemiology in certain populations and distinguishing relapses from re-infections among recurrent TB cases (Jagielski et al., 2014; Puustinen et al., 2003; Smit et al., 2014; van Soolingen et al., 1999).

2.2.1 Traditional genotyping methods

Several genotyping methods have been developed during the last decades. The most common methods used for *M. tuberculosis* are IS*6110*-restriction fragment length polymorphism (IS*6110*-RFLP), multilocus variable number tandem repeat genotyping (MIRU-VNTR) and spoligotyping (Amlerova et al., 2018). These methods are based on the analysis of differing numbers or locations of specific markers in the genome of *M. tuberculosis* (Bryant et al., 2013).

The first genotyping method, called fingerprinting, was based on polymerase chain reaction (PCR) amplification and restriction of certain sequences of *M. tuberculosis* followed by analysis by electrophoresis. IS*6110*-RFLP detects the distribution of markers that are insertion elements, i.e. fragments of genome capable of multiplying and inserting themselves anywhere in the genome. In MIRU-VNTR, the number of copies of tandem repeated sequences, which vary among strains, is detected by PCR. Polymorphism of a genomic region called direct repeat region, is analysed by spoligotyping (Amlerova et al., 2018; Ei et al., 2016; Jagielski et al., 2014).

The traditional genotyping methods have been internationally harmonised allowing for comparison of results obtained in different laboratories. However, traditional genotyping methods have major disadvantages. They detect only short parts of genomic DNA resulting in restricted data. Their ability to discriminate strains varies depending on the method and they have insufficient capability to distinguish strains that are genetically highly related. Furthermore, some methods are laborious, time consuming and require advanced technical equipment and software (Cabibbe et al., 2018; Quainoo et al., 2017). The cost-effectiveness of their use has also been questioned (Mears et al., 2014). Thus, better tools for genotyping are needed.

2.2.2 Whole genome sequencing

The DNA sequence of the whole genome of *M. tuberculosis* can be analysed using whole genome sequencing, except for the so-called repetitive sequences, which are hard to read. The sequence is compared to reference genome of *M. tuberculosis* and mutations in base pairs, i.e., single nucleotide polymorphism (SNPs), insertions and deletions can be detected (Cabibbe et al., 2018). WGS can be used to identify species of mycobacteria, to detect mixed infections and to detect mutations conferring drug resistance in addition to using WGS as a genotyping method (Cabibbe et al., 2018;

Pankhurst et al., 2016; van Soolingen et al., 2016; Zhang et al., 2013). WGS may also detect novel drug resistance mutations (Witney et al., 2017) .

Compared to traditional genotyping methods, WGS is more powerful for strain differentiation and gives more accurate information about transmission chains and clusters, thus avoiding erroneous assessment of clusters (Nikolayevskyy et al., 2019). The average mutation rate of *M. tuberculosis* genome varies between 0.3-1.1 SNPs/year (Bryant et al., 2013; Norheim et al., 2017; Walker et al., 2013). Among isolates from patients with confirmed epidemiologic links, the number of SNPs between isolates has varied from 0-5 (Bryant et al., 2013; Casali et al., 2016; Walker et al., 2013). Recent studies have proposed <6 SNPs and >12 SNPs as cut offs to indicate and exclude recent (in 3 years) transmission, respectively (Nikolayevskyy et al., 2019; Nikolayevskyy et al., 2016).

2.3 Treatment of tuberculosis

The standard treatment regimen for cases with drug-susceptible TB includes an intensive phase for 2 months with 4 anti-TB drugs, isoniazid, rifampicin, pyrazinamide and ethambutol. Pyrazinamide and ethambutol can be discontinued for consolidation phase lasting for four months once resistance to rifampicin and isoniazid is excluded (Migliori et al., 2018). In Finland, the use of a fourth drug, ethambutol, during the intensive phase was included in recommendations in 2013 (Sosiaali- ja terveysministeriö, 2013).

Recommendations for treatment prolongation vary, including cases with, e.g., cavitary or extensive pulmonary TB with persistently positive sputum culture at two months after treatment initiation, TB affecting the central nervous system, bone and joint TB and impaired immune system (American Thoracic Society, CDC, & Infectious Diseases Society of America, 2003; Horsburgh et al., 2015; Rajasekaran et al., 2018; Sosiaali- ja terveysministeriö, 2013; WHO, 2017b).

Adverse effects, such as nausea and vomiting, rash and hepatotoxic effects, are very common during TB treatment. A prospective cohort study, including cases with drug susceptible isolates with standard treatment, reported that at least one anti-TB drug had to be interrupted or discontinued due to adverse reaction for 15% of the cases (Lv et al., 2013).

WHO and European Center for Disease Prevention and Control (ECDC) currently recommend a patient-centered approach to treatment including the use of directly observed treatment (DOT), when appropriate (Migliori et al., 2018; WHO,

2017b). In Finland, the use of DOT has been recommended for certain risk groups since 2006 and for all patients since 2013 (Sosiaali- ja terveysministeriö, 2013).

2.3.1 Treatment for cases with drug resistant isolates

Treatment regimen and duration should be individualised on the basis of known or assumed drug susceptibility for cases with confirmed drug resistance or with a strong suspicion for drug resistance and necessitated treatment initiation without drug susceptibility results. Current guidelines by WHO for high-resource countries advise that cases with MDR-TB should be started with MDR regimens, which are based on a combination of at least 4-5 likely susceptible second-line drugs, including a newer drug, bedaquiline, and the treatment period should last for at least 18-20 months (WHO, 2019b).

2.3.2 Surgical interventions for treatment of TB

Anti-TB medication solely is not enough for permanent cure for some cases with extrapulmonary TB manifestations. Patients may benefit from drainage of pleural and pericardial effusions (Imazio et al., 2013; Shaw et al., 2019; Wiysonge et al., 2017). Surgery may be needed for some extrapulmonary TB manifestations, such as pleural empyema and spinal tuberculosis with neurological deficits, large abscesses or deformity (Dunn et al., 2018; Shaw et al., 2019). The introduction of anti-TB medications in the 1950s and 1960s almost replaced surgery in the treatment for pulmonary TB, but the emergence of MDR- and XDR-TB has brought surgery as an option. Surgery should be considered for pulmonary MDR-patients with unilateral disease and sufficient lung function, if medical treatment has failed or the risk of failure or TB relapse is high, e.g., XDR-cases (Dheda et al., 2017; Lange et al., 2019; Sharma et al., 2019).

2.3.3 Contact investigations and follow-up

The need for contact investigations should be assessed for each case to stop ongoing transmission (Migliori et al., 2018). An attempt to obtain sputum samples for smear microscopy and culture should be made for pulmonary TB cases to follow up response to treatment, at least at the end of the intensive phase. Obtaining sputum

samples monthly is recommended for all and especially for MDR-cases. Furthermore, clinical response to treatment should be assessed monthly for all TB cases. (Migliori et al., 2018; WHO, 2019b).

2.4 TB treatment outcomes

Adequate TB treatment, in addition to healing the patient, is a key element to decrease transmission together with early diagnoses and appropriate contact investigations. WHO set a global target in 1991 to cure 85% of pulmonary TB patients under treatment (WHO, 1991). A constitutive way to evaluate the functionality of a national TB control program is TB treatment outcome monitoring. Recommendations for treatment outcome monitoring in Europe were published by a Working Group of WHO and the International Union against Tuberculosis and Lung Disease (IUATLD) in 1998 (Veen et al., 1998). Notifying treatment outcomes to National Infectious Disease Register (TTR) for microbiologically confirmed pulmonary TB cases has been compulsory in Finland since 2007 (Sosiaali ja terveysministeriö, 2006).

2.4.1 Current guidelines for outcome evaluation

WHO recommends assigning treatment outcomes with six categories for all TB cases at the time of treatment cessation. Treatment outcomes should be assigned separately for cases with rifampicin resistant and MDR isolates. (WHO, 2014). Recommendations for European countries made by the ECDC together with the WHO office for the European Region are consistent with global recommendations, except that outcomes should be notified at the latest at 12 months after treatment initiation (ECDC/WHO regional office for Europe, 2013). Hence, an additional outcome group 'still on treatment' is subsumed. Table 1 presents the definitions for outcome groups by the WHO and ECDC/WHO regional office for Europe. Finnish guidelines for outcome reporting and grouping are consistent with European guidelines with the exception that treatment outcomes are not notified for extrapulmonary TB cases, and for pulmonary TB cases without microbiological confirmation outcomes have been notified only since 2017 (Jaakola et al., 2018).

Table 1. Definitions for treatment outcome grouping by WHO and ECDC/WHO regional office for Europe

Treatment outcome WHO 2013	Treatment outcome for Europe ECDC/WHO 2013	Definition for outcome
Cured[1]	Cured[1]	A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion
Treatment completed[1]	Treatment completed[1]	Treatment completed, but does not meet the criteria to be classified as cure or treatment failure
Failed	Failed	A TB patient whose sputum smear or culture is positive at month 5 or later during treatment
Lost to follow-up[2]	Lost to follow-up[3]	A TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more
Died	Died	A TB patient who dies for any reason before starting or during the course of treatment
Not evaluated[4]	Not evaluated[4]	A TB patient for whom no treatment outcome is assigned. This includes cases "transferred out" to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit
-	Still on treatment	Patient still on treatment at 12 months without any other outcome during treatment

[1]Cured+treatment completed=successful treatment outcomes
[2]Nominated as default in earlier versions of guidelines by WHO
[3]Nominated as treatment interrupted in earlier versions of guidelines by ECDC/WHO regional office for Europe
[4]Transferred out cases were notified separately in earlier versions of guidelines by WHO/ECDC
Modified from references (ECDC/WHO regional office for Europe, 2013; WHO, 2014)

2.4.2 Rates for successful treatment outcomes

Among the 6.4 million reported new and relapse TB cases globally in 2017, the average treatment success rate, i.e. proportions of cases with outcomes cured and treatment completed combined, was 85%. The treatment success rate reported by the WHO for the European region, 78%, is the second lowest among the six WHO regions. (WHO, 2019a). Many explanations may be presented for the widely differing success rates between regions, such as differences in demographics among TB patients, including the proportions of elderly patients, cases with risk factors (e.g., substance abuse and comorbidities) and cases with MDR isolates, as well as differences in coverage of outcome reporting (Guthmann et al., 2020; Karo et al., 2015). There may also be crucial differences in outcome evaluation procedures, e.g., who performs outcome evaluation, reflected by some countries with high TB incidences reporting astonishingly high treatment success rates, such as 96% in Ethiopia and 94% in Cambodia (WHO, 2019a).

Among European countries, recommendations for reporting and the coverage of routine outcome reporting vary widely (ECDC/WHO regional office for Europe,

2019). A study that included surveillance data from 18 European countries in 2002-2011 showed a distribution of treatment success rate from 56% in Hungary to 89% in Norway (Karo et al., 2015).

2.4.3 Rates for non-successful treatment outcomes

Among the 15% of cases in 2017 with other than successful treatment outcome notified globally, not evaluated was the most common outcome, followed by outcomes died, lost to follow-up and failed, respectively (WHO, 2019a). The most common non-successful outcome for cases notified in the WHO European region in 2016 was died with an 8% proportion, followed by outcomes not evaluated (7%), lost to follow-up (5%), failed (4%) and still on treatment (1%). The notified proportion of cases with outcome died varies widely from 0% in small countries with very few TB cases, Iceland and Lichtenstein, up to 16-20% in countries with a high proportion of elderly cases, Croatia, Czech Republic and Slovenia. (ECDC/WHO regional office for Europe, 2019).

2.4.4 Definitions for treatment outcome categories and exclusion criteria used in studies

A systematic review for treatment outcome studies from 10 European countries found that differing methods for treatment outcome evaluation limit international comparisons (van Hest et al., 2013). For example, 12 different outcome categories were used instead of the 7 suggested for European countries (ECDC/WHO regional office for Europe, 2013). Heterogeneity in terminology for outcomes is also confusing, e.g., outcome lost to follow-up currently used to describe premature interruption of treatment was used in some older studies to describe cases who disappeared before the end of treatment (Antoine et al., 2007; Ditah et al., 2008).

Most older and some newer studies report outcomes for culture-positive pulmonary TB cases solely, whereas a few newer studies report outcomes for all notified TB cases (Karo et al., 2015; Stosic et al., 2020). Furthermore, exclusion criteria among studies vary widely. Some studies excluded patients who did not receive treatment due to TB diagnosis established after death or due to disappearance, patients with unreported outcome and patients under 18 years of age (Guthmann et al., 2020; Pradipta et al., 2018).

Outcomes are usually combined into groups with varying combinations to evaluate risk factors for other than successful outcomes. Many studies compare cases with successful treatment outcomes to cases with all other treatment outcomes combined, called non-successful or unsuccessful outcomes (Antoine et al., 2007; Karo et al., 2015; Kherad et al., 2009). A common way is also to make three outcome groups by distinguishing cases with successful outcomes, cases with outcome died and cases with other unsatisfactory outcomes, including either all other outcomes or, e.g., outcomes lost to follow-up, failed and proportionately also a former outcome category transferred out before or during TB treatment, which is included in the outcome category not evaluated in current guidelines (Farah et al., 2005; Pradipta et al., 2018; Stosic et al., 2020; Vasankari et al., 2007). The rationale for this grouping is that cases with outcomes lost to follow-up, failed and transferred out may transmit the disease further when the site of disease is pulmonary.

Finally, cases with death as outcome have been discriminated into successful and non-successful outcome categories based on the assessment of whether TB was reported as a cause of death or not (Ditah et al., 2008; Guthmann et al., 2020). Furthermore, some studies have distributed cases with outcome still on treatment to successful and non-successful treatment groups based on the reason for treatment elongation (Cegolon et al., 2010; Ditah et al., 2008).

2.4.5 Death as outcome

WHO and ECDC recommend to report death as outcome for all TB cases who die before or during TB treatment irrespective of the cause of death. Two studies from the U.S. showed that among fatal cases early deaths are common, as a quarter died before and more than 40% died in two months after initiation of treatment (Nguyen et al., 2011; Pascopella et al., 2014).

Several studies using multivariable models have shown that increasing age is the strongest predictor for death as outcome (Stosic et al., 2020; Vasankari et al., 2007). Male gender has also been associated with a fatal outcome in a few studies (Horne et al., 2010; Vasankari et al., 2007). Immigrants and asylum seekers have been related to a lower risk for death as outcome, which may reflect differences in other risk factors, such as comorbidities and substance abuse as well as earlier suspicion for TB (Horne et al., 2010; Pradipta et al., 2018). Cases infected with HIV and cases receiving immunosuppressive medication have been associated with a higher risk for

death in some studies from low-incidence countries (Beavers et al., 2018; Nguyen et al., 2011; Pascopella et al., 2014; Vasankari et al., 2007).

A more severe state of TB infection, such as an infection affecting the central nervous system, disseminated or miliary disease, as well as coincidental pulmonary and extrapulmonary infection, increases the risk for death (Nguyen et al., 2011; Pascopella et al., 2014; Pradipta et al., 2018). Among pulmonary TB cases, cavitary disease and sputum smear positivity have been associated with both increased and decreased risk for death (Pascopella et al., 2014; Waitt et al., 2011). The former observation is probably due to a more severe state of disease and the latter due to enhanced diagnostic procedure. Furthermore, social risk factors, such as alcohol or drug abuse, unemployment and a low level of education, have been associated with the risk of death (Caylà et al., 2009; Pascopella et al., 2014; Sterling et al., 2006).

Comorbidities are more common with increasing age. Chronic diseases, such as diabetes, chronic obstructive pulmonary disease (COPD), malignancies and severe renal insufficiency have been associated with death as outcome (Oursler et al., 2002; Waitt et al., 2011; Walpola et al., 2003). Furthermore, elderly patients often have many chronic diseases concomitantly. The combined effect of comorbidities, assessed using, e.g., the Charlson comorbidity index, on the risk of death among TB cases has very rarely been published (Fløe et al., 2018). A case-control register study from Denmark showed that mortality among TB cases was higher than among matched controls in all age groups above 20 years and comorbidities were associated with the risk of death among TB cases (Fløe et al., 2018).

2.4.6 Risk factors for other non-successful outcomes

Many studies using multivariable models have described male gender as a risk factor for unsatisfactory outcomes including outcomes lost to follow-up, failed and in some studies also former outcome category transferred out before or during TB treatment (Antoine et al., 2013; Holden et al., 2019; Stosic et al., 2020). Other published demographic risk factors include younger age and foreign origin, especially recent immigration (Antoine et al., 2013; Baussano et al., 2008; Pradipta et al., 2018). However, a study from Geneva, Switzerland, showed that asylum seekers had a higher probability for successful outcomes, but the group of unsuccessful outcomes also included a few cases with death as outcome (Kherad et al., 2009).

Social risk factors, such as alcohol abuse, homelessness and being in prison have been associated with unsatisfactory outcomes (Baussano et al., 2008; Holden et al.,

2019; Pradipta et al., 2018). Furthermore, diabetes, mental disorders and anaemia at the time of diagnosis have predicted unsatisfactory outcomes (Holden et al., 2019; Pradipta et al., 2018).

The impact of the disease site is rarely published, because most studies evaluating risk factors for unsatisfactory outcomes have included solely pulmonary TB cases. A recent study from Serbia showed that having pulmonary TB increases the risk for outcome lost to follow-up (Stosic et al., 2020). Unsatisfactory outcomes have also been associated with cases having a history of former TB treatment (Antoine et al., 2013; Stosic et al., 2020). A recent multinational European study also showed that isoniazid resistance predicts non-successful outcomes, including all other outcomes except successful (Karo et al., 2019). Being infected with MDR isolate has also been associated with the risk for non-successful outcomes (Antoine et al., 2007; Karo et al., 2015).

Finally, a cohort study from Finland evaluating treatment regimens and duration showed that pause(s) in treatment and treatment regimen including ethambutol or/and streptomycin instead of pyrazinamide during the intensive phase were associated with an unsatisfactory outcome (Vasankari et al., 2007).

2.5 Recurrent tuberculosis

Recurrent tuberculosis means that a patient, after being treated for tuberculosis, gets the diseases again. Recurrence of TB increases the global burden of TB considerably. According to WHO, almost half a million recurrent TB episodes were notified in 2015 representing almost 8% of all notified TB cases worldwide (WHO, 2017a).

TB recurrence can occur in two ways: as re-infection and as relapse. Re-infection means that a person gets a new infection with a new strain of *M. tuberculosis* after being treated for tuberculosis. Relapse is due to endogenous reactivation with the same strain of *M. tuberculosis* as in the former TB episode.

Definitions made by WHO distinguish retreatment cases from new cases of TB by the case having received at least one month of TB medication in the past (WHO, 2014). Retreatment cases are further classified by the outcome of the preceding TB episode to relapses (successfully treated), treatment after failure, treatment after lost to follow-up and other previously treated patients (treatment outcome not known). WHO's definition for relapse thus also includes recurrent cases due to re-infection. This definition may be appropriate for countries with low resources and a high incidence of TB. High-income countries, where relapses can be distinguished from

re-infections using genotyping methods when isolates from both TB episodes are available, restrict the use of the term relapse to cases due to endogenous reactivation (Rosser et al., 2018). The term recurrent TB is used instead. All the data presented in this section are from studies concerning low TB-incidence countries, if not mentioned otherwise.

2.5.1 Definitions used in and inclusion criteria for studies of recurrent TB

The definitions used for recurrent TB in studies are heterogeneous. Table 2 presents characteristics and inclusion criteria for a selection of studies from countries with a low TB incidence. Notified completion of TB treatment in the preceding TB episode is necessitated in many studies (Dobler et al., 2009; Pettit et al., 2011; Schiroli et al., 2015). However, in some studies, all cases with a re-notified episode of TB are included regardless of TB treatment given or outcome of the preceding episode (Crofts et al., 2010; Dale et al., 2017). A recurrent TB episode is defined in a few studies as a patient notifying a previous diagnosis of TB (Afshar et al., 2019; Kim et al., 2013)

Furthermore, the earliest time point set for a recurrent episode varies. Some studies say that a recurrent TB episode can occur right after treatment for the preceding episode was completed (at the earliest 6 months after the notification of the preceding episode) (Bang et al., 2010; Pettit et al., 2011). In other studies, a 12-month period is necessitated to exist between TB episodes (Crofts et al., 2010). Some studies from the US especially require a 12-month period following completion of TB treatment in the preceding episode (Interrante et al., 2015; Kim et al., 2016; Millet et al., 2009). These are called late recurrences (Interrante et al., 2015; Pascopella, Deriemer, Watt, & Flood, 2011). Studies also vary by the length of the study period, as presented in Table 2.

Inclusion criteria for study cases also vary widely. A positive culture for *M. tuberculosis* in both episodes is required in studies using genotyping. Few studies include only episodes with pulmonary TB (Millet et al., 2013; Pascopella et al., 2011), while most studies also include extrapulmonary TB cases. Many studies are retrospective and based on only register data (Crofts et al., 2010; Kim et al., 2016) or include also medical records review (Avery et al., 2015; Bang et al., 2010). There are also few prospective interventional studies (Jasmer et al., 2004). Very few studies include assessment of TB treatments given in the episode preceding recurrence (Pascopella et al., 2011; Selassie et al., 2005).

Table 2. Selection of studies evaluating the frequency of TB recurrence and distinguishing relapses from re-infections

Author, year	Country, TB incidence /100 000	Co-hort size n	Inclusion criteria	Recur-rences n (%)	Study period, years	Incidence of recurrence/ 100 000 patient years	Genoty-ping performed for both isolates n/recurren t cases n (%)	Relapse n (%)	Method
Parvaresh 2018	Australia (New South Wales)	3700	Culture +, 12 months between TB episodes, treatment completed DOT for all	18 (0.5%)	10	NA	15/18 (83%)	13 (87%)	WGS MIRU-VNTR
Dale 2017	Australia, (Victoria) 6.7	4766	≥2 TB episodes during study period irrespective of treatment outcome	32 (0.7%)	13	NA	20/32 (63%)	18 (90%)	MIRU-VNTR
Interrante 2015	USA 9.7	312589	Culture+, treatment completed +1 year	3039 (1.0%)	19	NA	136/3039 (4.5%)	116 (85.3%)	RFLP, spoligot.
Schiroli 2015	Italy 9.5	4682	Culture+, treatment completed	83 (1.8%)	15	NA	83/83 (100%)	64 (77%)	spoligot., MIRU-VNTR
Pettit 2011	USA 5.8	1431	Treatment completed	20 (1.4%)	7	NA	15/20 (75%)	12 (80%)	RFLP, for cases with <6 bands→ spoligot., MIRU-VNTR
Bang 2010	Denmark 6.9-10.5	4154	Culture+, treatment completed for relapses, irrespective of outcome for re-infections	73 (1.8%)	13.5	NA	73/73 (100%)	54 (74%)	RFLP
Dobler 2009	Australia (New South Wales) 6.5	3731	Culture +, treatment completed DOT for all	15 (0.4%)	13	71	15/15 (100%)	11 (73%)	spoligot. MIRU-VNTR if identical isolates→ RFLP
Kim 2016	USA 9.7	142221	Treatment completed, +1 year	1714 (1.2%)	13	NA	NA	NA	NA
Pascopell 2011	USA (California) 9.7	23517	Culture+, pulmonary TB, treatment completed +1 year	148 (0.6%) .	15	NA	NA	NA	NA
Crofts 2010	England and Wales 10.9	53 214	12 months between TB episodes irrespective of treatment outcome	588 (1.1%)	8	410	NA	NA	NA

Modified from Rosser et al. 2018. WGS= whole genome sequencing, RFLP=IS6110RFLP, spoligot=spoligotyping

2.5.2 Frequency and incidence of recurrent TB

Comparison between studies is challenging due to varying study protocols. A recent state-of-the-art article reported that the overall proportion of recurrent TB cases following treatment completion of the preceding TB episode was 3.4 % (range 0.4%-16.7%) (Rosser et al., 2018). However, this article with 42 studies included studies from countries with a wide variation of TB-incidence (1.5-86/100 000). Five studies with only MDR cases were also included. Recurrence rates have varied from 0.4% up to 6.0% in studies from countries with low TB incidence (Dobler et al., 2009; Jasmer et al., 2004). The former was a retrospective cohort study from one Australian state with DOT for all TB cases. The latter was a prospective clinical trial with high-dose isoniazid and rifamycin administered with DOT twice or once a week during the continuation phase, and the study included a remarkable proportion of HIV-positive cases.

Several studies have demonstrated that the majority of recurrences occur during the first years of follow-up (Crofts et al., 2010; Dobler et al., 2009). The state-of-the-art article just mentioned reported that the median time to recurrence following completion of treatment of the preceding episode, estimated in 10 studies, was 1.4 years (IQR 1.1.-2.8) (Rosser et al., 2018). As a result of a wide variety of TB incidences, lengths of study periods and study protocols, a comparable way of evaluating rates of TB recurrence between countries would be to calculate the incidence of recurrent TB/100 000 patient years of follow-up in the cohort and to compare it with overall incidence of TB in the country. The incidence of recurrent TB has been published in only few studies from countries with TB-incidence<50/100 000 (Rosser et al., 2018). After excluding studies with mainly HIV-positive cases, the incidence of recurrent TB varies between 71/100 000 in New South Wales, Australia and 530/100 000 patient years in Madrid, Spain (Dobler et al., 2009; Millet et al., 2009). When these incidences are compared to overall TB incidences of the countries in question, the incidence of recurrent TB is 12-38 times higher (Crofts et al., 2010; Dobler et al., 2009).

A distinct way to evaluate the period with the highest risk of TB recurrence would be to calculate the incidence of recurrent TB separately for subsequent years of follow-up in the cohort. This is particularly important in countries with a high proportion of elderly cases resulting in a higher reduction of cases in follow-up due to deaths during follow-up. However, as far as we know, this has not been published previously.

2.5.3 Rates of relapses and re-infections among recurrent cases

Distinguishing relapses from re-infections is important, because they point out different challenges in TB control. Relapses reflect shortcomings in TB treatment programs, whereas re-infections reflect the epidemiologic situation in the country (Millet et al., 2013). *M. tuberculosis* isolates from both TB episodes need to be available for genotyping to distinguish relapses from re-infections. Isolates with identical or nearly identical genotypes are regarded as relapses and isolates with differing genotypes as re-infections (Bang et al., 2010; van Rie et al., 1999) The main attribute of the rate of re-infections is the incidence of tuberculosis in the population (Uys et al., 2009). The proportion of re-infections among recurrent cases with treatment completed in the preceding episode has been rated up to 75% in countries with high TB-incidence (van Rie et al., 1999). This study from South Africa was one of the earliest studies using genotyping to distinguish relapses from re-infections.

Most recurrences are due to relapses in countries with low TB incidence (Rosser et al., 2018). The proportion of cases with relapses varies between 73-90% (Dale et al., 2017; Dobler et al., 2009). However, re-infections may play a more important role in certain subpopulations. A study from the US reported that re-infection was 10-times more common as a cause of recurrence among Mexican-born immigrants than among natives (Interrante et al., 2015).

The results just described are from studies using traditional genotyping methods. These methods may erroneously classify re-infections as relapses when the strains are genetically highly related. Whole genome sequencing has been proven to discriminate strains with higher sensitivity during the last few years (Nikolayevskyy et al., 2019). The first studies for recurrent TB using WGS in addition to traditional genotyping methods were from countries with a high TB incidence and had very short study periods (Bryant et al., 2013; Guerra-Assuncao et al., 2015). They showed a clear polarisation of the number of SNPs between isolates of subsequent TB episodes to 0-8, regarded as relapses, and to >100, regarded as re-infections. Later studies have shown that isolates with 8-30 SNP difference cannot be reliably classified as relapse or re-infection (Folkvardsen et al., 2020; Parvaresh et al., 2018).

Recent studies from Australia and Canada concluded that 13-17% of cases classified as relapses using traditional genotyping methods seemed to be due to re-infections using WGS (Parvaresh et al., 2018; Tyler et al., 2017). Furthermore, the interpretation is even more unclear in settings with a higher TB incidence and with a high predominance of genetically close strains, because a re-infection due to a very similar strain results in a very low number of SNPs between episodes (Folkvardsen

et al., 2020; Nikolayevskyy et al., 2019). In addition to the very small number of published studies using WGS, factors affecting the number of SNPs among relapse cases, such as the adequacy of treatment, have not been studied. Thus, further studies are needed.

2.5.4 Risk factors for TB recurrence

Recurrence of TB is dependent on many factors, e.g., characteristics of the patient, TB treatment given, the strain of *M. tuberculosis* in the preceding episode and TB incidence in the population.

2.5.4.1 Patient characteristics

Male gender has been shown to increase the risk of recurrence up to 2-4 times, but this finding has been inconsistent (Hung et al., 2015; Millet et al., 2009; Pascopella et al., 2011). Data published on age groups with increased risk have been incompatible (Golub et al., 2008; Kim et al., 2016; Selassie et al., 2005). Several studies have shown that the risk of recurrence is higher among immigrants (Crofts et al., 2010; Interrante et al., 2015). A study from London established that this was due to increased rate of both re-infections and relapses (Afshar et al., 2019). However, natives have also been associated with a higher risk of TB recurrence in a study from California (Pascopella et al., 2011).

HIV infection has been reported to increase the risk of recurrence in several studies (Crofts et al., 2010; Driver et al., 2001; Unis et al., 2014). Golub et al. showed that the use of antiretroviral therapy decreases the risk of recurrence by half among HIV-positive TB cases (Golub et al., 2008). Studies from countries with high TB incidences have shown that the increased risk of TB recurrence among HIV-positive patients is due to re-infections, not relapses (Crampin et al., 2010; Sonnenberg et al., 2002). This was also supported by one study from the US (Pettit et al., 2011).

Furthermore, other diseases or medications that also impair the immune system may increase the risk of recurrence. An association between diabetes mellitus and TB recurrence has been published (Driver et al., 2001; Lee et al., 2014). Chronic lung disease, including COPD, asthma and interstitial lung diseases, has been shown to increase the risk of TB recurrence (Hung et al., 2015; Pettit et al., 2011). Hung et al. published that the increased risk was independent of smoking status, whereas Pettit et al. found an interaction between smoking and lung disease in the risk of recurrence

(Hung et al., 2015; Pettit et al., 2011). Smoking, per se, has also been associated with recurrent TB (Leung et al., 2015; Thomas et al., 2005).

A history of alcohol and drug abuse has been reported as a risk factor for TB recurrence (Driver et al., 2001; Kim et al., 2016; Millet et al., 2009; Selassie et al., 2005). A small study published by Cacho et al. showed that substance abuse increases the risk of relapses, not re-infections (Cacho et al., 2007). An interaction between poor treatment adherence and alcoholism has been published, which may, at least in part, explain the increased risk of relapse (Selassie et al., 2005). However, persons with substance abuse may also be more prone to become re-infected due to repeated exposure to TB and probably due to an impaired immune system. Other factors reflecting low social-economic status, e.g., unemployment and belonging to the lowest salary group have also been associated with recurrence (Anaam et al. 2012; El Sahly et al., 2004; Hung et al., 2015). It has been stated that the higher risk for recurrence among men might reflect other confounding risk factors more prevalent in men, such as smoking and alcohol abuse (Hung et al., 2015).

2.5.4.2 Characteristics and treatment of TB episodes preceding recurrence

Several factors concerning TB episodes preceding recurrence have been associated with recurrence, such as pulmonary TB as well as concomitant pulmonary and extrapulmonary TB (Crofts et al., 2010; Kim et al., 2016; Millet et al., 2009). A positive sputum culture at two months of treatment, cavitation at the time of diagnosis and residual cavitation at the end of TB treatment have been associated with recurrence in older studies with non-standard treatments (Aber et al., 1978; Benator et al., 2002; Mallory et al., 2000). In addition to reflecting more advanced disease at the time of diagnosis, persistent sputum culture positivity and residual cavitation may also result from inadequate treatment. Studies using genotyping have shown that recurrence among more advanced states of disease, such as pulmonary TB with cavities, miliary TB and TB affecting the central nervous system, is due to relapses (Bang et al., 2010; Cacho et al., 2007; Jasmer et al., 2004)

A few studies have analysed the effects of treatment regimens and supervision of treatment in the preceding episode. A study from South Carolina reported that the treatment regimen not containing rifampicin increased the risk of recurrence by 9-times, even when the duration of treatment with isoniazid+1-3 other anti-TB drugs was prolonged to 18-24 months (Selassie et al., 2005). In addition, a treatment regimen containing rifampicin only during the intensive phase and a treatment regimen containing only rifampicin and isoniazid without a third anti-TB drug during

the intensive phase have been shown to increase the risk of recurrence (Menzies et al., 2009; Pascopella et al., 2011).

Furthermore, an association between poor treatment adherence and the recurrence of TB has been shown (Cox et al., Drier et al., 2001; Selassie et al., 2005). Selassie et al. found an interaction between prolonged treatment duration and poor treatment adherence for all treatment regimen groups (Selassie et al., 2005). Conflicting results have been published concerning the effect of supervision of TB treatment to lower the risk for recurrence (El Sahly et al., 2004; Kim et al., 2016).

2.5.4.3 The phenotype of *M. tuberculosis* isolate in the preceding TB episode

Finally, the phenotype of *M. tuberculosis* isolate may also affect the risk of recurrence. Isolates with resistance to at least one TB drug, and particularly isolates with resistance to pyrazinamide, have been associated with TB recurrence (Brugueras et al., 2020; Pascopella et al., 2011). These findings may be due to shortcomings in altering treatment regimens and duration for cases with drug resistance. Conversely, Jasmer et al. reported that isoniazid mono-resistance protects from TB recurrence, which may reflect intensified treatment when resistance is recognised (Jasmer et al., 2004). Studies from countries with higher proportions of MDR cases have shown that being infected with an MDR isolate, and in particular being infected with pre-XDR and XDR isolates, increases the risk of recurrence (Blöndal et al. 2012; Sun et al. 2017). Furthermore, *M. tuberculosis* isolates belonging to the Beijing genotype have been associated with relapses in studies from the US and Vietnam (Burman et al., 2009; Huyen et al., 2013). Multidrug resistance is more prevalent among isolates with the Beijing genotype, but drug resistance was not related to this finding.

3 AIMS OF THE STUDY

This thesis was carried out to evaluate the quality of tuberculosis treatment in Finland by assessing treatment outcomes at one year and recurrence of tuberculosis in a longer period and to determine risk factors for non-successful treatment.

The specific aims were:

1. to describe treatment outcomes notified for microbiologically confirmed pulmonary TB cases (Study I)
2. to assess the frequency of recurrent TB (Study II)
3. to distinguish relapses, which reflect insufficient treatment in the TB episode preceding TB recurrence, from re-infections among recurrent cases by using genotyping methods (Study III)
4. to study risk factors for non-successful treatment by assessing risk factors for non-successful treatment outcomes and recurrent TB (Studies I and II)
5. to evaluate the validity of treatment outcomes notified and the adequacy of TB treatments prescribed in the episode preceding recurrence (Studies I and II)

4 MATERIALS AND METHODS

This dissertation is based on three articles. Studies I and II are retrospective cohort studies based on tuberculosis cases registered in the National Infectious Disease Register (TTR) in 2007-2014 and 1995-2013, respectively. Study III constituted a subpopulation of study II. Table 3 presents the study populations and data sources for studies I, II and III.

Table 3. Data sources for studies I, II and III

	Cohort	Study population	Subjects (n)	National infectious disease register	Medical records	Finnish hospital discharge register	Cause of death register	Genotyping
I	Pulmonary tuberculosis cases registered in TTR in 2007-2014	Microbiologically confirmed pulmonary TB cases	1416	Yes	For cases with unsatisfactory outcomes (n=50)	Yes	Yes	No
II	Tuberculosis cases registered in TTR in 1995-2013	Recurrent tuberculosis cases: ≥2 TB episodes registered in TTR during 1995-2013	50	Yes	Yes	No	No	No
III	Tuberculosis cases registered in TTR in 1995-2013	Recurrent TB cases with *M. tuberculosis* isolates available for genotyping from two episodes	21	Yes	Yes	No	No	Spoligotyping: For all isolates WGS: For isolates with identical spoligotypes in both episodes

4.1 Study protocols

4.1.1 Study I

All pulmonary TB cases registered in TTR during 2007-2014 were retrieved (n=1785). TTR is maintained at the Finnish Institute for Health and Welfare (THL). Cases with errors in registration (27), TB diagnosed outside Finland (8) and one TB episode registered twice (2) were excluded after assessing data in TTR. Cases with only clinical diagnosis with a decision to give TB treatment (306), for which treatment outcomes were not collected by TTR during the study period, and cases with MDR isolates (25), for which treatment outcomes are recommended by ECDC/WHO to be notified at 24 moths, were also excluded. All data in TTR were collected for the remaining 1416 study cases. Comorbidity data from the Finnish Hospital Discharge Register (HILMO), maintained by THL, were also collected to evaluate the impact of comorbidities on the risk of death.

For cases with death as outcome, the structured data on the causes of death from the Cause of death register, maintained at the Statistics Finland, were collected to evaluate whether deaths were registered as TB related.

Medical records were reviewed, when necessary, for cases with outcomes failed, lost to follow-up and notified as not known, to evaluate the reason for and adequacy of the outcome notified.

4.1.2 Study II

The study cohort included all TB cases registered in TTR during 1995-2013 (n=8084). A case with recurrent TB was defined as one patient registered in TTR with at least two episodes of TB at least 360 days apart (n=84). Cases with errors in registration (13), laboratory contamination (1), no culture positivity in either episode (1), TB treatment given for <1 month or treatment outcome failed in the first episode (5) and only one long continuous TB episode (14), were excluded after assessing the data in TTR and medical records. All data in TTR and medical records were collected for the remaining 50 recurrent cases.

4.1.3 Study III

Study III constituted a subpopulation of study II. Section 4.1.2 describes the study cohort, definition for recurrent TB cases and exclusion criteria with two exceptions: patients not receiving TB treatment in the first episode (3) were not excluded from study III while cases with only one culture-positive TB episode were excluded (6) from study III. Among the 42 patients with more than one culture-positive TB episodes during the study period, isolates from two TB episodes were available for 21 patients. Every tenth *M tuberculosis* isolate was stored frozen before year 2000 and all isolates since 2000. Spoligotyping was performed for the isolates of these 21 patients. Whole genome sequencing was performed for the isolates when spoligotypes in the two isolates were non-identical (n=18).

4.2 Register data

4.2.1 National infectious disease register data (Studies I, II, III)

The National Infectious Diseases Register is maintained in Finland by THL for prevention and control of communicable diseases, based on Communicable Disease Act (Ministry of Social Affairs and Health, 2016). TTR collects data on TB cases from the treating physician and clinical laboratory of the hospital district in which the patient is diagnosed and treated, from the mycobacterial reference laboratory at THL and from the national population register. Data from different sources are connected as a case using the unique identifier or, when it is absent (recent immigrants and newborns), using the name and date of birth.

Notifying TB cases to TTR is mandatory for physicians and laboratories. Laboratories notify positive culture and nucleic acid amplification test (NAAT) results for *M tuberculosis* (Kansanterveyslaitos, 2008). Since 2001, all laboratories have sent *M tuberculosis* isolates to the mycobacterial reference laboratory at THL for species confirmation, drug susceptible testing and genotyping. For physicians, it has been obligatory to notify TB cases with a positive culture, sputum smear or histology since 1995 and to notify clinically diagnosed TB cases with a decision to give full TB treatment since 2007 (Hulkko et al., 2010) .

The registration date in TTR is the date when the first positive culture or NAAT sample is taken or, if these are absent, the date of the physician's notification. A case

is registered as pulmonary TB when (1) sputum culture or NAAT is positive for *M. tuberculosis* or (2) the physician's notification includes an ICD10-code for pulmonary TB, a remark of pulmonary TB or a positive sputum smear microscopy result. All TB notifications for the same patient are combined as a case when the dates of notifications are within 12 months for non-MDR cases and within 36 months for MDR cases.

Drug resistance and genotyping data are obtained from mycobacterial reference laboratory and data on positive HIV test results by connecting data in TTR. The country of birth and date of death are retrieved from the national population register. Table 4 details the information collected in TTR from different sources.

Table 4. Data sources for TTR and notified data on tuberculosis cases

Variable	Physician notification	Clinical laboratory notification	Mycobacterial reference laboratory	Treatment outcome notification	Population register
name	x	x	x	x	
unique identifier	x	x	x	x	x (a must)
gender	x	x	x	x[1]	x
place of residence	x				x
nationality	x				x
country of birth	x				x
date of death					x
ICD-10 code	x			x[1]	
primary culture result		x			
NAA-test result		x			
sample type for the positive finding (culture or NAAT)		x			
sample date (culture or NAAT)		x			
treatment organisation in which positive sample taken		x			
histological diagnosis	x				
sputum smear result (positive/negative)	x				
isolate species confirmation			x		
isolate drug susceptibility			x		
isolate genotype			x		
a history of earlier TB diagnosis (since 1950)	x				
in which year was earlier TB diagnosed	x				
was TB treatment given for ≥ 1 month in the earlier TB episode	x				
was full treatment given in the earlier TB episode	x				
has the decision been made to give full TB treatment in the current episode	x				
date of TB treatment commencement				x[1]	
date of TB treatment cessation				x[1]	
total duration of treatment interruptions (days)				x[1]	
INH and RIF concomitantly given (months)				x[1]	
who decided to stop treatment				x[1]	
treatment outcome				x	
patient transferred before cessation of treatment				x[1]	
date and receiving hospital district/city/country				x[1]	
additional information	x	x		x	
notifier information	x	x	x	x[1]	

[1]notified for cases registered in 2007-2014

4.2.1.1 Data on treatment outcomes in TTR (Study I)

Notifying treatment outcomes and data on a given TB treatment for pulmonary TB cases with a positive culture, NAAT or sputum smear result has been compulsory for hospital districts since 2007. Treatment outcome categories are based on recommendations by WHO/IUATLD and ECDC/WHO regional office for Europe and are the following: cured, treatment completed, failed, lost to follow-up, died, still on treatment and not known (including transferred out cases) (ECDC/WHO regional office for Europe, 2009; Veen et al., 1998). Table 1 shows the definitions for these outcomes. TTR recommends outcomes in Finland to be evaluated by one physician in each hospital district. Table 4 shows data collected in TTR from treatment outcome notifications until year 2015.

4.2.2 Finnish hospital discharge register data (Study I)

Hospitals are required to collect and send patient information to HILMO for inpatients and outpatients, including name and unique identifier, diagnoses, admission and discharge dates; this has been maintained by THL with current information for hospital patients since 1995 (Terveyden ja hyvinvoinnin laitos, 2012). All diagnoses in HILMO for study cases were retrieved three years preceding and one year following TB diagnosis to evaluate the impact of comorbidities on the risk of death. Data were not available for 132 cases with an incorrect or temporary unique identifier.

4.2.3 Causes of death register data (Study I)

The structured data on the causes of death from the Cause of death register, maintained at the Statistics Finland, was collected to define whether death was registered as TB related. Death was classified as TB related in this study for cases with TB as the immediate or underlying cause of death or as a significant condition contributing to death.

4.3 Medical records data

4.3.1 Study I

Medical records data were obtained for those patients with outcomes failed, lost to follow-up and notified as not known, for whom the reason for the notified outcome was not mentioned or was unclear in the outcome notification (n=12). Only the reason for and adequacy of the notified outcome were assessed from patient records.

4.3.2 Studies II and III

Data from medical records were obtained for the 84 patients registered in TTR at least twice during the study period, as described in section 4.1.2. All medical records for at least 6 months preceding and 18 months following TB episodes were ordered from hospital districts. Exclusion of cases without a true recurrent TB episode was assessed by the primary investigator and one other TB expert in the study group. The data collected from medical records for the 50 recurrent cases were demographics, pulmonary/extrapulmonary disease manifestations, radiological, microbiological, histological and laboratory, including HIV, test results, comorbidities, the use of immunosuppressive medication and substance abuse. Prescribed TB treatments and adverse events for the first TB episodes were also collected in detail, as well as application of directly observed treatment. Medical records for two study cases were not received.

4.4 Categorisation of treatment outcomes (Study I)

Outcomes were combined into 4 categories to evaluate risk factors for non-successful treatment outcomes. Successful treatment outcome consisted of cases with outcomes cured and treatment completed. Unsatisfactory outcomes included cases with outcomes failed, lost to follow-up and not known. Cases with non-defined outcomes were either still on treatment at 12 months or the outcome was not notified to TTR. Outcome died included fatal cases. Treatment outcome categorisation for each case was based on the original outcome notified.

4.5 Categorisation of comorbidities (Study I)

A model invented by Charlson et al. and modified by Deyo et al. was used to calculate comorbidity scores for chronic diseases to evaluate the impact of comorbidities on the risk of death (Charlson et al., 1987; Deyo et al., 1992). The model includes 17 diagnostic groups for chronic diseases with an index weighted for severity of diseases. The index has been shown to be associated with 1-year mortality and can be used with register data and ICD-10 codes (Quan et al., 2011). The Charlson indexes were categorised into 4 groups: index 0 (no comorbidities), index 1-2, index 3-4 and index ≥5, for this study.

4.6 Categorisation of treatment regimens and durations (Study II)

Described treatment regimens were categorised into four groups to evaluate adequacy of TB treatments given in TB episodes preceding recurrent TB (Table 5). The criteria used for adequate duration of treatment for standard treatment groups 1 and 2 were based on an earlier publication (Vasankari et al., 2007). Adequacy of treatment regimens and durations for treatment groups 3 and 4 were assessed individually by the primary investigator and one other TB expert in the study group. Treatment outcomes for TB episodes preceding recurrence were defined by the same two experts using the criteria described earlier (section 4.2.1.1).

Table 5. Criteria for evaluation of adequacy of treatment regimen and duration

Treatment group	Treatment regimen in intensive phase	Treatment regimen in continuation phase	Adequate duration of treatment
Group 1	INH, RMP, PZA[1]	INH, RMP	≥5.5 months
Group 2	INH, RMP, EMB[1] (Group 2)	INH, RMP	≥8 months
Group 3	Other probably effective combination of drugs[2] (Group 3)	NA	NA
Group 4	Probably ineffective combination of drugs[2] (Group 4)	NA	NA

INH=isoniazid, RMP=rifampicin, PZA=pyrazinamide, EMB=ethambutol
[1]±an additional TB drug
[2]adequacy assessed by two experts of the study group
NA=not applicable

4.7 Genotyping methods (Study III)

Stored isolates were cultivated on Löwenstein–Jensen medium in order to receive a pure culture for genotyping. A commercially available kit (Isogen Bioscience BV, Maarssen, The Netherlands) was used to perform spoligotyping. The spoligotyping results were compared to the SITVIT2 database of the Institute Pasteur of Guadeloupe. Isolates with non-identical spoligotypes in the two episodes were stored frozen and re-cultured on 7H11 agar plates. A DNeasy blood and Tissue kit (Qiagen, Hilden, Germany) was used to isolate Genomic DNA for whole genome sequencing. Nextera XT kit (Illumina, San Diego, CA, USA) was used to prepare paired-end multiplex libraries and MiSeq platform (Illumina) to sequence the libraries. Single nucleotide polymorphisms (SNPs) in difficult to map regions (especially in annotated repeat regions) were excluded after processing sequence reads. Custom scripts were used to extract variant sites passing quality filters and SNP distances were determined between isolates.

4.8 Statistical methods

Statistical analyses were performed using the IBM SPSS Statistics for Windows, versions 22.0 and 25.0 (Armonk, NY: IBM Corp.) and StataCorp. 2015 (Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). For categorical variables Chi-squared test and Fisher's exact test were used, when appropriate. Mann-Whitney U-test was used for continuous variables with non-normal distribution. The cut point for a statistically significant p-value was set at <0.05.

In study I, multinomial logistic regression model was used to estimate relative risk ratios (RRR) with 95% confidence intervals (95% CI) for non-successful outcome groups (died, unsatisfactory and non-defined) compared to the successful outcome group for potential risk factors with data available for the cohort, e.g., gender (males compared to females) and age as a continuous variable. Variables with $p<0.20$ in the univariate analysis were included in multivariable analysis. Multi-imputation with MICE-algorithm was used for missing data of variables drug resistance, sputum smear microscopy and the Charlson comorbidity index (van Buuren, 2007). Akaike information criteria with backward selection were used to obtain the most parsimonious model, its explanatory variables and their appropriate interactions (Akaike, 1974).

In study II, a follow-up time from 360 days after notification date in TTR until re-notification (recurrent episode of TB), death or the end of the study period, was calculated individually for all cases in the cohort. The incidence of recurrent TB was calculated as the number of recurrent episodes/the sum of follow-up years in the cohort and described as the number of recurrent episodes/100 000 follow-up years, separately for the first year, the second year and the overall follow-up time. Log-linear regression model was used for the pseudo-observations, which were generated using cumulative incidence functions under competing risks (Parner et al., 2010). Risk ratios (RR) with 95% CI for potential risk factors with data available for the cohort, e.g., gender (males compared to females) and age (at the registration date of the first episode) as a continuous variable, were estimated. Time dependent effects were allowed for variables gender and anatomical disease site (pulmonary/extrapulmonary TB) in the model. Variables with $p<0.20$ in the univariate analysis were included in multivariable analysis.

4.9 Ethical considerations

This is a retrospective cohort study without contact with patients. Therefore, approval by Ethics Committee and informed consent to participate was not required. Permission for collecting data from national registers and medical records was given by the National Institute for Health and Welfare.

5 RESULTS

5.1 Treatment outcomes for microbiologically confirmed pulmonary TB cases (Study I)

5.1.1 Characteristics of the cohort

The final study cohort consisted of 1416 microbiologically confirmed pulmonary TB cases diagnosed in Finland during 2007-2014. Among the cases, 64.6% were males and 72.7% were of Finnish origin (Table 6). The foreign-born cases were significantly younger than the Finnish-born cases (median ages 28 years vs. 70 years, $p<0.001$). The median age of all cases was 60 years (range, 0-98 years). TB diagnosis was confirmed by positive culture for 1388 (98.0%), positive NAA-test result for 16 (1.1%), and positive sputum smear and the treating physician's intent to give full TB treatment for 12 (0.9%) cases. The proportions of fully susceptible, mono-resistant and poly-resistant isolates were 89.1%, 6.5% and 1.8%, respectively. Besides the 28 culture negative cases, drug resistance results were not known for eight culture positive cases. Sputum smear was positive for 694 (49.0%) and not notified for 93 (6.6%) cases. HIV positivity was notified for 25 cases (1.8%). Five cases (0.4%) were registered with two separate TB episodes during the study period.

Table 6. Characteristics of the cohort and risk factors for non-successful outcomes in univariate analysis

Variable	Category, n	Successful (1063) n (%)	Died (231) n (%)	Univariate RRR[1] for death[2] (95% CI) p	Unsatisfactory[3] (50) n (%)	Univariate RRR[1] for unsatisfactory[2] (95% CI) p	Non-defined[4] (72) n (%)	Univariate RRR[1] for non-defined[2] (95% CI) p
Age	Age/10 years	Median 57 years	Median 79 years	1.77 (1.53-2.06) **<0.001**	Median 27.5 years	0.65 (0.59-0.72) **<0.001**	Median 51.5 years	0.96 (0.87-1.05) 0.341
Gender	Female (501)	387 (77.2%)	68 (13.6%)	1	17 (3.4%)	1	29 (5.8%)	1
	Male (915)	676 (73.9%)	163 (17.8%)	1.37 (1.06-1.77) **0.015**	33 (3.6%)	1.11 (0.68-1.82) 0.677	43 (4.7%)	0.85 (0.49-1.46) 0.552
Origin	Finnish (1030)	744 (72.2%)	225 (21.8%)	1	13 (1.3%)	1	48 (4.7%)	1
	Foreign (386)	319 (82.6%)	6 (1.6%)	0.06 (0.04-0.11) **<0.001**	37 (9.6%)	6.64 (3.68-11.98) **<0.001**	24 (6.2%)	1.17 (0.70-1.93) 0.551
Drug resistance non-MDR[5]	No (1262)	966 (76.5%)	207 (16.4%)	1	38 (3.0%)	1	51 (4.0%)	1
	Yes (118)	74 (62.7%)	12 (10.2%)	0.76 (0.33-1.74) 0.511	12 (10.2%)	4.12 (2.02-8.41) **<0.001**	20 (16.9%)	5.12 (3.23-8.11) **<0.001**
Sputum smear[6]	Negative (629)	481 (76.5%)	96 (15.3%)	1	23 (3.7%)	1	29 (4.6%)	1
	Positive (694)	527 (75.9%)	105 (15.1%)	0.998 (0.82-1.21) 0.986	24 (3.5%)	0.95 (0.48-1.87) 0.888	38 (5.5%)	1.20 (0.80-1.79) 0.387
Study period	2007-2010 (760)	570 (75.0%)	118 (15.5%)	1	31 (4.1%)	1	41 (5.4%)	1
	2011-2014 (656)	493 (75.2%)	113 (17.2%)	1.11 (0.88-1.39) 0.383	19 (2.9%)	0.71 (0.51-0.99) **0.045**	31 (4.7%)	0.87 (0.43-1.77) 0.709
Charlson comorbidity index[7]	0 (631)	550 (87.2%)	30 (4.8%)	1	17 (2.7%)	1	34 (5.4%)	1
	1-2 (462)	322 (69.7%)	111 (24.0%)	6.32 (4.57-8.74) **<0.001**	9 (2.0%)	0.90 (0.30-3.73) 0.859	20 (4.3%)	1.00 (0.60-1.67) 0.985
	3-4 (172)	109 (63.4%)	54 (31.4%)	9.08 (5.63-14.66) **<0.001**	1 (0.6%)	0.30 (0.04-2.36) 0.251	8 (4.7%)	1.19 (0.50-2.82) 0.697
	≥5 (85)	52 (61.2%)	28 (32.9%)	9.87 (4.63-21.06) **<0.001**	1 (1.2%)	0.62 (0.11-3.67) 0.600	4 (4.7%)	1.24 (0.55-2.79) 0.596

[1]RRR=ratio of relative risks

[2] compared to successfully treated cases

[3] including cases with outcomes failed, lost to follow-up (treatment interrupted) and not known (transferred out)

[4]including cases with outcome still on treatment and cases without an outcome notification

[5]Information missing for 36 cases (23 successfully treated cases, 12 fatal cases, 1 non-defined case)

[6]Information missing for 93 cases (55 successfully treated cases, 30 fatal cases, 3 other defined unsuccessfully treated cases, 5 non-defined outcome)

[7]Information missing for 66 cases (30 successfully treated cases, 8 fatal cases, 22 other defined unsuccessfully treated cases, 6 non-defined outcome)

5.1.2 Treatment outcomes

Treatment outcome was notified as successful for 1063 (75.1%), as death for 231 (16.3%), as unsatisfactory for 50 (3.5%) and as non-defined for 72 (5.1%) cases (Figure 1). Among the 50 unsatisfactory outcomes, outcome notified as not known was the main outcome with 35 (70%) cases, followed by outcome lost to follow-up with 12 (24%) and outcome failed with 3 (6%) cases. Treatment outcome was not notified for 20 (1.4%) cases, and 52 (3.7%) cases were still on treatment at twelve months after treatment initiation.

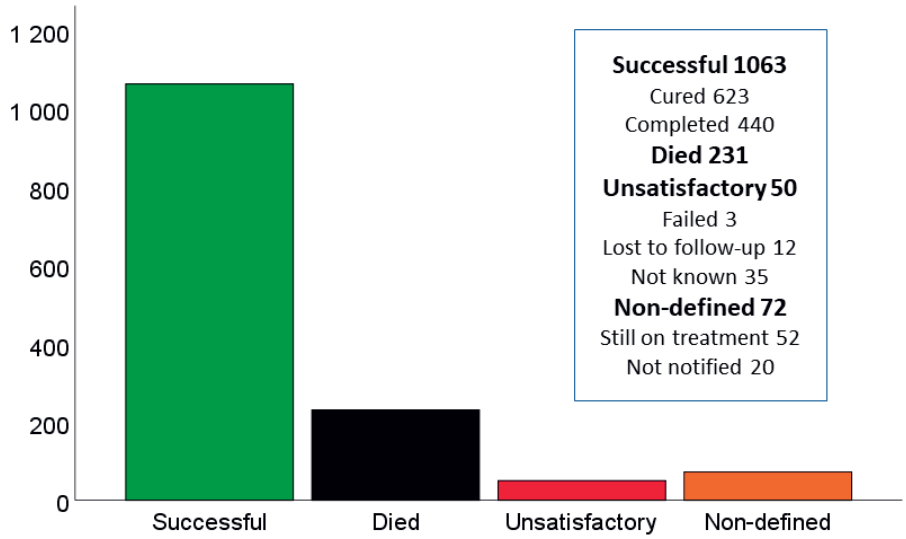

Figure 1. Treatment outcome categories among study cases

5.1.3 Duration of treatment

Treatment duration was notified for 1204 (85.0%) cases, and median duration was 204 days (interquartile range (IQR), 183-278 days). Treatment duration for cases notified with a successful outcome was analysed in detail to evaluate the validity of treatment outcome assessment in notifications (Table 7). The duration of treatment for 6 cases (0.6%) was too short (less than 5.5 months), and 91 cases (8.6%) received treatment for more than 12 months. Hence, treatment outcome assessment for cases

notified with a successful outcome was incorrect in 97 (9.2%) cases according to treatment durations notified.

Table 7. Distribution of treatment durations for successfully treated and fatal cases

Treatment duration	Successful (n=1063) n (%)	Died (n=231) n (%)
No treatment	0 (0%)	58 (25.1%)
Treatment duration <5.5 months	6 (0.6%)	137[1] (59.3%)
Treatment duration 5.5-12 months	913 (85.9%)	12 (5.2%)
Treatment duration >12 months	91 (8.6%)	1 (0.4%)
Treatment duration not notified	53 (5.0%)	23 (10.0%)

[1]81 cases (35.1%) received treatment for <2 months

5.1.4 Cases with death as outcome

Death was notified as outcome for 225 cases (21.8%) among Finnish-born and for 6 cases (1.6%) among foreign-born cases. Fatal cases were significantly older than cases with a successful outcome (median age 79 years (range 0-96) versus 57 years (range 0-98), $p<0.001$). Among cases with death as outcome, 25.1 % did not receive any TB medication and 35.1% received treatment for less than 2 months (early deaths with TB 60.2%) (Table 7). TB was not registered as a cause of death in death certificates for 64 (27.7%) of the fatal cases. Death was classified as TB related for the rest. TB was registered as the immediate/underlying cause and as a significant condition contributing to death in 101 (43.7%) and 64 (28.6%) cases, respectively. Cases classified with a TB-related death did not have significantly different Charlson comorbidity indexes than cases classified with a non-TB-related death ($p=0.13$).

Among all cases with death as outcome, death was associated with Finnish origin, increasing Charlson comorbidity index, increasing age and male gender in univariate analysis (Table 6). These risk factors, except Finnish origin, were also predictors for death in the multivariable logistic regression model (Table 8). The risk for death among men was higher compared to women, but the risk ratio decreased with increasing age (interaction between gender and age).

Table 8. Risk factors for non-successful treatment outcomes and TB recurrence in multivariable models

Risk factors	Death (n=231) RRR[1] (95% CI), p	Unsatisfactory outcomes (n=50) RRR[1] (95% CI), p	Non-defined outcomes (n=72) RRR[1] (95% CI), p	TB recurrence (n=50) RR[2] (95% CI), p
Age/+10 years	Finnish male 1.5 (1.3-1.8), **p<0.001** Finnish female 1.9 (1.4-2.7), **p<0.001** Foreign origin male 1.2 (0.9-1.6), p=0.18 Foreign origin female 1.6 (1.04-2.3), **p=0.03**	0.6 (0.3-1.1), p=0.09	0.9 (0.7-1.1), p=0.19	0.8 (0.7-0.99), p=**0.04**
Male gender	9.5 (1.4-66.7), **p=0.02**	1.4 (0.4-4.4), p=0.62	1.2 (0.4-4.0), p=0.34	5.9 (2.2.-15.7) **p<0.001**[3]
Charlson comorbidity index>0	Charlson 1-2: 3.0 (2.1-4.4), **p<0.001** Charlson 3-4: 3.6 (2.2-5.8), **p<0.001** Charlson ≥5: 5.9 (2.7-13.3), **p<0.001**	NA	NA	NA
Drug resistance	0.8 (0.3-2.1), p=0.71	2.6 (1.2-5.8), **p=0.02**	5.6 (3.8-8.1), **p<0.001**	Data not analysed[4]
Pulmonary TB	NA	NA	NA	15.2 (5.0-46.1), **p<0.001**[3]

[1]RRR=ratio of relative risks, compared to successfully treated cases
[2]RR=relative risk, compared to cases with no recurrence
[3] at 18 years of follow-up
[4]5/50 recurrent cases (10%) had a drug-resistant isolate in the first episode (INH R)
NA=not applicable
Modified from Korhonen et al., 2017

5.1.5 Cases with unsatisfactory outcomes

Among the 50 cases with unsatisfactory outcomes, the reason for the notified outcome was available in outcome notifications for 38 cases, and medical records data were reviewed for the remaining 12 cases. Table 9 shows in detail the reasons and incorrectly assessed treatment outcomes. Risk factors in the univariate model for unsatisfactory outcomes were foreign origin, non-MDR drug resistance, younger age and TB registered in the first study period (Table 6). An interaction between origin and age was observed. In the multivariable model, an independent association was observed with non-MDR drug resistance (Table 8) and TB registered in the first study period (ratio of relative risks, (RRR) 1.5; 95%CI, 1.1-2.1, p=0.02). Restricting

analysis to outcomes notified as failed and lost to follow-up combined yielded non-MDR drug resistance as a risk factor (univariate RRR, 4.8; 95% CI 2.1-11.0, p<0.001; multivariate RRR, 4.9; 95% CI 2.3-10.7, p<0.001) (data not shown).

5.1.6 Cases with non-defined outcomes

The reasons for prolonged treatment among the 52 cases with outcome still on treatment were notified for 36 cases (69%), shown in Table 9. The only risk factor associated with non-defined outcome was non-MDR drug resistance in univariate (Table 6) and multivariable regression analysis (Table 8).

Table 9. Explanations for unsatisfactory and non-defined outcomes

Notified outcome, n	**Explanation for correctly notified outcomes, n**	**Incorrectly notified outcome**[1], **n (%)**	**Correct outcome, n**
Failed 3	Positive sputum culture at 5 months or later during treatment 2	1 (33.3%)	Treatment completed 1
Lost to follow-up 12	Severe substance abuse 4 Severe adverse reaction 3 Positive culture missed by physician 1	4 (33.3%)	Not known 3 Still on treatment 1
Not known 35[2]	Case transferred to another country 25[3] Case disappeared 4[3] Case transferred in Finland 3[4]	3 (8.6%)	Still on treatment 1 Treatment completed 1 Lost to follow-up 1
Still on treatment 52	Non-MDR drug-resistant isolate 19 Miliary/disseminated disease 5 Advanced cavitary disease 4 Pauses on treatment 4 Bone and joint tuberculosis 2 Recurrent TB 1 Prolonged sputum culture positivity 1 Explanation not notified 16	NA	NA

[1]evaluation based on additional information in notifications and patient chart review
[2]origins: 33 foreign, 2 Finnish
[3] before or during treatment
[4] and the final outcome not notified by the receiving hospital district
NA=not applicable

In summary, treatment outcome was successful in 75% of cases and 16% of cases died with TB. Risk factors for death were male gender, comorbidities and increasing age. Drug resistance was a risk factor for non-successful outcomes other than death. Notified treatment outcome was incorrect in 16% of cases notified with unsatisfactory outcomes and in 9% of cases notified with successful outcomes.

5.2 Recurrent TB (Study II)

5.2.1 The cohort

There were 8084 tuberculosis cases registered in TTR during the study period of 1995-2013. Among the cases, 57.1% were males and 84.6% of Finnish origin (Table 10). Finnish-born cases were significantly older than cases with foreign origins (median ages 70 years versus 30 years, $p<0.001$).

5.2.2 Characteristics of recurrent TB cases

The study population consisted of 50 patients with at least two verified TB episodes at least 360 days apart. Two of these had 3 TB episodes and 48 had 2 TB episodes during the study period. Table 10 presents demographic data as well as data on some potential risk factors for recurrence. Pulmonary TB was significantly more common among males than females (97.4% vs 54.5%, $p<0.001$) in the first episode. Two cases were HIV positive among the 18 recurrent cases (36% of all) with HIV test result available on patient charts. In the first episode, 43 cases (86%) had a fully susceptible isolate, 5 cases (10%) were infected with an isoniazid-resistant isolate, and 2 cases (4%) were culture negative. Substance abuse, mostly alcohol, was registered in patient records for 23 (59%) males and for none of the females. Compliance with TB treatment could not be assessed, because this was rarely mentioned in patient records.

Table 10. Characteristics of recurrent TB cases and the whole cohort of TB cases in Finland 1995-2013

Characteristics	TB recurrence (n=50) n (%)	All (n=8084) n (%)
Age, median	51.5 years[1]	66 years
Gender male	39 (78.0%)	4616 (57.1%)
Origin Finnish	41 (82.0%)	6838[2] (84.6%)
Culture positive	48 (96.0%)[1]	6680 (82.6%)
Pulmonary TB	44 (88.0%)[1]	5605 (69.3%)
Study period 1995-2006	45 (90.0%)[1]	5807 (71.8%)

[1]data of the first TB episode during the study
[2]origin not known for 172 cases
Modified from Korhonen et al, 2017

5.2.3 Treatment regimens and treatment outcomes for TB episodes preceding recurrence

Adequacy of TB treatment could be evaluated for 48 cases (96%) with complete patient records (Table 11). The described treatment regimen was adequate for 42 cases, but for 7 of these, the treatment period was too short. Treatment regimen was inadequate for 6 cases. Among the 35 cases with an adequate treatment regimen for long enough, treatment outcome was successful for 27, and 8 cases were still on treatment at 12 months. Among the 14 cases with outcome lost to follow-up, premature treatment cessation was due to the patient in two cases, due to the treating physician's decision in 6 cases, and the treating physician had prescribed an inadequate treatment regimen in 6 cases. The treatment outcome was successful in 27 (55%), lost to follow-up in 14 (29%) cases, and 8 (16%) cases were still on treatment at 12 months from treatment initiation. Successful treatment outcome was more common among males than females (61.5% vs 27.3%, p=0.04) and among pulmonary TB than extrapulmonary TB (59.1% vs 16.7%, p=0.05). Directly observed therapy was implemented for 6 cases (12%).

Table 11. Adequacy of treatment and evaluation of treatment outcomes for recurrent cases

	Treatment period adequate	Treatment period inadequate	Treatment outcome
Treatment regimen adequate 42	35	7	Successful 27 Still on treatment 8 Lost to follow-up 7
Treatment regimen inadequate 6	NA	NA	Lost to follow-up 6
Adequacy of treatment regimen not evaluated 1[1]	NA	1[1]	Lost to follow-up 1

[1]treatment outcome evaluated based on additional information on treatment outcome notification
NA=not applicable

5.2.4 Incidence of and risk factors for TB recurrence

Median follow-up time among the cohort of 8084 TB cases was 6.1 years (IQR 2.7-11.1 years). Maximal follow-up time was 18 years. Half of the recurrences among all cases and all recurrences among women and extrapulmonary cases occurred within the first 2 years of follow-up (Figure 2). The incidences for TB recurrence for the first year of follow-up, the second year of follow-up and for overall follow-up time were 236 (95%CI 140-399), 207 (95%CI 115-373) and 113 (95%CI 86-149) per 100 000 person years, respectively.

Older age decreased the risk of TB recurrence in univariate (RR/+10 years 0.9; 95%CI 0.8-0.98, p=0.03) and multivariate analysis (Table 8). Male gender, compared to women, and pulmonary TB, compared to extrapulmonary TB, were not predictors for recurrence at 1 and 2 years of follow-up (Figure 2). However, at 18 years of follow-up TB recurrence was associated with male gender (RR 3.9; 95% CI 2.0-7.9, p<0.001) as well as pulmonary TB (RR 5.5; 95% CI 2.2-14.1, p<0.001) in univariate analysis. In multivariate analysis, male gender was an independent risk factor for recurrence with almost a six-fold risk ratio and pulmonary TB with a 15-fold risk ratio (Table 8).

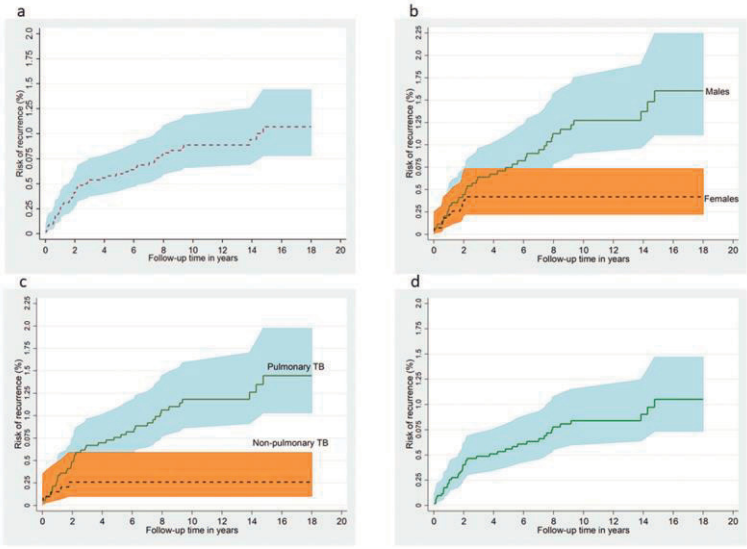

Figure 2. Cumulative risk of recurrence of TB by follow-up time (and its 95% confidence limits). a. For overall cohort b. By gender c. By anatomical site of disease d. For culture positive cases only. Modified from Korhonen et al., 2017.

5.2.5 Very early recurrences (data not published)

Cases with re-notification in TTR by a physician or laboratory 6-12 months (183-359 days) after the notification date of the first episode were retrieved to assess the number of very early recurrences, which are missed by our study protocol. Among these 22 cases, there were errors in notification dates in 9 cases, other registration errors in 2 cases, only 1 long continuous TB episode in 2 cases, and 1 case did not receive TB treatment in the first episode. One case had a true very early recurrence, because treatment outcome was notified as lost to follow-up for the first episode. The reason for re-notification for 9 cases with no additional information in NIDR could not be evaluated, because a patient chart review was not carried out.

In summary, 0.6% of TB cases were recurrent in our study with a median follow-up time of 6 years in the cohort. Risk factors for TB recurrence were pulmonary TB, male gender and younger age. Among recurrent cases, treatment outcomes for TB episodes preceding recurrence were assessed as successful in 54%, lost to follow-up in 28% and still on treatment in 16% of cases.

5.3 Genotyping data for recurrent TB cases (Study III)

Among the 42 patients with >1 culture-positive TB episodes during the study period of 1995-2013, isolates from two episodes were available for genotyping in 21 patients. *M. tuberculosis* isolates from the first and second episodes had unidentical spoligotypes in 3 (14%) patients, meaning that TB recurrence was due to re-infection. One of these patients was from Somalia and one from the former Soviet Union. The third patient's origin was Finland, but a new TB contact before the recurrent TB episode was recognised according to a patient records review. Eighteen (86%) patients with *M. tuberculosis* isolates with identical spoligotype in both episodes were suspected of having relapse TB.

5.3.1 Characteristics of cases with suspected relapse TB

Among the cases, 68.7% were males and 83.3% were of Finnish origin. The mean age was 63 years (range, 25-95 years) at the first episode's registration time. Based on drug-susceptible testing, two patients had isoniazid-resistant isolates in both episodes, but for one of them, the isolate was also resistant to streptomycin in the second isolate. One patient with a fully susceptible isolate in the first episode had a pyrazinamide-resistant isolate in the second episode. The interval between registration dates of TB episodes was 1-2 years in 9 (50%) cases, 2-4 years in 7 (39%) cases and >4 years in 2 (11%) cases. The treatment outcome in the first episode was successful in 10 (55.6%) cases, lost to follow-up in 4 (22.2%) cases and still on treatment in 3 (16.7%) cases. The treatment outcome could not be evaluated due to lacking patient records for one (5.6%) case.

5.3.2 Whole genome sequencing data of cases with identical spoligotypes in both episodes

Whole genome sequencing was performed for *M. tuberculosis* isolates from both episodes in 18 patients suspected of having relapse TB. The median number of SNPs, i.e. mutations in base pairs, between isolates from two episodes of a same patient was 1 (IQR 0-5). This strongly suggests that TB recurrence in these cases was due to relapse, except for 1 outlier with 38 SNPs (Figure 3). Compared to the reference sequence (*M. tuberculosis* H37Rv), the first isolate of the outlier had 20 unique SNPs and the second isolate had 18 unique SNPs. This infers that the second isolate did not evolve directly from the first, suggesting that the second episode was due to re-infection. When SNPs were calculated relative to the time interval between episodes, the median number of mutations per genome per year was 0.4 (IQR 0-2.5). The outlier had 17.3 SNPs/year, which also supports the idea of recurrence due to re-infection. The number of SNPs did not seem to be directly related to the interval between TB episodes or the first episode's treatment outcome (Figure 3).

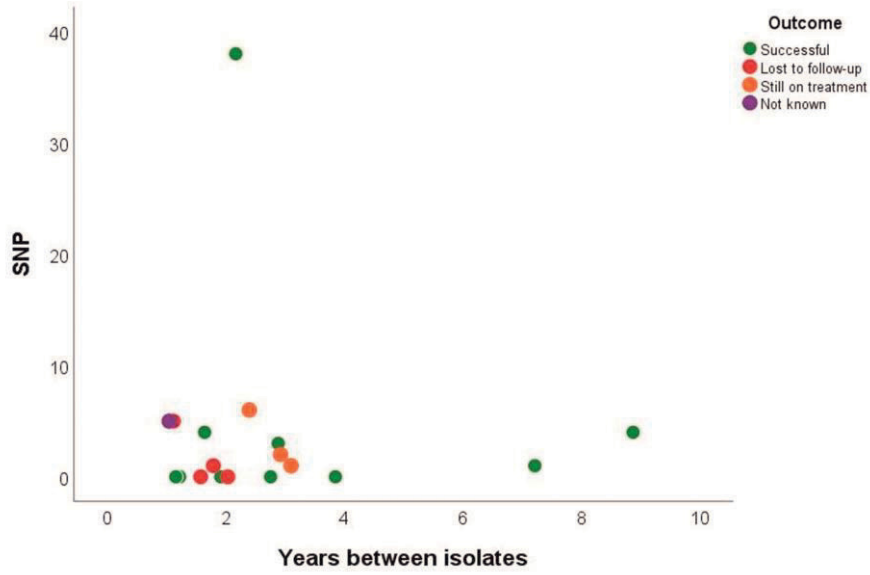

Figure 3. Treatment outcome and number of years between TB episodes in relate to number of SNPs between isolates of the same patient. Modified from Korhonen et al., 2016

In summary, 14% of recurrent TB cases had unidentical spoligotypes in isolates from the first and the second episodes, meaning that recurrence was due to re-infection. Whole genome sequencing was performed for both isolates from the 18 patients suspected of having relapse TB. In 17/18 cases there were 0-6 mutations between isolates of a same patient strongly suggesting that recurrence was due to relapse. In 1 case, the number of SNPs was 38. It is probable, that this case had been re-infected by *M. tuberculosis* isolate with identical spoligotype with the isolate in the first episode.

6 DISCUSSION

6.1 Study populations

This dissertation included two national cohorts of TB cases registered in TTR. We had long retrospective study periods of eight (Study I) and 19 years (Studies II and III) to ensure adequate study populations because of the rather low annual number of TB cases registered in Finland. Study I presents treatment outcomes for microbiologically confirmed pulmonary TB cases, except for cases with MDR-isolates. The notification rate is very high in Finland, with treatment outcomes notified for 99% of the cases. Treatment outcomes in Finland are not notified for extrapulmonary cases and are notified for pulmonary cases without a microbiological confirmation only since 2017. Most former studies also restrict outcome reporting to non-MDR pulmonary TB cases, whereas a few newer studies also report outcomes for extrapulmonary cases (Guthmann et al., 2020; Stosic et al., 2020). Our results cannot be generalised for extrapulmonary and MDR cases even though our cohort was large with 1416 cases.

Studies II and III were based on the cohort of all notified TB cases during 19 years consisting of 8084 cases. Our study period is among one of the longest, but due to the small number of TB cases diagnosed annually, the size of the cohort and the number of recurrent cases is average compared to other studies published (Table 2).

6.2 Non-successful treatment outcomes and recurrent TB

The rate of successful treatment outcome in Finland, 75%, is far from the 85% target set by WHO, mostly because of a high 16% proportion of cases with death as outcome. However, the rate of cases with unsatisfactory outcomes, which have a potential to transmit the disease, is rather low, 3.5%. Recurrent TB cases are rare, constituting only 0.6% of all TB cases. More than 80% of recurrent cases are due to relapses.

6.2.1 Death as outcome

Among pulmonary TB cases in Finland, death as outcome has decreased from 19% in the mid-1990s to current 16%, but the proportion is still remarkably higher than the overall 7% found in the 16 European Union/European Economic Area (EU/EEA) countries between 2002-2011 (Karo et al., 2015; Vasankari et al., 2007). Nevertheless, the proportion is in line with some other European countries, e.g., Czech Republic (18%) and Slovenia (14%), with somewhat similar demographics among TB patients (Karo et al., 2015). Comparison of death rates between countries with differing distributions of risk factors, such as the proportion of elderly cases and cases with comorbidities, is of limited value, because all deaths before or during TB treatment are notified irrespective of the cause of death. The mean age among Finnish-born TB cases is exceptionally high, such as 71 years in 2017, the highest among the EU/EEA countries (ECDC/WHO regional office for Europe, 2019).

Our observation that almost two thirds of cases with death as outcome die early (before or during the first two months of treatment) is in line with two studies from the U.S. (Nguyen et al., 2011; Pascopella et al., 2014). Our observations of a high proportion of early deaths and a high proportion of comorbidities, together with earlier observations of more severe forms of TB resulting in death as outcome, indicate that many cases who die present at a state when their disease has already become difficult to treat (Pascopella et al., 2014; Pradipta et al., 2018). The diagnostic delays by the health-care system may be remarkable, in addition to delays in patients seeking care. A case-control study from the U.S. showed that TB-related deaths were associated with an alternative diagnosis, e.g., pneumonia, before the TB diagnosis (Beavers et al., 2018). Non-infective chronic comorbidities may also increase the risk for death not only as a cause of death but also due to causing delays in diagnostics by offering an alternative explanation for symptoms.

As far as we know, our study was the first to compare the combined effect of comorbidities, using the Charlson comorbidity indexes, between cases with TB-related and non-TB-related deaths categorised by the causes of death on death certificates. We did not find significant differences between these groups. This may be explained by the facts that assessing the impact of TB on death in patients with comorbidities is not always clear, and cases with early deaths may not have a confirmed TB diagnosis before death. A retrospective study from the U.S. categorised cases with death as outcome as having a TB-related or non-TB-related death by the study group performing an intensive medical records review (Beavers et al., 2018). They observed that the sensitivity and specificity of death certificate

data to detect TB-related deaths was only 55% and 75%, respectively, and the definition for TB-related death was similar to our definition.

6.2.2 Other non-successful treatment outcomes

Among cases with unsatisfactory outcomes, outcomes failed and lost to follow-up combined are rare in Finland with only a 1% proportion compared to the 8% proportion in the study of 16 EU/EEA countries between 2002-2011 (Karo et al., 2015). However, the 2% proportion of cases notified as outcome not known, mainly because they transferred to another country before or during treatment, is higher than in many studies from high-income European countries (Holden et al., 2019; Karo et al., 2015). These cases may have a potential to transmit the disease further if treatment is interrupted; thus, staying in Finland until treatment completion or continuation of treatment in the receiving country should be ensured.

The main outcome among non-defined outcomes was still on treatment at 12 months with almost a 4% proportion, which is slightly higher than the average proportion in the study of 16 EU/EEA countries between 2002-2011 (Karo et al., 2015). Treatment outcome was not notified for only 1% of cases.

6.2.3 Recurrent TB

The proportion of recurrent TB cases in Finland, 0.6%, is in line with other studies from countries with low TB incidences (Table 2). However, differing study protocols, lengths of study periods and incidence of TB make comparisons less reliable. Unlike many other studies, we did not include cases with very early recurrences (in 6-12 months after the notification date of the first episode), because the standard cut-off point for treatment outcome evaluation set by the ECDC is at twelve months, and distinguishing very early recurrences from treatment failures is challenging, especially in extrapulmonary cases and in pulmonary cases when sputum samples are not obtained during follow-up (Dale et al., 2017; Pettit et al., 2011). However, including very early recurrences would have increased the rate of recurrent TB at most by 10 cases to 0.7%. Furthermore, excluding cases who did not complete treatment in the former TB episode, which has been done in many other studies, would have decreased our amount of recurrent TB cases by almost one third (Parvaresh et al., 2018; Schiroli et al., 2015).

A more comparable way of evaluating the rate of recurrent TB is calculating the incidence of recurrent TB/100 000 patient years of follow-up and comparing it with the overall incidence of TB, but this has been published very rarely (Rosser et al., 2018). Even though a complex way due to its need for information of individual follow-up times for each case in the cohort, this is necessary for cohorts with a high proportion of elderly patients that result in high mortality during follow-up. The overall incidence of TB recurrence in our study, 113/100 000 patient years, is the second lowest published so far (Dobler et al., 2009). We found that the first two years of follow-up have the highest risk for TB recurrence with incidences higher than 200/100 000 patient years of follow-up. As far as we know, this is the first study to publish the incidence of TB recurrence at different time points of follow-up. Persons with an earlier episode of TB should be recognised as a high-risk group for TB, because we found that TB incidence among them is 10-20 times higher than in the population during the study period. A very high-risk period for TB recurrence is the first two years after a TB episode.

6.3 Distinguishing relapses from re-infections among recurrent TB cases and the use of whole genome sequencing

Our observation of more than 80% of recurrent cases resulting from relapses is in line with other studies published from low-incidence countries using traditional genotyping methods (Dobler et al., 2009; Rosser et al., 2018). We used a novel genotyping method, whole genome sequencing, which has lately been shown to discriminate strains with a higher sensitivity (Nikolayevskyy et al., 2019). Our study was the first published from a low-incidence country using WGS in this setting. We showed that the number of mutations between isolates of subsequent TB episodes is not always as clearly polarised as was shown in two earlier studies from countries with high TB incidences (Bryant et al., 2013; Guerra-Assuncao et al., 2015). In our study, one case classified as relapse based on similar spoligotypes in both episodes was assessed as a re-infection based on WGS data. Recent studies have shown that distinguishing relapses from re-infections is not always possible, even when using WGS. Becoming re-infected with a similar or very closely related strain is probable in settings with a high predominance of genetically close strains (Folkvardsen et al., 2020; Nikolayevskyy et al., 2019). However, a wide distribution of strains has been observed in Finland (Smit et al., 2013).

Whole genome sequencing is expected to replace variable earlier molecular and genotyping methods used for, e.g., identification of species and strains, detection of transmission chains and laboratory cross contaminations and for rapid identification of drug resistant isolates, and it can be established with accurate speed and with reasonable costs (Cabibbe et al., 2018; Nikolayevskyy et al., 2019; Pankhurst et al., 2016). Some major limitations, such as the lack of international standardisation, the need for culture of *M. tuberculosis* isolates and advanced bioinformatics, have to be noticed (Pankhurst et al., 2016; Satta et al., 2018; van Soolingen et al., 2016). Very few studies with a very low number of *M. tuberculosis* isolates using whole genome sequencing to distinguish relapses from re-infections have been published so far. Our study found no relation between the number of mutations and the interval between TB episodes as well as the treatment outcome of the first episode among relapse cases. Other studies evaluating factors affecting the number of mutations among relapse cases have not been published to our knowledge, so further studies are needed to determine factors affecting the mutation rate.

6.4 Risk factors for non-successful treatment

Male gender is a risk factor for both death as outcome and for TB recurrence. Older age increases the risk for death, whereas younger age increases the risk for TB recurrence. The combined effect of comorbidities on the risk of death, using the Charlson comorbidity index, has been published very rarely earlier (Fløe et al., 2018). Drug resistance predicts non-successful treatment outcomes other than death. A pulmonary disease site strongly correlates with the risk of TB recurrence in the long run.

6.4.1 Male gender

Our observation of male gender as a risk factor for both death as outcome and for TB recurrence has been published in earlier studies, but the risk ratios between genders are exceptionally high in our study (Fløe et al., 2018; Kim et al., 2016). Other studies have also associated male gender with non-successful outcomes other than death (Holden et al., 2019; Stosic et al., 2020). We did not find this association, but the number of cases with other non-successful outcomes was low in our study.

We could not evaluate reasons for the risk differences between genders, because we did not have data on other risk factors, such as adherence to treatment, substance abuse and smoking, the severity of TB and the use of immunosuppressive medications, for the cases in the cohorts. An unexpected finding was that among recurrent TB cases, the treatment outcomes of the episodes preceding recurrence were successful among males more than twice as commonly as among females. This means that other risk factors may be more common among men. Poor treatment adherence and substance abuse may at least partly explain this. We found that among recurrent cases, almost two thirds of males and no females had a history of substance abuse, mostly alcohol. The risk of alcohol use disorders in the Finnish population is about four fold among men compared to women and decreases with increasing age above age group 30-44 years (Peña et al., 2018). Alcohol abuse has been associated with poor treatment adherence in recurrent TB (Selassie et al., 2005). Furthermore, other studies have shown an association between alcohol abuse and death with TB (Volkmann et al., 2016; Waitt et al., 2011).

6.4.2 Age

We found that older age increases the risk for death with TB, whereas younger age increases the risk for TB recurrence. Increasing age has been associated with the risk of death with TB in several studies, but our observation of a greater risk increase with age among females than males has not been published earlier, as far as we know (Stosic et al., 2020; Vasankari et al., 2007). This may reflect that differences in other risk factors between genders are more prevalent among younger age groups. Even though death as outcome was 10 times more common among Finnish-born than foreign-born cases, origin was not an independent risk factor for death due to strikingly differing age distributions between these two groups.

We observed that younger age increases the risk for TB recurrence, whereas two earlier studies have shown that patients aged >65 years and between 25-44 years have a higher risk for TB recurrence (Kim et al., 2016; Selassie et al., 2005). We are unable to evaluate the reasons for this finding, but it may be explained by more prevalent risk factors among younger TB cases, as described earlier.

6.4.3 Comorbidities

Comorbidities are common when the proportion of elderly patients is high. Several non-infective comorbidities, such as diabetes, COPD and severe kidney disease, have been associated with death as outcome among TB cases (Fielder et al., 2002; Oursler et al., 2002; Walpola et al., 2003). We assessed the combined effect of comorbidities on the risk of death, using the Charlson comorbidity index, and found that comorbidities were associated with death as outcome in all age groups. In addition, the higher the comorbidity index, the higher the risk for death. The only earlier study assessing the combined effect of comorbidities, using the Charlson comorbidity index, is from Denmark and shows that almost 90% of TB cases in their cohort had a Charlson index of zero at the time of TB diagnosis (Fløe et al., 2018). Less than half of the cases in our cohort have a Charlson index of zero, meaning that our TB patients have substantially more comorbidities. This, together with the high proportion of elderly patients in our cohort, at least partially explains the high proportion of death as outcome in our study.

6.4.4 Drug resistance

We found that drug resistance predicts non-successful treatment outcomes other than death. Among the group of non-defined treatment outcomes, this is explained by the justifiable extension of treatment for cases with drug resistance, resulting in categorisation in outcome group still on treatment at 12 months.

Our observation of drug resistance as a risk factor for unsatisfactory outcomes as well as for solely outcomes failed and lost to follow-up combined is alarming, because cases with these outcomes may have potential to further transmit the disease. A recent multi-center European study described isoniazid resistance as a risk factor for non-successful treatment outcomes, also including cases with death as outcome, but this finding has been inconsistent (Bang et al., 2010; Cattamanchi et al., 2009; Karo et al., 2019). Our finding underscores the need for training for physicians and for updating national guidelines for treatment of drug resistant cases.

Data on drug susceptibility were not available for all cases in the cohort until year 2001, so we did not evaluate the impact of drug resistance on TB recurrence. Other studies have shown incompatible results concerning the risk of TB recurrence among cases with drug resistance, excluding MDR cases (Brugueras et al., 2020; Jasmer et al., 2004).

6.4.5 Pulmonary disease site

We observed that the pulmonary disease site correlates with the risk for TB recurrence, which has also been shown in some earlier studies (Crofts et al., 2010; Kim et al., 2016). Over 40% of the recurrent cases in our study had coincidental pulmonary and extrapulmonary disease in the first episode (data not shown). Having both pulmonary and extrapulmonary infection has been associated with the risk of TB recurrence and outcome as death in earlier studies (Millet et al., 2009; Pradipta et al., 2018). Our study for treatment outcomes included only pulmonary TB cases, in line with most other studies; thus, we could not evaluate the risk differences for outcomes between pulmonary and extrapulmonary TB cases. A recent study from Serbia showed that death as outcome and outcome lost to follow-up are more common among pulmonary TB cases than extrapulmonary TB cases (Stosic et al., 2020). However, in a Finnish cohort from the mid-1990s, pulmonary TB cases showed a higher treatment success rate due to lower proportions of outcomes lost to follow-up and still on treatment, even though death as outcome was more common among pulmonary cases (Vasankari et al., 2010; Vasankari et al., 2007).

6.5 Validity of treatment outcomes notified and adequacy of prescribed treatment regimens in TB episodes preceding recurrence

We evaluated the validity of treatment outcomes notified in the cohort of pulmonary TB cases and the adequacy of prescribed treatment regimens in TB episodes preceding recurrence and found substantial deviations from the guidelines. The actual treatments of the first episode among recurrent TB cases have rarely been published, and the adequacy of treatment regimens and the validity of notified treatment outcomes have not been assessed in earlier publications to our knowledge (Pascopella et al., 2011; Selassie et al., 2005).

The median duration of TB treatment in our cohort of microbiologically confirmed pulmonary TB cases was almost 7 months. This is clearly longer than the standard treatment duration of 6 months recommended by WHO, which was the median duration of treatment in a recent study from Denmark (Holden et al., 2019). We used 5.5 months as minimum duration for adequate standard treatment in line with an earlier study from Finland (Vasankari et al., 2007). A high majority of studies on treatment outcomes do not present the duration of treatments, but in some

countries, such as in France, receiving at least 80% of the standard TB treatment, which means less than 5 months, is demanded for successful treatment outcomes (Guthmann et al., 2020). Less than 1% of cases with a successful outcome had received treatment for less than 5.5 months in our study, according to treatment durations notified. However, we found that almost 10% of cases with successful outcomes should have been classified with outcome still on treatment according to the national guidelines. The treatment outcome still on treatment is thus much more common than the 4% notified. In addition, almost one fifth of cases originally notified with an unsatisfactory treatment outcome were misclassified. Even though not published from other countries, shortcomings in treatment outcome evaluation are probably present globally considering the fact that in Finland, treatment outcome evaluation is recommended to be evaluated by one trained physician in each hospital district instead of the treating physician as in many countries.

Almost one third of our recurrent cases had been prescribed with inadequate treatment in the TB episode preceding recurrence. In addition, almost 1 in 6 cases were still on treatment at twelve months. Suboptimal TB treatment has been associated with the risk of recurrence earlier, and treatment lasting for more than 12 months has been associated with poor treatment adherence, a factor related to the risk of recurrence (Pascopella et al., 2014; Selassie et al., 2005). We could not evaluate the fulfillment of prescribed treatments, because this was a retrospective study, but DOT was implemented for only about 10% of cases in TB episodes preceding recurrence.

A need exists to review national guidelines and provide further training for physicians treating TB cases and evaluating treatment outcomes due to shortcomings observed in allocation of treatment outcomes in the cohort and in prescribed treatment regimens in episodes preceding recurrence among recurrent cases. These shortcomings probably are widely present and should be taken into consideration when comparing the quality of TB treatment between countries, even though they are not published from other countries.

6.6 Strengths and limitations

The study's strengths are the use of comprehensive national cohorts with large numbers of cases and the long study periods. The reporting rate for treatment outcomes was very high, 99%. We carried out careful validation for cases with suspected recurrent TB by assessing medical records data, which is rarely done in

register-based studies of recurrent TB. Furthermore, we used a novel, more discriminating genotyping method, whole genome sequencing, to distinguish relapses from re-infections.

The main limitations of the study result from the retrospective, register-based cohort study design. We miss TB cases that are not notified to TTR, but the register has been shown to have high sensitivity and specificity for TB (Kokki et al, 2005). Only a limited amount of data is collected in TTR; therefore, we do not have cohort data on some factors that may have an effect on treatment success, such as substance abuse, the severity of the TB infection and the implementation of DOT. Some results are only descriptive, because we did not have a case-control study design. Additionally, only positive HIV test results are notified to TTR.

Furthermore, our evaluation of the validity of notified treatment outcomes is not complete, because we did not have data on treatment regimens prescribed for cases notified with a successful outcome. Among recurrent cases, we could not evaluate adherence to treatment in the episodes preceding recurrence, because this was rarely mentioned in the medical records. The Charlson comorbidity index misses some disease groups which may have an impact on the treatment outcome, such as inflammatory bowel disease and vasculitis, in which immunosuppressive medication is commonly used.

Finally, relapses were distinguished from re-infections among recurrent TB cases when isolates from both TB episodes were available for genotyping. The analysis could be performed for only half of the cases with at least two culture positive TB episodes. Until the year 2000, only every tenth *M. tuberculosis* isolate was stored frozen in the culture collection. The bias caused in the analysis is acceptable, because the decision to store frozen *M. tuberculosis* isolates in our national reference laboratory was random.

6.7 Future considerations

Treatment outcomes are notified for only pulmonary TB cases in Finland, so our study of treatment outcomes did not include extrapulmonary cases. A study with a medical records review is needed to evaluate treatment outcomes and current risk factors for non-successful outcomes among extrapulmonary TB cases. An additional study for MDR-cases, including evaluation of prescribed treatments and treatment outcomes, is in progress by our research group.

Only a few retrospective studies have classified cases with death as outcome as TB-related deaths and non-TB-related deaths (Beavers et al., 2018; Guthmann et al., 2020). Even though complex, evaluating the importance of TB as a cause of death among fatal cases, preferably in a prospective study, would be particularly beneficial in Finland due to the high rate of death as outcome, as well as the high rate of confounding factors such as comorbidities and older age. Additionally, early deaths with TB are common, so the delays in TB diagnostics and reasons for the delays by the patient and the health care system should be evaluated to expedite and improve the diagnostics. A systematic review of delays in the diagnosis and treatment of tuberculosis found a wide variation in the length of the delays and the risk factors for diagnostic delays (Storla et al., 2008).

Only a few studies, with low numbers of patients, distinguish relapses from re-infections among recurrent cases (Rosser et al., 2018). The conclusions to be drawn for national TB control programs depend on the background of TB recurrence, because relapses and re-infections reflect clearly different situations. Differences in study designs, TB incidences and demographics also make interpreting published results difficult. Further prospective or case-control cohort studies for the risk factors of recurrent TB distinguishing relapses from re-infections are needed.

Finally, the epidemiologic situation in Finland is changing gradually. TB among natives is decreasing with the decline of those generations, that were infected with TB decades ago. This, together with increasing immigration from countries with high TB incidences, is leading to a higher proportion of immigrants among TB cases (Räisänen et al., 2015). Consequently, TB cases are expected to be younger and have fewer comorbidities. The importance of other risk factors may rise, instead. It is therefore essential to continuously assess TB situation in Finland.

7 SUMMARY AND CONCLUSIONS

Adequate TB treatment is important both to cure the patient and to decrease transmission together with early diagnoses and appropriate contact investigations. WHO recommends assigning treatment outcomes for all TB patients. Evaluation of treatment outcomes presents the efficacy of treatment in a shorter period. A more permanent cure can be assessed by evaluating TB recurrence. It is important to distinguish the two ways of recurrences, relapses and re-infections when evaluating the quality of TB treatment, because only relapses reflect shortcomings in the treatment of the previous TB episode.

This study evaluated the quality of tuberculosis treatment in Finland by assessing treatment outcomes at one year and recurrence of tuberculosis in a longer period and determined some risk factors for non-successful treatment.

The main findings and conclusions are as follows:

1. The proportion of successful treatment outcomes is 75% among microbiologically confirmed pulmonary TB cases. The proportion of death as outcome is high (16%), whereas outcomes failed (0.2%), lost to follow-up (0.8%) and not known (2.5%), presenting cases that may have a potential to transmit the disease, are rare.
2. Recurrent TB is rare in Finland (0.6% of the cases). However, persons with an earlier episode of TB constitute a high-risk group with 10-20 times higher incidence of TB than in the general population.
3. A high majority (>80%) of recurrent cases are due to relapses.
4. Male gender is a risk factor for both death as outcome and for TB recurrence. Older age increases the risk for death, whereas younger age increases the risk for TB recurrence. Comorbidities are common among TB cases and are associated with the risk of death in all age groups. Drug resistance predicts non-successful treatment outcomes other than death. A pulmonary disease site strongly correlates with the risk for TB recurrence in the long run.

5. There are substantial deviations from the guidelines in notified treatment outcomes and prescribed treatment regimens in TB episodes preceding recurrence.

In conclusion, the rate of successful treatment outcomes is clearly below the target of 85% set by WHO. This is mainly due to the high proportion of death as outcome. The high mean age among Finnish-born cases and the presence of comorbidities at least partially explain this situation. However, because most fatal cases die early, better awareness of TB among the population and health-care professionals is needed to reach early suspicion and timely diagnosis and treatment of TB. Younger male patients show high relative risks for death as outcome as well as for TB recurrence, and cases with drug-resistant strains show high relative risks for non-successful outcomes other than death; thus, special attention should be paid to choosing the correct treatment regimens for them and to promoting treatment adherence using DOT. Finally, shortcomings observed in allocation of treatment outcomes in the cohort and in prescribed treatment regimens in episodes preceding recurrence show that a need exists to review national guidelines and to further train physicians treating TB cases.

8 KIITOKSET (ACKNOWLEDGEMENTS)

Tämä väitöskirjatutkimus on toteutettu Tampereen Yliopiston lääketieteen ja terveysteknologian tiedekunnan, Tampereen yliopistollisen sairaalan keuhkoklinikan, Terveyden ja hyvinvoinnin laitoksen (THL) sekä Filha ry:n yhteistyönä.

Esitän nöyrimmät kiitokseni ohjaajilleni, työelämäprofessori Tuula Vasankarille, dosentti Hanna Soinille sekä apulaisprofessori Lauri Lehtimäelle. Tuula, tietomääräsi yhdistettynä vankkaan käytännön kokemukseesi tuberkuloosista tekee sinusta raudanlujan osaajan. Olet innostunut ja innostava ohjaaja, jonka kanssa on aina mukava keskustella sekä asiasta että asian vierestä. Hanna, tätä tutkimusta ei olisi syntynyt ilman opastustasi tartuntatautirekisterin syövereihin. Kykysi kuvata monimutkaisia asioita ymmärrettävästi sekä ohjaajana, luennoitsijana että kirjoittajana on ilmiömäinen. Lauri, ohjauksesi sekä tohtoriopintojen että käytännön työn saralla on merkinnyt minulle paljon. Olet pyöräilevä esimerkki siitä, miten päättäväisellä asenteella, olennaiseen keskittymisellä ja ripauksella huumoria pystyy toimimaan sekä menestyvänä tutkijana, innostavana luennoitsijana että taitavana kliinikkona.

Kiitän väitöskirjani esitarkastajia, dosentti Kaisu Rantakokko-Jalavaa Turun yliopistosta sekä dosentti Tuula Toljamoa Oulun yliopistosta, rakentavista kommenteista ja korjausehdotuksista.

Lämpimät kiitokseni kuuluvat väitöskirjani seurantaryhmän jäsenille, emeritus tutkimusprofessori Petri Ruudulle sekä LT Iiris Rajalahdelle. Petri, panoksesi tämän väitöstyön osajulkaisuissa on ollut korvaamaton! Olet hiihtävä esimerkki siitä, miten laajasti sivistynyt ja asioista innostuva kollega säteilee energiaa ympärilleen. Iiris, arvostan suuresti sinulta saamaani tukea sekä kliinisen työn haasteissa että tutkimustyön vastatuulissa. Ilman sinua en olisi löytänyt tietäni mykobakteerien ihmeelliseen maailmaan.

Kiitän kaikkia väitöskirjani osatöissä mukana olleita henkilöitä, erityisesti THL:n tutkimusprofessori Outi Lyytikäistä, tilastotutkija Jukka Ollgrenia, sekä erikoistutkija Marjo Haanperää avusta monissa yllättävissäkin käänteissä. Jukka on tarvittaessa yön pimeinäkin tunteina rakentanut tilastotieteellisiä malleja, jotka jaksavat hämmästyttää kliinikkoa.

Haluan kiittää Tays:n keuhkoklinikan johtoa, dosentti Seppo Saarelaista, LT Kirsi Laasosta sekä LT Hannele Hasalaa tutkimustyön teon mahdollistamisesta. Kirsi, kannustit minua valitsemaan tutkimusaiheeksi tämän harvinaisen, mutta niin monimuotoisen infektion, vaikka suoraviivaisempiakin reittejä tutkimuksen saloihin olisi ollut tarjolla. Ajattelen lämmöllä Tays:n keuhkoklinikan muita kollegoja ja ammattiryhmiä, joiden kanssa työskentely on aina mielenkiintoista ja tapahtumarikasta. Erityisesti LT Sirpa Leivo-Korpelan ja LL Anu Kojon kanssa käydyt ammatilliset ja muut monenkirjavat keskustelut antavat minulle voimaa ja toivoa. Kiitos että olette!

Haluan osoittaa kiitokseni myös monille muille kannustaville ja inspiroiville ystävilleni. LT Pipsa Peromaa-Haavisto ja dosentti Tanja Skyttä, olette esimerkillänne osoittaneet kliinisen työn, tutkimustyön ja hyvän perhe-elämän yhdistämisen mahdolliseksi. Pitkä ystävyytemme on näyttänyt, että teihin voi tukeutua sekä myötä- että vastamäissä.

Kiitän vanhempiani Stinaa ja Erkkiä tuesta, kannustuksesta ja rakkaudesta. Olette opettaneet, että vaikka eteenpäin pyrkiminen on tärkeää, silti tärkeintä on huolehtia läheisistään. Kiitän veljeäni Kimmoa rinnalla kulkemisesta sekä hänen lapsiaan Kristiania, Jenniä ja Markusta ilosta, jota tuotte elämääni. Ajattelen lämmöllä tätiäni Mairea, jota parempaa kuuntelijaa ei maailmasta löydy. Suurimmat kiitokseni kuuluvat Korkeille eli puolisolleni Jannelle sekä lapsillemme Juliukselle, Moonalle ja Vilhelmille. Julius, sanavalmiutesi ja rennon letkeä asenteesi vie sinut vielä pitkälle. Moona, sinusta näkee, miten lahjakas ja tarmokas nuori nainen loistaa, kun saa tehdä rakastamiaan asioita. Vilkku, yleistietosi, reiluutesi ja kokintaitosi jaksavat hämmästyttää minua. Ja vielä Janne, ikuinen optimismisi, tietotekniset taitosi ja suuri panoksesi perhe-elämässämme ovat mahdollistaneet väitöstyöni valmistumisen. Rakkautemme on vankka peruskallio, jonka päälle tämä hyvä elämä on rakentunut. Kuljemme kumpikin omilla poluillamme, mutta aina samaan suuntaan.

Tätä tutkimusta ovat tukeneet Tampereen tuberkuloosisäätiö, Pirkanmaan sairaanhoitopiirin kuntayhtymä, Tampereen yliopisto, Väinö ja Laina Kiven säätiö, Tuberkuloosin vastustamisyhdistyksen säätiö sekä Hengityssairauksien tutkimussäätiö.

Tampereella 20.8.2020

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10 PUBLICATIONS

PUBLICATION I

Risk factors affecting treatment outcomes for pulmonary tuberculosis in Finland 2007-2014: a national cohort study

Korhonen V., Lyytikäinen O., Ollgren J., Soini H., Vasankari T., Ruutu P.

BMC Public Health 20: 1250-1258
DOI 10.1186/s12889-020-09360-7

Korhonen et al. BMC Public Health (2020) 20:1250
https://doi.org/10.1186/s12889-020-09360-7

BMC Public Health

RESEARCH ARTICLE Open Access

Risk factors affecting treatment outcomes for pulmonary tuberculosis in Finland 2007–2014: a national cohort study

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Abstract

Background: Major transition in tuberculosis (TB) epidemiology is taking place in many European countries including Finland. Monitoring treatment outcome of TB cases is important for identifying gaps in the national TB control program, in order to strengthen the system. The aim of the study was to identify potential risk factors for non-successful TB treatment outcomes, with a particular focus on the impact of comorbidities. We also evaluated the treatment outcome monitoring system.

Methods: All notified microbiologically confirmed pulmonary TB cases in Finland in 2007–2014 were included, except multi-drug resistant (MDR) cases. Nationwide register data were retrieved from: Infectious Diseases Register, Population Register, Cause of Death Register and Hospital Discharge Register. Non-successful outcomes were divided into three groups: death, unsatisfactory outcomes and non-defined outcomes. Logistic regression analyses were used to identify risk factors for non-successful outcomes.

Results: Treatment outcomes were notified for 98.6% of study cases ($n = 1396/1416$). Treatment success rate was 75%. The main reason for non-successful outcome was death (16%), whereas outcomes failed and lost to follow-up were rare (1% together). In a multivariable model, risk factors for death as outcome were increasing age, male gender and Charlson comorbidity index ≥1, for unsatisfactory outcomes non-MDR drug resistance and TB registered in the first study period, and for non-defined outcomes non-MDR drug resistance. Among 50 cases with unsatisfactory outcomes, we observed false outcome allocations in eight (16%), and > 2% of the cases transferred to another country or disappeared before or during treatment.

Conclusions: With a high proportion of older population among tuberculosis cases, death is a common treatment outcome in Finland. Comorbidity is an important factor to be incorporated when interpreting and comparing outcome rates. There was a considerable inconsistency in outcome allocation in the monitoring system, which implies that there is need to review the guidelines and provide further training for outcome assessment.

Keywords: Treatment outcome, Treatment, Mortality, Cohort analysis, Surveillance, Tuberculosis

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Background

Adequate TB treatment is important both for the recovery of the patient and prevention of transmission, together with early diagnoses and appropriate contact investigations. Tuberculosis treatment outcome monitoring is essential for evaluating the function of a national tuberculosis control program. European Centre for Disease Prevention and Control (ECDC) [1] and World Health Organization (WHO) [2] recommend assessing outcomes for all TB cases with different criteria and timing for drug-susceptible and multidrug-resistant (MDR) TB. Recommendations by ECDC for European Union/European Economic Area (EU/EEA) countries are consistent with WHO recommendations, except that the outcomes are assessed at the latest at 12 months after treatment initiation and hence an outcome group of 'still on treatment' is included [1].

Treatment success rates in most European countries do not reach the global target of 85% set by WHO, varying widely from 56% in Hungary to 89% in Norway during 2002–2011 [3]. In 2015, WHO has set more ambitious targets for treatment outcome, including success of at least 90% by year 2025, as well as reducing tuberculosis deaths by 75% [4]. In routine outcome monitoring from European region countries for cases registered in 2016, treatment success rate was 75% and proportion of fatal cases was 8% [1].

In Finland, with a current TB incidence rate of 4.1/100000, the incidence among Finnish-born has decreased and immigration from high-TB-incidence countries has increased raising the proportion of TB cases with foreign origins from 4% in 1995–1996 [5] to 40% in 2017 [6]. The majority of TB among the Finnish-born population is in the elderly persons from reactivation of a latent TB infection, reflected by the mean age of 71 years among Finnish-born TB cases in 2017, the highest in the EU/EEA countries [1]. In countries with a high proportion of elderly cases, comorbidities are common, probably contributing to higher mortality observed in TB outcome monitoring [7] and complicating the interpretation of treatment non-success rates between countries.

A previous cohort study of culture-confirmed pulmonary TB cases registered in 1995–1996 in Finland showed that there was a wide variety of TB treatment combinations and durations [5]. Treatment success rate was 65, and 19% of cases died before or during TB treatment [5]. Thereafter, a National TB Control Program has been published, with recommendations for TB treatment and the use of directly observed treatment (DOT) for risk groups in 2006 [8] and for all patients in 2013 [9].

The aim of our study was to identify potential risk factors for non-successful TB treatment outcomes, with a particular focus on the impact of comorbidities. We also evaluated the treatment outcome monitoring system in order to identify ways to improve the treatment success rate, and to strengthen the TB monitoring program in the changing epidemiologic environment.

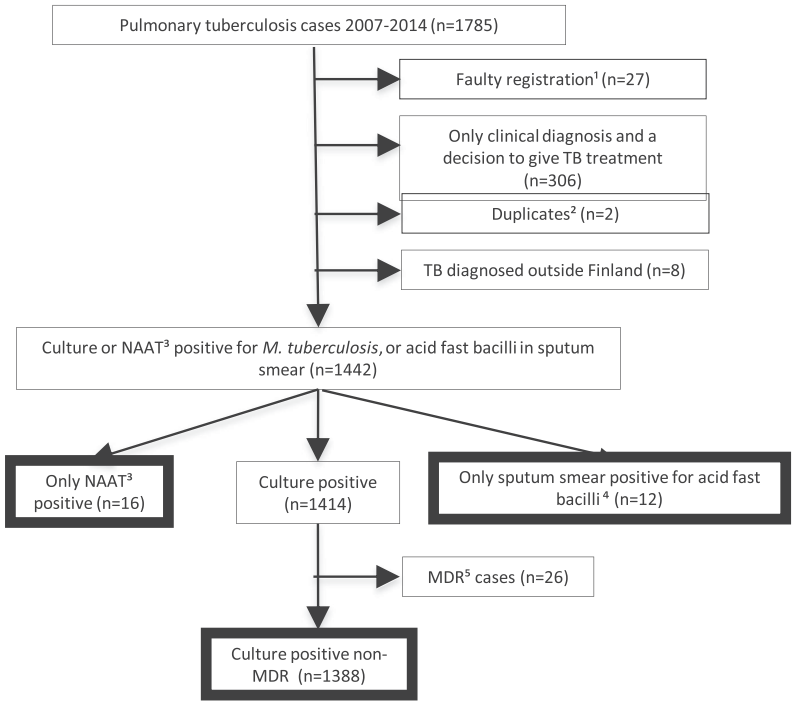
Methods

Data sources

Our cohort study was based on the data of National Infectious Diseases Register (NIDR), National Population Register, Cause of Death Register and Finnish Hospital Discharge Register. This study included all microbiologically confirmed pulmonary TB cases, except MDR-TB cases, notified from January 1, 2007, to December 31, 2014, to the National Infectious Disease Register (NIDR) (Fig. 1).

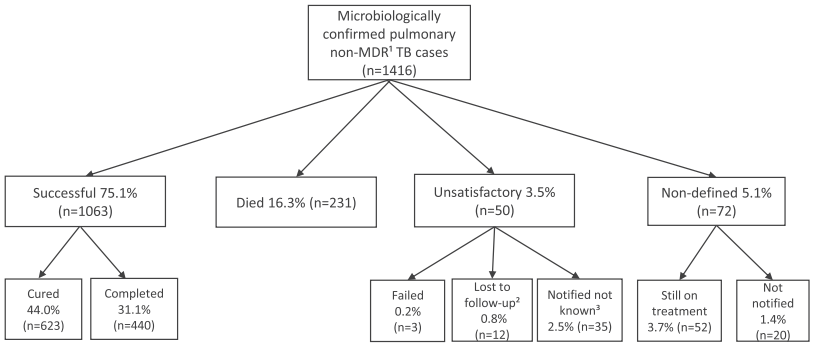
Reporting of TB cases to NIDR is mandatory to all physicians and laboratories, and laboratories send an automated reminder to the treating physician of the obligation to notify, when TB is microbiologically confirmed. The date of death is retrieved from the national population registry and HIV cases are linked to TB cases in NIDR. Data from the different sources are automatically linked as a case by the unique national identifier. Following data was collected from NIDR: patient identification (name and unique national identifier), age, gender, origin (country of birth or if unknown, nationality), place of residence, a history of earlier TB diagnosis (since 1950) and its treatment (year, did the patient receive (full) treatment), ICD10 codes (International Classification of Diseases, 10th Revision), microbiological test results including the results of drug susceptibility testing in the national reference laboratory, date of registration in NIDR, and the intent to give full TB treatment.

Since 2007, treatment outcomes for all microbiologically confirmed pulmonary TB cases in Finland are notified to NIDR according to the WHO/IUATLD recommendation for EU/EEA countries [10] with two additional categories 'Still on treatment' [1] and 'Notified not known' (Fig. 2). At 12 months from the date of registration, NIDR sends a request for outcome notification to the hospital district ($n = 20$), from which the TB case was originally notified. Outcome notification includes data on treatment of the current TB episode (dates on treatment commencement and cessation, total duration of treatment interruptions, duration of rifampicin plus isoniazid given concomitantly), the patient moving to another hospital district during treatment and the district to which the case moves. One trained physician notifies all outcomes in each hospital district. For cases with outcomes failed, lost to follow-up and notified as not known, we acquired additional information from the notifying health care facility to assess whether the outcomes had been notified correctly.



[1]M. bovis BCG registered as M. tuberculosis (n=2), LTBI registered as pulmonary TB (n=1), extrapulmonary TB originally registered as pulmonary TB (n=24)
[2]Only one long continuous TB episode registered as two episodes
[3]NAAT=nucleic acid amplification test
[4]and the treating physician reporting an intent to give full TB treatment
[5]multi-drug resistant

Fig. 1 Steps in identifying cases for treatment outcome evaluation, Finland 2007–2014



[1]multi-drug resistant
[2]=Treatment interrupted
[3]including transferred out cases

Fig. 2 Treatment outcomes in 1416 microbiologically confirmed pulmonary non-MDR TB cases, 2007–2014

For cases with death as outcome, we obtained the structured data on the causes of death from the Cause of Death Register at Statistics Finland, in order to determine whether death was TB-related. A death was classified as TB-associated, if TB was the immediate or underlying cause of death or a significant condition contributing to death.

To assess comorbidities, all diagnoses, coded in ICD-10 for study cases, were obtained from National Hospital Discharge Register [11] three years preceding and one year following TB registration date. Comorbidity scores were calculated using the model developed by Charlson et al. [12]. The Charlson-Deyo [13] comorbidity index contains 17 diagnostic groups for chronic diseases with a weighted score that is associated with 1-year mortality, and the model has been used with administrative register data, and with ICD-10 diagnostic codes [14].

Analysis and statistics

According to the WHO criteria [2], successful treatment outcome includes cases with completed treatment, with or without microbiological evidence of cure. For statistical analysis, we combined failed cases, with a positive culture or sputum smear at 5 months or later, cases with outcomes lost to follow-up (treatment interrupted) and notified not known, including transferred out cases, as a group of unsatisfactory outcome (Fig. 2). Cases with outcomes still on treatment and not notified were combined as a group of non-defined outcome. Death as outcome includes also cases diagnosed post mortem (e.g. at autopsy).

The allocation of each case in outcome grouping in Fig. 2 is based on the original routine outcome notification. For the study, patient chart review was performed for cases with outcomes failed, lost to follow-up and notified not known. We used 5.5 months as the minimum duration for full treatment, as described in our previous study [5]. We used logistic regression analyses for modelling the outcome of tuberculosis treatment. Apart from well-known risk factors, we incorporated in the multivariable analysis the variables with a 20% likelihood in the univariate analysis, as well as the Charlson comorbidity score. We used multi-imputation of the missing data of some variables with the MICE algorithm [15] assuming that the missing values were randomly distributed (MAR). In the model, the shape of the variable age was selected using Akaike information criteria (AIC) [16] because of the high risk of death among the very young children (J-shaped curve). We found that the inverse of age (1/age) was a parsimonious way of modelling the young age effect on the mortality. Furthermore, we incorporated into the model the variables with interactions with age, for which statistical evidence was seen with e.g. AIC. Due to possible clustering in hospital district level, robust standard error estimators were used in the model.

Results

Of the 1785 pulmonary TB cases notified during 2007–2014, we identified 1416 bacteriologically confirmed non-MDR cases (Fig. 1). The proportion of males was 64.6% and those of foreign origin 27.3%. Three most frequent countries of birth among foreign-born cases were Somalia, Vietnam and countries of the former Soviet Union. The median age of cases was 60 years (range, 0–98 years). Finnish-born cases were significantly older (median age 70 vs. 28 years, $p < 0.001$) and had more comorbidities (Charlson comorbidity index> 0 in 61.9% vs. 22.1%, $p < 0.001$) than cases with foreign origins. Of the *M. tuberculosis* isolates, 89.1% were fully susceptible. The proportions of mono-resistant and non-MDR poly-resistant isolates were 6.5 and 1.8%, respectively. Twenty-five cases (1.8%) were HIV positive.

Treatment outcomes and treatment duration

Treatment outcome was notified for 1396 cases (98.6%) (Fig. 2). Successful treatment outcome was achieved in 1063 cases (75.1%). When calculated for only cases with a notified outcome, treatment success rate was 76.1%. The main reason for non-successful outcome was death in 231 cases (16.3%). Outcomes 'failed' and 'lost to follow-up' together accounted for 15 cases (1.0%).

Among the 1204 cases with data on treatment duration, median duration was 204 days (interquartile range (IQR), 183–278 days). Among cases notified as successfully treated and with data on treatment duration (n = 1010/1063), 6 cases (0.6%) received treatment for less than 5.5 months, 913 cases (90.4%) for 5.5 months to one year, and 91 cases (9.0%) for more than a year. Among cases with outcome lost to follow-up (treatment interrupted) and with data on treatment duration (n = 10/12), one (10%) did not receive any medication, five (50%) received treatment for less than 5.5 months, three (30.0%) for 5.5 months to one year and one (10%) for more than one year. Data on the duration of pauses in treatment was available for 773 (64.2%) of the 1204 cases. Among these, TB treatment was discontinued for at least one day for 242 cases (31.3%) and the mean duration of the sum of pauses was 6.7 days per patient.

Fatal cases

The proportions of death as outcome for Finnish-born and foreign-born cases were 21.8 and 1.6%, respectively. The median time from the date of registration to death was 44 days (IQR, 12–121 days). Among the 150 cases who received TB medication, with data on treatment duration, 81 cases (54.0%) received medication for less than 2 months, 56 (37.3%) for two to less than 5.5 months, 12 (8.0%) for 5.5 months to a year and one (0.7%) for more than a year. Fifty-eight (25.1%) fatal cases did not receive any TB medication.

Table 1 Univariate analysis for risk factors for non-successful outcomes in 1416 pulmonary non-MDR TB cases

Variable	Category	Successful (*n* = 1063)	Died (*n* = 231)	Univariate RRR[a] for death[b] (95%CI) p	Unsatisfactory[c] (*n* = 50)	Univariate RRR[a] for unsatisfactory[b] (95%CI) p	Non-defined (*n* = 72)	Univariate RRR[a] for non-defined[b] (95%CI) p
Age	Age/10 years	Median 57 years	Median 79 years	1.77 (1.53–2.06) **< 0.001**	Median 27.5 years	0.65 (0.59–0.72) **< 0.001**	Median 51.5 years	0.96 (0.87–1.05) 0.341
Gender	Female (*n* = 501)	*n* = 387 (77.2%)	*n* = 68 (13.6%)	1	*n* = 17 (3.4%)	1	*n* = 29 (5.8%)	1
	Male (*n* = 915)	*n* = 676 (73.9%)	*n* = 163 (17.8%)	1.37 (1.06–1.77) **0.015**	*n* = 33 (3.6%)	1.11 (0.68–1.82) 0.677	*n* = 43 (4.7%)	0.85 (0.49–1.46) 0.552
Origin	Finnish (*n* = 1030)	*n* = 744 (72.2%)	*n* = 225 (21.8%)	1	n = 13 (1.3%)	1	*n* = 48 (4.7%)	1
	Foreign (*n* = 386)	*n* = 319 (82.6%)	n = 6 (1.6%)	0.06 (0.04–0.11) **< 0.001**	*n* = 37 (9.6%)	6.64 (3.68–11.98) **< 0.001**	*n* = 24 (6.2%)	1.17 (0.70–1.93) 0.551
Drug resistance non-MDR[d]	No (*n* = 1262)	*n* = 966 (76.5%)	*n* = 207 (16.4%)	1	n = 38 (3.0%)	1	*n* = 51 (4.0%)	1
	Yes (*n* = 118)	n = 74 (62.7%)	n = 12 (10.2%)	0.76 (0.33–1.74) 0.511	n = 12 (10.2%)	4.12 (2.02–8.41) **< 0.001**	n = 20 (16.9%)	5.12 (3.23–8.11) **< 0.001**
Sputum smear[e]	Negative (*n* = 629)	*n* = 481 (76.5%)	n = 96 (15.3%)	1	n = 23 (3.7%)	1	n = 29 (4.6%)	1
	Positive (*n* = 694)	*n* = 527 (75.9%)	*n* = 105 (15.1%)	0.998 (0.82–1.21) 0.986	n = 24 (3.5%)	0.95 (0.48–1.87) 0.888	n = 38 (5.5%)	1.20 (0.80–1.79) 0.387
Study period	2007–2010 (*n* = 760)	*n* = 570 (75.0%)	n = 118 (15.5%)	1	n = 31 (4.1%)	1	*n* = 41 (5.4%)	1
	2011–2014 (*n* = 656)	*n* = 493 (75.2%)	*n* = 113 (17.2%)	1.11 (0.88–1.39) 0.383	*n* = 19 (2.9%)	0.71 (0.51–0.99) **0.045**	n = 31 (4.7%)	0.87 (0.43–1.77) 0.709
Charlson[f]	0 (*n* = 631)	*n* = 550 (87.2%)	*n* = 30 (4.8%)	1	n = 17 (2.7%)	1	*n* = 34 (5.4%)	1
	1–2 (*n* = 462)	*n* = 322 (69.7%)	*n* = 111 (24.0%)	6.32 (4.57–8.74) **< 0.001**	n = 9 (2.0%)	0.90 (0.30–3.73) 0.859	n = 20 (4.3%)	1.00 (0.60–1.67) 0.985
	3–4 (*n* = 172)	*n* = 109 (63.4%)	*n* = 54 (31.4%)	9.08 (5.63–14.66) **< 0.001**	n = 1 (0.6%)	0.30 (0.04–2.36) 0.251	*n* = 8 (4.7%)	1.19 (0.50–2.82) 0.697
	≥5 (*n* = 85)	n = 52 (61.2%)	*n* = 28 (32.9%)	9.87 (4.63–21.06) **< 0.001**	n = 1 (1.2%)	0.62 (0.11–3.67) 0.600	n = 4 (4.7%)	1.24 (0.55–2.79) 0.596

[a]ratio of relative risks
[b]compared to successfully treated cases
[c] including cases transferred out
[d]Information missing for 36 cases (23 successfully treated cases, 12 fatal cases, 1 non-defined case)
[e]Information missing for 93 cases (55 successfully treated cases, 30 fatal cases, 3 other defined unsuccessfully treated cases, 5 non-defined outcome)
[f]Information missing for 66 cases (30 successfully treated cases, 8 fatal cases, 22 other defined unsuccessfully treated cases, 6 non-defined outcome)

According to death certificates, death was classified as TB-associated in 167 fatal cases (72.3%); in 101 (43.7%) TB was as the immediate or underlying cause of death, and in 64 (28.6%) as a significant condition contributing to death. Charlson comorbidity scores for cases with TB-associated death did not differ from scores for non-TB-associated deaths ($p = 0.13$).

In univariate analysis, risk factors for death were increasing age, male gender, Finnish origin and Charlson comorbidity index ≥1 (Table 1). In multivariate logistic regression model, independent association for death as outcome was observed with increasing age, male gender and Charlson comorbidity index (Table 2). Interaction was observed between age and gender. The association

Table 2 Multivariable analysis for risk factors for death in 1416 pulmonary non-MDR TB cases

Variable	Multivariable RRR[a] for death (95% CI)	p
Age/10 years[b]		
Finnish male	1.50 (1.25–1.80)	**< 0.001**
Finnish female	1.94 (1.42–2.65)	**< 0.001**
Foreign origin male	1.20 (0.92–1.57)	0.183
Foreign origin female	1.55 (1.04–2.30)	**0.030**
Male	9.54 (1.36–66.70)	**0.023**
Foreign origin	1.01 (0.20–5.21)	0.991
Drug resistance non-MDR[c]	0.84 (0.33–2.11)	0.705
Study period 2011–2014	1.06 (0.80–1.40)	0.695
Charlson 1–2[d]	3.03 (2.11–4.35)	**< 0.001**
Charlson 3–4[d]	3.55 (2.20–5.75)	**< 0.001**
Charlson ≥ 5[d]	5.94 (2.65–13.33)	**< 0.001**

[a]ratio of relative risks
[b]1/age was also included in the model to explain the increase in the risk of death among very young children (curve J-shaped), $p < 0.001$.
[c]Information imputed for 23 successfully treated cases, 12 fatal cases
[d]Information imputed for 30 successfully treated cases, 8 fatal cases

of higher Charlson index with increased risk for death was seen in all age groups (data not shown).

Unsatisfactory outcomes

Among the 12 cases with outcome lost to follow-up, premature treatment cessation was due to an adverse reaction in three cases, non-compliance because of substance abuse in four, and in one, a positive TB culture had been missed. Among the 35 cases notified as outcome not known, 33 cases were of foreign origin. Out of the 35, 25 transferred to another country before or during TB treatment, three transferred within Finland and four cases disappeared. Among the 50 cases with unsatisfactory outcomes, we observed false outcome allocations in eight cases (16%).

In univariate analysis, risk factors for unsatisfactory outcome (failed, lost to follow-up and notified not known) were younger age, foreign origin, non-MDR drug resistance and TB registered in the first study period (years 2007–2010) (Table 1). In multivariable logistic regression model, an independent association was observed with non-MDR drug resistance (ratio of relative risks (RRR), 2.6; 95% confidence interval (CI), 1.2–5.8) and TB registered during the first study period (RRR, 1.5; 95%CI, 1.1–2.1). Interaction was observed between age and origin. When we restricted analysis to only outcomes 'failed' and 'lost to follow-up' combined, we found that non-MDR drug resistance was a clear predictor for these outcomes (univariate RRR, 4.75; 95%CI, 2.05–11.02, multivariate RRR, 4.93; 95%CI, 2.26–10.74).

Non-defined outcomes

Among the 52 cases with outcome still on treatment at 12 months, the reasons for extension of treatment was reported for 36 cases (69%) and were following: having non-MDR drug resistant isolate ($n = 19$), miliary/disseminated disease ($n = 5$), advanced cavitary disease ($n = 4$), pauses on treatment because of adverse drug reactions ($n = 4$), bone and joint tuberculosis ($n = 2$), recurrent TB ($n = 1$) and prolonged sputum culture positivity ($n = 1$). Only non-MDR drug resistance was associated with non-defined outcome in univariate (Table 1) and multivariable regression analysis (RRR, 5.6; 95%CI, 3.8–8.1).

Quality of treatment outcome allocations and treatment follow-up

Based on the patient chart review and additional information in the notifications of the 50 cases with an unsatisfactory outcome, we observed false outcome allocations in eight cases (16%): four among cases with outcome lost to follow-up, three among cases with outcome not known and one among cases with outcome failed. Among these eight, two should have been categorized as successful outcome, two still on treatment, one not known (transferred) and three lost to follow-up, including one refusal. According to the additional data mentioned above, 26 (1.8% of all) cases transferred to another country and seven (0.5%) cases disappeared before or during TB treatment. Among the 31 cases who were originally notified as not known (transferred to another hospital district in Finland during TB treatment), the final treatment outcome was notified for 28 (90.3%) (2 still on treatment, 1 died, 25 successful). Among cases notified as successfully treated and with data on treatment duration (1010/1063, 95%), 91 cases (9.0%) had received treatment for more than a year according to the outcome notification.

Discussion

Our population-based cohort study on risk factors for non-successful treatment outcomes of 1416 pulmonary TB cases during 2007–2014 in Finland indicates that comorbidities, in addition to age and male gender, have an independent contribution to death as outcome. Death constituted two thirds of the non-successful outcomes and was the main reason for the proportion of successful outcomes remaining far below the 85% success rate objective set by the WHO.

Although there has been a minor decline in death as outcome in pulmonary TB in Finland from 19% in 1995–1996 [5] to current 16%, the proportion of death as outcome is still remarkably higher than the overall 7 % found in the 16 EU/EEA countries in 2002–2011 [3]. However, this is in line with some other European countries, e.g. Czech Republic (18%) and Slovenia (14%), with

similar demographics among TB patients [17, 18]. Earlier studies have shown several non-infective comorbidities, e.g. diabetes [19, 20], liver [21] and kidney disease [19–21] as well as COPD [19], to be associated with death as outcome, but the combined effect of comorbidities on death with TB has rarely been studied [7]. In a case-control register study from Denmark [7], comorbidities were shown to be associated with death, and mortality among TB cases was significantly higher than among matched controls for all age groups above twenty years. With a median age as high as 70 years among Finnish-born patients, comorbidities are common. Using the Charlson comorbidity index [12, 14] we found an association between comorbidity and the risk of death; this was seen in all age groups. Beside the fact that comorbidities may directly cause death in a patient with TB, they also may cause delays in TB diagnostics by offering alternative explanations for patients' symptoms. In a study from the US, tuberculosis-related deaths were associated with patients having an alternative diagnosis, e.g. pneumonia, before TB diagnosis [22]. In our study, two thirds of fatal cases with data on TB treatment duration died before treatment was started or received treatment for less than two months (early deaths with TB). This is in line with previous studies from California [23] and North Carolina [24], emphasizing that TB diagnosis is often missed or delayed with current diagnostic tools. We did not find a clear difference in Charlson comorbidity scores between cases with TB-associated and non-TB-associated death according to data on death certificates. It has been stated that the diagnostic accuracy of death certificate data is often poor [25, 26], especially when death takes place outside hospital [27].

We found a striking difference between Finnish-born cases and those with foreign origins: over one-fifth of Finnish-born cases versus less than 2 % of those with foreign origins had death as outcome, but origin was not independently associated with death in multivariable analysis. Male gender was a risk factor for death as outcome, consistent with our earlier study in Finland [28] as well as previous reports from other low-incidence countries [3, 7, 29]. We were not able to evaluate reasons for the large risk difference between genders particularly at young age groups, because we did not have data on several risk factors, such as alcohol and substance abuse, the use of immunosuppressive medications and the implementation of directly observed therapy (DOT). Alcohol abuse has been associated with death among TB cases in several studies [23, 24, 30] and especially among patients younger than 65 years [31].

Outcomes 'failed' and 'lost to follow-up' combined accounted for only 1% of outcomes in our study, which is far less than the 8% found in the 16 EU/EEA countries in 2002–2011 [3]. We observed that non-MDR drug resistance was a risk factor for outcomes 'failed' and 'lost to follow-up' combined, as well as for the group of unsatisfactory outcomes, which includes also cases with outcome notified as not known. Isoniazid resistance has been described as a risk factor for non-successful treatment, including also fatal cases, in earlier studies in low-incidence countries [32, 33], but this finding has been inconsistent [34, 35]. Our finding underscores the need for training physicians and having a special focus in the national guidelines on appropriate treatment of drug resistant cases and the importance of routine pheno- and genotypic drug-susceptibility testing. Even though our study period did not include the year 2015, when large numbers of asylum seekers arrived Finland, more than 2% of cases transferred to another country or disappeared before or during TB treatment. This results at a high risk of treatment interruption, and the potential for continued transmission, and stresses the need to develop a system to inform the known or probable receiving country when a TB patient emigrates or disappears.

When evaluating the quality of treatment outcome notifications, we observed that 16% of cases originally notified with an unsatisfactory outcome had been misclassified. Furthermore, almost 10% of cases notified as successfully treated, whose treatment duration was reported in the outcome notification, should have been categorized as still on treatment according to the national guidelines, which sets the evaluation at 12 months from case registration. This suggests that outcome assessment has a considerable rate of deviations from guidelines, even when it is performed by one trained specialist in each hospital district. This, together with our observations on the effect of comorbidity, should be taken into consideration when comparing countries and developing European statistics on TB.

The strengths of this nationally comprehensive cohort study include the observed high reporting rate at almost 99% for pulmonary tuberculosis in the mandatory TB outcome reporting. NIDR sends a request for outcome notification to the hospital district at 12 months from the date of registration, and actively follows up for missing notifications. In addition, the outcome surveillance system was efficient in monitoring transfer of cases within Finland during treatment. We have previously shown a high coverage for the TB surveillance system [36], after which integration of automated mechanisms for laboratory notifications and automated laboratory reminder of obligation to notify TB to the treating physician have been added, ensuring a high coverage and representativeness of the data used. Comorbidities were retrieved from National Hospital Discharge Register, which has a high sensitivity and specificity for the common disease groups [11] constituting a major part of the Charlson comorbidity index.

Limitations of our study include the fact that we did not have data on some known risk factors, particularly substance abuse. Furthermore, we did not have data on concomitant severe extrapulmonary TB manifestations, such as meningeal TB. Until 2013, DOT was recommended in national guidelines [8] for only certain risk groups, e.g. alcohol or substance abuse, elderly patients and patients with many comorbidities, but the absence of data on DOT use is unlikely to introduce a major bias on our analysis. Additionally, Charlson comorbidity index does not contain all disease groups, which may influence TB treatment outcome, such as inflammatory bowel disease and vasculitis, in which immunosuppressive medication is commonly used. Less than 2% of TB cases were HIV positive, and as a country with a very low incidence for HIV [6], the absence of HIV test results is unlikely to introduce a bias. Treatment outcomes for solely clinically diagnosed TB cases have been notified only since year 2015 in Finland.

Conclusions

With a high proportion of older population among TB cases, death is a common treatment outcome in Finland. Comorbidities contribute to death with TB in all age groups, and two thirds of deaths occur within two months after TB registration. Increased awareness of TB as well as a low threshold for TB suspicion and diagnostics is needed. In addition, early empiric TB treatment when the suspicion of TB is strong and sufficient material for TB diagnostics have been collected, as well as adequate treatment of comorbidities during TB treatment should be emphasized. Moreover, a special focus should be given in the national guidelines on the treatment of non-MDR drug resistant cases, as these are at risk for other non-successful outcomes than death. Furthermore, as we identified errors in outcome allocation, there is a need to review guidelines and provide further training in outcome assessment.

Abbreviations

TB: Tuberculosis; ECDC: European Centre for Disease Prevention and Control; WHO: World Health Organization; MDR: Multi drug resistant; EU/EEA: European Union/European Economic Area; NIDR: National infectious disease register; ICD10: International Classification of Diseases, 10th Revision; IUATLD: International Union Against Tuberculosis and Lung Disease; AIC: Akaike information criteria; IQR: Interquartile range; RRR: Ratio of relative risks; CI: Confidence interval; DOT: Directly observed therapy.

Acknowledgements

We thank associate professor Lauri Lehtimäki at the Faculty of Medicine and Health Technology, Tampere University, for critically reviewing this article.

Authors' contributions

VK, OL, JO, HS, TV and PR designed the study. VK and JO collected the data. VK, OL, JO, TV and PR contributed to data analysis and interpretation of the results. VK, OL, JO and PR drafted, and all authors finalized the manuscript. All authors revised the manuscript critically and approved the final version for publication.

Funding

Virve Korhonen was supported by the Tuberculosis Foundation of Tampere. The funder had no role in study design, data collection and analysis, interpretation of data or writing the manuscript.

Availability of data and materials

The data sources of this study were National Infectious Diseases Register (https://thl.fi/fi/web/infektiotaudit-ja-rokotukset/seurantajarjestelmat-ja-rekisterit/tartuntatautirekisteri) and Finnish Hospital Discharge Register (https://thl.fi/fi/tilastot-ja-data/ohjeet-tietojen-toimittamiseen/hoitoilmoitusjarjestelma-hilmo), maintained by the Finnish Institute for Health and Welfare (www.thl.fi), and Cause of Death Register (https://www.stat.fi/meta/til/ksyyt.html) maintained by Statistics Finland (www.stat.fi). Permissions required in order to access these register data can be applied from the Finnish Institute for Health and Welfare (www.thl.fi). The datasets generated and/or analyzed during the current study are not publicly available due to possibility of recognition of a patient even though data does not include personal level data.

Ethics approval

The ethics approval for the study was given by the Ethics Committee of Tampere University Hospital, Tampere, Finland. Consent to participate: Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 19 January 2020 Accepted: 6 August 2020
Published online: 17 August 2020

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Publisher's Note

PUBLICATION II

Recurrent tuberculosis in Finland 1995-2013: a clinical and epidemiological cohort study

Korhonen V., Soini H., Vasankari T., Ollgren J., Smit P.W., Ruutu P.

BMC Infectious Diseases 17: 721-727
DOI 10.1186/s12879-017-2818-6

Korhonen et al. BMC Infectious Diseases (2017) 17:721
DOI 10.1186/s12879-017-2818-6

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Recurrent tuberculosis in Finland 1995–2013: a clinical and epidemiological cohort study

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Abstract

Background: We investigated the epidemiology and prevalence of potential risk factors of tuberculosis (TB) recurrence in a population-based registry cohort of 8084 TB cases between 1995 and 2013.

Methods: An episode of recurrent TB was defined as a case re-registered in the National Infectious Disease Register at least 360 days from the date of the initial registration. A regression model was used to estimate risk factors for recurrence in the national cohort. To describe the presence of known risk factors for recurrence, patient records of the recurrent cases were reviewed for TB diagnosis confirmation, potential factors affecting the risk of recurrence, the treatment regimens given and the outcomes of the TB episodes preceding the recurrence.

Results: TB registry data included 84 patients, for whom more than 1 TB episode had been registered. After a careful clinical review, 50 recurrent TB cases (0.6%) were identified. The overall incidence of recurrence was 113 cases per 100,000 person-years over a median follow up of 6.1 years. For the first 2 years, the incidence of recurrence was over 200/100000. In multivariate analysis of the national cohort, younger age remained an independent risk factor at all time points, and male gender and pulmonary TB at 18 years of follow-up. Among the 50 recurrent cases, 35 patients (70%) had received adequate treatment for the first episode; in 12 cases (24%) the treating physician and in two cases (4%) the patient had discontinued treatment prematurely. In one case (2%) the treatment outcome could not be assessed.

Conclusions: In Finland, the rate of recurrent TB was low despite no systematic directly observed therapy. The first 2 years after a TB episode had the highest risk for recurrence. Among the recurrent cases, the observed premature discontinuation of treatment in the first episode in nearly one fourth of the recurrent cases calls for improved training of the physicians.

Keywords: Tuberculosis recurrence, Tuberculosis epidemiology, Tuberculosis treatment, Tuberculosis

Background

Tuberculosis (TB) remains a major global health problem with estimated 10.4 million new TB cases worldwide in 2015. In 2013, 0.3 million TB cases were reported as recurrent [1]. After successful treatment, recurrent TB is estimated to occur in 0–14% of all TB patients within 1–3 years [2]. Recurrence of TB following treatment of an initial disease episode can occur due to endogenous re-activation with the same strain of *Mycobacterium tuberculosis* (relapse) or exogenous infection with a new strain (re-infection). In low-incidence countries, recurrence rates have varied between 0.4% and in a prospective clinical trial up to 6% [3–5]. The proportion due to re-infection has been reported to vary between 4 and 27% [3, 4]. In high-incidence countries the majority of recurrent cases, up to 77%, are caused by re-infection [6].

Finland is a low-TB-incidence (<10/100000) country since 2001, and in 2015 TB incidence was 5/100000 [7]. In 2015, 1% of TB cases had HIV infection. However, emerging challenges for the TB control program include gradually increasing resistance of *M. tuberculosis*, with 3% of all isolates multi drug resistant (MDR) in 2015 [7], concomitantly with a rapid increase in the proportion of TB cases occurring in immigrants [8]. Finland did not implement a comprehensive DOT (directly observed therapy) strategy in patient management until 2013 [9].

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Our previous study shows, that in Finland more than 80% of recurrent cases during 1995–2013 were relapses [10]. The aim of the present study was to investigate in a national, population-based TB cohort the occurrence of recurrent TB and a few potential factors affecting the risk of recurrence in Finland during the years 1995–2013. In recurrent cases, we describe treatment regimens administered and treatment outcome in the first episode, and other potential factors affecting the risk of recurrence, in order to strengthen the TB treatment program in the changing epidemiologic environment.

Methods

Surveillance system and study population

The study population consisted of all TB cases in Finland reported from January 1, 1995, to December 31, 2013, to the National Infectious Disease Register (NIDR), maintained at the National Institute for Health and Welfare (THL). Clinical microbiology laboratories mandatorily notify new *M. tuberculosis* isolations to NIDR and submit isolates to the Mycobacterial Reference Laboratory (NRL) at THL for drug susceptibility testing (ethambutol, isoniazid, pyrazinamide, rifampicin and streptomycin). Physicians mandatorily notify to NIDR laboratory-confirmed cases of TB: the laboratory report of a positive test result to the clinician automatically includes a reminder to notify the case. Since 2007, also clinically diagnosed TB cases, when a decision to give a full course of TB treatment is made, are notified. Information on HIV positivity is obtained by linking data within NIDR. Data on the country of origin and the date of death were retrieved from the national population registry. Data from the different sources are automatically linked as a case by a unique person identifying number.

Case definitions and data collection for the subgroup of recurrent cases

An episode of recurrent TB was defined as a case re-registered in NIDR at least 360 days from the date of the initial registration of a TB infection episode. For the cases who had a recurrent episode in the register, data on anatomical site of disease (pulmonary/extrapulmonary), radiological, histological and microbiological results, HIV test results, substance abuse, the drug regimen in the first episode and adverse effects were extracted from patient charts. In pulmonary TB cases, also sputum smear and culture results at months 0 and 2, and at the end of the treatment were obtained. Based on careful review of these data, a number of cases were excluded from further analysis as recurrent cases, as they did not meet the criteria for recurrent TB (Fig. 1). In culture negative episodes, the diagnoses were based on clinical criteria [11], including radiological findings in combination with either histological confirmation or positive nucleic acid amplification test results, except for one case in which the diagnosis was only clinical and radiological.

Management of the TB episode preceding recurrence

The treatment regimen, free of charge for the patient, administered in the first TB episode was grouped into six categories, based on the national recommendations: until 2006 as described earlier [12, 13], and from 2006 in the National TB Control Program [9]. The treatment regimens and the outcomes of the first TB episodes of the recurrent cases, including culture negative and extrapulmonary cases, were assessed by two clinical TB experts, and classified [11] as cured, completed treatment, lost to follow-up and not evaluated (no fatal cases nor failures). 'Lost to follow-up' was further divided into subgroups: physician's decision to stop prematurely (including cases that received ineffective treatment), and defaulted (interruption due to the patient). Group 'not evaluated' was further divided into subgroups: still on treatment at 12 months, and treatment outcome not assessed. In two cases the details of treatment in the first episode were not available, but in one of these cases the treatment outcome could be assessed.

Statistical analysis

To calculate the incidence of recurrent TB among all reported cases of the national cohort, follow-up time in days was calculated for all cases in the national TB cohort from 360 days after they were notified until an event (re-notification), death or censored (December 31, 2013). The date of death was acquired by linkage to population register using person identifier. The regression model employed, uses pseudo-observations to model censored data [14] with Stata version 14.02 (StataCorp LLC, 4905 Lakeway Drive, Collage Station, TX 77845 USA) to estimate effects (their relative risks) of risk factors. The censoring was assumed to be independent, conditional on the covariates [15]. Gender and anatomical site of disease were allowed to have time dependent effects, and predictive margins were calculated to estimate cumulative risk differences between genders and anatomical sites of disease, respectively, adjusting for other factors in the model. Results were qualitatively checked using the extended time dependent Cox model. All the explanatory variables with univariate p-values <0.2 were included in the multivariate model, and included gender, anatomical site of disease and age. Cause specific cumulative risks for time points 1 year, 2 years and 18 years were calculated, as the chosen early time points had most recurrent events for explanatory variables, and it has been previously reported that the first 1–2 years have the highest hazard for recurrence [3, 16]. The maximum surveillance time point set at 18 years gives the final overall difference estimate for

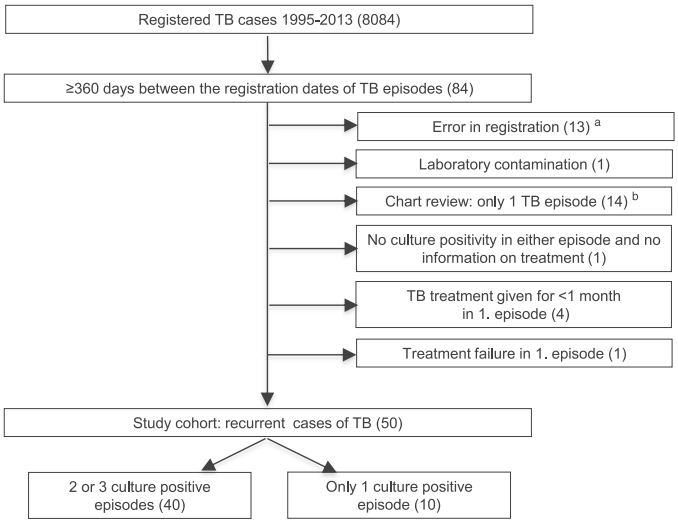

Fig. 1 Steps in identifying recurrent cases of tuberculosis, Finland, 1995–2013. [a] Non-tuberculous mycobacterium registered as M. tuberculosis ($N = 2$), registration for false identity ($N = 1$) or incorrect registration date by the notifier ($N = 10$). [b] Only one long continuous TB episode ($N = 5$), or treating physician decided to give TB treatment without specific evidence of TB and in subsequent expert assessment by the study team there was another more likely cause for the disease ($N = 9$)

the cumulative risk of recurrence. The distribution of continuous variables between groups was compared using Wilcoxon rank-sum test.

Ethics

The ethics approval for the study was given by the Ethics Committee of Tampere University Hospital, Tampere, Finland.

Results

A total of 8084 TB cases were registered in Finland during the study period (Table 1): 43% were female, and 13.6% of foreign origin (increased from 4.8% in 1995 to 32.2% in 2013). The median age was 70 years (interquartile range [IQR], 56–79 years) for Finnish-born and 30 years (IQR 23–40 years, $p < 0{,}001$) for foreign-born cases.

Table 1 Distribution of demographic and potential risk factors for recurrent tuberculosis in a national TB cohort of 8084 cases, Finland 1995–2013

Variable	TB recurrence ($n = 50$)	No TB recurrence ($n = 8034$)	All ($n = 8084$)
Median age, years	51, 5	66	66
Gender female *n* (%)	11 (22%)	3457 (43%)	3468 (42.9%)
Foreign origin *n* (%)	9 (18%)	1066 (13.6%)[a]	1074 (13.6%)[b]
Pulmonary site of disease *n* (%)	44 (88%)[c]	5599 (69.2%)	5605 (69.3%)
Culture positive *n* (%)	48 (96%)[c]	6631 (82.5%)	6680 (82.6%)
Prior to year 2007 *n* (%)	45 (90%)[c]	5762 (71.7%)	5807 (71.8%)

[a] Origin known for 7862 cases
[b] Origin known for 7912 cases
[c] Data of the first episode

Characteristics and incidence of recurrent TB

After a careful review of the 84 cases with more than one episode registered, 50 TB cases (0.6% of all cases in the cohort) were classified as recurrent (Fig. 1). The mean overall incidence of recurrence was 112.9 (95% confidence interval [CI], 85.6–148.9); for the first year of follow-up the overall incidence was 236.4 (95%CI 140.0–399.2) and for the second year of follow-up 206.7 (95%CI 114.5–373.2) per 100,000 person-years. Out of the 50 recurrent cases, two had three disease episodes and 48 two disease episodes. Forty cases were culture positive in all episodes. The median age was 51.5 years (range 6–95 years) at the registration of the first episode; eleven cases (22%) were female. In the first episode 44 cases (88%) and in the second episode 39 cases (78%) were classified as pulmonary TB. Two cases were HIV positive; for 64% of cases HIV had not been tested. Nine recurrent cases (18%) were of foreign origin. A history of substance abuse, mostly alcohol, was registered in the patient records in at least 1 TB episode of 59% of males and none of females; 49% of males had substance abuse recorded in both episodes.

Management of the TB episodes preceding recurrence

Among the 48 recurrent cases (96%) with complete patient records, 36 cases (75%) received standard treatment in the first episode (Table 2). Among these, the duration of

Table 2 Distribution of treatment regimens in the first episodes of 50 TB cases with a recurrence

Treatment group	Total in group	Intensive phase short	Intensive phase adequate	Duration of treatment short	Duration of treatment adequate
Standard treatment A[a]	15	NA	15	1	14
Standard treatment B[b]	4	NA	4	2	2
Standard treatment with short intensive phase C[c]	3	3	NA	2	1
Standard treatment D[d]	14	0	14	1	13
Other probably effective combination of anti-TB drugs[e]	6	NA	NA	1	5
Other probably ineffective combination of anti-TB drugs[f]	6	NA	NA	NA	NA
Not evaluated	2	–	–	–	–

NA not applicable, *H* isoniazid, *R* Rifampicin, *Z* pyrazinamide, *E* ethambutol
[a] HRZ in intensive phase, HR in continuation phase, adequate duration of treatment ≥ 5.5 months
[b] HRE in intensive phase, HR in continuation phase, adequate duration of treatment ≥ 8 months
[c] Short intensive phase <54 days in standard treatment A or B
[d] ≥ 4 anti-TB drugs, including HRZ (adequate duration of treatment ≥ 5.5 months) or HRE (adequate duration of treatment ≥ 8 months)
[e] Non-standard combinations guided by drug resistance or due to adverse effects, the adequacy of treatment duration assessed by the study group
[f] Drug resistance ignored or inappropriate dosing
References [9, 12, 13]

treatment was short (<5.5 months) in six cases, and the intensive phase (<54 days) in three cases. Twelve cases (25%) received non-standard treatment due to drug resistance, adverse effects, or the treating physician's decision. Of these, six cases received TB treatment regimens that were assessed as probably effective, one of them for a too short time period. Treatment regimens of six cases were assessed as probably ineffective. Six cases (12.5%) received directly observed therapy (DOT). Among the 49 cases with treatment outcome, it was successful in 27 cases (55.1%). In 12 cases (24.5%) the treating physician had stopped the treatment prematurely, and in two cases (4.1%) interruption was due to the patient. Eight cases (16.3%) were still on treatment at 12 months (all finally completed treatment).

There were no recurrent cases with an MDR isolate in either episode. Five cases were initially infected with isoniazid-resistant isolates, but in three of these cases, the treatment regimen was not modified accordingly. In two cases, additional resistance to streptomycin and in one case resistance to pyrazinamide developed during treatment. In one case, initially fully susceptible isolate developed resistance to pyrazinamide.

Risk factors for recurrence in the national cohort

The median follow-up time of cases in the cohort of 8084 TB cases was 6.1 years (IQR 2.7–11.1 years). The recurrence occurred within less than 2 years in 25 (50%), two to less than 4 years in 8 (16%), and later in 17 cases (34%) (Fig. 2a). No recurrences occurred in females and for extrapulmonary cases after the first 2 years (Fig. 2b and c). In univariate analysis of variables available for the national cohort, the cumulative risks of recurrence between males and females, and between pulmonary and extrapulmonary TB did not differ statistically significantly at 1 and 2 years of follow-up (Table 3). However, at 18 years of follow-up, the cumulative risk for males was nearly fourfold compared to females (Fig. 2b), and more than fivefold for pulmonary TB compared to extrapulmonary TB (Fig. 2c). The risk of recurrence decreased with every additional 10 years of age (Table 3). When only cases that were culture positive in all episodes were included, the recurrence rate was similar to that seen in the whole recurrent cases cohort (Fig. 2d). Whether the first episode occurred prior to versus after 2007 did not have a significant association with the risk of recurrence (Table 3).

In the multivariate analysis (Table 3) younger age remained an independent risk factor for cumulative risk of recurrence at all time points, and male gender and pulmonary TB at 18 years of follow-up.

Discussion

We investigated the epidemiology and the prevalence of risk factors associated with recurrence of TB in Finland in a comprehensive national, register-based cohort of 8084 TB cases from 1995 to 2013, and found in a follow-up of up to 18 years that 0.6% of cases during the study period were recurrent. The overall incidence of recurrence is 10–20 times higher, and for the first 2 years of follow-up 20–40 times higher than the incidence of TB in the general population in Finland during the study period [7]. Patient chart review of the recurrent cases revealed that in nearly one fourth of the recurrent cases, the physician had discontinued the treatment of the first episode prematurely.

In register-based studies on recurrent TB, validation of the data requires considerable effort to ensure data

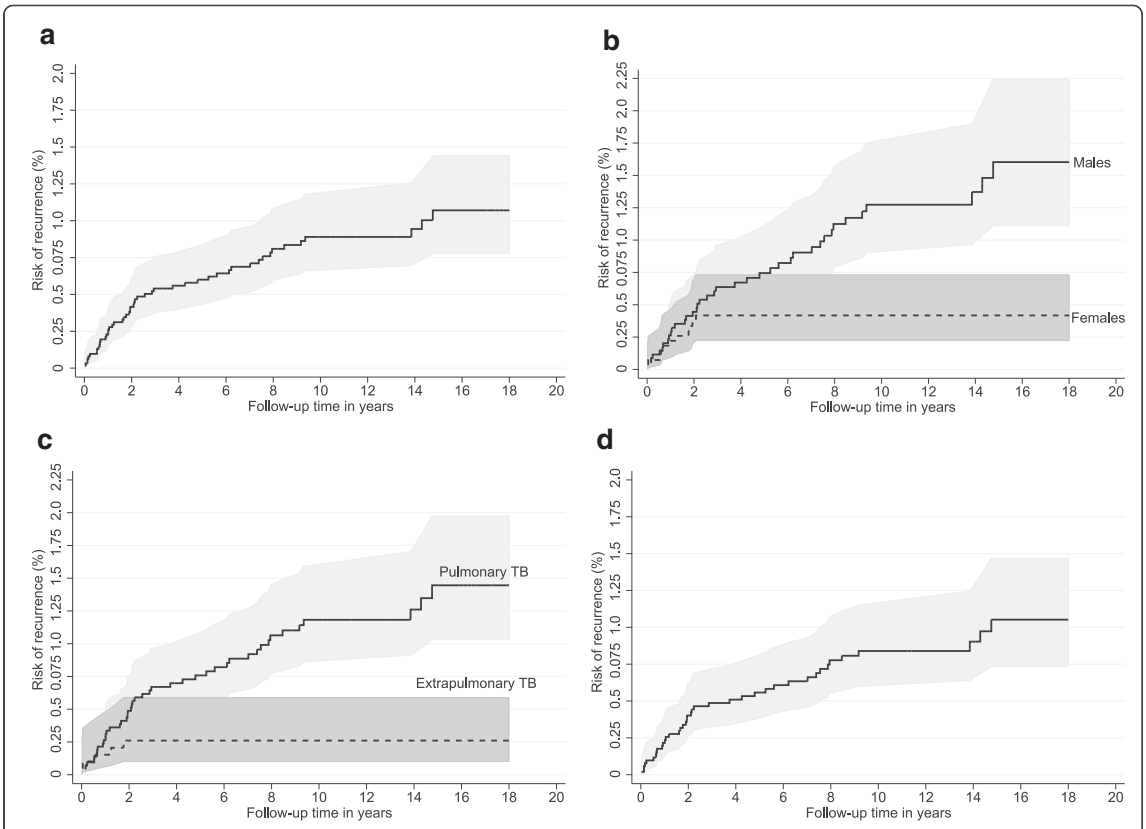

Fig. 2 Cumulative risk of recurrence of TB by follow-up time (and its pointwise 95% confidence limits). **a** Overall cohort; (**b**) By gender; (**c**) By anatomical site of disease; (**d**) Only culture positive TB

quality, as demonstrated in our study using a register with proven high sensitivity and specificity for TB [17], after which automated notification from the laboratories and mandatory reminders from the laboratory to the clinician on the need to notify were introduced. We observed that out of the 84 cases initially identified in the register data as recurrent, only 50 cases were truly recurrent. Major reasons for inaccuracies in our register data included incorrect notification dates, and notifying clinical TB cases without microbiological confirmation, which the chart review revealed to be incorrect. Without validation of the data for recurrent TB cases, the proportion of recurrent

Table 3 Univariate and multivariate analysis for risk factors of TB recurrence in a national cohort of TB cases in Finland, 1995–2013

Variables	Univariate RR	95% CI	*p*	Multivariate RR	95% CI	*p*
Males at 1 year [a]	1,43	0,48–4,26	0,52	1,93	0,40–9,31	0,41
Males at 2 years [a]	1,18	0,53–2,62	0,69	1,39	0,44–4,38	0,58
Males at 18 years [a]	3,92	1,95–7,91	< 0,001	5,88	2,21–15,66	< 0,001
Pulmonary TB at 1 year [b]	1,72	0,48–6,19	0,40	4,13	0,58–29,67	0,16
Pulmonary TB at 2 years [b]	1,87	0,70–4,99	0,21	2,00	0,43–9,33	0,38
Pulmonary TB at 18 years [b]	5,54	2,17–14,14	< 0,001	15,15	4,98–46,08	< 0,001
Age + 10 years	0,87	0,77–0,98	0,03	0,83	0,70–0,99	0,04
Finnish origin	0,64	0,31–1,33	0,23	–	–	–
1.episode before 2007	1,33	0,81–2,21	0,26	–	–	–

[a] Reference females. Overall *p*-value for male gender in univariate analysis < 0,0001 and in multivariate analysis 0,0015
[b] Reference extrapulmonary TB. Overall *p*-value for pulmonary TB < 0,0001

cases out of the total cohort in our study would have been almost double, stressing the need for rigid patient chart review when assessing TB recurrence.

Over 80% of recurrent cases in Finland are relapses of the previous infection [8], as elsewhere in low-incidence countries [3, 18, 19]. In register-based investigations of recurrent TB in low incidence-countries, careful review of the actual treatment in the first TB period of recurrent cases has rarely been reported [20, 21]. We observed that approximately two thirds, including those who received treatment lasting over 12 months, had received an adequate treatment. Just over one half among the recurrent cases had in the first episode a successful outcome according to WHO criteria [11]. Important for training policy was the finding that clearly more frequently than interruption due to the patient, the treating physician had discontinued treatment prematurely, as described in our previous report [12]. Inadequate treatments were caused by the presence of drug resistance without appropriate treatment regimen modification, absence of appropriate extension of duration of treatment when treatment was modified, adverse effects, or the physician's decision to stop without the reason being documented in the patient record.

The overall incidence of recurrence in the national cohort, with a median follow-up period of 6 years, and up to 18 years, was 113/100000, 10–20 times higher than for the general population in Finland, in line with long-term follow-up in low-incidence countries (71–410/100000) [3, 16]. For the first year of follow-up, starting at 12 months from the registration of the first episode, the incidence of recurrence was 236/100000, of the same magnitude as in recent studies from Australia [3] and Denmark [18], but clearly lower than in a number of earlier studies from industrialised countries [22].

Our observation in the national cohort of male gender as a risk factor for recurrence is in line with some previous reports from low-incidence countries [23–25], but this finding has been inconsistent [20, 21]. Substance abuse data is not collected in the NIDR for the national cohort, but we found in nearly 60% of the recurrent male cases a history of substance abuse in patient charts, but none in females, which could contribute to the excess risk seen in males. An association between treatment adherence and alcoholism has been reported in recurrent TB in the USA [20]. We found in the national cohort the risk of recurrence higher for pulmonary than for extrapulmonary TB, in line with earlier studies [16, 23, 26]. More than 40% of our recurrent cases (data not shown) had both pulmonary and extrapulmonary infection in the first episode, which has been reported to be a risk factor for recurrences [25]. An unexpected finding in the national cohort was that the risk of recurrence was associated with younger age, whereas in two earlier studies, age > 65 years [20] or age between 25 and 64 years [23] have been reported as risk factors for recurrence. In earlier studies, either being an immigrant [16, 20, 25] or being borne in the country [21] were reported as risk factors, while origin was not a risk factor for recurrence in our national cohort study.

Limitations of our cohort study include the fact that we may fail to identify some recurrences, as recurrences before 360 days from the date of the initial episode (early recurrences) were not analysed from the register data. The standard cut-off time recommended by WHO, at which treatment outcome is recorded, is 12 months [11]. Therefore, we chose this timepoint as a cut-off for recurrence, in line with eg a large UK cohort [16]. The proportion of early recurrences in retrospective studies is small [3, 18]. In addition, in retrospective studies, it may be difficult to distinguish between treatment failures and early recurrences as sputum samples are not systematically collected during treatment, and in our study we also included culture negative and extrapulmonary cases. Almost one third of our recurrent cases do not meet the WHO treatment regimen description and outcome criteria for a recurrent case [11]. However, the careful validation process of our register data demonstrates that the same challenges are likely to be present in other register-based studies, unless careful validation has been performed. As a country with very low incidence for HIV [7], the absence of systematic testing of all TB cases for HIV is unlikely to introduce a bias in the risk analysis.

The observations on the shortcomings of treatment among the first episodes of the recurrent cases are important for guiding training and system development for the integrated TB control program. In 2013–2015, treatment outcome in Finland was successful (cure or completed treatment) in 75–78% of pulmonary TB cases [7], as in the European Region on average [27].

Conclusions

In the absence of a comprehensive DOT strategy, the rate of TB recurrence was found to be low in Finland. An important finding was that in one fourth of the recurrent cases, the physician had discontinued the treatment prematurely, which implies that training of physicians needs to be improved and, as TB becomes rare, treatment should possibly be provided in fewer centers. The first 2 years after a TB episode is a very high-risk period for recurrence: this could be incorporated as an automated high-risk signal in the developing integrated electronic patient management systems for reducing the delays in implementing TB diagnostics and treatment.

Abbreviations

CI: Confidence interval; DOT: Directly observed therapy; HIV: Human immunodeficiency virus; IQR: Interquartile range; MDR: Multi drug resistant;

NIDR: National infectious disease register; NRL: Mycobacterial reference laboratory; TB: Tuberculosis; THL: National Institute for Health and Welfare

Acknowledgements
We thank Pirjo Turtiainen, Jan-Erik Löflund and Teemu Möttönen at the National Institute for Health and Welfare for technical assistance and Professor Lauri Lehtimäki at the School of Medicine, University of Tampere for critically reviewing this article.

Funding
The study was supported by the Tampere Tuberculosis Foundation, Väinö and Laina Kivi Foundation and The Research Foundation of the Pulmonary Diseases. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials
All data generated or analysed during this study are included in this published article.

Authors' contributions
VK participated in designing the study, collected the clinical data, and was involved in analysing the data and writing the manuscript. HS participated in designing the study, supervised the laboratory work and was involved in analysing the data and writing the manuscript. TV participated in designing the study, and was involved in analysing especially the clinical data and writing the manuscript. JO participated in designing the study and performed the statistical analysis. PS participated in designing the study and writing the manuscript. PR participated in designing the study, and was involved in analysing the data and had a major role in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The ethics approval for the study was given by the Ethics Committee of Tampere University Hospital, Tampere, Finland. Consent to participate: Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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Received: 13 April 2017 Accepted: 5 November 2017
Published online: 16 November 2017

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PUBLICATION III

Whole genome analysis of *Mycobacterium tuberculosis* isolates from recurrent episodes of tuberculosis, Finland, 1995-2013

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Clinical Microbiology and Infection 22(6): 549-554
DOI 10.1016/j.cmi.2016.03.014

Whole genome analysis of *Mycobacterium tuberculosis* isolates from recurrent episodes of tuberculosis, Finland, 1995–2013

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Abstract

Recurrent tuberculosis (TB) is caused by an endogenous re-activation of the same strain of *Mycobacterium tuberculosis* (relapse) or exogenous infection with a new strain (re-infection). Recurrence of TB in Finland was analysed in a population-based, 19-year study, and genotyping was used to define relapse and re-infection. The *M. tuberculosis* isolates from patients with suspected relapse were further analysed by whole genome sequencing (WGS) to determine the number and type of mutations occurring in the bacterial genome between the first and second disease episodes. In addition, publicly available tools (PhyResSE and SpolPred) were used to predict drug resistance and spoligotype profile from the WGS data. Of the 8299 notified TB cases, 48 (0.6%) patients had episodes classified as recurrent. Forty-two patients had more than one culture-confirmed TB episode, and isolates from two episodes in 21 patients were available for genotyping. In 18 patients, the *M. tuberculosis* isolates obtained from the first and second TB episodes had identical spoligotypes. The WGS analysis of the 36 *M. tuberculosis* isolates from the 18 suspected relapse patients (average time between isolates 2.8 years) revealed 0 to 38 single nucleotide polymorphisms (median 1, mean 3.78) between the first and second isolate. There seemed to be no direct relation between the number of years between the two isolates, or treatment outcome, and the number of single nucleotide polymorphisms. The results suggest that the mutation rate may depend on multiple host-, strain- and treatment-related factors.

Keywords: Re-infection, recurrent, relapse, single nucleotide polymorphism, tuberculosis, whole genome sequencing
Original Submission: 14 December 2015; **Revised Submission:** 10 February 2016; **Accepted:** 13 March 2016
Editor: M. Drancourt
Article published online: 26 March 2016

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Introduction

Tuberculosis (TB) remains a major global health problem with 9 million new TB cases notified worldwide in 2013. TB ranks as the second leading cause of death from an infectious disease with an estimated 1.5 million TB deaths in 2013 [1]. Recurrent TB is defined as an episode of TB occurring in the same person after successful treatment [2–4]. Two processes can cause recurrence: endogenous re-activation with the same strain of *Mycobacterium tuberculosis* (relapse) or exogenous infection with a new strain (re-infection). Recurrent TB episodes, of which 0.3 million were reported in 2013, contribute considerably to the burden of TB [1]. Recurrent TB has been reported to occur in 0%–14% of patients after successful treatment over 1–3 years of follow up [5]; in low-incidence countries, rates of recurrence have varied between 0.4% and 7% [4–6].

Genotyping methods can be used to aid in the differentiation between relapse and re-infection. As *M. tuberculosis* isolates from both disease episodes need to be analysed

http://dx.doi.org/10.1016/j.cmi.2016.03.014

with the same molecular marker, results are available from only a few studies. Studies have shown that in recurrent TB the proportion of disease due to re-infection varies from 4% [5] to >60% [6,7]. A high proportion of apparent re-infections may be due to mislabelling, cross-contamination, mixed infections (the presence of more than one strain), or to differences in patient populations, length of follow up, and background risk of infection with *M. tuberculosis* [8]. Recently, whole genome sequencing (WGS) has proved to be useful and more discriminatory than standard genotyping for the distinction of re-infection and relapse [8,9]. As differentiation between relapse and re-infection is crucial in terms of patient treatment and public health response, a discriminatory method could aid in this challenging differentiation.

Finland has been a low-TB-incidence country since 2001. In 2013, 271 new TB cases were notified and TB incidence was 5/100 000. Although the majority of cases are still detected among elderly, native Finns, the number and proportion of immigrant cases is increasing. In 2013, 17 (6%) of the newly notified TB cases were reported to have had a previous episode of TB since 1950. In 2013, a favourable outcome of treatment (cure or completed treatment) was achieved in only 68% of pulmonary TB cases in Finland, and 16% of TB patients died before or during TB treatment [10].

The aim of the study was to analyse how many recurrent TB cases were diagnosed in Finland during the years 1995–2013, and to define, by using molecular methods, how many of the cases were caused by relapse versus re-infection. The *M. tuberculosis* isolates from relapse cases were further analysed by WGS to determine the number and type of mutations occurring in the bacterial genome between the first and second disease episodes. In addition, publicly available tools were used to predict drug resistance and spoligotype profile from the WGS data.

Materials and methods

Clinical microbiology laboratories notify new *M. tuberculosis* isolations directly to the National Infectious Disease Register and submit isolates to the Mycobacterial Reference Laboratory at National Institute for Health and Welfare (THL) for drug susceptibility testing (ethambutol, isoniazid, pyrazinamide, rifampicin, streptomycin) and genotyping. In addition, physicians notify clinically suspected or confirmed TB cases. Data from the different sources were linked as a case by the unique person-identifying number used in all Finnish healthcare systems. Data on country of origin were retrieved from the national population registry.

Recurrent TB was defined as a case with a registration date of a new TB episode in the same patient >360 days after the registration date of a first episode, irrespective of the treatment and treatment outcome in the initial episode. For the present report, all episodes of TB had to be culture confirmed and occur during 1995–2013. The registration date in culture-positive cases is the sampling date of the first culture that was positive for *M. tuberculosis*. Based on reviewing laboratory data and patient charts of all initially identified recurrent episodes, known laboratory contaminations and registration errors (non-tuberculous mycobacterium registered as *M. tuberculosis* or registration for false identity or false date) were excluded, as well as cases in which there was only one definite TB episode. Treatment outcome for the first episode was defined according to WHO criteria [1]. Treatment outcome was classified as successful, if treatment outcome was 'cured' or 'completed treatment' at 12 months; unsuccessful, if treatment outcome was 'died, failed, or lost to follow up'; or not known, if treatment outcome was not evaluated.

Clinical specimens, obtained from persons suspected of having TB, were decontaminated and cultured in a BACTEC MGIT TB system (Becton Dickinson, Franklin Lakes, NJ, USA) following standard procedures. The isolates were re-grown on Löwenstein–Jensen medium to obtain a pure culture for genotyping and were characterized by spoligotyping [11]. After initial genotyping, isolates were stored frozen (−20°C) and were re-cultured on 7H11 agar plates for WGS analysis. Whether *M. tuberculosis* isolates from both disease episodes were available for analysis, depended on the time of the disease episode. Before the year 2000, only every tenth *M. tuberculosis* isolate was stored in the culture collection.

Genomic DNA was isolated using a DNeasy blood and Tissue kit (Qiagen, Hilden, Germany) and paired-end multiplex libraries were prepared with a Nextera XT kit (Illumina, San Diego, CA, USA) at the Bacterial Infections Unit of the National Institute for Health and Welfare (Turku, Finland). The libraries were sequenced at the Finnish Microarray and Sequencing Centre (Turku, Finland) using the MiSeq platform (Illumina) according to the manufacturer's recommendations. Sequence reads were processed as described previously [12]. The average coverage of the reference genome (percentage bases >15) was 95.2% (range 71.8%–99.3%). SNPs were considered valid if supported by at least two and >70% of mapped reads on each strand with a minimum mapping quality of 45. Single nucleotide polymorphisms (SNPs) in annotated repeat regions and other notoriously difficult to map regions, comprising 9.3% of the genome, were excluded from further analysis. Variant sites that passed quality filters in all isolates were extracted using custom scripts [12] and used to determine SNP distances between isolates.

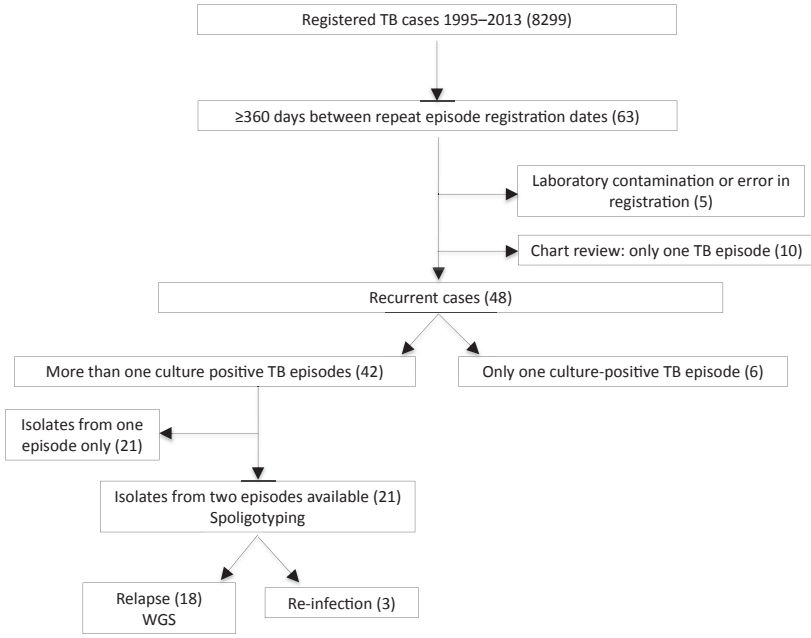

FIG. 1. Steps in identifying recurrent cases of tuberculosis, Finland, 1995–2013.

Sequences were analysed using the PhyResSE and Mykrobe online tools (v. 0.2.1) for detection of drug-resistance-conferring mutations (27 October 2015). Spoligotypes were predicted from the WGS data using the SpolPred tool (downloaded in July 2015) [13–15].

The ethics approval for the study was given by the Ethics Committee of Tampere University Hospital, Tampere, Finland.

Results

A total of 8299 TB cases were notified in Finland during 1995–2013. Analysis of the national TB registry data identified 63 patients, for whom more than one TB episode had been registered. After careful review of all laboratory data and patient charts, 48 TB cases were classified as being recurrent (0.6% of all registered cases) (Fig. 1). Of the 42 patients with more than one culture-confirmed TB episode, for 21 patients isolates from repeat episodes were available for genotyping.

In 18 of the 21 (86%) patients, the *M. tuberculosis* isolates obtained from first and second TB episodes had identical spoligotypes and were suspected of having relapse TB (Table 1). The average age of the patients was 62.8 years (range 25–95 years) at the time of the registration of the first TB episode, 13 were men and five were women. The country of origin was Finland for 15 patients, and Cameroon, Russia and Somalia, each for one patient. The average period between the registration dates of the first and second TB episodes was 2.8 years (range 1.0–9.8 episodes). The relapse occurred in the first 2 years from the registration date of the first episode in nine cases (50%), between 2 and 4 years in seven cases (39%), and after >4 years in two cases (11%). The first TB disease episode was pulmonary in 13 cases and extrapulmonary in five cases. One case was confirmed to be in an HIV-positive patient. Treatment outcome of the first episode was classified as successful (completed treatment) in ten cases (56%), unsuccessful (lost to follow up) in four cases (22%), and not known in four cases (22%). The second episode was pulmonary in 12 cases, extrapulmonary in four cases, and both in two cases.

The 36 *M. tuberculosis* isolates obtained from the consecutive episodes of the 18 patients with suspected relapse were submitted for WGS. The number of SNPs between the first and second isolates varied from 0 to 38 (median 1, mean 3.78). In the majority of pairs (17 of 18) the number of SNPs was between 0 and 6 (median 1, mean 1.76), strongly suggesting that it was a relapse case. However, in one pair the SNP difference was 38, developed in a 2.2-year time between the isolation dates, leaving the interpretation unclear. The number of mutations per genome per year ranged from 0 to 17.3 for all isolates, the average being 1.37. When excluding the outlier, the average number of mutations per genome per year was 0.63. There seemed to be no direct relation between the number of years between the two isolates, or treatment outcome, and the number of SNPs (Fig. 2). The genomic location and type of

TABLE 1. Demographic, clinical, drug treatment and genotyping characteristics of 18 cases of relapsed tuberculosis, Finland, 1995–2013

				First episode			Second episode			
Nr	Age	Gender	Country of birth	Disease	Drug resistance[a]	Treatment outcome[b]	Drug resistance	Year difference[c]	Spoligotype	SNPs between isolates
1	70	Male	Finland	Pulmonary	INH	Unsuccessful	INH+SM	2	SIT47	0
2	71	Male	Finland	Pulmonary and other (pleura)	No	Successful	No	2.2	SIT46	38
3	95	Female	Finland	Other (lymph node)	No	Not known	No	1	SIT53	5
4	52	Male	Finland	Pulmonary	No	Successful	No	7.2	SIT49	1
5	47	Male	Finland	Pulmonary and other (pleura)	No	Unsuccessful	No	1.1	SIT262	5
6	78	Female	Finland	Pulmonary and other (pleura)	INH	Not known	INH	2.9	SIT917	2
7	40	Male	Finland	Pulmonary and other (pleura)	No	Successful	No	8.9	SIT50	4
8	64	Male	Finland	Pulmonary	No	Successful	No	3.8	SIT56	0
9	81	Female	Russia	Other (uterus)	No	Unsuccessful	No	1.8	SIT20	1
10	72	Female	Finland	Pulmonary	No	Successful	No	2.9	SIT53	3
11	70	Male	Finland	Pulmonary	No	Unsuccessful	No	1.6	SIT47	0
12	48	Male	Finland	Pulmonary	No	Not known	No	3.1	SIT383	1
13	78	Male	Finland	Other (pleura)	No	Successful	No	1.9	SIT871	0
14	87	Male	Finland	Other (bone & joint)	No	Not known	No	2.5	SIT50	6
15	25	Male	Cameroon	Pulmonary	No	Successful	No	1.6	SIT20	2
16	68	Male	Finland	Pulmonary	No	Successful	PZA	1.2	F343	0
17	25	Female	Somalia	Other (lymph node)	No	Successful	No	2.8	F427	0
18	45	Male	Finland	Pulmonary	No	Successful	No	1.2	SIT42	0

[a]Antibiotic abbreviations: INH, isoniazid; SM, streptomycin; PZA, pyrazinamide.
[b]Treatment outcome classified according to the WHO standard.
[c]Indicates the time difference between the sampling dates of the first positive *M. tuberculosis* culture in the first and second disease episode.

mutation for each SNP detected is listed in the Supplementary material (Table S1).

In predicting spoligotype from WGS data by SpolPred, the results were identical for 28 isolates and discordant for eight isolates. The eight discordant spoligotype profiles all had the same misclassification, an exact match to spacer 31 was detected by SpolPred, but not by blotting. However, spacer 31 was detected in 14 other isolates by both blotting and SpolPred. Rechecking of the original spoligotype blots did not resolve the discrepancy. Recurrent isolates belonged to Haarlem (n = 11, 61%), T (n = 5, 28%), LAM (n = 4, 22%), and Ural (n = 1, 6%) lineages. The distribution of spoligotype lineages was similar to what has been observed for *M. tuberculosis* isolates in Finland previously [11].

Based on conventional drug susceptibility testing, three patients were infected with drug-resistant isolates. Isolates from one patient were resistant to isoniazid (first episode) and isoniazid combined with streptomycin (second episode), and isolates from the second patient were resistant to isoniazid alone (both episodes). In the third patient, the isolate from the second episode was resistant to pyrazinamide by the phenotypic method. This was confirmed by PhyResSE analysis. Moreover, the isolate from the first episode of the same patient was found to carry the same high-probability pyrazinamide resistance mutation encoding a His51Gln amino acid change in the PncA. However, the pyrazinamide resistance mutations could not be confirmed with the Mykrobe tool (23 November 2015). Additional mutations indicating resistance were not detected from the WGS data.

Discussion

Recurrent TB, and particularly relapsed cases can be seen as a proxy for the quality of the national TB programme [16]. To our knowledge, this is the first population-based study in a low-TB-incidence country that used WGS for the investigation of recurrent TB. The overall rate of recurrence (0.6%) detected during our 19-year study period is in line with other low-TB-incidence countries [4,17,18], even if not standardized for the duration of follow up.

The difference in SNPs between the majority of the consecutive isolates in relapse cases observed in our study (0 to 6) was comparable to a study from Malawi finding the number of SNPs between 0 and 8 [8]. In another study from three high-incidence countries, the average number of mutations observed among relapse cases was 0.47 SNPs [9], which is lower than was found in our study (1.76 SNPs). This is most likely a result of the shorter time period between the two isolates used in their study (17–60 weeks) compared with ours (1.0–9.8 years). However, we found no direct relation between the number of years and number of SNPs in isolates from the same patient. This suggests that there is no steady mutation rate per year but the rate is probably dependent on factors such as patient characteristics, treatment regimen, treatment compliance, strain lineage or type. Due to the small number of relapse cases observed in Finland, it was not possible to assess statistically the correlation between the time and SNPs observed in relapse isolates.

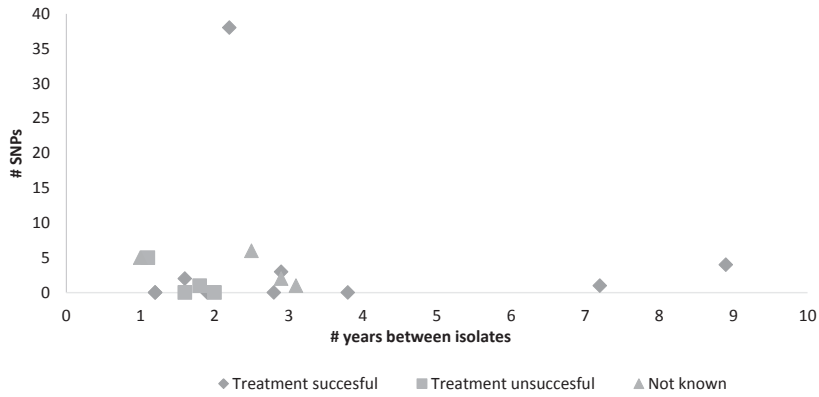

FIG. 2. The number of single nucleotide polymorphisms (SNPs), number of years between *Mycobacterium tuberculosis* isolates, and treatment outcome of 18 cases of relapsed tuberculosis, Finland, 1995–2013.

In most cases, TB relapse versus re-infection was distinctly defined by both WGS and spoligotyping. However, in those cases where the second disease episode was caused by an *M. tuberculosis* isolate that is dominant in the Finnish population (such as SIT53 or SIT47), re-infection cannot be definitely ruled out. In one case, the difference between the two *M. tuberculosis* isolates was 38 SNPs, although both episodes occurred within 2 years. This difference exceeds the average number of SNPs detected in this and other studies comparing consecutive isolates of the same patient or in a transmission chain (0–10 SNPs) [9,12,19,20]. Based on medical records, appropriate treatment was prescribed for this patient, but compliance was poor, and the patient received high-dose corticosteroids for several months during TB treatment, which may have made the patient more susceptible to *M. tuberculosis* relapse or re-infection. The *M. tuberculosis* isolate of the first disease episode (H0901/98) had 20 unique SNPs and the second isolate (H1015/00) had 18 unique SNPs compared with reference sequence (*M. tuberculosis* H37Rv). This suggests that the second isolate did not evolve directly from the first, making the relapse theory less likely. This patient developed a third culture-positive disease episode 3 years after the second episode, but unfortunately, no isolate was available for analysis from the third episode.

As WGS is likely to become the standard for typing of *M. tuberculosis* isolates in the near future, it is important to maintain comparability with data obtained with previously used genotyping methods, such as spoligotyping. When the SpolPred tool was used to predict spoligotype profiles from sequences, results were identical except for one spacer. Further studies are needed to assess whether this is a systematic error either in the WGS analysis tool or in the interpretation of the traditional spoligotype blots.

Two publicly available online tools, PhyResSE and Mykrobe, were used to search drug-resistance-related mutations from WGS data. However, only the mutation conferring resistance to pyrazinamide was identified with the PhyResSE tool, whereas neither tool was able to detect drug-resistance-conferring mutations in any of the four isolates phenotypically resistant to streptomycin or isoniazid. This may be a result of resistance caused by previously unknown mutations, incorrect interpretation of the phenotypic assays, or low coverage of the sequence data. However, the latter was checked and, according to PhyResSE, 100% coverage for all regions known to be associated with drug resistance was obtained for all of the 36 isolates.

To conclude, in our population-based study spanning 19 years of national surveillance on recurrent TB cases, WGS seemed to concur well with spoligotyping in discriminating between relapse and re-infection in a low-incidence country. The results suggest that there is no steady mutation rate per year but it may depend on multiple host-, strain- and treatment-related factors.

Acknowledgements

Toni Huovinen, Tiina Nurmio, Tuula Rantasalo, Arto Talvitie, and the staff of the Finnish Microarray and Sequencing Centre, especially Ville Mäkelä, are thanked for excellent technical assistance. The study was supported by the Tampere Tuberculosis Foundation.

Transparency declaration

The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary materials related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2016.03.014.

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